Sunil K. Deshmukh
Mukund V. Deshpande
Kandikere R. Sridhar *Editors*

Fungal Biopolymers and Biocomposites

Prospects and Avenues

Springer

Fungal Biopolymers and Biocomposites

Sunil K. Deshmukh • Mukund V. Deshpande •
Kandikere R. Sridhar
Editors

Fungal Biopolymers and Biocomposites

Prospects and Avenues

Springer

Editors
Sunil K. Deshmukh
Research and Development Division
Greenvention Biotech Pvt. Ltd.
Pune, Maharashtra, India

Mukund V. Deshpande
Research and Development Division
Greenvention Biotech Pvt. Ltd.
Pune, Maharashtra, India

Kandikere R. Sridhar
Department of Biosciences
Mangalore University
Mangalore, Karnataka, India

ISBN 978-981-19-0999-3 ISBN 978-981-19-1000-5 (eBook)
https://doi.org/10.1007/978-981-19-1000-5

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Foreword

Plastics have revolutionized the modern world. Suffice to mention that life without polymers would be one of unimaginably and unbearably lower quality. Natural macromolecules including proteins, polysaccharides and polynucleotides are the basis of life. Humans have exploited these naturally occurring polymers in several ways even much before they were recognized, on a scientific basis, as macromolecules. For instance, cellulose in wood and cotton has been used to build structures and create clothing, and proteins from silkworms have been made into silk for centuries.

The spectacular growth of the petrochemical industry in the mid-twentieth century played a crucial role in the development of polymers in that period of time, providing inexpensive and abundant access to monomers (e.g. olefins). These synthetic macromolecules not only replaced the existing materials such as wood, metals and glass in specific applications, but entirely new polymeric materials were developed thanks to their low cost, versatility and outstanding mechanical properties.

Nevertheless, plastics are a double-edged sword: a boon to the modern society but at the same time a major source of environmental pollution. Indeed, one of the grand challenges of the twenty-first century is to solve the environmental problem caused by the plastic litter—including single-use plastics used as packaging materials. The magnitude and the complexity of the problem created by plastic litter—white pollution—is of global proportion. It is estimated that 8300 million tonnes of plastics was cumulatively produced globally, from 1950 to 2015, of which 6300 million tonnes ended up as waste. Roughly 800 million tonnes (12%) of this waste was incinerated and 600 million tonnes (9%) recycled, only 10% of which was recycled more than once. Roughly 60% (4900 million tonnes) of all the plastics ever produced was discarded, ending up as a landfill or as litter in the natural environment. If current practices regarding plastic use and waste management are perpetuated, this will lead, inevitably, to the accumulation of 12,000 million tonnes of plastics in the environment by 2050.

The above-cited numbers make it abundantly clear that the socially and economically redundant linear 'take-make-use-dispose' economy is urgently in need of replacement by a circular economy. Against this backdrop, the bio-based economy has garnered an upsurge of interest in both academic and industrial laboratories. The bio-based economy is concerned with the use of renewable biomass to replace the unsustainable use of fossil resources—coal, oil and natural gas—as raw materials for the manufacture of plastics. This must also mesh well with conditions for sustainable development: (1) natural resources should be used at rates that do not unacceptably deplete supplies over the long term, and (2) residues should be generated at rates of no higher than can be readily assimilated by the natural environment. Indeed, sustainable polymers from renewable resources can be viewed as the macromolecular materials of the twenty-first century.

The primary motivation for making bio-based plastics is to reduce GHG emissions. However, the use of renewable resources should not compete with food production, as is the case with the first-generation (1G) biomass, or result in deforestation and loss of biodiversity. The key is to use an unavoidable waste of agro-processing industry as the feedstock. For example, second-generation (2G) biomass comprising lignocellulosic waste in agricultural and forestry residues and food supply chain waste can serve as a raw material for the production of plastics. Annual plants, typical of agricultural operations, are most significant to the future of bioeconomy since they grow considerably faster and involve more frequent harvests. The utilization of associated side stream has a major socio-economic appeal given in the increase in the land footprint of the respective supply chain and disposal costs, if not consumed. Sourcing from agricultural side-streams also opens the possibility for strong economic growth and supports agriculture-centred economies. The potential sources of polymer building blocks and polymers also includes by-products from fisheries and livestock industries as well as those found in downstream biotechnological processes.

Although there have been great advances in efforts to reengineer biopolymers into sustainable materials, processability and performance remain challenging compared to synthetic alternatives. Nonetheless, innovative approaches were demonstrated to enable new property spaces, opening new opportunities for biopolymer constructs. It must be recognized that the replacement of synthetic systems might not be the main goal; instead, new classes of materials can be designed by taking advantage of the inherent multiscale hierarchies already present in the biopolymeric building blocks derived from plant and animal biomass.

The book entitled *Fungal Biopolymers and Biocomposites: Prospects and Avenues* addresses comprehensively the topic of biopolymers that are readily available at industrial scale as sources of sustainable materials for the bioeconomy. Highlighted in the book are the building blocks with low environmental footprint with a performance that matches or competes favourably compared to the selected synthetic counterparts in certain applications.

The fungal cell wall polysaccharides, viz. chitin, chitosan, β-glucan and mannan, and different exopolysaccharides find wide-ranging applications in various industries. The fungal exopolysaccharides (EPSs) such as pullulan, scleroglucan and

botryosphaeran are recognized as high-value bio-macromolecules for pharmaceutical, medicine, food and other industries. Fungal waste generated in mushroom industry, yeast lees from wineries, enzymes and antibiotic industries, to name a few, can be used for the isolation of cell wall polymers and thus serves a purpose of value addition to the ongoing industry. Whereas, the number of fungi have been studied extensively for chitin and/or chitosan, glucan and mannan production solely. Usually, the fungal strains, solid or submerged fermentation and nutritional parameters decide the quality of the biopolymers produced. Furthermore, nowadays, fungal mycelial biocomposite is an upcoming industry for packaging materials. A couple of chapters on biocomposites, laccase-mediated green composite synthesis, bio-surfactants and bio-emulsifiers add value to the contents of the book.

The chapters incorporated in the book meet the criteria such as (1) high standard of scientific writing, (2) relevance and current importance of the topic under discussion, (3) thoroughness of the literature coverage, (4) rigorous, critical, creative and thoughtful assessment of the published data, (5) compelling and thought-provoking discussions and (6) clear identification of future research needs and remaining challenges along with directions for further advancing the topics related to biopolymers.

The Editors have made conscious efforts to select authors of great prestige from different parts of the world, representing diverse disciplines including microbial sciences, chemistry, chemical engineering, polymer science, material chemistry and so on and showcasing the truly multidisciplinary efforts in the burgeoning field of biopolymers. The diversity of concepts, approaches and tactics employed to advance the field of fungal biopolymers is indeed astonishing. The collective efforts of the authors of the chapters presented in the book will surely serve to broaden the foundation that supports future growth in fungal biopolymers and the influx of a new idea that growth brings.

It is my firm belief that the book dealing with critical analysis of science, technology and sustainability aspects of fungal biopolymers will prove to be a real advancement in comparison to previous publications/articles on this topic, which is mostly scattered and make the book genuinely worth reading. The collection of information and knowledge incorporated in the book will surely inspire the students and scientific community as well as entrepreneurs and technologists by way of introduction to fundamentals, exposure to state-of-the-art developments and (potential) applications.

Polymer Science and Engineering Division, CSIR-National Chemical Laboratory, Pune, India

Prakash P. Wadgaonkar

Preface

Fungi are multifaceted microorganisms, standing next to insects in biodiversity. They are mainly responsible for recycling organic matter and also cause diseases in plants, humans and animals. This group is less explored, pertaining to applications in various biotechnological, industrial, sustainable agriculture and environmental protection. However, fungi are fairly explored for the production of bioactive metabolites for pharmaceutical/agricultural relevance, biofuels and enzymes in food/textile industries. They are also one of the principal organisms necessary for sustainable environment owing to their multiple roles in the production of biocomposites. The vegetative growth of filamentous fungi (mycelium) has attracted increasing academic and commercial attention over the past decades as a new form of low-energy biofabrication and waste recycling. Mycelium has the capacity to bind the organic matter through a network of hyphal microfilaments as a natural biological process able to be exploited to produce both low-value materials (packaging) and high-value composite materials (building materials) from vastly available agricultural and industrial residues as raw materials with meagre commercial value. The mycelium binder constituent interfaces a dispersed phase of agricultural residues (as substrate filler) and functions as a load transfer medium between the typically fibrous agricultural residues within the composite similar to the matrix phase of a polymer composite. These polymer composites could be useful as packaging material, mycelial bricks, materials for shoe sole, leather-like material for biofabrication, alternative insulation material, termite-resistant composites, furniture and fixations and so on. Besides, mycelial biopolymers are also useful in oil recovery, production of bakery products, for advanced electrocatalysis, bioemulsifiers, bioprotectants and biosurfactants. Fungi are also well known to produce a large number of polysaccharides useful in various pharmaceutical, nutraceutical and industrial purposes.

In brief, the book discusses the applications of filamentous fungi in sustainable industrial applications and industrial developments with environmental safety. It describes the fungal biopolymers, bio-composites and other macromolecules in three parts. Part I includes some aspects of biopolymers from fungi, fermentation processes for the production of fungal biopolymers with industrial applications, fungal

hydrophobins, the economic, non-seasonal source for the production of chitin and chitosan, fungal exopolymeric substances and their applications, production and application of nanofibres from pullulan, using fungal biopolymers for enhanced oil recovery and polysaccharides from filamentous fungi as effective biosurfactants and bioemulsifiers. Part II describes the challenges and advantages of building with mycelium-based composites, growth factors that affect the material properties, applications of fungal mycelium-based functional biomaterials, fungal biopolymers as an alternative construction material, packaging applications of fungal mycelium-based biodegradable composites, fungi for material futures with role and design. Part III covers topics like the production of bioresins from fungal mycelia, laccase-mediated green composite synthesis, marine fungi as a source of biosurfactants and bioemulsifiers, and lignin fungal depolymerization from substrate characterization to oligomer valorization, and one chapter covers various aspects of blue pigment xylindein, in green technology. The contributors are experts in their respective fields from countries, namely Australia, Brazil, China, France, India, Italy, Oman, Turkey, the United States of America and the United Kingdom. We are thankful to all the contributors for their meticulous correspondence, timely submission and revision of the chapters. We are also grateful to reviewers for their critical comments in a short period. Our gratitude is to Springer Nature for publishing the book.

Pune, India — Sunil K. Deshmukh
Pune, India — Mukund V. Deshpande
Mangalore, India — Kandikere R. Sridhar

Contents

Part I Fungal Biopolymers

Biopolymers from Fungi and Their Applications 3
S. G. Tupe, Sunil K. Deshmukh, R. B. Zambare, A. A. Tripathi, and Mukund V. Deshpande

Fermentation Processes for Production of Fungal Biopolymers with Industrial Applications 15
M. C. Veiga, A. C. F. Piazzi, and M. A. Mazutti

Fungal Hydrophobins 33
Vandana Ghormade, S. G. Tupe, Ejaj Pathan, and Mukund V. Deshpande

Fungal Exopolymeric Substances and Their Applications 49
Murat Topal and E. Işıl Arslan Topal

Production and Application of Nanofibres from Pullulan 73
N. Vigneshwaran and Komal Saraf

Using Fungal Biopolymers for Enhanced Oil Recovery 85
S. H. Al-Araimi, S. N. Al-Bahry, and Y. M. Al-Wahaibi

Fungal Polysaccharides as Biosurfactants and Bioemulsifiers 105
Luciana Luft

Part II Bio-composites

Challenges and Advantages of Building with Mycelium-Based Composites: A Review of Growth Factors that Affect the Material Properties 131
Ali Ghazvinian and Benay Gürsoy

Applications of Fungal Mycelium-Based Functional Biomaterials 147
Sehrish Manan, Omer Muhammad Atta, Ajmal Shahzad, Mazhar Ul-Islam, Muhammad Wajid Ullah, and Guang Yang

Fungal Biopolymers as an Alternative Construction Material 169
Sabu John

Packaging Applications of Fungal Mycelium-Based Biodegradable Composites 189
Rejeesh C. Rajendran

Fungi for Material Futures: The Role of Design 209
N. Ivanova

Part III Other Macromolecules

Production of Bioresins from Fungal Mycelia 255
Lai Jiang

Laccase Mediated Green Composite Synthesis: A Name Synonymous with Each Other 295
Komal Agrawal, Shiva Vaishnavi, and Pradeep Verma

Marine Fungi as a Source of Biosurfactants and Bioemulsifiers 313
Rossana Pitocchi, Alessandra Piscitelli, and Paola Giardina

Lignin Fungal Depolymerization: From Substrate Characterization to Oligomers Valorization 329
Shruthi Meenakshisundaram, Estelle Léonard, Claire Ceballos, and Antoine Fayeulle

From Blue Pigment to Green Technology: Properties and Applications of Fungi-Derived Pigment Xylindein 393
G. Giesbers, M. J. Brodeur, R. C. Van Court, S. C. Robinson, and O. Ostroverkhova

Editors and Contributors

About the Editors

Sunil K. Deshmukh received his Ph.D. in Mycology from Dr. H.S. Gour University, Sagar (M.P.) in 1983. He is a veteran industrial mycologist who spent a substantial part of his career at Hoechst Marion Roussel Limited (now Sanofi India Ltd.), Mumbai, and Piramal Enterprises Limited, Mumbai, in drug discovery. He has to his credit eight patents, 135 publications and 14 books on various aspects of fungi and natural products of microbial origin. He is a president of Association of Fungal Biologists and the past president of the Mycological Society of India (MSI). He is a fellow of MSI, the Association of Biotechnology and Pharmacy, the Society for Applied Biotechnology and Maharashtra Academy of Science. He was a Fellow at Nano Biotechnology Centre, TERI, New Delhi, and Adjunct Associate Professor in Deakin University, Australia, till Jan 2019 and had been working towards the development of natural food colours, antioxidants, and biostimulants through nanotechnology intervention. He is also an advisor to Agpharm Bioinnovations LLP, Patiala, Punjab, India, Greenvention Biotech, Pune, India.

Mukund V. Deshpande obtained his Ph.D. in 1982 in Biochemistry and D.Sc. in Microbiology from the University of Pune in 1994. He has been working extensively on the use of fungi and fungal products in Biotechnology. Dr. Deshpande successfully completed more than 35 research projects funded by national and international funding agencies like Indo-Swiss Collaboration in Biotechnology (ISCB) on the development of mycoinsecticide, Indo-Belarus programme of DBT on biopesticides, Indo-Mexico programme on fungal dimorphism, to name a few. He is an elected fellow of Maharashtra Academy of Sciences (FMASc 1994) and the Society for Biocontrol Advancement (FSBA 2010). He has to his credit more than 150 research papers, reviews and chapters, eight patents, eight books and a number of popular articles. He has his own start-up Greenvention Biotech located in Uruli Kanchan, Pune, for the translational activities in agricultural biotechnology.

Kandikere R. Sridhar is an adjunct professor in the Department of Biosciences, Mangalore University and Yenepoya (Deemed to be University). His main areas of research is 'Diversity and Ecology of Fungi of the Western Ghats, Mangroves and Marine Habitats'. He was an NSERC postdoctoral fellow/visiting professor in Mount Allison University, Canada; Helmholtz Centre for Environmental Research-UFZ and Martin Luther University, Germany; Center of Biology, University of Minho, Portugal. He was a recipient of The Shome Memorial Award (2004), became Vice-President (2013), President (2018) and Lifetime Achievement Awardee (2019) by the Mycological Society of India. He was a recipient of The Fellow of Indian Mycological Society, Kolkata (2014), Distinguished Asian Mycologist (2015) and Outstanding Leader in Education and Research, Association of Agricultural Technology of Southeast Asia (2016). He was awarded UGC-BSR Faculty Fellowship (2014–2017). He is one of the world's top 2% scientists in the field of mycology (2020–2021). He has over 450 publications and edited eight books.

Contributors

Komal Agrawal Bioprocess and Bioenergy Laboratory, Department of Microbiology, Central University of Rajasthan, Kishangarh, Ajmer, Rajasthan, India

S. H. Al-Araimi Department of Biology, College of Science, Sultan Qaboos University, Muscat, Sultanate of Oman

S. N. Al-Bahry Department of Biology, College of Science, Sultan Qaboos University, Muscat, Sultanate of Oman

Y. M. Al-Wahaibi A'Sharqiyah University, Ibra, Sultanate of Oman

Omer Muhammad Atta Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan, People's Republic of China

M. J. Brodeur Department of Physics, Oregon State University, Corvallis, OR, USA

Claire Ceballos Université de technologie de Compiègne, ESCOM, TIMR (Integrated Transformations of Renewable Matter), Centre de Recherche Royallieu, CS 60 319, Compiègne Cedex, France

R. C. Van Court Department of Wood Science and Engineering, Oregon State University, Corvallis, OR, USA

Sunil K. Deshmukh Research and Development Division, Greenvention Biotech Pvt. Ltd., Pune, Maharashtra, India

Mukund V. Deshpande Research and Development Division, Greenvention Biotech Pvt. Ltd., Pune, Maharashtra, India

Antoine Fayeulle Université de technologie de Compiègne, ESCOM, TIMR (Integrated Transformations of Renewable Matter), Centre de Recherche Royallieu, CS 60 319, Compiègne Cedex, France

Benay Gürsoy Department of Architecture, Penn State University, University Park, PA, USA

Ali Ghazvinian Department of Architecture, Penn State University, University Park, PA, USA

Vandana Ghormade Nanobioscience, Agharkar Research Institute, Pune, Maharashtra, India

Paola Giardina Department of Chemical Sciences, University Federico II, Naples, Italy

G. Giesbers Department of Physics, Oregon State University, Corvallis, OR, USA

N. Ivanova Inclusive Design for Business Impact, The Helen Hamlyn Centre for Design, Royal College of Art, London, UK

Lai Jiang Department of Mechanical Engineering, Prairie View A&M University, Prairie View, TX, USA

Sabu John School of Engineering, RMIT University, Bundoora East Campus, Bundoora, VIC, Australia

Estelle Léonard Université de technologie de Compiègne, ESCOM, TIMR (Integrated Transformations of Renewable Matter), Centre de Recherche Royallieu, CS 60 319, Compiègne Cedex, France

Luciana Luft Department of Chemical Engineering, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil

Sehrish Manan Biofuels Institute, School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang, People's Republic of China
Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan, People's Republic of China

M. A. Mazutti Departamento de Engenharia Química, Centro de Tecnologia, Universidade Federal de Santa Maria, Santa Maria, Brazil

Shruthi Meenakshisundaram Université de technologie de Compiègne, ESCOM, TIMR (Integrated Transformations of Renewable Matter), Centre de Recherche Royallieu, CS 60 319, Compiègne Cedex, France

O. Ostroverkhova Department of Physics, Oregon State University, Corvallis, OR, USA

Ejaj Pathan Department of Biotechnology, Indian Institute of Technology-Bombay, Mumbai, India

A. C. F. Piazzi Departamento de Engenharia Química, Centro de Tecnologia, Universidade Federal de Santa Maria, Santa Maria, Brazil

Alessandra Piscitelli Department of Chemical Sciences, University Federico II, Naples, Italy

Rossana Pitocchi Department of Chemical Sciences, University Federico II, Naples, Italy

Rejeesh C. Rajendran Department of Mechanical Engineering, Federal Institute of Science and Technology, Angamaly, Kerala, India

S. C. Robinson Department of Wood Science and Engineering, Oregon State University, Corvallis, OR, USA

Komal Saraf ICAR-Central Institute for Research on Cotton Technology, Mumbai, India

Ajmal Shahzad Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan, People's Republic of China

Murat Topal Department of Chemistry and Chemical Processing Technologies, Tunceli Vocation School, Munzur University, Tunceli, Turkey

E. Işıl Arslan Topal Department of Environmental Engineering, Faculty of Engineering, Fırat University, Elazığ, Turkey

A. A. Tripathi Research and Development Division, Greenvention Biotech Pvt. Ltd., Pune, Maharashtra, India

S. G. Tupe Research and Development Division, Greenvention Biotech Pvt. Ltd., Pune, Maharashtra, India

Mazhar Ul-Islam Department of Chemical Engineering, College of Engineering, Dhofar University, Salalah, Sultanate of Oman

Muhammad Wajid Ullah Biofuels Institute, School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang, People's Republic of China
Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan, People's Republic of China

Shiva Vaishnavi Bioprocess and Bioenergy Laboratory, Department of Microbiology, Central University of Rajasthan, Kishangarh, Ajmer, Rajasthan, India

M. C. Veiga Departamento de Engenharia Química, Centro de Tecnologia, Universidade Federal de Santa Maria, Santa Maria, Brazil

Pradeep Verma Bioprocess and Bioenergy Laboratory, Department of Microbiology, Central University of Rajasthan, Kishangarh, Ajmer, Rajasthan, India

N. Vigneshwaran ICAR-Central Institute for Research on Cotton Technology, Mumbai, India

Guang Yang Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan, People's Republic of China

R. B. Zambare Research and Development Division, Greenvention Biotech Pvt. Ltd., Pune, Maharashtra, India

Part I
Fungal Biopolymers

Biopolymers from Fungi and Their Applications

S. G. Tupe, Sunil K. Deshmukh, R. B. Zambare, A. A. Tripathi, and Mukund V. Deshpande

Abstract The cell wall polysaccharides of fungi *viz.* β-glucan, chitin, chitosan, and mannan and different fungal exopolysaccharides find wide-ranging applications in various industries. The fungal exopolysaccharides (EPSs) such as pullulan (*Aureobasidium*), scleroglucan (*Sclerotium*), and botryosphaeran (*Botryosphaeria*) are recognized as high value bio-macromolecules for pharmaceuticals, medicine, foods and other industries. The fungal waste is generated in mushroom industry, wineries (*Saccharomyces* and non-*Saccharomyces* yeasts), enzyme (*Aspergillus, Trichoderma*), and antibiotic (*Penicillium*) industries, to name a few. Whereas, the genera like *Absidia, Benjaminiella, Gongronella, Rhizopu, Saccharomyces* and others have been studied extensively for chitin and/or chitosan, glucan, mannan production. Usually the fungal strains, solid or submerged fermentation, nutritional parameters decide the quality of the biopolymers produced.

Keywords Biopolymers · Cell wall · Chitin/chitosan · Commercial potential · Exopolysaccharides · Glucans hydrophobins · Mannans

1 Introduction

Every year up to 180 m tons of different polymers are generated for their use in different fields (Castillo et al. 2015). The plant as well as petroleum -based polymers though widely used have the disadvantage of limited resources and the negative impact on the environment of latter is a concern too. In this regard, use of microbial sources can be a viable option (Donot et al. 2012). Fungal biopolymers have attracted researchers for their variety of applications owing to their biological and biophysical characteristics. In general, biopolymers being complex chains of exo-polysaccharides of microbial origin and have capacity to form viscous solutions or gels in aqueous system compatibility with different salts for a varied range of

S. G. Tupe · S. K. Deshmukh · R. B. Zambare · A. A. Tripathi · M. V. Deshpande (✉)
Research and Development Division, Greenvention Biotech Pvt. Ltd., Pune, Maharashtra, India
e-mail: greenventionbiotech@gmail.com

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_1

pH and temperature, high solubility water, and compatibility along with other polysaccharides. In addition to the exopolysaccharides, in case of fungi the polymers of cell wall like chitin, chitosan, glucan and mannans have also significant applications in industry, agriculture and in healthcare too (Ghormade et al. 2017). The fungal biopolymers have not been sufficiently explored for their commercial potential. Furthermore, the increasing interest in biopolymers production is because of availability of excess of glycerol generated in biodiesel production which could be employed as a substrate for fungal growth for biopolymer production (Araújo et al. 2016).

The following section will give the overview of fungal biopolymers.

1.1 The Fungal Cell Wall Polymers

The fungal cell wall is a protective cover with immense plasticity and is indeed essential for the cellular integrity and viability. It facilitates interactions with the exterior environment through adhesins as well as signal transduction complexes. The cell wall of fungi is specifically designed in different layers with main innermost structural component, chitin, β-1,4- linked *N*-acetylglucosamine polymer and its deacetylated form chitosan (mainly glucosamine), while matrix components like glucans and mannans and glycosylated proteins (Table 1). Glucan and chitin are synthesized at the plasma membrane (PM). Usually, the nascent enzyme complexes are transported to the PM through secretory vesicles and eventually activated when insertion into the PM. While endoplasmic reticulum and Golgi are sites for the synthesis of mannans and other glycoconjugates. Then they possibly conjugate to proteins in cell wall, and subsequently brought to the cell wall by secretory vesicles. Nucleoside diphosphate-sugars are substrates for all synthases. Indeed, enzymes

Table 1 Common fungal cell wall components

Division	Structural/fibrous polymers	Matrix/gel-like polymers
Chytridiomycota (*Allomyces*)	Chitin (> 50%), Glucan (>16%)	Glucan
Zygomycota (*Absidia, Benjaminiella, Mucor, Rhizopus*)	Chitin (10%), Chitosan (30%)	Polyglucuronic acid, α-1,2/ α-1,6-mannosyl and β-1,5/β-1,6-Glucuro nomannoproteins, Polyphosphate
Ascomycota (*Aspergillus,* yeasts)	Chitin (1–40%), β-1,3 and β-1,6, Glucan (40–60%)	α-1,2/ α-1,6- Mannosyl and β-1,5/β-1,6-galacto-furanosyl residues in Galactomannoproteins, α-1,3-Glucan
Basidiomycota, (mushroom)	Chitin (5–30%) β -(1–3), β-(1–6) Glucan (40–60%)	Xylomannoproteins, α-1,3-Glucan

Modified from Gooday (1990), Gow et al. (2017), Khale and Deshpande (1992)

involved in the synthesis of nucleoside sugars are found to be necessary for the construction of the cell wall as well as for rate-limiting too (Gow et al. 2017).

1.1.1 Chitin and Chitosan

Naturally abundant chitin is a polysaccharide, it was first isolated from the cell wall of mushrooms by Braconnot, where it was designated as fungine. During 1823, fungine was re-designated as chitin by Odier. It is a polymer of β 1,4-linked *N*-acetyl glucosamine (GlcNAc) abundantly present in crustaceans, insects, fungi, including a few algae as a main structural component (Deshpande 1986; Ghormade et al. 2017).

Chitin exists in three forms in nature: alpha (α), beta (β) and gamma (γ). The first one has antiparallel chains of β-1,4-linked GlcNAc. Most of the natural chitin is in α-form. The β-chitin has parallel alignment of chains while γ-chitin, which is exists in insects, which has two chains those are parallel in one direction and the third chain is antiparallel. The β-chitin is not common in living entities and reported in spines of diatoms and squid pens to name a few. In nature, the exocellular β-chitin spines of *Thalassiosira fluviatilis*, a centric diatom, are the only in pure form of chitin *i.e.* 100% degree of acetylation. Otherwise in rest of the organisms, including fungi, chitin is a polymer of both GlcN and GlcNAc with variable percentages. In general, chitosan is >70% in deacetylated form. Chemically chitin is a linear polymer of β-1,4-linked *N*-acetylglucosamine (GlcNAc) units, whereas its deacetylated form, chitosan contains glucosamine (GlcN) residues (Ghormade et al. 2017).

The content of chitin in the fungal wall differs (2–61%) depending on the vegetative morphological phase (unicellular yeast or filamentous) of the fungus (Khale and Deshpande 1992). In chitin synthesis, the enzymes from carbon as well as nitrogen metabolism are brought together by an enzyme glucosamine 6 phosphate synthase (EC 2.6.1.16) to synthesize uridine diphosphate-*N*-acetyl glucosamine (UDP-GlcNAc) from glucose and glutamine. The chitin synthase (CS, EC 2.4.1.16) is an important enzyme for further synthesis of chitin from UDP-GlcNAc.

Briefly, glucosamine 6 phosphate is synthesized with the help of glucosamine 6 phosphate synthase (EC 2.6.1.16) which is further acetylated to form *N*-acetylglucosamine 6 phosphate by an enzyme glucosamine 6 phosphate *N*-acetyl transferase (EC 2.3.1.4). UDP-N-GlcNAc was further synthesized in two steps with the involvement of 2 enzymes, namely phosphoacetyl glucosmine mutase (EC 5.4.2.3) and UDP *N*-acetylglucosamine phosphorylase (EC 2.7.7.23). The final step of chitin synthesis involves sequential transfer of GlcNAc from UDP-GlcNAc to non-reducing end of growing polymer, which is catalysed by CS. The zymogenic CS, transported to the plasma membrane through a vesicle (chitosome), is activated by proteolysis (Bartnicki-Garcia 2006; Deshpande et al. 1997; Phadatare et al. 1989). Multiplicity of CSs is a common occurrence. Chitnis et al. (2002) reported eight discrete CS genes in *B. poitrasii* as confirmed based on DNA sequence as well as Southern analysis. Earlier six CSs were reported in *Ustilago maydis*, while three are present in *S. cerevisiae* and a dimorphic *Sporothirx*

schenckii. Ten CSs were reported in a zygomycetous fungus *Phycomyces blakesleeanus* (from Chitnis et al. 2002). The role of chitin metabolism in the morphogenesis was explicitly studied using *B. poitrasii* protoplast regeneration in presence of different cell wall polymer synthesis inhibitors (Chaudhary et al. 2013; Chitnis and Deshpande 2002).

The chitosan is not directly synthesized in nature. Chitin deacetylase (CDA; E.C. 3.5.1.41) drives the hydrolysis of acetamido groups of GlcNAc in chitin, thus promoting the conversion to chitosan (Ghormade et al. 2010).

For the hyphal tip elongation the old cell wall chitin is hydrolysed to supply GlcNAc to the growing tip. There are two different pathways to hydrolyze chitin (Chavan and Deshpande 2013). In the first one, a chitinase complex consisting of: endo chitinases (EC 3.2.1.14), *N*-acetyl hexosaminidase (EC 3.2.1.52) and exo-chitinase (EC 3.2.1.201). The second pathway is comprised of two enzymes namely, chitin deacetylase (CDA, EC 3.5.1.41) hydrolyses acetamide group in GlcN by removing acetic acid. Chitosanase (EC 3.2.1.132) perform endo hydrolysis of β-1,4 linkage between GlcN residue in partially acetylated chitosan while exo chitosanase (GlcNase, EC 3.2.1.165) successively removes GlcN residues from non-reducing end of chitosan oligosaccharides (Chavan and Deshpande 2013).

1.1.2 Glucans

Glucan is one of the most abundant structural polysaccharides which represents 40–60% of the cell walls in ascomycetous fungi. Most of the glucans are β- 1,3 and β-1,6 polymers of glucose. In some fungi glucans have α-1,3 and -1,4 linkages too. They are either linear or branched, and also either microfibrillar or amorphous (Ruiz-Herrera and Ortiz-Castellanos 2019). The α-1,3-glucans are copious in the fungal cell walls. While in glycogen, α-1,4 and α-1,6 bonds are present between glycosyl units. The structure of β-glucans is highly complex, linear as well as branched mainly β-1,3 and β-1,6. The glucans and chitin are major structural components of the cell wall of fungi. The most studied β 1,3- glucans come from *Saccharomyces cerevisiae*. Another polysaccharide in *S. cerevisiae* is made up of glucosyl units having β- 1,6 linkages, is called pustulan. While in lichens, lichenan, a polysaccharide having β -1,3 as well as β- 1,4 linkages is mainly present.

Most of the secreted polysaccharides have β-1,6 branches of different lengths and characteristics: botryosphaeran extracted from *Botryosphaeria* sp., grifolan from *Grifola frondosa*, schizophylan from *Schizophylum commune* and scleroglucan from *Sclerotium* sp. The basidiocarp of *Calocybe indica* contains calocyban, chain of glucosyl units linked by β 1,3-bonds with β 1,4- linked branches (For more details refer Ruiz-Herrera and Ortiz-Castellanos 2019). The plasma membrane bound β-glucan synthase (EC 2.4.1.34) is involved in the synthesis of β-1,3-glucan (Garcia-Rubio et al. 2020).

The synthesis of α-1,3 glucan was very well studied in *Schizosaccharomyces pombe*.

The multiplicity of α -1,3- glucan synthases (EC 2.4.1.183) was reported in *S. pombe* (three members), four in *Rhizoctonia solani* and up to seven in *Aspergillus niger* (Ruiz-Herrera and Ortiz-Castellanos 2019).

Many fungi produce extracellularly β-(1,3)- and β-(1,6)-glucans -hydrolysing enzymes, β-(1,3)- and β-(1,6)-glucanases (Kéry et al. 1991). These enzymes are O-glycoside hydrolases (EC 3.2.1-58 and EC 3.2.1.75, respectively), catalyzing the hydrolysis of glycosidic bonds (Martin et al. 2007). *Trichoderma harzianum* produces at least ten different forms of endo-acting β-1,3- glucanases. While *Acremonium persicinum* produced three β-(1,3)-glucanases (two exo- and 1 endo-acting) which were attributed to the expression of three vried genes. Though multiple β-1,6-glucanases were rarely reported, one of the exception is *T. harzianum*, which produces three different β-(1,6)-glucanases coded by three different genes (Martin et al. 2007). It was reported further by Martin et al. (2007) that post-synthesis glycosylation may generate multiple isoforms. For instance, extracellular β-glucanases (Exo1a and Exo1b) in *S. cerevisiae* were reported. All these enzymes have applied value in the food industries, wine industries (to lower viscosity) and also in treating feed grains and serve as a tool for the structural analysis of β-glucans.

1.1.3 Mannans

Mannans represent about 35–40% of fungal cell wall as matrix components. As compared to chitin and β-glucans, mannans are less rigid. Mannans have low permeability as well as porosity, which contribute to the resistance of the fungus against antifungal drugs and other host defence mechanisms. In fungal cell wall short *O*-linked and *N*-linked mannans along with glycoproteins as well as long mannan chains are present. The *O*-linked mannans contain mannose residues linked to the hydroxyl group of either threonine or serine residues, while in *N*-linked mannans, highly branched, are connected to amide group of aspargine residues. The phospholipomannan (PLM) is mainly comprised of long linear chain of β-1,2 mannose unit and inositol covalently linked through a phosphate diester bond to a lipid moiety. In filamentous fungi, galactomannan consists of α-1,2/ α-1,6-mannosyl and β-1,5/β-1,6- galactofuranosyl residues, is observed at the cell wall surface.

Synthesis of *O*- and *N*- linked mannans is similar in both unicellular yeasts and filamentous fungi. However, a difference among unicellular yeasts and filamentous fungi is the structural organization of the long mannan chains in the cell walls. For instance, in yeasts highly branched *N*-linked mannans (100 mannosyl residues) are bound per molecule of protein. Interestingly, mannans without covalent bonding with polysaccharide core of the cell wall cover the surface of the cell wall. On the other hand in*Aspergillus fumigatus*, the long mannans (average, 50 mannose residues/chain) bind to glucan and chitin and thus become an integral part of the cell wall (Henry et al. 2016).

Mannosylation of proteins occurs in endoplasmic reticulum and then in Golgi. Some protein mannosyltransferases are important in the first step of *O*-linked

mannan biosynthesis, addition of mannose residue to a threonine or serine residue. Additional mannose residues are added by the α-1,2 mannosyltransferases (EC 2.4.1.131) which result in a short α-1,2 mannose chains and addition of an α-1,3 mannose by α-1,3 mannosyltransferases (EC 2.4.1.258). While an attachment of α-1,6 mannose residue to core structure is done by the action of α-1,6 mannosyltransferase (EC 2.4.1.232). The enzyme mannosylphosphate transferase (EC 2.4.1.B72) is involved in attachment of phosphomannan group to the branches. For the synthesis of phospholipomannan, mannose inositolphosphoceramide mannosyltransferase (EC 2.4.1.370) is needed (Garcia-Rubio et al. 2020).

Hydrolysis of mannan is catalysed mainly by endo-acting β-mannanases (EC 3.2.1.78) and axo-acting β-mannosidase (EC 3.2.1.25) (Moreira and Filho 2008).

1.1.4 Proteins

Fungi are considered as a promising source for novel proteins with exceptional features. These include: lectins, enzymes and enzyme inhibitors and hydrophobins. These have applications in several medical and biotechnological processes (Erjavec et al. 2012). Number of proteinases have role in fungal morphogenesis (Deshpande 1992; Phadatare et al. 1989).

Heavily glycosylated proteins are usually anchored on cell membrane and cell wall. These proteins play a significant role in morphogenesis, adhesion, and pathogenicity. Mannoproteins are made up of several classes. For instance, glycosyl phosphatidyl inositol (GPI)-modified proteins (almost 88%) are responsible for cell wall synthesis, organisation, remodelling, and virulence too (Ibe and Munro 2021; Pitarch et al. 2008).

1.1.5 Hydrophobins

Hydrophobins are amphipathic proteins play significant role in fungal growth as well as differentiation. The classification of hydrophobins in two classes (I and II) is based on distribution of the cysteines and the clustering of both hydrophobic and hydrophilic residues which affect the solubility and morphology. The aggregates of class I hydrophobins are highly insoluble even in sodium dodecyl sulfate (2% SDS at 100 °C) and can only be dissociated with high concentration of formic acid or trifluoracetic acid. The Class II hydrophobins will be readily dissolved with ethanol (60%) or SDS (2%). Under the microscope, class I hydrophobins show rodlet structures outside the fungal cell wall. Hydrophobins are well characterized with eight conserved cysteine residues (Wösten 2001). The characteristic spacing between cysteine residues distinguishes hydrophobins of Class I and Class II (Wösten and Wessels 1997). According to Kershaw et al. (1998) in the class II hydrophobins the eight residues of cysteine form four disulphide linkages resulting in the formation of four loop-like structures. The characteristic order of cysteine

residues is useful to distinguish the fungal hydrophobins from other cysteine rich proteins.

1.2 Fungal Exopolysaccharides

Number of exopolysaccharides with different structural complexities are synthesized by microorganisms. Some of them remain attached to the cell wall or found in the extracellular medium. The exopolysaccharides are high molecular weight, long-chain polysaccharides consist of branched and repeating units of sugars linked either by β- 1, 4 or β-1, 3linkage or α-1,2 or α- 1,6 linkage. They have distinct physiological roles which include: communication, defence against predation, detoxification of chemicals, to name a few (Osemwegie et al. 2020, Tang and Zhong 2002).

A bacterium, *Leuconostoc mesenteroides* was the first organism identified as exopolysaccharide (EPS) producer (Mahapatra and Banerjee 2013). While different fungi such as *Agaricus blazi*, *Cordyceps* sp., *Ganoderma lucidum*, Grifola *frondosa* and *Lentinus edodes* were reported initially to produce different exopolysaccharides in submerged fermentation. Presently number of fungi are being reported to produce EPS in the fermentation under defined nutritional and fermentation parameters. Mahapatra and Banerjee (2013) have given exhaustive list of EPS producing fungi from all the classes. Only few fungi have been studied to understand the biosynthesis pathway for EPS. Donot et al. (2012) reviewed microbial EPS for their synthesis, secretion, genetics and extraction. The main fungal EPS are: pullulan (*Aureobasidium pullulans*), scleroglucan (*Sclerotium* sp.), schizophyllan (*Schizophyllum commune*), galactan (*Sporobolomyces* sp) and glucan (*Cryptococcus* sp., *Ganoderma lucidum*, *Rhodotorula* sp., *Tremella aurantia, T. fusiformes* and *T. mesenterica*). The synthesis of EPS is not very well understood. Osemwegie et al. (2020) have reviewed current status of EPS from fungi and bacteria with respect to biosynthesis, production and structural studies. According to the literature, the biosynthesis of homopolysaccharides is theoretically differs from that of heteropolysaccharides synthesis. In case of bacteria, heteropolysaccharides are synthesized intracellularly followed by transport into the extracellular environment. On the other hand homopolysaccharides are produced extracellularly. The enzymes such as hexokinases (involved in phosphorylation), enzymes involved in formation of sugar nucleotides (UDP-glucose pyrophosphorylase); glycosyl transferases and finally enzymes required for acylation, acetylation, sulphation and methylation are involved in EPS synthesis, in general. Then EPSs are exported to the exterior surface with the help of specific enzymes or transporters (flippase, permease and ABC transporters) (Osemwegie et al. 2020). The identifiable characteristics of any particular exopolysaccharide are consistent with monomeric units, the kind of linkage between the monomers, and the sequence of linkage.

1.2.1 Pullulan

Bauer (1938) observed the viscous culture broth when a fungal isolate, the then *Pullularia* was grown with excess glucose. In 1958, Bernier suggested the viscosity is due to the exopolysaccharide produced in the medium (from Kelkar 1991). Eventually it was named as pullulan. It is a polymer composed of malto-triose units with linkages as Glu-α-(1–4)- Glu-α-(1–4)-Glu-α-(1–6) (Fig.1a). Many other types of linkages also found in pullulan as a succession of maltotetraose units (-(1–4)-Glu-_-(1–4)-Glu-_-(1–6)-Glu-_-(1–6)) (Kelkar 1991). *Aureobasidium pullulans* (Black yeast) produces high concentrations of pullulan (Jiang 2010; Wu et al. 2009). In fact, *A. pullulans* produces three extracellular glucan components. Major is pullulan (α-1–4 and α-1–6) while acidic β-linked glucan containing β-1,3 and β-1,6 linkages and an acidic heteropolysaccharide containing galactose, glucose, mannose and hexuronic acid are quantitatively less. Pullulan synthesis is not possible by all the strains of *A. pullulans* (Mishra et al. 2018).

The mechanism of pullulan synthesis is not completely understood LeDuy et al. (2014). Simon et al. (1998) suggested that the pullulan and others too are synthesized within the cell in *A. pullulans*, and then are secreted out. Three major enzymes are reported to play crucial role in the biosynthesis of pullulan: α-phosphoglucose mutase, (UDPG, uridine 5′-diphosphate glucose EC 5.4.2.2), which facilitates the interconversion of glucose 1 phosphate and glucose 6 phosphate, while pyrophosphorylase (EC 2.7.7.9) involved in the synthesis of UDP-glucose from glucose 1 phosphate and glucosyltransferase (EC 2.4.1.B64), which catalyzes glycosidic bond formation using sugar donors.

The lipid phosphate is involved in pullulan synthesis from UDPGlc. The lipid phosphate receives two successive glucosyl moieties from UDPGlc to form an

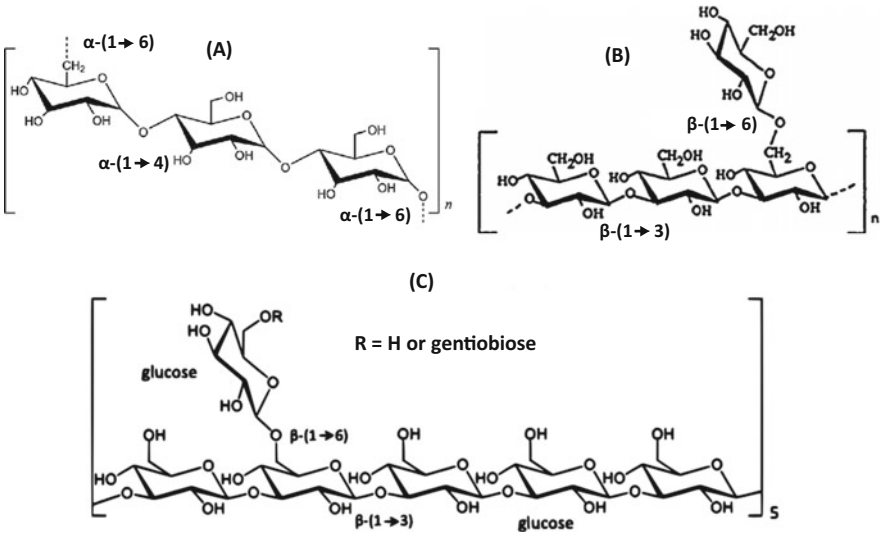


Fig. 1 Structures of Pullulan (**a**), Scleroglucan (**b**) and Botryosphaeran (**c**)

isomaltose molecule (Lipid-P-Glc-α-1-6-Glc), finally receiving a glucosyl unit from UDPG to form an isopanosyl intermediate (Lipid-P-Glc-α-1-6-Glc-α-1-4 Glc). These isopanosyl moieties are further proceed for polymerization to form the pullulan.

The enzymes, namely pullulanase, isopullulanase, neopullulanase, and glucoamylase hydrolyze pullulan. Pullulanase (Pullulan 6-glucanhydrolase, EC 3.2.1.41) can hydrolyse pullulan into maltotriose. It has linkage specificity to α-(1–6) linkage only. Isopullulanase (Pullulan 4-glucanohydrolase, EC 3.2.1.57) hydrolyses pullulan into isopanose (maltosyl- α-1,4-glucose) as the sole product. From pullulan the enzyme produces a mixture of isopanose, isomaltose, and glucose as end products. Neopullulanse (Pullulan 4-glucanohydrolase, EC 3.2.1.135) attacks the α-(1–4) glycosidic linkages on the non-reducing end of the α-(1–6) linkage in pullulan at random and produces panose (D-glucosyl-α-1,6-maltose). Glucoamylase (1,4-α-glucan glucohydrolase, EC 3.2.1.3) hydrolyses pullulan from the non-reducing point producing glucose as a sole product of hydrolysis (Kelkar and Deshpande 1993).

1.2.2 Scleroglucan

A fungus from mycelia sterilia group (fungi which do not produce any sexual or asexual spores), *Sclerotium* sp. produces a β- 1,3 and -1,6 glucan known as Scleroglucan, having diverse branching frequencies, side chain length as well as molecular weight relaying on the strain and /or growth conditions (Fig. 1b). Due to its water-solubility, viscosity and stability against a wide range of temperature, pH and salinity, it has lot of applications as food additives, for enhanced oil recovery, and in cosmotic and pharmaceutical products (Castillo et al. 2015).

The biosyntheses of scleroglucan and oxalic acid are closely linked (Schmid et al. 2011). The uptake of glucose into the cell will be by facilitated diffusion as well as phosphorylation by hexokinase (EC 2.7.1.1) to glucose-6-phosphate (G6P), which is then converted in to G1P by phosphoglucomutase (EC 5.4.2.2) which eventually starts scleroglucan synthesis (Schmid et al. 2011). UTP-glucose-1-phosphate uridylyltransferase synthesizes UDP-glucose from glucose-1- phosphate and UTP. A (1 → 3)-β-glucan synthase (EC 2.4.1.34) polymerizes the backbone chain. The last few steps to the definite branching at every third glucose unit are still unclear.

1.2.3 Botryosphaeran

Botryosphaeria rhodina causes a stem canker in *Eucalyptus* tree. It produces β-glucan type EPS in submerged fermentation on glucose. Structurally it is β-glucan with (1 → 3) (1 → 6)-linkages (Dekker and Barbosa 2019). According to Barbosa et al. (2003) botryosphaeran is a (1 → 3)-β-D-glucan with almost 22% side branching at C-6 (Fig.1C). Furthermore, partial acid hydrolysis studies indicated that on the side branches a single (1 → 6)-β-linked glucosyl, and

(1 → 6)-β-linked gentiobiosyl residues are present. When grown on varied carbohydrates, *B. rhodina* produced botryosphaerans with a same1,3-linked backbone chain but with different levels of branching on C-6 with glucose as well as gentiobiose. Dekker and Barbosa (2019) reported that when *B. rhodina* was grown on glucose or sucrose a branch point occurred on every fifth glucose residue along the backbone chain for the botryosphaeran, while grown on fructose one in every third glucose residues branch point occurred. The membrane-modifying agents such as Tween 80 and soybean oil enhanced production of botryosphaeran without changing its chemical structure. The botrypsphaeran has the status as GRAS (Generally Regarded As Safe). The biopolymer exhibits strong antimutagenic/anticlastogenic activity as chemoprotective agent against many cancer chemotherapy drugs. Similar to other β-glucans, botryosphaeran is also synthesized through complex enzymatic pathway from UDP-glucose (Utama et al. 2020).

Botryosphaeran, is hydrolysed by β-1,3-glucanases mainly produced by *B. rhodina* and *Trichoderma harzianum* (Giese et al. 2006). The hydrolysis products mainly were glucose as well as gentiobiose with some trisaccharide, however, no laminaribiose or tetrasaccharide when hydrolysed by *T. harzianum* glucanase. On the other hand, *B. rhodina* β-1,3-glucanases generated predominantly glucose during botryosphaeran hydrolysis.

1.3 Future Perspectives

The fungal cell wall polymers especially chitosan, glucan and mannan have a lot of applications especially in healthcare industry. A number of fungi produce exopolyssacharides which have been extensively studied using advanced analytical techniques. However, the predictable uniformity in the structural features and functional characteristics is lacking. The structural features are dependent on the composition and combination of monosaccharides used to grow the fungal organism. It is also relevant that in addition to cultivation conditions, the downstream processing to ensure a high recovery yield, with respect to molecular weight, degree of polymerization, purity is also important. The continuous efforts are necessary to understand structure-function relationship and eventually application potential and cost-effective production of fungal biopolymers.

References

Araújo TR, De Oliveira PCL, Cardoso VL, Filho UC, Vieira PA (2016) Biopolymer production using fungus *Mucor racemosus* Fresenius and glycerol as substrate. Polímeros 26:144–151

Barbosa AM, Steluti RM, Dekker RFH, Cardoso MS, Corradi da Silva ML (2003) Structural characterization of botryosphaeran: a (1→3;1→6)-β-d-glucan produced by the ascomyceteous fungus, *Botryosphaeria* sp. Carbohydr Res 338:1691–1698

Bartnicki-Garcia S (2006) Chitosomes: past, present and future. FEMS Yeast Res 6:957–965

Bauer R (1938) Physiology of Dematium Pullulans de Bary. Zentralbl Bacteriol Parasitenkd Infektionskr Hyg Abt2 98:133–167

Castillo NA, Valdez AL, Farina JI (2015) Microbial production of scleroglucan and downstream processing. Front Microbiol 6:1106. https://doi.org/10.3389/fmicb.2015.01106

Chaudhary PM, Tupe SG, Deshpande MV (2013) Chitin synthase inhibitors as antifungal agents. Mini-Rev Med Chem 13:222–236

Chavan SB, Deshpande MV (2013) Chitinolytic enzymes: an appraisal as a product of commercial potential. Biotechnol Progress 29:833–846. https://doi.org/10.1002/btpr.1732

Chitnis M, Deshpande MV (2002) Isolation and regeneration of protoplasts from the yeast and mycelial form of a dimorphic fungus *Benjaminiella poitrasii*: role of chitin metabolism for morphogenesis during regeneration. Microbiol Res 157:29–37

Chitnis M, Munro CA, Brown AJP, Gooday GW, Gow NAR, Deshpande MV (2002) The zygomycetous fungus, *Benjaminiella poitrasii* contains a large family of differentially regulated chitin synthase genes. Fungal Genetics Biol 36:215–223

Dekker RFH, Barbosa A (2019) Botryosphaeran – A fungal exopolysaccharide of the (1→3)(1→6)-β-D-glucan kind: structure and biological functions. In: Extracellular Sugar-Based Biopolymers Matrices, Biologically Inspired Systems, vol 12. Springer Nature, Cham, pp 433–484. https://doi.org/10.1007/978-3-030-12919-4_11

Deshpande MV (1986) Enzymatic degradation of chitin and its biological applications. J Sci Ind Res 45:273–281

Deshpande MV (1992) Proteinases in fungal morphogenesis. World J Microbiol Biotechnol 8:242–250

Deshpande MV, O'Donnell R, Gooday GW (1997) Regulation of chitin synthase activity in the dimorphic fungus *Benjaminiella poitrasii* by external osmotic pressure. FEMS Microbiol Lett 152:327–332

Donot F, Fontana A, Baccou JC, Schorr-Galindo S (2012) Microbial exopolysaccharides: Main examples of synthesis, excretion, genetics, and extraction. Carbohydr Polym 87:951–962

Erjavec J, Kos J, Ravnikar M, Dreo T, Sabotič J (2012) Proteins of higher fungi – from forest to application. Trends Biotechnol 30:259–273

Garcia-Rubio R, de Oliveira HC, Rivera J, Trevijano-Contador N (2020) The fungal cell wall: *Candida, Cryptococcus,* and *aspergillus* species. Front Microbiol 10:2993. https://doi.org/10.3389/fmicb.2019.02993

Ghormade V, Kulkarni S, Doiphode N, Rajamohanan PR, Deshpande MV (2010) Chitin deacetylase: A comprehensive account on its role in nature and its biotechnological applications. In: Mendez-Vilas A (ed) Technology and education topics in applied microbiology and microbial biotechnology. Current Research, Formatex Research Center, Badajoz, pp 1054–1066

Ghormade V, Pathan EK, Deshpande MV (2017) Can fungi compete with marine sources for chitosan production? Int J Biol Macromolec 104(B):1415–1421. https://doi.org/10.1016/j.ijbiomac.2017.01.112

Giese EC, Covizzi LG, Dekker RFH, Monteiro NK, Da Silva CM d L, Barbosa AM (2006) Enzymatic hydrolysis of botryosphaeran and laminarin by β-1,3-glucanases produced by *Botryosphaeria rhodina* and *Trichoderma harzianum* Rifai. Process Biochem 41:1265–1271. https://doi.org/10.1016/j.procbio.2005.12.023

Gooday GW (1990) Inhibition of chitin metabolism. In: Kuhn PJ, Trinci APJ, Jung MJ, Goosey MW, Copping LG (eds) Biochemistry of cell walls and membranes in fungi. Springer, Berlin, pp 60–79

Gow NAR, Latge J-P, Munro CA (2017) The fungal cell wall: structure, biosynthesis, and function. Microbiol Spectrum 5(3) FUNK-0035-2016. https://doi.org/10.1128/microbiolspec

Henry C, Fontaine T, Heddergott C, Robinet P, Aimanianda V, Beau R, Beauvais A, Mouyna I, Prevost M-C, Fekkar A, Zhao Y, Perlin D, Latge J-P (2016) Biosynthesis of cell wall mannan in the conidium and the mycelium of *aspergillus fumigatus*. Cell Microbiol 18:1881–1891

Ibe C, Munro CA (2021) Fungal cell wall: an underexploited target for antifungal therapies. PLoS Pathog 17:e1009470. https://doi.org/10.1371/journal.ppat.1009470

Jiang L (2010) Optimization of fermentation conditions for pullulan production by *Aureobasidium* pullulan using response surface methodology. Carbohydr Polym 79:414–417
Kelkar HS (1991) Studies on pullulan hydrolysing activity from *Sclerotium rolfsii*. A PhD thesis submitted to Pune University, Pune
Kelkar HS, Deshpande MV (1993) Purification and characterization of a pullulan-hydrolysing glucoamylase from *Sclerotium rolfsii*. Stärke/Starch 45:361–368
Kershaw MJ, Wakley G, Talbot NJ (1998) Complementation of the *Mpg1* mutant phenotype in *Magnaporthe grisea* reveals functional relationships between fungal hydrophobins. EMBO J 17:3838–3849
Kéry V, Kogan G, Zajacová K, Slámová K, Masler L, Alföldi J (1991) Hydrolysis of yeast cell-wall glucan by extracellular (1→3)-β-glucanases from *aspergillus Niger*. Enz Microb Technol 13: 87–90
Khale A, Deshpande MV (1992) Dimorphism in Benjaminiella *poitrasii*: cell wall chemistry of parent and two stable yeast mutants. Anton van Leeuwenhoek 62:299–307
LeDuy A, Choplin L, Zajic J, Luong J (2014) Pullulan: properties, synthesis, and applications. In: Encyclopedia of polymer science and technology. https://doi.org/10.1002/0471440264.pst620
Mahapatra S, Banerjee D (2013) Fungal exopolysaccharide: production, composition and applications. Microbiol Insights 6:1–16. https://doi.org/10.4137/MBI.S10957
Martin K, McDougall BM, Mcllroy S, Chen JJ, Seviour RJ (2007) Biochemistry and molecular biology of exocellular fungal β-(1,3)- and β-(1,6)-glucanases. FEMS Microbiol Rev 31:168–192
Mishra B, Zamare D, Manikanta A (2018) Selection and utilization of agro-industrial waste for biosynthesis and hyper-production of pullulan: A review. In: Varjani S, Parameswaran B, Kumar S, Khare S (eds) Biosynthetic technology and environmental challenges. Springer Nature, Singapore, pp 89–103. https://doi.org/10.1007/978-981-10-7434-9_6
Moreira LR, Filho EX (2008) An overview of mannan structure and mannan-degrading enzyme systems. Appl Microbiol Biotechnol 79:165–178
Osemwegie OO, Adetunji CO, Ayeni EA, Adeiobi OI, Arise RO, Nwonuma CO, Oghenekaro AO (2020) Exopolysaccharides from bacteria and fungi: current status and perspectives in Africa. Heliyon 6:e04205. https://doi.org/10.1016/j.heliyon.2020.e04205
Phadatare S, Srinivasan MC, Deshpande MV (1989) Evidence for the involvement of serine protease in the conidial discharge of *Conidiobolus coronatus*. Arch Microbiol 153:47–49
Pitarch A, Nombela C, Gil C (2008) Collection of proteins secreted from yeast protoplasts in active cell wall regeneration. Meth Mol Biol 425:241–263
Ruiz-Herrera J, Ortiz-Castellanos L (2019) Cell wall glucans of fungi. A review. Cell Surface 5: 100022. https://doi.org/10.1016/j.tcsw.2019.100022
Schmid J, Meyer V, Volker S (2011) Scleroglucan: biosynthesis, production and application of a versatile hydrocolloid. Appl Microbiol Biotechnol 91:937–947. https://doi.org/10.1007/s00253-011-3438-5
Simon L, Bouchet B, Bremond K, Gallant DJ, Bouchonneau M (1998) Studies on pullulan extracellular production and glycogen intracellular content in *Aureobasidium pullulan*. Can J Microbiol 44:1193–1199
Tang YJ, Zhong JJ (2002) Exopolysaccharide biosynthesis and related enzyme activities of the medicinal fungus, *Ganoderma lucidum*, grown on lactose in a bioreactor. Biotechnol Lett 24: 1023–1026
Utama GL, Dio C, Lembong E, Cahyana Y, Balia RL (2020) Microorganism-based β-glucan production and their potential as antioxidant. Sys Rev Pharm 11:868–873
Wösten H, Wessels J (1997) Hydrophobins, from molecular structure to multiple functions in fungal development. Mycoscience 38:363–374
Wösten HAB (2001) Hydrophobins: multipurpose proteins. Ann Rev Microbiol 55:625–646
Wu S, Jin Z, Tong Q, Chen H (2009) Sweet potato: a novel substrate for pullulan production by *Aureobasidium pullulans*. Carbohydr Polym 76:645–649

Fermentation Processes for Production of Fungal Biopolymers with Industrial Applications

M. C. Veiga, A. C. F. Piazzi, and M. A. Mazutti

Abstract The production of fungal biopolymers has stood out in the scientific community due to its properties. Its applications can be made in different areas; however, its most prominent applications are found in the agricultural and food industries. Scientific studies have used different approaches to produce and apply fungal biopolymers. Therefore, this chapter brings together information about the production of fungal biopolymers, as well as their properties, applications and perspectives. In addition, this chapter covers information on the initial steps of the process, such as, selection of culture media, parameter definition, extraction, recovery and purification, to assess the ideal conditions for scaling up. Scaling up is still one of the biggest challenges of biotechnology, however, experiments and operational production could solve this challenge.

Keywords Fungal biopolymers · Recovery and purification · Scale up

1 Introduction

Biopolymers are organic molecules that contain covalently linked monomeric units. These molecules can be produced by biological systems such as animals (Ranganathan et al. 2019), plants (Kora et al. 2012), microorganisms (Quintana-Quirino et al. 2019) or extracted from renewable raw materials (Joye 2019). The growing interest in biopolymers in biotechnology encompasses environmental and socioeconomic aspects, such as the reduction of environmental impacts obtained in oil extraction and refining processes (Luft et al. 2020). Furthermore, due to their faster decomposition, unlike polymers produced from petroleum, biopolymers have a great potential for replacement by polymers obtained from fossil sources (Brito et al. 2011).

M. C. Veiga · A. C. F. Piazzi · M. A. Mazutti (✉)
Departamento de Engenharia Química, Centro de Tecnologia, Universidade Federal de Santa Maria, Santa Maria, Brazil
e-mail: mazutti@ufsm.br

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_2

Microorganisms synthesize a wide variety of biopolymers for their function and survival (Vijayendra and Shamala 2013), being of great importance in the production of these biomolecules. Among microorganisms, fungi have stood out in food (Manzi and Pizzoferrato 2000) and nutraceutical (Camelini et al. 2005) applications, as well as in the environmental area, acting in the detoxification of compounds in contaminated environments (Kaewdoung et al. 2016). The application of these microorganisms can be made from their biomass or isolation of their cellular components, as well as enzymes (Couto and Toca-Herrera 2007; Schneider et al. 2018), phenolic compounds (Dulf et al. 2016) and polysaccharides (Li et al. 2016).

Due to the found components and fungal biopolymers, researchers' interest in these compounds as natural sources of active compounds has increased markedly. However, more studies are needed despite its biological activity and great chemical and structural diversity (Freitas et al. 2017). Based on this context, this chapter focuses on the production of biopolymers from different strains of fungi by submerged fermentation.

2 Fungi

Fungi are eukaryotic and heterotrophic organisms that obtain nutrients through the absorption of organic compounds, such as carbon sources. The fungal cell has a lipoprotein cytoplasmic membrane, whose function is to regulate exchanges with the medium. In addition, it has a rigid cell wall, which provides resistance to osmotic and mechanical pressures (Borzani et al. 2008; Madigan et al. 2016). The cell wall of fungi is composed of fibrous polysaccharides, such as chitin and glucans, and glycoproteins that determine the fibrillar structure of the wall (Serrano-Carreón et al. 2015).

Fungi are chemorganotrophic, most of which are aerobic and generally have simple nutritional requirements. These microorganisms feed by secreting extracellular enzymes digesting polymeric materials, such as polysaccharides or proteins, into monomers that are assimilated as a source of carbon and energy. As decomposers, fungi digest dead animals and plant material. As parasites, fungi use the same mechanism of nutrition but capture their nutrients from the living cells of plants and animals that infect and invade instead of what occurs with dead organic material (Madigan et al. 2016).

The most modern classification of fungi recognizes the phyla: *Chitridiomycetes, Zygomycetes, Glomeromycetes, Ascomycetes, and Basidiomycetes.* This classification is based on the comparative sequencing of the 18S ribosomal RNA and can be used to determine relatively close relationships between eukaryotes (Madigan et al. 2016; Takahashi et al. 2017).

As these organisms manage to survive at the expense of a wide variety of organic compounds, they can inhabit diverse environments such as soil, seawater, freshwater, and live associated with animals, insects, plants, and debris (Adams 2004; Takahashi et al. 2017).

Fungi have application potential in different industrial areas, such as food, nutraceuticals, medicines, chemicals, agriculture and energy (Bilal and Iqbal 2011; Camelini et al. 2005; Manzi and Pizzoferrato 2000). Its application can be made from its biomass or isolation of its cellular components (Couto and Toca-Herrera 2007; Schneider et al. 2018; Dulf et al. 2016; Li et al. 2016).

3 Cultivation

The versatility of the fungi has in adapting to different habitats, makes wide the way of cultivation. Microorganisms can use several metabolic pathways and their variations, and their fermentative products can be derived from pyruvate or other intermediates. In addition, there are two ways for a microorganism to go through such a process: solid-state fermentation or submerged fermentation.

The solid-state fermentation (SSF) aims to simulate the natural habitat of microorganisms that use this fermentation medium, since many of these microorganisms grow and function better in SSF conditions than in submerged conditions (Pandey 2003; Thomas et al. 2013). For the development of the fruit body of some fungi, such as *Lentinula edodes*, the natural habitat is wood, therefore, several studies have been carried out in order to optimize the process conditions for solid state fermentation, mainly with regard to the use of agro-industrial residues such as substrate.

On the other hand, submerged fermentation (SF) can be considered a change in the natural habitat for some microorganisms, such as filamentous fungi. However, in this type of fermentation, it is easier to control process conditions, such as heat and mass transfer, gas diffusivity, temperature, humidity, pH, availability of nutrients. While in a SSF there are challenges regarding scaling up, biomass estimation, and product purification (Singhania et al. 2009).

When referring to the cultivation of fungi, the production of mycelium in submerged fermentation is, in terms of study and practical application, relatively recent when compared to the solid-state. However, the advantage of submerged cultivation has the advantage of producing large amounts of mycelium, in a short period of time and at any time of the year, obtaining products of uniform quality, since the cultivation conditions, such as pH, temperature, the concentration of nutrients, agitation, among others can be controlled. The submerged cultivation of *Basidiomycetes* has few studies, and the main existing studies deal only with the optimization of polysaccharide production and cultivation parameters since these parameters decisively interfere in the cultivation of these microorganisms (Souza 2009).

The submerged cultivation of filamentous fungi has been used industrially to produce a wide variety of metabolites of economic and social importance, such as antibiotics, enzymes, mycotoxins, vitamins, among others. The works found in the literature report the production of polysaccharides constituting the mycelial mass of fungi produced in a submerged form (Wu and Hansen 2006), as well as the

production of exopolysaccharides excreted in the liquid culture medium (Feng et al. 2010) and therapeutic activity (Enman et al. 2012; Heuger et al. 2015).

Cultures of *Basidiomycetes* in bioreactors have different objectives, such as biomass production, maximizing the production of metabolites of interest, and optimization of culture conditions, to understand how different variables affect the production of these microorganisms (Chicatto et al. 2014; Li et al. 2018; Tsivileva et al. 2010).

4 Polysaccharides

Possessing a wide variety of complex chemical structures, polysaccharides obtained from living organisms have several applications. Among the industrial applications of these polysaccharides are the food, plastics, agronomy and fuel industries. Due to their characteristics, these polymers are involved in several biological processes (Kumar et al. 2018).

Polysaccharides are sugar polymers, mainly hexoses and pentoses, with hundreds or thousands of monosaccharide units. They can be divided into heteropolysaccharides, containing different monomeric species or homopolysaccharides made up of a single sugar with varying content (Laroche and Michaud 2007; Nelson and Cox 2011). They are mainly composed of glucose, mannose and galactose (Ismail and Nampoothiri 2010).

Biotechnological production of polysaccharides occurs by submerged fermentation intracellularly (intra-polysaccharides, IPS) or extracellularly (exo-polysaccharides, EPS) (Akila 2014). Under unfavorable environmental conditions, these polysaccharides develop a metabolic survival strategy and are usually synthesized and accumulated after the growth phase (Banerjee and Banerjee 2013). EPS and IPS are polysaccharides of sugar or derivatives, such as glucose, fructose, galactose and mannose, which have repeated and branched units (Ismail and Nampoothiri 2010). The fungus wall is a structure composed of polysaccharides and lipids, such as chitin, 1,3-β-glucan and 1,6-β-glucan (Lee et al. 2013).

4.1 Glucans

B-glucans are polysaccharides of D-glucose monomers linked by glycosidic bonds. They can be found in yeast, filamentous fungi, mushrooms, bacteria, algae and some cereals. B-glucans from different sources have specific branching patterns, binding type and molecular weight (Rieder and Samuelsen 2012), so the biological activities of β-glucans are affected (Zekovic et al. 2005; Zhu et al. 2016). Mushroom β-glucans have antitumor and immunostimulating properties (Du et al. 2013), while β-glucans from some cereals help to lower cholesterol and blood sugar (Zhu et al. 2015). Due to the bioactivity found in these polysaccharides, β-glucans have received special

attention, especially with regard to immunomodulation. When recognized by the body, β-glucan modifies its biological response, triggering a series of events in the immune response (Magnani and Castro-Gómez 2008; Maity et al. 2013; Wang et al. 2017).

In the cell wall of Saccharomyces cerevisiae is found between 55 and 65% β-glucan (Klis et al. 2002). Furthermore, the polysaccharide extracted from this source has high viscosity, water retention, oil fixation and emulsion stabilization capacity. The β-glucans from the yeast Saccharomyces cerevisiae consist of β-(1 → 3) and β-(1 → 6) bonds (Magnani and Castro-Gómez 2008), the rigidity and shape of the cell is given by the intertwined glucan layer with chitin fibrils, adjacent to the plasma membrane (Kopecká et al. 1974; Kapteyn et al. 1996).

Mushroom polysaccharides are mainly glucans with multiple glycosidic bonds, such as β-glucans-(1 → 3), β-glucans-(1 → 6), α-glucans-(1 → 3), α-glucans-(1 → 6) and heteroglycans (Wasser et al. 2003). Structurally, the β-glucans of the fungus fruit body (mushrooms) consist of β-(1 → 3) linear structures with side chains connected by β-(1 → 6). These structures can form complex tertiary structures stabilized by hydrogen bonds of variable length and distribution (Brown and Gordon 2005). Mushroom β-glucan has high health promoting properties (Wani et al. 2010). The percentage of β-glucans in mushrooms are insoluble, varies from 54 to 82%, while soluble β-glucans are between 16 and 46% (Manzi and Pizzoferrato 2000). Furthermore, its composition depends on the growing conditions and the mushroom origin (Gern et al. 2008).

Fungal glucans have a great diversity of molecular mass and configuration. They are constituents of the mycelium cell walls, fruiting bodies or other parts of different micro (*Penicillium*, *Aspergillus*, *Cladosporium* and *Rhodotorula*) and macromycetes (*Lentinus edodes*, *Pleurotus ostreatus*, *Piptoporus betulinus* and *Laetiporus sulphureus*). Despite its simple monosaccharide composition, there is diversity in relation to the number and anomeric configuration of the D-glucopyranose units, in addition to modifications in the sequence of glycosidic bonds, in position, in the degree of branching and in the conformation along a chain. Branched glucans with one or more monosaccharide units can contain several side chains linked at different positions (Synytsya and Novák 2013).

Growing conditions, species, total dietary fiber content, among other factors, influence the β-glucan content of mushrooms (Zhu et al. 2015). Depending on the structure and molecular mass, fungal glucans or their derivatives can exert different biological activities. Studies are being focused on describing the antioxidant, immunological and antitumor properties of high molecular weight polysaccharides (Smirnou et al. 2014; Zhao et al. 2016a, b; Huang et al. 2012; Ina et al. 2013; Ishikawa et al. 2001).

According to Wang et al. (2019) polysaccharides produced from *Antrodia cinnamomea* have anti-inflammatory, anti-hepatitis B virus and anti-cancer effects, in addition to also presenting bactericidal and phagocytic activities. These polysaccharides demonstrated a decrease in lipopolysaccharide-induced inflammation due to the water-soluble polysaccharide composed of β-D-glucan units and antioxidant activity in human hepatocytes (Wang et al. 2019). Free radicals act in various

reactions in food systems, through the oxidation of biomolecules can result in cell death and tissue damage. This contributes to cell aging and appears to be associated with degenerative diseases (Ames et al. 1993). Mushrooms have been reported as sources of natural antioxidants which are correlated with the medicinal value of their polysaccharides (Klaus et al. 2011; Mau et al. 2005). *Hericium erinaceus* extract showed anti-fatigue polysaccharide properties, opening the possibility of using fungal polysaccharides in sports nutrition (Liu et al. 2015). Furthermore, Chiu et al. (2014) claim that the use of polysaccharides obtained from *Cordyceps sobolifera* can protect against kidney damage.

4.2 Exopolysaccharides

Exopolysaccharides (EPS) can be obtained more efficiently through submerged cultures, compared to solid state fermentation (SSF). This is because through submerged fermentation (SF) it is possible to monitor cultivation parameters and physical, chemical and biological factors more efficiently, as these factors directly interfere in the production of EPS (García-Cruz et al. 2019). In addition, the SF time is shorter than SSF obtaining the product more quickly and the recovery of EPS from the culture medium is easier compared to FES (Osinska-Jaroszuk et al. 2015; Mahapatra and Banerjee 2013).

Due to its specific rheological and physicochemical properties, EPS can be used in the food, cosmetic and pharmaceutical industries, as stabilizers, emulsifiers and texture modifiers. In addition, they have excellent biological functions (antioxidant, antitumor, anti-inflammatory and immunomodulatory activities), which makes them considered as potent bioactive molecules. The properties of EPS can be altered depending on the growing conditions or the strain used, for example, while *Rhodotorula mucilaginosa* has antitumor activity, *Rhodotorula glutinis* has antiviral, antioxidant and antitumor activities (Gientka et al. 2016). In the study by Du et al. (2013) an EPS of *S. commune* was extracted, isolated and purified and demonstrated anti-inflammatory potential.

As they are renewable raw materials and meet environmental protection standards, EPS have relevant possibilities in agricultural and environmental applications, protecting the cell against adverse conditions (Gientka et al. 2016; Luft et al. 2020). Some applications relate to biofertilization, bioremediation, plant bioprotection, sewage treatment, soil preservation and desertification prevention (Osinska-Jaroszuk et al. 2015; Chang et al. 2015). In order to be produced commercially, it is necessary to have knowledge about the regulation of biosynthetic pathways, in addition to finding strains that efficiently produce EPS, as well as optimizing the conditions of cultivation and purification of the product. Some studies show that the increase in bioherbicidal activity is associated with the increase in EPS in the medium, suggesting that this compound may increase toxicity. This effect was found in studies using the fungus *Fusarium fujikuroi* (Todero et al. 2019) and also when using *Phoma* sp. (Luft et al. 2019).

EPS synthesis can be divided into three stages: carbon assimilation, intracellular synthesis, and excretion into the extracellular medium (He et al. 2014; Donot et al. 2012). And its production is associated with the secondary metabolism of the fungus. Furthermore, its physical, chemical and biological properties depend on the culture parameters used (culture medium, temperature, pH, agitation, aeration, among others) (Gientka et al. 2016). Two hypotheses can explain the presence of EPS, the first one refers to the presence of vesicles synthesized inside the cell, these vesicles transport the macromolecules to the extracellular medium through the cell wall. The second hypothesis is related to the polymerization of EPS in the extracellular space (Rodrigues et al. 2011, 2013). Secreted EPS do not show toxicity. Many polysaccharides are secreted in large amounts at a low cost, which makes them attractive for biotechnological use (Lei and Feng 2017; Luft et al. 2020).

4.3 Chitin/Chitosan

Chitin is a homopolymer of *N*-acetyl-D-glucosamine with β-1,4 linkages obtained mainly from crustaceans, algae and marine invertebrates and is one of nature's most insoluble compounds (Roca et al. 2012). Furthermore, it is found in fungi such as *Ascomycetes*, *Basidiomycetes* and *Zygomycetes*.

As a component of the fungus cell wall and structural membranes, chitin provides rigidity to the fungus cell wall to withstand adversities such as internal osmotic pressure and external moisture (Akila 2014; New et al. 2011). Although chitin is an important structural component, it represents only 1–2% of the dry mass of yeast cell walls and 10–20% of the cell walls of filamentous fungi. The most abundant structural component of fungal cell walls is β-glucan, which corresponds to 50–60% of dry mass (Fesel and Zuccaro 2016). Chitin occurs in two ways in the fungal cell wall, as free aminoglucoside and covalently linked to β-glucan (New et al. 2011).

The deacetylated derivative of chitin, chitosan, is biocompatible, biodegradable and non-toxic. Furthermore, it has filmogenic properties that form matrices for the transport of active substances (Cota-Arriola et al. 2013). Due to its properties, the use of fungal chitosan as a bioemulsifier is advantageous compared to the chemical process, as it can be produced from renewable sources and at low cost. Because it provides superior physicochemical properties, there is an increasing demand for the production of chitosan from fungi (Abdel-Gawad et al. 2017).

Commercial chitosan is obtained from crustaceans such as crabs, lobsters and shrimp (Akila 2014), however, due to the seasonal supply of shells and crustaceans, its high cost and inconsistent physicochemical properties, the production process has limited industrial acceptance. In contrast, the production of fungal e chitosan can be achieved through controlled conditions, either by submerged fermentation or solid state fermentation.

Fungal chitosan production processes are more consistent and of high quality compared to existing processes for industrial-scale production as they offer a stable

source with non-seasonal raw materials. Due to its extremely versatile properties, this biopolymer can be used in areas such as food (Abdel-Gawad et al. 2017; Wu et al. 2019), agriculture (Alshubaily 2019), water and sewage treatment (Alsharari et al. 2018), medicine (Almutairi et al. 2020; Zhu et al. 2019), cosmetics (Farias et al. 2019) and biotechnology (Kucera 2004). In addition, fungal chitosan can be used as a biofertilizer (Stamford et al. 2015).

5 Recovery of Biopolymers

In several centific areas, microbial biopolymers are research objects with a focus on optimizing their obtaining processes. However, for efficiency in the production process, attention and care must be taken during all stages of cultivation, from the initial stages of selection of the producing strain, culture media and definition of process parameters. The choice of these factors is extremely important since the obtaining of fungal biopolymers is highly affected by growing conditions. However, there are no guarantees of high yield for all microorganisms through proper growing conditions. Obtaining fungal biopolymers is more viable when compared to production them from other natural sources, as the production and extraction time are short, and it is a simple process (Freitas et al. 2017).

The fermentation processes consist of several steps, and the synthesis of products of biological origin is not the only part of the process, as shown in Fig. 1. The fermentation process is followed by a separation process in which the biomass and the supernatant are separated (Hubbuch and Kula 2007). To be applied, the final

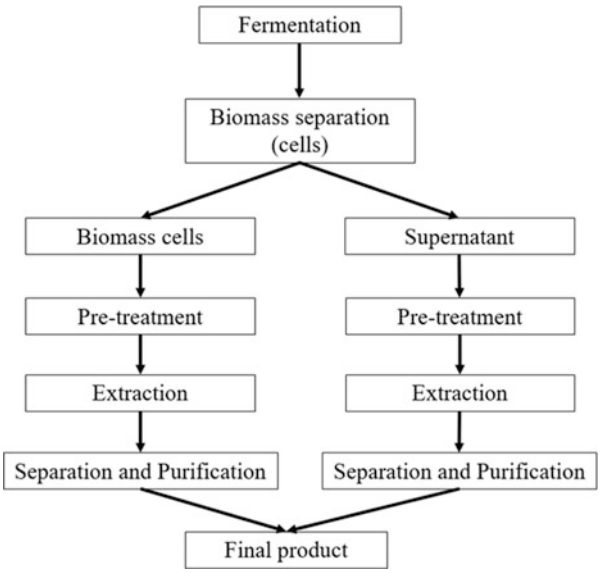


Fig. 1 Diagram for obtaining, separating and purifying products via microbiological synthesis

product needs to be free of impurities, therefore, after separation from the biopolymer, the next step is purification (Coutte et al. 2017).

Different unit operations can be used to isolate, separate and purify the final product so that it meets the final product requirements (Hubbuch and Kula 2007). The diversity of the origin of biotechnological products means that there is no technique of general application, but several techniques for each case (Bonomi and Schmidell 2001). The separation and purification processes are used in sequences adapted to a specific process or product (Kalyanpur 2002).

The further processing of products accounts for up to 60% of the total cost and must be chosen based on the purity of the bioproduct for a given application, which requires a large investment for these processes (Luft et al. 2020) Product recovery is performed according to cell location, solubility, and ionic charge (Satpute et al. 2010).

Biopolymer extraction or purification techniques commonly rely on precipitation, which in some cases is optional, in addition to the use of organic solvents according to their hydrophobicity and hydrophilic-lipophilic balance of the compounds.

Through the steps of extracting the supernatant, by precipitation with ammonium sulfate and subsequent purification by dialysis, it is possible to obtain bioemulsifiers (Banat et al. 2010). Other extraction techniques are acid precipitation, precipitation using trichloroacetic acid and acetone, alcohol precipitation, foam fractionation, solvent extraction, and assisted ultrasound extraction (Osinska-Jaroszuk et al. 2015; Zheng et al. 2019; Coutte et al. 2017).

There are strategies such as: ion exchange chromatography, membrane ultrafiltration techniques and adsorption-desorption on resins or activated carbon for purification. Furthermore, techniques developed such as HPLC and gel filtration are used to fractionate and purify these compounds. The use of a multi-stage recovery strategy is indicated to obtain a product in any required degree of purity. In multi-stage recovery, purification steps followed by product concentration steps are used (Mnif and Ghribi 2016).

The recovery of biopolymers takes place after the freeze-drying step. Mycelium extraction techniques require more drastic methods, such as the use of hot water (Zhao et al. 2016a, b; Wang et al. 2017) and inorganic (Fang and Zhong 2002) or organic (Geys et al. 2014) solvents. Ultrasound-assisted extraction is an effective, economical, fast, and simple methodology when compared to conventional methods, as it reduces process time (Sourki et al. 2017).

The balance between extraction efficiency and reproducibility as well as cost, time, and safety determine which extraction method will be used. However, some bottlenecks need to be resolved for the implementation of the process, such as purification yields, scalability, use or not of toxic solvents, and the cost of implementation.

The extraction procedure affects the functional behavior of the molecule, due to the profound effect on structure and molecular weight (Zhu et al. 2015). Different extraction techniques are available for β-glucan extraction, including hot water extraction, solvent extraction, enzymatic extraction and alkaline extraction (Smiderle et al. 2006; Ahmad et al. 2009), in addition, new extraction methods are

Table 1 Extraction and purification methods of β-glucans from mushrooms

Microorganism	Polysaccharide	Extraction	Purification	Reference
Lentinula edodes	Lentinan	High pressure dynamics by microfluidization	–	Huang et al. (2012)
Lentinula edodes	Lentinan	Enzymatic treatment (150 U g^{-1} of cellulose) + ultrasound	–	Ke (2015)
Lentinula edodes	β-glucan	Ultrasound and hot water	–	Zhao et al. (2018)
Agaricus brasiliensis	β-glucan	Hot water	DEAE-cellulose column chromatography	Camelini et al. (2005)
Agaricus blazei	β-glucan	Hot water	Sephadex A-25 column chromatography	Kim et al. (2005)
Schizophyllum commune	Schizophylan	Hot water and NaOH at 121 °C	Thin layer chromatography	Klaus et al. (2011)
Ganoderma lucidum	Ganoderan	Hot water and ethanol precipitation	DEAE-cellulose column chromatography	Dong et al. (2012)
Pleurotus nebrodensis	Pleuran	Methanol + saline solution	–	Cha et al. (2012)
Pleurotus florida e *Volvariella volvacea*	β-glucan	Hot water	DEAE-cellulose column chromatography	Das et al. (2010)
Pleurotus florida e *Volvariella volvacea*	β-glucan	Alkaline extraction	Sepharose 6B column chromatography	Maity et al. (2013)

available. Being states, such as the use of high pressure (Huang et al. 2012) and ultrasound (Zhao et al. 2018). Differences in β-glucan molecular weight estimates may arise from the methods used for extraction and purification, aggregation phenomena and depolymerization events that occur during the extraction step (Larazidou and Biliaderis 2007). Table 1 shows different techniques used for the extraction and purification of β-glucans.

6 Scale-Up of Bioreactors

In the case of fermentative processes, research on equipment on a production scale is not feasible, so small-scale bioreactors become more common. The small scale was defined as between 1 and 20 L (Mathews 2008), and its application is security, viable and economic option for the scaling up, developing a culture medium, costs reduce

and exploring ways to optimize the fermentation process (Burke 2008; Bareither and Pollard 2010).

The scale-up involves the small-scale and an intermediate step, the pilot-scale. This step is bigger than the small-scale and involves more study about the process. The scale expansion project is carried out to explore the possibility of producing natural products on a larger scale through fermentation. This process involves studying to understand the process as a whole and to a large extent, to know the final product and the physiology of the microorganisms used (Burke 2008).

The biggest challenge in moving from small scale to industrial scale is to ensure that the conditions of the fermentation process are maintained. This is because, the increase in scale does not always allow the conditions to be maintained and ensured throughout the process and, it may happen that the increase does not present the same behavior (Formenti et al. 2014). Some variables are difficult to control in scale-up, like an aeration, agitation, pH, sterilization of system, the ratio microorganism/cultivation medium, temperature, mass transfer, viscosity and others (Burke 2008).

When it comes to filamentous microorganisms, the scale increase complexity increases (Ishaq et al. 2015; Seviour et al. 2011), due to the morphology that ends up being an obstacle to the transfer of mass and aeration, besides that, depending on the culture conditions, the morphology can be drastically altered (Formenti et al. 2014).

One of the parameters to be taken into account is the safety of choosing the location of the bioreactor so that the health of those who operate it and the industries are guarantee (Mathews 2008).

7 Epilogue

The cultivation of filamentous fungi emerged as attractive alternatives for the production of biopolymers. These biopolymers accumulate in fungal cells due to stresses caused during the process. Because of this, the optimization of cultivation through the use of low-cost renewable substrates (agribusiness waste) and efficient methods of biopolymer recovery can make its production more economically viable, increasing its productivity and reducing environmental damage. Through the development of biotechnology, with a focus on research to optimize cultivation parameters on a large scale, it will allow fungal biopolymers to be commercialized in the future.

References

Abdel-Gawad KM, Hifney AF, Fawzy MA, Gomaa M (2017) Technology optimization of chitosan production from *Aspergillus niger* biomass and its functional activities. Food Hydrocoll 63: 593–601

Adams DJ (2004) Fungal cell wall chitinases and glucanases. Microbiology 150:2029–2035

Ahmad A, Anjum FM, Zahoor T, Nawaz H, Din A (2009) Physicochemical and functional properties of barley β-glucan as affected by different extraction procedures. Int J Food Sci Technol 44:181–187

Akila RM (2014) Fermentative production of fungal chitosan, a versatile biopolymer (perspectives and its applications). Adv Appl Sci Res 5:157–170

Almutairi FM, El Rabey HA, Tayel AA, Alalawy AI, Al-Duais MA, Sakran MI, Zidan NS (2020) Augmented anticancer activity of curcumin loaded fungal chitosan nanoparticles. Int J Biol Macromol 155:861–867

Alsharari SF, Tayel AA, Moussa SH (2018) Soil emendation with nano-fungal chitosan for heavy metals biosorption. Int J Biol Macromol 118:2265–2268

Alshubaily FA (2019) Enhanced antimycotic activity of nanoconjugates from fungal chitosan and Saussurea costus extract against resistant pathogenic *Candida* strains. Int J Biol Macromol 141: 499–503

Ames BN, Shigenaga MK, Hagen TM (1993) Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A 90:7915–7922

Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, Smyth TJ, Marchant R (2010) Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotechnol 87:427–444

Banerjee MM, Banerjee D (2013) Fungal exopolysaccharide: production, composition and applications. Microbiol Insights 6:1–16

Bareither R, Pollard D (2010) A review of advanced small-scale parallel bioreactor technology for accelerated process development: current state and future need. Biotechnol Process 27:2–14

Bilal M, Iqbal MN (2011) Naturally-derived biopolymers: potential platforms for enzyme immobilization. Int J Biol Macromol 130:462–482

Bonomi A, Schmidell W (2001) Modelagem matemática e simulação de processos fermentativos. In: Biotecnologia Industrial: Engenharia Bioquímica. Edgard Blücher, São Paulo, pp 123–178

Borzani W, Schmidell W, Lima UA, Aquarone E (2008) Elementos de microbiologia. In: Biotecnologia Industrial - Fundamentos. Blucher, São Paulo, pp 10–12

Brito GF, Agrawal P, Araújo EM, Mélo TJA (2011) Biopolímeros, polímeros biodegradáveis e polímeros verdes. Ver Eletron Mat Proc 2:127–139

Brown GD, Gordon S (2005) Immune recognition of fungal β-glucans. Cell Microbiol 7:471–479

Burke F (2008) Scale up and scale down for fermentation process. In: Practical fermentation technology. Wiley, New York, pp 231–270

Camelini CM, Maraschin M, Mendonça MM, Zucco C, Ferreira AG, Tavares LA (2005) Structural characterization of β-glucans of *Agaricus brasiliensis* in different stages of fruiting body maturity and their use in nutraceutical products. Biotechnol Lett 27:1295–1299

Cha YJ, Alam N, Lee JS, Lee KR, Shim MJ, Lee MW, Kim HY, Shin PG, Cheong JC, Yoo YB, Lee TS (2012) Anticancer and immunopotentiating activities of crude polysaccharides from *Pleurotus nebrodensis* on mouse sarcoma 180. Mycobiology 40:236–243

Chang I, Kharis A, Im J, Shin HD, Cho GC (2015) Soil treatment using microbial biopolymers for anti-desertification purposes. Geoderma 253–254:39–47

Chicatto JA, Costa A, Nunes H, Helm CV, Tavares LBB (2014) Evaluation of hollocelulase production by Lentinula edodes (Berk.) Pegler during the submerged fermentation growth using RSM. Braz J Biol 74:243–250

Chiu C, Chyau C, Chen C, Lin C, Cheng C, Mong M (2014) Polysaccharide extract of Cordyceps sobolifera attenuates renal injury in endotoxemic rats. Food Chem Toxicol 69:281–288

Cota-Arriola O, Cortez-Rocha MO, Burgos-Hernández A, Ezquerra-Brauer JM, Plascencia-Jatomea M (2013) Controlled release matrices and micro/nanoparticles of chitosan with antimicrobial potential: development of new strategies for microbial control in agriculture. J Sci Food Agric 93:1525–1536

Couto SR, Toca-Herrera JL (2007) Laccase production at reactor scale by filamentous fungi. Biotechnol Adv 25:558–569

Coutte F, Lecouturier D, Dimitrov K, Guez JS, Delvigne F, Dhulster P, Jacques P (2017) Microbial lipopeptide production and purification bioprocesses, current progress and future challenges. Biotechnol J 12:1600566

Das D, Mondal S, Roy SK, Maiti D, Bhunia B, Maiti TK, Sikdar SR, Islam SS (2010) A (1→6)-β-glucan from a somatic hybrid of *Pleurotus florida* and *Volvariella volvacea*: isolation, characterization, and study of immunoenhancing properties. Carbohydr Res 345:974–978

Dong Q, Wang Y, Shi L, Yao J, Li J, Ma F, Ding K (2012) A novel water-soluble β-D-glucan isolated from the spores of *Ganoderma lucidum*. Carbohydr Res 353:100–105

Donot F, Fontana A, Baccou JC, Schorr-Galindo S (2012) Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction. Carbohydr Polym 87:951–962

Du B, Bian Z, Xu B (2013) Skin health promotion effects of natural beta-glucan derived form cereals and microorganisms: a review. Phytother Res 28:159–166

Dulf FV, Vodnar DC, Socaciu C (2016) Effects of solid-state fermentation with two filamentous fungi on the total phenolic contents, flavonoids, antioxidant activities and lipid fractions of plum fruit (*Prunus domestica* L.) by-products. Food Chem 209:27–36

Enman J, Hodge D, Berglund KA, Rova U (2012) Growth promotive conditions for enhanced eritadenine production during submerged cultivation of *Lentinus edodes*. J Chem Technol Biotechnol 87:903–907

Fang QH, Zhong JJ (2002) Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*. Process Biochem 37:769–774

Farias JM, Stamford TCM, Resende AHM, Aguiar JS, Rufino RD, Luna JM, Sarubbo LA (2019) Mouthwash containing a biosurfactant and chitosan: an eco-sustainable option for the control of cariogenic microorganisms. Int J Biol Macromol 129:853–860

Feng YL, Li WQ, Wu XQ, Cheng JW, Ma SY (2010) Statistical optimization of media for mycelial growth and exo-polysaccharide production by *Lentinus edodes* and a kinetic model study of two growth morphologies. Biochem Eng J 49:104–112

Fesel PH, Zuccaro A (2016) β-glucan: crucial component of the fungal cell wall and elusive MAMP in plants. Fungal Genet Biol 90:53–60

Formenti LR, Nørregaard A, Bolic A, Hernandez DQ, Hagemann T, Heins A, Larsson H, Mears L, Mauricio-Iglesias M, Krühne U, Gernaey KV (2014) Challenges in industrial fermentation technology research. Biotechnol J 9:727–738

Freitas F, Torres CAV, Reis MAM (2017) Engineering aspects of microbial exopolysaccharide production. Bioresour Technol 245(Pt B):1674–1683

García-Cruz F, Durán-Páramo E, Garín-Aguilar MA, Valencia del Toro G, Chairez I (2019) Parametric characterization of the initial pH effect on the polysaccharides production by Lentinula edodes in submerged culture. Food Bioprod Process 119:170–178

Gern RM, Wisbeck E, Rampinelli J, Ninow JL, Furlan SA (2008) Alternative medium for production of Pleurotus ostreatus biomass and potential antitumor polysaccharides. Bioresour Technol 99:76–82

Geys R, Soetaert W, Van Bogaert I (2014) Biotechnological opportunities in biosurfactant production. Curr Opin Biotechnol 30:66–72

Gientka I, Bzducha-Wróbel A, Stasiak L, Bednarska AA, Blazejak S (2016) The exopolysaccharides biosynthesis by *Candida* yeast depends on carbon sources. Electron J Biotechnol 22:31–37

He Y, Ye M, Du Z, Wang H, Wu Y, Yang L (2014) Purification, characterization and promoting effect on wound healing of an exopolysaccharide from Lachnum YM405. Carbohydr Polym 105:169–176

Heuger AH, Fraatz MA, Zorn H (2015) Biotechnological production of C_8 aroma compounds by submerged cultures of shiitake (*Lentinula edodes*), ACS symposium series. American Chemical Society, Washington, DC

Huang X, Tu Z, Jiang Y, Xiao H, Zhang Q, Wang H (2012) Dynamic high pressure microfluidization-assisted extraction and antioxidant activities of lentinan. Int J Biol Macromol 51:926–932

Hubbuch J, Kula M (2007) Isolation and purification of biotechnological products. J Non-Equil Thermody 32:99–127

Ina K, Kataoka T, Ando T (2013) The use of lentinan for treating gastric cancer. Anti Cancer Agents Med Chem 13:681–688

Ishaq U, Akram MS, Iqbal Z, Rafiq M, Akrem A, Nadeem M, Shafi F, Shafiq Z, Mahmood S, Baig MA (2015) Production and characterization of novel self-assembling biosurfactants from Aspergillus flavus. J Appl Microbiol 119:1035–1045

Ishikawa NK, Kasuya MCM, Vanetti MCD (2001) Antibacterial activity of *Lentinula edodes* grown in liquid medium. Braz J Microbiol 32:206–210

Ismail B, Nampoothiri KM (2010) Exopolysaccharide production and prevention of syneresis in starch using encapsulated probiotic *Lactobacillus plantarum*. Food Technol Biotechnol 48:484–489

Joye IJ (2019) Cereal biopolymers for nano- and microtechnology: a myriad of opportunities for novel (functional) food applications. Trends Food Sci Technol 83:1–11

Kaewdoung B, Sutjaritvorakul T, Gadd GM, Whalley AJS, Sihanonth P (2016) Heavy metal tolerance and biotransformation of toxic metal compounds by new isolates of wood-rotting fungi from Thailand. Geomicrobiol J 33:283–288

Kalyanpur M (2002) Downstream processing in the biotechnology industry. Mol Biotechnol 22: 87–98

Kapteyn JC, Montijn RC, Vink E, de la Cruz J, Llobell A, Shimoi H, Lipke P, Klis FM (1996) Glycobiology 6:337–345

Ke LQ (2015) Optimization of ultrasonic extraction of polysaccharides from *Lentinus edodes* based on enzymatic treatment. J Food Process Preserv 35:254–259

Kim YW, Kim KH, Choi HJ, Lee DS (2005) Anti-diabetic activity of β-glucans and their enzymatically hydrolyzed oligosaccharides from *Agaricus blazei*. Biotechnol Lett 27:483–487

Klaus A, Kozarski M, Niksic M, Jakovljevic D, Todorovic N, Van Griensven LJLD (2011) Antioxidative activities and chemical characterization of polysaccharides extracted from the basidiomycete *Schizophyllum commune*. Food Sci Technol 44:2005–2011

Klis FM, Mol P, Hellingwerf KJ, Brul S (2002) Dynamics of cell wall structure in Saccharomyces cerevisiae. FEMS Microbiol Rev 26:239–256

Kopecká M, Phaff HJ, Fleet GH (1974) Demonstration of a Wbril-lar component in the cell wall of the yeast *Saccharomyces cerevisiae* and its chemical nature. J Cell Biol 62:66–76

Kora AJ, Sashidhar RB, Arunachalam J (2012) Aqueous extract of gum olibanum (*Boswellia serrata*): a reductant and stabilizer for the biosynthesis of antibacterial silver nanoparticles. Process Biochem 47:1516–1520

Kucera J (2004) Fungal mycelium—the source of chitosan for chromatography. J Chromatogr B Anal Technol Biomed Life Sci 808:69–73

Kumar CG, Mongolla P, Pombala S (2018) Lasiosan a new exopolysaccharide from Lasiodiplodia sp. strain B2 (MTCC 6000): structural characterization and biological evaluation. Process Biochem 72:162–169

Larazidou A, Biliaderis CG (2007) Molecular aspects of cereal β-glucan functionality: physical properties, technological applications and physiological effects. J Cereal Sci 46:101–118

Laroche C, Michaud P (2007) New developments and prospective applications for beta(1,3) glucans. Recent Pat Biotechnol 1:59–73

Lee CM, Cho EM, Yang SI, Ganbold E, Jun J, Cho K (2013) Raman spectroscopy and density functional theory calculations of β-glucans and chitins in fungal cell walls. Bull Korean Chem Soc 34:943–945

Lei S, Feng ET (2017) Polysaccharides, microbial. In: Roitberg BD (ed) Reference module in life sciences. Elsevier, Amsterdam, pp 482–494

Li Y, Meng S, Shi M, Hu X, Yang Y, Zhang Z (2016) Bioactivity evaluation of crude polysaccharide from rice bran fermented by Preussia aemulans and the changes in its nutritional contents. J Food Biochem 40:664–672

Li S, Wang A, Tian G, Wei S, Xu F (2018) Evaluation of nutritional values of shiitake mushroom (Lentinus edodes) stipes. J Food Meas Charact 12:2012–2019

Liu Z, Yi J, Xiao X (2015) Study on the effective mechanism of Plumula nelumbinis extract against hepatic fibrosis in rats Chin. J Clin Pharmacol 31:1749–1753

Luft L, Confortin TC, Todero I, Chaves Neto JR, Tonato D, Felimberti PZ, Zabot GL, Mazutti MA (2019) Different techniques for concentration of extracellular biopolymers with herbicidal activity produced by *Phoma* sp. Environ Technol 42:1–10

Luft L, Confortin TC, Todero I, Zabot GL, Mazutii MA (2020) An overview of fungal biopolymers: bioemulsifiers and biosurfactants compounds production. Crit Rev Biotechnol 40(8): 1059–1080. https://doi.org/10.1080/07388551.2020.1805405

Madigan M, Martinko JM, Bender KS, Buckley DH, Stahl DA (2016) Fungos. In: Microbiologia de Brock. Artmed, São Paulo, pp 556–558

Magnani M, Castro-Gómez RJH (2008) β-glucana from *Saccharomyces cerevisiae*: constitution, bioactivity and obtaining. Semi Cienc Agrar 29:631–650

Mahapatra S, Banerjee D (2013) Fungal exopolysaccharide: production, composition and applications. Microbiol Insights 6:1–16

Maity KK, Patra S, Dey B, Bhunia SK, Mandal S, Bahera B, Maiti TK, Sikdar SR, Islam SS (2013) A β-glucan from the alkaline extract of a somatic hybrid (*PfloVv5FB*) of *Pleurotus florida* and *Volvariella volvacea*: structural characterization and study of immunoactivation. Carbohydr Res 370:13–18

Manzi P, Pizzoferrato L (2000) Beta-glucans in edible mushrooms. Food Chem 68:315–318

Mathews G (2008) Fermentation equipment selection: laboratory scale bioreactor design considerations. In: Practical fermentation technology. Wiley, New York, pp 3–37

Mau JL, Tsai SY, Tseng YH, Huang SJ (2005) Antioxidant properties of hot water extracts from Ganoderma tsugae Murrill. LWT Food Sci Technol 38:589–597

Mnif I, Ghribi D (2016) Glycolipid biosurfactants: main properties and potential applications in agriculture and food industry. J Sci Food Agric 96:4310–4320

Nelson DL, Cox MM (2011) Princípios de bioquímica de Lehninger. Artmed, Porto Alegre

New N, Furuike T, Tamura H (2011) Production, properties and applications of fungal cell wall polysaccharides: chitosan and glucan. Adv Polym Sci 244:187–207

Osinska-Jaroszuk M, Jarosz-Wilkołazka A, Jaroszuk-Sciseł J, Szałapata K, Nowak A, Jaszek M, Ozimej E, Majewska M (2015) Extracellular polysaccharides from Ascomycota and Basidiomycota: production conditions, biochemical characteristics, and biological properties. World J Microbiol Biotechnol 31:1823–1844

Pandey A (2003) Solid-state fermentation. Biochem Eng J 13:81–84

Quintana-Quirino M, Morales-Osorio C, Ramírez GV, Vázquez-Torres H, Shirai K (2019) Bacterial cellulose grows with a honeycomb geometry in a solid-state culture of *Gluconacetobacter xylinus* using polyurethane foam support. Process Biochem 82:1–9

Ranganathan S, Balagangadharan K, Selvamurugan N (2019) Chitosan and gelatin-based electrospun fibers for bone tissue engineering. Int J Biol Macromol 133:354–364

Rieder A, Samuelsen AB (2012) Do cereal mixed-linkage β-glucans possess immune-modulating activities? Mol Nutr Food Res 56:536–547

Roca C, Chagas B, Farinha I, Freitas F, Mafra L, Aguiar F, Oliveira R, Reis MAM (2012) Production of yeast chitin-glucan complex from biodiesel industry byproduct. Process Biochem 47:1670–1675

Rodrigues ML, Nosanchuk JD, Schrank A, Vainstein MH, Casadevall A, Nimrichter L (2011) Vesicular transport systems in fungi. Future Microbiol 6:1371–1381

Rodrigues ML, Franzen AJ, Nimrichter L, Miranda K (2013) Vesicular mechanisms of traffic of fungal molecules to the extracellular space. Curr Opin Microbiol 16:414–420

Satpute SK, Banpurka AG, Dhakephalkar PK, Banat IM, Chopade BA (2010) Methods for investigating biosurfactants and bioemulsifiers: a review. Crit Rev Biotechnol 30:127–144

Schneider WDH, Fontana RC, Mendonça S, Siqueira FG, Dillon AJP, Camassola M (2018) High level production of laccases and peroxidases from the newly isolated white-rot basidiomycete

Marasmiellus palmivorus VE111 in a stirred-tank bioreactor in response to different carbon and nitrogen sources. Process Biochem 69:1–11

Serrano-Carreón L, Galindo E, Rocha-Valadéz JA, Holguín-Salas A, Corkidi G (2015) Hydrodynamics, fungal physiology, and morphology. Adv Biochem Eng Biotechnol 149:55–90

Seviour RJ, McNeil B, Fazenda ML, Harvey LM (2011) Operating bioreactors for microbial exopolysaccharide production. Crit Rev Biotechnol 31:170–185

Singhania RR, Patel AK, Soccol CR, Pandey A (2009) Recent advances in solid-state fermentation. Biochem Eng J 44:13–18

Smiderle FR, Carbonero ER, Mellinger CG, Sassaki GL, Gorin PAJ, Iacomini M (2006) Structural characterization of a polysaccharide and a β-glucan isolated from the edible mushroom *Flammulina velutipes*. Phytochemistry 67:2189–2196

Smirnou D, Hrubosová D, Kulhánek J, Svík K, Bobková L, Moravcova V, Krcmár M, Franke L, Velebný V (2014) Cryptococcus laurentii extracellular biopolymer production for application in wound management. Appl Biochem Biotechnol 174:1344–1353

Sourki AH, Koocheki A, Elahi M (2017) Ultrasound-assisted extraction of β-d-glucan from hull-less barley: assessment of physicochemical and functional properties. Int J Biol Macromol 95: 462–475

Souza CMCO (2009) Production of metabolites with antimicrobial and antioxidant activity from *Lentinula edodes* in submerged culture. Dissertation (Master in biotechnological processes), Federal University of Paraná, Paraná

Stamford NP, Figueiredo MVB, Silva Junior S, Freitas ADS, Santos CERES, Lima Junior MA (2015) Effect of gypsum and sulfur with Acidithiobacillus on soil salinity alleviation and on cowpea biomass and nutrient status as affected by PK rock biofertilizer. Sci Hortic 192:287–292

Synytsya A, Novák M (2013) Structural diversity of fungal glucans. Carbohydr Polym 92:792–809

Takahashi JA, Lima GS, Dos Santos GF, Lyra FH, Da Silva-Hughes AF, Gonçalves FAG (2017) Fungos filamentosos e química: Velhos conhecidos, novos aliados. Rev Virtual Quim 9:2351–2382

Thomas L, Larroche C, Pandey A (2013) Current developments in solid-state fermentation. Biochem Eng J 81:146–161

Todero I, Confortin TC, Luft L, Seibel J, Kuhn RC, Tres MV, Zabot GL, Mazutii MA (2019) Concentration of exopolysaccharides produced by *Fusarium fujikuroi* and application of bioproduct as an effective bioherbicide. Environ Technol 41:2742–2749

Tsivileva OM, Pankratov AN, Nikitina VE (2010) Extracellular protein production and morphogenesis of *Lentinula edodes* in submerged culture. Mycol Prog 9:157–167

Vijayendra SVN, Shamala TR (2013) Film forming microbial biopolymers for commercial applications—a review. Crit Rev Biotechnol 8551:1–20

Wang KF, Yan Sui K, Guo C, Liu C (2017) Improved production and antitumor activity of intracellular protein polysaccharide from *Trametes versicolor* by the quorum sensing molecule-tyrosol. J Funct Foods 37:90–96

Wang T, He W, Liu X, Liu C, Liang Y, Mei Y (2019) Mycelial polysaccharides of *Lentinula edodes* (shiitake mushroom) in submerged culture exert immunoenhancing effect on macrophage cells via MAPK pathway. Int J Biol Macromol 130:745–754

Wani IA, Sogi DS, Wani AA, Gill BS, Shivhare US (2010) Physico-chemical properties of starches from Indian kidney bean (Phaseolus vulgaris) cultivars. Int J Food Sci Technol 45:2176–2185

Wasser SP, Elisashvili VI, Tan KK (2003) Effects of carbon and nitrogen sources in the medium on *Tremella mesenterica* Retz.:Fr. (Heterobasidiomycetes) growth and polysaccharide production. Int J Med Mushrooms 5:49–56

Wu XJ, Hansen C (2006) Effects of whey permeate-based medium on the proximate composition of *Lentinus edodes* in the submerged culture. J Food Sci 71:174–179

Wu J, Niu Y, Jiao Y, Chen Q (2019) Fungal chitosan from *Agaricus bisporus* (Lange) Sing. Chaidam increased the stability and antioxidant activity of liposomes modified with biosurfactants and loading betulinic acid. Int J Biol Macromol 123:291–299

Zekovic DB, Kwiatkowski S, Vrvic MM, Jakovljevic D, Moran CA (2005) Natural and modified (1→3)-β-D-glucans in health promotion and disease alleviation. Crit Rev Biotechnol 25:205–230

Zhao H, Li S, Zhang J, Che G, Zhou M, Liu M, Zhang C, Xu N, Lin L, Liu Y, Jia L (2016a) The antihyperlipidemic activities of enzymatic and acidic intracellular polysaccharides by *Termitomyces albuminosus*. Carbohydr Polym 151:1227–1234

Zhao Y, Wang J, Wu Z, Yang J, Li W, Shen L (2016b) Extraction, purification and anti-proliferative activities of polysaccharides from *Lentinus edodes*. Int J Biol Macromol 93:136–144

Zhao YM, Yang JM, Liu YH, Zhao M, Wang J (2018) Ultrasound assisted extraction of polysaccharides from *Lentinus edodes* and its anti-hepatitis B activity *in vitro*. Int J Biol Macromol 107:2217–2223

Zheng H, Hao M, Liu W, Zheng W, Fan YS, Wu Z (2019) Foam fractionation for the concentration of exopolysaccharides produced by repeated batch fermentation of *Cordyceps militaris*. Sep Purif Technol 210:682–689

Zhu F, Du B, Bian Z, Xu B (2015) Beta-glucans from edible and medicinal mushrooms: characteristics, physicochemical and biological activities. J Food Compos Anal 41:165–173

Zhu F, Du B, Xu B (2016) A critical review on production and industrial applications of beta-glucans. Food Hydrocoll 52:275–288

Zhu L, Li J, Mai J, Chang M (2019) Ultrasound-assisted synthesis of chitosan from fungal precursors for biomedical applications. Chem Eng J 357:498–507

Fungal Hydrophobins

Vandana Ghormade, S. G. Tupe, Ejaj Pathan, and Mukund V. Deshpande

Abstract Hydrophobins are cysteine-rich amphipathic proteins found throughout the fungal kingdom and play important functions in the fungal life cycle. They are known to be involved in the conidiogenesis and formation of appressorium in *Magnapothe oryzae* and *Metarhizium anisopliae*, the growth of microsclerotia in *Verticillium dahlia*, and the development of microconidia in *Fusarium verticillioides*. Hydrophobins are also involved in disseminating *Cladosporium fulvum* spores and the production of aerial hyphae in *Schizophyllum commune*. Moreover, fungal hydrophobins have been well studied at the biochemical and molecular levels. Their role in the life cycles of *Agaricus bisporus, Aspergillus fumigatus, A. nidulans, Magnaporthe grisea, Neurospora crassa, Ophiostoma ulmi, Schizophyllum commune, Trichoderma harzianum*, and *T. reesei* have also been studied. Furthermore, the amphipathic characteristics of fungal hydrophobins have been used to solubilize and deliver hydrophobic medicines, as well as for purification and immobilization of proteins, as a antimicrobial coats, in biosensor development, and also as emulsifying agents.

Keywords Adhesion · Drug delivery · Fungal hydrophobins · *Metarhizium* · Tissue engineering

1 Introduction

Hydrophobins, a small cysteine-rich proteins known to form a hydrophobic surface, are produced by the filamentous fungi. Upon exposure to hydrophilic-hydrophobic or water-air interfaces, they are self-assembled for the formation of amphipathic

V. Ghormade (✉)
Nanobioscience, Agharkar Research Institute, Pune, Maharashtra, India
e-mail: vandanaghormade@aripune.org

S. G. Tupe · M. V. Deshpande
Research and Development Division, Greenvention Biotech Pvt. Ltd., Pune, Maharashtra, India

E. Pathan
Department of Biotechnology, Indian Institute of Technology-Bombay, Mumbai, India

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_3

membranes. They are first reported from a white-rot basidiomycete, *Schizophyllum commune* (Linder et al. 2005).

These proteins moisten hydrophobic surfaces such as gas bubbles, liquids, and solid objects; on the other hand, they change hydrophilic surfaces into hydrophobic. Therefore, the hydrophobins are useful in dispersing hydrophobic materials, stabilizing foam in food products, and immobilizing antibodies, cells, and enzymes. Furthermore, they have therapeutic value as immunomodulators. The following sections will discuss biochemical and molecular studies of hydrophobins, their functions in the life cycle of fungi and their applications in various fields.

2 Hydrophobins

Hydrophobins are low molecular weight ($\leq$20 kDa) hydrophobic self-assembling fungal proteins having characteristic eight conserved cysteine (Cys) residues (Bayry et al. 2012; Wösten 2001). Though hydrophobins are unique to fungi, some orthologs were reported from *Streptomyces*. Hydrophobins gene families usually contain 2–10 members, while *Coprinus cinereus* shows 33 members (Bayry et al. 2012).

Hydrophobins are classified into two types based on the distribution of cysteines and the clustering pattern of hydrophobic and hydrophilic residues. The solubility and shape of the aggregates generated by these two types may be differentiated. Class I hydrophobins create very insoluble membranes that can only be decomposed by a high concentration of acids (even in 2% SDS at 100 °C) (such as formic acid or trifluoroacetic acid). Hydrophobins of class II are less stable and may be easily dissolved in ethanol (60%) or SDS (2%).

Outside the fungal cell wall, the majority of hydrophobins class I form a microscopically detectable rodlet structure (Asgeirsdattir et al. 1995, 1997). Hydrophobins of class II create assemblies with no distinguishing rodlet shape. Despite these physical distinctions, no clear differentiation between the roles of class I and class II hydrophobins in the life cycle of fungi has yet to be discovered.

Based on characteristic spacings between their cysteine residues, hydrophobins class I and class II can be distinguished (Wösten and Wessels 1997). In both hydrophobin classes, however, the cysteine pattern is similar: the second and third cysteine residues, the sixth and seventh cysteine residues, are always located adjacent to each other. It has been observed that the eight cysteine residues in class II hydrophobins are engaged in four disulphide interactions, resulting in the development of four loop-like structures (Kershaw et al. 1998). The linkages of cysteine have been identified in *Ceratocystis ulmi* (Cys1–2, Cys3–4, Cys5–6 and Cys7–8). Two of the four "loops" were primarily made up of hydrophobic residues. Figure 1 depicts a schematic illustration of *C. ulmi* hydrophobins (Yaguchi et al. 1993).

While Wösten and Scholtmeijer (2015) reported that Hydrophobins range in length from 70 to 350 amino acids (along with signal sequence). They all have eight conserved cysteine residues that link C1–C6, C2–C5, C3–C4, and C7–C8

Fig. 1 Based on intramolecular disulphide connections found for *Ceratocystis ulmi*, a schematic depiction of probable hydrophobin structures was created (originally constructed by Yaguchi et al. 1993)

Class I hydrophobin: X25-158-C-X5-9-C-C-X4-44-C-X7-23-C-X5-7-C-C-X6-18-C-X2-13

Class II hydrophobin:X17-165-C-X7-10-C-C-X11-C-X15/16-C-X 6-9-C-C-X10/11-C-X3-8

Fig. 2 Schematic representation of amino acid sequence lengths between eight cysteine residues in hydrophobins Class I and Class II (X, amino acid other than cysteine; sub-index denotes the number of amino acids)

through four disulfide linkages (Fig. 2). De Vocht et al. (2000) described that the cysteine residues in class I hydrophobin SC3 from *S. commune* are essential for the solubility of protein. The characteristic order of cysteine residues differentiates the fungal hydrophobins from the cysteine-rich proteins.

3 Molecular Studies

Multiple hydrophobin genes were reported from filamentous ascomycetes and basidiomycetes fungi (Wösten 2001). *C. cinereus*, for example, has 33 hydrophobin genes in its genome (Bayry et al. 2012), and *S. commune* has at least 5 genes coding for hydrophobins (Wessels 1997). Hydrophobin genes, on the other hand, are missing from the genomes of *Candida albicans, Cryptococcus neoformans, Kluyveromyces lactis, Saccharomyces cerevisiae, Schizosaccharomyces pombe*, and *Yarrowia lipolytica*. The hydrophobin genes from these fungi were most likely lost during the evolution. Teertstra et al. (2006) suggested that *Ustilago maydis,* a dimorphic basidiomycete, contain hydrophobin genes, however the resultant protein have been functionally replaced partially by the repellents. It was further suggested that in terms of the involvement of hydrophobins in the life cycle, *U. maydis* appears to be somewhere between filamentous fungi and yeasts. Another dimorphic fungus, *Paracoccidiodes brasiliensis,* an etiologic agent of paracoccidioidomycosis also exhibited two mycelial specific hydrophobin genes, *Pbhyd1* and *Pbhyd2* (Albuquerque et al. 2004).

4 Role of Hydrophobins in the Fungal Life-Cycle

Fungal hydrophobins are known to be highly surface-active globular proteins that have a variety of roles in growth and differentiation of filamentous fungi. For instance, hydrophobins play an important role in conidiogenesis as well as appressorium formation in *Magnaporthe oryzae*, *Metarhizium anisopliae*, development of microsclerotia in *Verticillium dahlia* as well as *Fusarium verticillioides* (Talbot et al. 1993; Fuchs et al. 2004; Klimes and Dobinson 2006; Li et al. 2006). They are also involved in spore dispersal of *Cladosporium fulvum*, aerial hyphal development in *S. commune* (Wosten et al. 1994; Van Wetter et al. 1996; Whiteford and Spanu 2001). The mutation studies in *Agaricus bisporus, Aspergillus fumigatus, A. nidulans, Claviceps pupurea, M. grisea, Neurospora crassa, Ophiostoma ulmi, S. commune, Trichoderma harzianum* and *Trichoderma reesei* were reported to understand their role in the life cycle (Kershaw et al. 1998).

Hydrophobins are key components of filamentous fungi's conidial cell membrane, polymerizing to create bundles or fascicles with rodlet-like structures. They assist fungi in escaping the watery environment, offer hydrophobicity to fungal surfaces in contact with air, and promote hyphal adherence to hydrophobic surfaces, which results in morphogenetic signals (Fig. 3). The latter is critical in the early stages of pathogenesis, when the fungus must connect to the host's hydrophobic

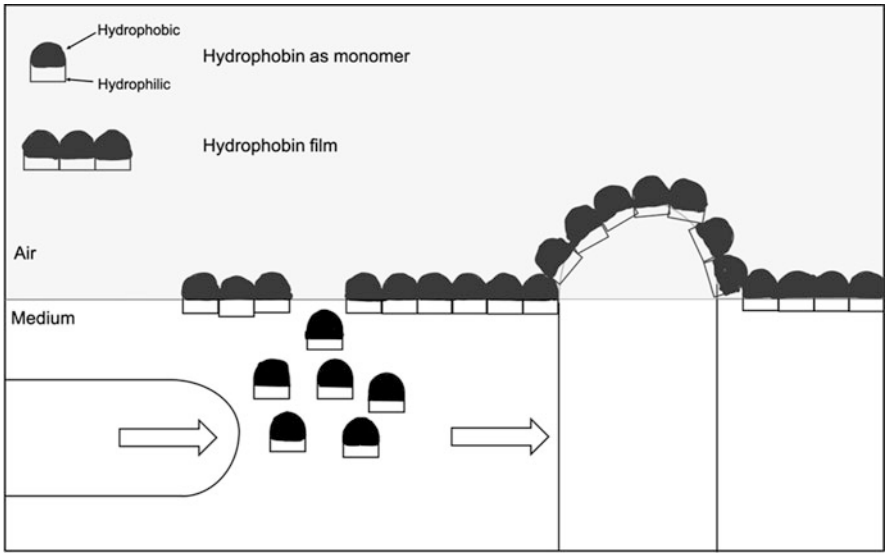


Fig. 3 Schematic diagram representing the assembly of aerial hyphae in *M. anisopliae* (adapted from Wessels 1997; Wösten 2001). The hydrophobic and hydrophilic regions of a hydrophobin molecule are denoted by the colours black and white, respectively. Hydrophobin molecules injected into the liquid culture medium produce a hydrophobin layer at the water-medium interface, lowering the liquid's surface tension and allowing the hyphae to grow into the air. Cell wall's rodlet-patterned hydrophobin layer makes the aerial hyphae hydrophobic

Table 1 Hydrophobins reported from fungi

Hydrophobin from	Number of amino acid (kDa)	Mr accession number	Reference or EMBL
Class I Hydrophobins			
Ascomycetes			
Aspergillus fumigatus RodB	140	14	Paris et al. (2003)
Aspergillus nidulans RodA	157	–	Stringer et al. (1991)
Cladosporium fulvum HCf-1	105	10	Spanu (1997)
Magnaporthe grisea MPG	112	15	Talbot et al. (1993)
Metarhizium anisopliae SSGA	96	–	St. Leger et al. (1992)
Neurospora crassa EAS	108	8.2	Bell-Pedersen et al. (1992)
Basidiomycete			
Agaricus bisporus ABH3	119	9.1	Lugones et al. (1996)
Dictyonema glabratum DGH1	130	14	Trembley et al. (2002)
Class II Hydrophobins			
Ascomycetes			
Claviceps fusiformis CFTH1	394	36.5	De Vries et al. (1999)
Fusarium verticillioides Hyd4	100	–	Fuchs et al. (2004)
Magnaporthe grisea MPH1	102	–	Kim et al. (2001)
Trichoderma harzianum SRHI	89	–	Munaoz et al. (1997)
Trichoderma reesei HFBI	97	7.5	Nakari-Setala et al. (1996)
Trichoderma viride SRH1	89	–	Linder et al. (2001)

surface prior to penetration and infection can begin. De Vries et al. (1999) were the first to conduct substantial research on SC3 hydrophobins from *S. commune*. Heterologous expression and purification of *Beauveria bassiana* hydrophobin (hyd2) from *E. coli* were performed recently (Kirkland and Keyhani 2011). Table 1 summarises the hydrophobins reported from different fungi.

Paris et al. (2003) demonstrated that insoluble protein complexes form rodlet layers on the surface of conidia of *A. nidulans* as well as *A. niger*. Further, hydrophobins hyd1 and hyd2 were involved in the formation of a rodlet layer on the conidial surface in *B. bassiana* (Zhang et al. 2011). These hydrophobins have unique physiological functions in processes such as rodlet layer formation, thermostability conidia, and influencing cell surface characteristics that contribute to the insect pathogenicity. Kapoor (2012) extensively studied the topological features of *M. anisopliae* conidia by Atomic force microscopy (AFM). This study demonstrated the assembled hydrophobin rodlet layer on the rough surface of *M. anisopliae* conidia. The roughness of a cell surface is a direct assessment of its hydrophobicity; the rougher the surface, the higher the hydrophobicity (Holder et al. 2007). The presence of the rodlets on conidial surface of *M. anisopliae* suggests their possible role in attachment to the insect cuticle during fungus-insect interaction.

5 Production of Hydrophobins

Various methods for the production of hydrophobins have been reported, including the use of fungal strains isolated from hydrophobic environment, use of bioreactors for submerged and solid-substrate fermentation scale-ups, and use of recombinant microbial strains. Kulkarni et al. (2017) have extensively reviewed the scale-up methods for fungal hydrophobin production. The hydrophobin production has been scale-up using heterologous and homologous expression in various vectors. For instance, *S. commune* (SC3) hydrophobin was produced by heterologous expression in *T. reesei* and *A. niger* having yield of ~60 mg/L. The heterologous expression of hydrophobins Class I from *A. nidulans* in *T. reesei* and *Grifola frondosa* in *Pichia pastoris* showed similar yields (Wang et al. 2010; Schmoll et al. 2010). Expression of fungal hydrophobin in the bacterial system reduced the yields, however expression of bacterial Class I hydrophobin DewA from *Bacillus subtilis* in *E. coli* yielded a kilogram scale of hydrophobins from the inclusion bodies, although it required further purification (Wohlleben et al. 2010). Expression of class II hydrophobins to levels of 120 mg/L was achieved in *P. pastoris* (Niu et al. 2012). *Aspergillus* Class I hydrophobins RodA and RodB were heterologously produced in *Pichia* between 200 and 300 mg/L (Pedersen et al. 2011; Song et al. 2016).

Homologous expression of hydrophobin HFB1 class II in *T. reesei* produced 600 mg/L yield, however it required further purification as most proteins were mycelium bound (Askolin et al. 2001). In *S. commune*, homologous over-expression of hydrophobin SC3Class I, led to methylation induced gene silencing (Schuurs et al. 1997).

The self-assembling nature of hydrophobins is a highly desired quality that offers advantages such as immobilization, improved stability and better surface interaction when fused to proteins. Several authors have utilized this property by developing recombinants with fusing either class I or II hydrophobin domains to the protein/peptide of choice for developingbiosensors. Sorrentino et al. (2019) combined the self-assembling properties of hydrophobin class I and laccase enzyme from *Plurotus ostreatus* to develop a stabilized fusion protein coating on polystyrene surfaces as a biosensor for L-DPOA and caffeic acid estimation in food and pharmaceutics. Soikkeli et al. (2016) fused the hydrophobin domain of hydrophobin HFBI class II to Protein A or a small peptide ZE that led to significant stabilization and successful biosensor development. Döring et al. (2019) fused the hydrophobin Ccg2 from *Neurospora crassa* with glyphosate binding enzyme 5-enolpyruvylshikimate-3-phosphatesyhthase (EPSPS) to fabricate a photometric biosensor for pesticide sensing. Similarly, Mirzaei et al.(2019) demonstrated improved enzyme stability of recombinant lactate dehydrogenase enzyme fused with hydrophobin. The recombinant fusion protein of a thermophilic arsenate reductase from *Thermus thermophilus* with a hydrophobin from *P. ostreatus* having self-assembling property, was produced and successfully employed as biosensor in Arsenic detection (Puopolo et al. 2021).

Suspension culture and transgenic plants are emerging as plant-based heterologous hosts for hydrophobin production. The expression of GFP-HFBI was reported from *Nicotiana benthamiana* (tobacco) that achieved 3.7 mg/g productivity of fresh leaf weight, constituting around half of the total soluble proteins (Joensuu et al. 2010). Expression of GFP-HFBI was reported in suspension culture with a high-expressing BY-2 clone with a yield of 1.1 g/L. Suspension cultures may contribute to the industrial scale-up of hydrophobin production technology (Häkkinen et al. 2018). The high yields of hydrophobins were obtained by stable expression of GFP-HFBI fusion protein in the leaves of *Nicotiana benthamiana* (Joensuu et al. 2010; Reuter et al. 2016; Gutiérrez et al. 2013) and tobacco BY-2 suspension cells. The intriguing growth of hydrophobin-induced protein aggregates in leaf cells was attributed to the improved concentration of recombinant proteins, which kept them shielded from proteases in the cytoplasm (Gutiérrez et al. 2013).

The unique self-assembling and amphipathic properties of hydrophobin are harnessed for several applications.

6 Applications of Hydrophobins

Fungal hydrophobins are utilized to solubilize and deliver hydrophobic medicines, as well as for purification and immobilization of proteins, as a antimicrobial coats, in biosensor development, and also as emulsifying agents.

6.1 Drug Delivery and Improved Solubility

The amphipathic nature of hydrophobins is conducive for their application for drug delivery of hydrophobic drugs. Valo et al. (2010) synthesize hydrophobin-coated drug nanoparticles (NPs) by inserting a lipophilic drug beclomethasone dipropionate into the cavity formed by hydrophobin. Beclomethasone dipropionate NPs showed improved solubility, biocompatibility and were stable for ~5 h in suspension followed by longer after freeze-drying. Recently, glucagon-like peptide-1 (GLP-1), a promising drug candidate for treating type 2 diabetes, was stabilized against protease degradation encapsulation of the drug in the cavity formed by the MH protein (Zhao et al. 2016a, b).

For coating docetaxel (DTX) NPs to be used for intra-venous drug delivery, a novel hydrophobin H star protein B (HPB) was used (Fang et al. 2014). Reuter et al. (2017) used porous silicon NPs coated with a HFBIV fused transferrin protein produced in plants for enhanced uptake of the drug by the cells of human breast cancer. Purified *Trichoderma reesei* HFBI and HFBII were coated on PSi nanoparticles to improve their biodistribution when administered intravenously to rats (Valo et al. 2010; Sarparanta et al. 2012). Further, the two cellulose-binding

domains were conjugated to HFB-coated itraconazole nanoparticles to facilitate their oral administration.

Recently, Politi et al. (2015) synthesized a stable protein–gold NPs hybrid for drug targeting and delivery by conjugating HFB to gold NPs for sensing and glucose monitoring. A Vmh2, a hydrophobin derived from *Pleurotus ostreatus,* was modified with dicarboxylic acid-terminated polyethylene glycol (PEG) and used to adorn gold nanoparticles, PEG-HFB-AuNPs (Politi et al. 2015, 2016). Coating hydrophobins on the surface of nanocarriers has a substantial influence on their behaviour when introduced into biological fluids, preventing the formation of protein corona that impacts the NPs in biological environments.

Reuter et al. (2017) recently created a fusion protein of *T. reesei* hydrophobins class II to human transferrin protein. They coated it on porous silicon nano-proteins to enhance uptake in cancer cells. Furthermore, highly conserved, stable disulfide bonds in the class II hydrophobins were used as a drug-release mechanism for improved drug delivery. For drug loading in the blood, dodecanethiol-coated gold nanoparticles were stabilized by a coating of class II hydrophobin HFBII. The uptake of these NPs by tissues resulted in the reduction of disulphides by cytoplasmic glutathione, allowing the drug to be released into the cytoplasm (Maiolo et al. 2017).

Hydrophobin coated niosomes (non-ionic surfactant based vesicles) were developed to deliver drugs to cancer cells to evade the recognition by human immune system (Barani et al. 2020). HFBI coated niosomes were used to enhance the efficacy and slow release of doxorubicin delivery to cancer cells. HFBI is more stable coating agent as compared to the commonly used PEG (polyethylene glycol).

Thus, hydrophobins are effective adjuvants for better drug (hydrophobic) solubility, stability, and bioavailability for drug-formulations. Overall, hydrophobin-based drug formulations are possible. There is a need to better understand hydrophobin structure as well as function to develop new hydrophobins as biocompatible coatings for increased drug bioavailability.

6.2 Biosensor Applications

The ordered self-assembly of hydrophobins make them suitable for electrochemical biosensor applications as well. Hydrophobin class I from *S. commune* immobilized redox enzymes for their catalytic activity on glassy carbon electrodes (Corvis et al. 2005).

Using hydrophobin HFBI class II as an enzyme immobilised matrix on platinum electrodes, a sensitive and effective glucose biosensor with high electroactivity and longevity was developed (Zhao et al. 2007). HFBI was also utilised to increase the wettability of a gold surface and immobilise the enzyme choline oxidase in an amperometric choline biosensor (Zhao et al. 2009). The sensor was stable for up to 7 weeks (Zhao et al. 2009). Recently, gold nanoparticles complexed with a

hydrophobin Vmh2 class I, which binds to carbohydrates naturally, were used to make a glucose biosensor in a one-pot synthesis (Spadavecchia et al. 2016). The unique binding properties of hydrophobins can be used for specific applications. A yeast pheromone detection biosensor was reported using recombinant class I hydrophobin, combined with or without alpha-factor, to wet a hydrophobic polystyrene surface (Hennig et al. 2016). The biosensor was robust, stable and can be used many times without loss of sensitivity (Hennig et al. 2016). In another interesting application, patterning genetically engineered hydrophobin HFBI with an N-terminal cysteine residue on gold nanoparticles in a pH-controlled manner resulted in a functional electrical interface for biosensors (Laaksonen et al. 2009). The self-assembling characteristic of the hydrophobin domain of the recombinant hydrophobin HFBI class II coupled to Protein A or a short peptide ZE was exploited to build a highly sensitive label free graphene biosensor (Soikkeli et al. 2016). Further, they demonstrated that the biosensor was stable, the monolayers survive drying, and is compatible with the small as well as large analytes (charged peptide and immunoglobin) (Soikkeli et al. 2016).

A photometric biosensor was constructed by surface functionalizing and fusing the hydrophobin Ccg2 from *Neurospora crassa* with the glyphosate binding enzyme 5-enolpyruvylshikimate-3-phosphatesyhthase (EPSPS) (Döring et al. 2019). The biosensor inhibits enzyme activity by glyphosate, a harmful pesticide, which is measured colourimetrically with malachite green to calculate inorganic phosphate. Duan et al. (2020) designed a fibre-optic biosensor to detect label-free antigen-antibody interaction by coating the fibre surface with a nanolayer of self-assembled class I hydrophobin from *Grifola frondosa*. The hydrophobin layer facilitated the immobilization of antibodies on the fibre biosensor surface. Mirzaei et al.(2019) reported a biosensor for pyruvate determination by coating glassy carbon electrodes with a fusion protein of hydrophobin and lactate dehydrogenase enzyme. The hydrophobin layer improved enzyme stability and demonstrated low detection limits (~8.69 nM). An electrochemical biosensor for arsenic (AsIII) detection was developed by coupling the thermophilic arsenate reductase from *Thermus thermophilus* with hydrophobin from *Plurotus ostreatus* with self-assembling properties (Puopolo et al. 2021). The chimeric protein ensured self-assembly of protein on the gold and polystyrene electrodes sensor surfaces, immobilization and proper orientation of the enzyme for detection of AsIII. Hydrophobin anchorage led to improved catalytic activity and was re-usable three times.

Sorrentino et al. (2019) created a fusion protein combining self-assembling properties of hydrophobin class I and laccase enzyme from *P. ostreatus*. The fusion protein was coated on polystyrene surfaces of plates and used for detection of L-DOPA ((*S*)2-amino-3-(3,4-dihydroxyphenol) propanoic acid) and caffeic acid (3-(3,4-dihydroxyphenyl)-2-propenoic acid) for estimation for food and pharmaceutics. The method was based on the inhibition of laccase-mediated ABTS oxidation by the analytes as competing inhibitors.

6.3 Tissue Engineering and Biomedical Applications

Hydrophobins enhance the biocompatibility of surfaces that are employed for tissue engineering and as biomedical implants. Invasive medical devices like catheters and guide wires require low friction surfaces to decrease tissue injury. Hydrophobin SC3 from recombinant *S. cerevisiae* was coated on polystyrene with spin coating and altered the surfaces, reduced the friction and rendered them biocompatible for use as medical device coatings (Misra et al. 2006). Devine et al. (2019) coated SC3 on a medical-grade nitric oxide (NO) releasing polymer, which provided antibacterial and antiplatelet properties to the medical devices.

Hydrophobin coatings are used to enhance cell attachment and growth on various surfaces. HFB1-collagen and HFB1-serum layer promote attachment and growth, respectively (Li et al. 2009). Scholtmeijer et al. (2002) stated that hydrophobin coated Teflon improved the biocompatibility and growth of fibroblast cells. The immobilization of class II hydrophobins on solid surfaces promoted the growth of human embryogenic kidney cells as well as neural stem cells (Hou et al. 2009).

Similarly, Zhang et al. (2011) improved the hydrophilicity and designed bioactive surfaces of electrospun PCL grafts for tissue engineering by using hydrophobin HFBI. Coating the HFBI layer on the PCL surface promoted attachment of anti-CD31 antibody and the retention of endothelial cells to the grafts. The introduction of a hydrophobin HGFI class I linked to vascular endothelial growth factor synthesized by the yeast Pichia pastoris increased the cellularization as well as vascularization of PCL scaffolds (Zhao et al. 2016a, b). Furthermore, to promote mesenchymal stem cell, osteoblast, fibroblast, and chondrocyte adhesion, orthopaedic implant surfaces were modified with a recombinant hydrophobin DewA class I coupled to an integrin-binding Arginine-Glycine-Aspartic acid motif (RGD) or laminin domain (Boeuf et al. 2012). The antibacterial activities of electrospun PCL grafts were enhanced by adding the class IIa antibacterial peptide, bacteriocin pediocin PA-1, linked to the hydrophobin HGFI class I produced in *S. cerevisiae* (Wang et al. 2017).

Adsorption of proteins on dental surfaces leads to steady deterioration. Melcher et al. (2016) demonstrated the reconstruction of eroded teeth by binding class I DewA hydrophobin fusion proteins with hydroxyapatite and calcium phosphate. Bile stents require replacement due to clogging caused by microbial growth. Weickert et al. (2011) reported that coating the stents with hydrophobin DewA-His fusion protein reduced the absorption of proteins and clogged.

6.4 Stabilization of Emulsions, Ointments, Foam

Hydrophobins stabilize emulsions like creams or ointments due to their assembly at the interface of the hydrophilic as well as hydrophobic liquids. Stabilization of foam formation is useful in beer brewing and sparkling wine production. However,

excessive hydrophobins in beer can promote primary 'gushing' or extensive foaming with spillovers (Mastanjevic et al. 2017). Primary gushing is induced by the presence of fungal hydrophobins in the malt, especially of *Fusarium* species. Other species such as *Rhizopus, Aspergillus, Penicillium* and *Nigrospora* are also associated with the primary gushing. The chemical or technological processes influence secondary gushing during malt brewing. Losses during malting and brewing can be reduced by avoiding or controlling this phenomenon by biological, physical or chemical methods. Biological methods include reduction of *Fusarium* infections in the field by lactic acid bacteria dispersion. Physical methods involve monitoring storage time of grains, mixing different batches, sorting infected grain, filtration, radiation, sonication, while chemical methods include treatments with hydrogen peroxide, polar/non-polar molecules or hop compounds. High emulsifying capacity of the class II hydrophobins showed longer stabilization effect on oil than the class I hydrophobins (Niu et al. 2012).

7 Conclusions

The remarkable cysteine-rich amphipathic hydrophobin proteins are found throughout the fungal kingdom and play important roles in the growth as well as development of fungi. These well-characterized fungal hydrophobins are produced mostly by the filamentous fungi and have several applications due to their amphipathic nature. Yeast-like fungi are mostly known to produce emulsifiers that may be useful in maintaining their single celled entity. The unique surface-activity of fungal hydrophobins can be employed to solubilize and deliver hydrophobic medicines, as well as for purification and immobilization of proteins, as a antimicrobial coats, in biosensor development, and also as emulsifying agents.

However, the large-scale production of native and engineered hydrophobins are required to realize their commercial potential. Further studies to understand the structure-function relationship of hydrophobins may result in enhanced surface activity, binding and solubilisation of these interesting proteins.

Acknowledgement The financial support from the Department of Biotechnology, New Delhi is gratefully acknowledged.

References

Albuquerque A, Kyaw CM, Saldanha RR, Brigido MM, Felipe MMS, Silva-Pereira I (2004) *Pbhyd1* and *Pbhyd2*: two mycelium-specific hydrophobin genes from the dimorphic fungus *Paracoccidioides brasiliensis*. Fungal Genet Biol 41:510–520

Asgeirsdattir SA, Van Wetter MA, Wessels JGH (1995) Differential expression of genes under control of the mating-type genes in the secondary mycelium of *Schizophyllum commune*. Microbiology 141:1281–1288

Asgeirsdattir SA, Halsall JR, Casselton LA (1997) Expression of two closely linked hydrophobin genes of Coprinus cinereus is monokaryon-specific and down-regulated by the oid-1 mutation. Fungal Genet Biol 22:54–63

Askolin S, Nakari-Setälä T, Tenkanen M (2001) Overproduction, purification, and characterization of the *Trichoderma reesei* hydrophobin HFBI. Appl Microbiol Biotechnol 57:124–130

Barani M, Mirzaei M, Torkzadeh-Mahani M, Lohrasbi-Nejad A, Nematollahi M (2020) A new formulation of hydrophobin-coated niosome as a drug carrier to cancer cells. Mater Sci Eng C 113:110975

Bayry J, Aimanianda V, Guijarro JI, Sunde M, Latgé J-P (2012) Hydrophobins—unique fungal proteins. PLoS Pathog 8:e1002700

Bell-Pedersen D, Dunlap JC, Loros JJ (1992) Clock-controlled genes isolated from Beurospira crassa are late night-to morning specific. Genes Dev 6:2382–2394

Boeuf S, Throm T, Gutt B, Strunk T, Hoffmann M, Seebach E, Mühlberg L, Brocher J, Gotterbarm T, Wenzel W, Fischer R, Richter W (2012) Engineering hydrophobin DewA to generate surfaces that enhance adhesion of human but not bacterial cells. Acta Biomater 8: 1037–1047

Corvis Y, Walcarius A, Rink R, Mrabet NT, Rogalska E (2005) Preparing catalytic surfaces for sensing applications by immobilizing enzymes via hydrophobin layers. Anal Chem 77:1622–1630

De Vocht ML, Reviakine I, Wösten HAB, Brisson A, Wessels JGH, Robillard GT (2000) Structural and functional role of the disulphide bridges in the hydrophobin SC3. J Biol Chem 275:28428–28432

De Vries OMH, Moore S, Arntz C, Wessels JGH, Tudzynski P (1999) Identification and characterization of a tri-partite hydrophobin from *Calviceps fusifomis*: a novel type of class II hydrophobin. Eur J Biochem 262:377–385

Devine R, Singha P, Handa H (2019) Versatile biomimetic medical device surface: hydrophobin coated, nitric oxide-releasing polymer for antimicrobial and hemocompatible applications. Biomater Sci 7:3438–3449

Döring J, Rettke D, Rödel G, Pompe T, Ostermann K (2019) Surface functionalization by hydrophobin-EPSPS fusion protein allows for the fast and simple detection of glyphosate. Biosensors 9:104

Duan S, Wang B, Qiao M, Zhang X, Liu B, Zhang H, Song B, Wu J (2020) Hydrophobin HGFI–based fibre-optic biosensor for detection of antigen–antibody interaction. Nanophotonics 9:177–186

Fang G, Tang B, Liu Z, Gou J, Zhang Y, Xu H, Tang X (2014) Novel hydrophobin-coated docetaxel nanoparticles for intravenous delivery: *in vitro* characteristics and *in vivo* performance. Eur J Pharm Sci 60:1–9

Fuchs U, Czymmek KJ, Sweigard JA (2004) Five hydrophobin genes in *Fusarium verticillioides* include two required for microconidial chain formation. Fungal Genet Biol 41:852–864

Gutiérrez SP, Saberianfar R, Kohalmi SE, Menassa R (2013) Protein body formation in stable transgenic tobacco expressing elastin-like polypeptide and hydrophobin fusion proteins. BMC Biotechnol 13:40

Häkkinen ST, Reuter L, Nuorti N, Joensuu JJ, Rischer H, Ritala A (2018) Tobacco BY2 media component optimization for a cost-efficient recombinant protein production. Front Plant Sci 9: 45

Hennig S, Rödel G, Ostermann K (2016) Hydrophobin-based surface engineering for sensitive and robust quantification of yeast pheromones. Sensors (Basel) 16:602

Holder DJ, Kirkland BH, Lewis MW, Keyhani NO (2007) Surface characteristics of the entomopathogenic fungus *Beauveria* (*Cordyceps*) *bassiana*. Microbiology 153:3448–3457

Hou S, Li X, Li X, Feng X-Z, Wang R, Wang C, Yu L, Qiao M-Q (2009) Surface modification using a novel type I hydrophobin HGFI. Anal Bioanal Chem 394:783–789

Joensuu JJ, Conley AJ, Lienemann M, Brandle JE, Linder MB, Menassa R (2010) Hydrophobin fusions for high-level transient protein expression and purification in *Nicotiana benthamiana*. Plant Physiol 152:622–633

Kapoor M (2012) Host-pathogen interaction in *Metarhizium anisopliae* and its host *Helicoverpa armigera*. PhD thesis, Savitribai Phule Pune University, Pune

Kershaw MJ, Wakley G, Talbot NJ (1998) Complementation of the *Mpg1* mutant phenotype in *Magnaporthe grisea* reveals functional relationships between fungal hydrophobins. EMBO J 17:3838–3849

Kirkland BH, Keyhani NO (2011) Expression and purification of a functionally active class I fungal hydrophobin from the entomopathogenic fungus Beauveria bassiana in *E. coli*. Ind J Microbiol Biotechnol 38:327–335

Kim S, Ahn IP, Lee YH (2001) Analysis of gene expressed during rice Magnaporhte grisea interactions. Mol Plant-Microbe Interact 14:1340–1346

Klimes A, Dobinson KF (2006) A hydrophobin gene, *VDH1*, is involved in microsclerotial development and spore viability in the plant pathogen *Verticillium dahlia*. Fungal Genet Biol 43:283–294

Kulkarni S, Nene S, Joshi K (2017) Production of hydrophobins from fungi. Process Biochem 61: 1–11

Laaksonen P, Kivioja J, Paananen A, Kainlauri M, Kontturi K, Ahopelto J, Linder MB (2009) Selective nanopatterning using citrate-stabilized Au nanoparticles and cystein-modified amphiphilic protein. Langmuir 25:5185–5192

Li S, Myung K, Guse D, Donkin B, Proctor RH, Grayburn WS, Calvo AM (2006) *FvVE1* regulates filamentous growth, the ratio of microconidia to macroconidia and cell wall formation in *Fusarium verticillioides*. Mol Microbiol 62:1418–1432

Li X, Hou S, Feng X, Yu Y, Ma J, Li L (2009) Patterning of neural stem cells on poly(lactic-co-glycolic acid) film modified by hydrophobin. Colloids Surf B Biointerfaces 74:370–374

Linder MB, Szilvay GR, Nakari-Setälä T, Penttilä ME (2005) Hydrophobins: the protein-amphiphiles of filamentous fungi. FEMS Microbiol Rev 29:877–896

Lugones LG, Bosscher JS, Scholtmeijer K, De Vries OMH, Wessels JGH (1996) An abundant hydrophobin (ABH1) forms hydrophocin rodlet layers in Agaricus bisporus fruiting bodies. Microbiology 142:1321–1329

Maiolo D, Pigliacelli C, Sánchez Moreno P, Violatto MB, Talamini L, Tirotta I, Piccirillo R, Zucchetti M, Morosi L, Frapolli R, Candiani G, Bigini P, Metrangolo P, Baldelli BF (2017) Bioreducible hydrophobin-stabilized supraparticles for selective intracellular release. ACS Nano 11:9413–9423

Mastanjevic K, Mastanjevic K, Krstanovic V (2017) The gushing experience—a quick overview. Beverages 3:25

Melcher M, Facey SJ, Henkes TM, Subkowski T, Hauer B (2016) Accelerated nucleation of hydroxyapatite using an engineered hydrophobin fusion protein. Biomacromolecules 17: 1716–1726

Mirzaei F, Mirzaei M, Torkzadeh-Mahani M (2019) A hydrophobin-based-biosensor layered by an immobilized lactate dehydrogenase enzyme for electrochemical determination of pyruvate. Bioelectrochemistry 130:107323

Misra R, Li J, Cannon GC, Morgan SE (2006) Nanoscale reduction in surface friction of polymer surfaces modified with Sc3 hydrophobin from *Schizophyllum commune*. Biomacromolecules 7: 1463–1470

Munaoz G, Nakari-Setälä T, Agosin E, Penttilä M (1997) Hydrophobin gene srh1, expressed during sporulation of the biocontrol agent Trichoderma harzianum. Curr Genet 32:225–230

Nakari-Setala T, Aro N, Kalkkinen N, Alatalo E, Penttila M (1996) Genetic and biochemical characterization of the Trichoderma reesei hydrophobin HFB1. Eur J Biochem 235:248–255

Niu B, Wang D, Yang Y, Xu H, Qiao M (2012) Heterologous expression and characterization of the hydrophobin HFBI in *Pichia pastoris* and evaluation of its contribution to the food industry. Amino Acids 43:763–771

Paris S, Debeaupuis JP, Crameri R, Carey M, Charlès F, Prevost MC, Schmitt C, Philippe B, Latge JP (2003) Conidial hydrophobins of *Aspergillus fumigatus*. Appl Environ Microbiol 69:1581–1588

Pedersen MH, Borodina I, Moresco JL, Svendsen WE, Frisvad JC, Søndergaard I (2011) High-yield production of hydrophobins RodA and RodB from *Aspergillus fumigatus* in *Pichia pastoris*. Appl Microbiol Biotechnol 90:1923–1932

Politi J, Stefano LD, Longobardi S, Giardina P, Rea I, Methivier C, Pradier CM, Casale S, Spadavecchia J (2015) The amphiphilic hydrophobin Vmh2 plays a key role in one step synthesis of hybrid protein-gold nanoparticles. Colloids Surf B Biointerfaces 136:214–221

Politi J, Stefano LD, Rea I, Gravagnuolo AM, Giardina P, Methivier C, Casale S, Spadavecchia J (2016) One-pot synthesis of a gold nanoparticle-Vmh2 hydrophobin nano-biocomplex for glucose monitoring. Nanotechnology 27:195701

Puopolo R, Sorrentino I, Gallo G, Piscitelli A, Giardina P, LeGof A, Fiorentino G (2021) Self-assembling thermostable chimeras as new platform for arsenic biosensing. Sci Rep 11:2991

Reuter L, Ritala A, Linder M, Joensuu J (2016) Novel hydrophobin fusion tags for plant-produced fusion proteins. PLoS One 11:e0164032

Reuter LJ, Shahbazi MA, Mäkilä EM, Salonen JJ, Saberianfar R, Menassa R, Santos HA, Joensuu JJ, Ritala A (2017) Coating nanoparticles with plant produced transferrin–hydrophobin fusion protein enhances their uptake in cancer cells. Bioconjug Chem 28:1639–1648

Sarparanta M, Bimbo LM, Rytkonen J, Makila E, Laaksonen TJ, Laaksonen P, Nyman M, Salonen J, Linder MB, Hirvonen J, Santos HA, Airaksen AJ (2012) Intravenous delivery of hydrophobin-functionalized porous silicon nanoparticles: stability, plasma protein adsorption and biodistribution. Mol Pharm 9:654–663

Schmoll M, Seibel C, Kotlowski C, Wöllert Genannt Vendt F, Liebmann B, Kubicek CP (2010) Recombinant production of an *Aspergillus nidulans* class I hydrophobin (*DewA*) in *Hypocrea jecorina* (*Trichoderma reesei*) is promoter-dependent. Appl Microbiol Biotechnol 88:95–103

Scholtmeijer K, Janssen MI, Gerssen B, de Vocht ML, van Leeuwen BMM, van Kooten TG, Wösten HAB, Wessels JGH (2002) Surface modifications created by using engineered hydrophobins. Appl Environ Microbiol 68:1367–1373

Schuurs TA, Schaeffer EA, Wessels JG (1997) Homology-dependent silencing of the *SC3* gene in *Schizophyllum commune*. Genetics 147:589–596

Soikkeli M, Kurppa K, Kainlauri M, Arpiainen S, Paananen A, Gunnarsson D, Joensuu JJ, Laaksonen P, Prunnila M, Linder MB, Ahopelto J (2016) Graphene biosensor programming with genetically engineered fusion protein monolayers. ACS Appl Mater Interfaces 8:8257–8264

Song D, Gao Z, Zhao L, Wang X, Xu H, Bai Y, Zhang X, Linder MB, Feng H, Qiao M (2016) High-yield fermentation and a novel heat-precipitation purification method for hydrophobin HGFI from *Grifola frondosa* in *Pichia pastoris*. Protein Expr Purif 128:22–28

Sorrentino I, Giardina P, Piscitelli A (2019) Development of a biosensing platform based on a laccase-hydrophobin chimera. Appl Microbiol Biotechnol 103:3061–3071

Spadavecchia JP, De Stefano L, Rea I, Gravagnuolo AM, Giardina P, Methivier C, Casale S, Politi J (2016) One-pot synthesis of a gold nanoparticle–Vmh2 hydrophobin nano-biocomplex for glucose monitoring. Nanotechnology 27:195701

Spanu P (1997) HCF-1, a hydrophobin from the tomato pathogen Cladosporium fulvum. Gene 193: 89–96

Stringer MA, Dean RA, Sewall TC, Timberlake WE (1991) Rodlet less, a new Aspergillus developmental mutant induced by directed gene inactivation. Genes Dev 5:1161–1171

St. Leger RJ, Staples RC, Roberts DW (1992) Cloning and regulatory analysis of a starvation strss gene Ssga, encoding a hydrophobin-like protein from the entomopahtogenic fungus, Metarhizium anisopliae. Gene 120:119–124

Talbot NJ, Ebbole DJ, Hamer JE (1993) Identification and characterization of *MPG1*, a gene involved in pathogenicity from the rice blast fungus *Magnaporthe grisea*. Plant Cell 5:1575–1590

Teertstra WR, Deelstra HJ, Vranes M, Bohlmann R, Kahmann R, Kämpe J, Wosten HAB (2006) Repellents have functionally replaced hydrophobins in mediating attachment to a hydrophobic surface and in formation of hydrophobic aerial hyphae in *Ustilago maydis*. Microbiology 152: 3607–3612

Trembley ML, Ringli C, Honegger R (2002) Hydrophobin DGH1, DGH2 and DGH3 in the lichenforming basidiomycete Dictyonema glabratum. Fungal Genet Biol 35:247–259

Valo H, Laaksonen P, Peltonen L, Linder M, Hirvonen J, Laaksonen T (2010) Multifunctional hydrophobin: toward functional coatings for drug nanoparticles. ACS Nano 4:1750–1758

Van Wetter MA, Schuren FHJ, Schuurs TA, Wessels JGH (1996) Targeted mutation of the *SC3* hydrophobin gene of *Schizophyllum commune* affects formation of aerial hyphae. FEMS Microbiol Lett 140:265–269

Wang Z, Feng S, Huang Y, Li S, Xu H, Zhang X, Bai Y, Qiao M (2010) Expression and characterization of a *Grifola frondosa* hydrophobin in *Pichia pastoris*. Protein Expr Purif 72: 19–25

Wang X, Mao J, Chen Y, Song D, Gao Z, Zhang X, Bai Y, Saris PEJ, Feng H, Xu H, Qiao M (2017) Design of antibacterial biointerfaces by surface modification of poly(ε-caprolactone) with fusion protein containing hydrophobin and PA-1. Colloids Surf B Biointerfaces 151:255–263

Weickert U, Wiesend F, Subkowski T, Eickhoff A, Reiss G (2011) Optimizing biliary stent patency by coating with hydrophobin alone or hydrophobin and antibiotics or heparin: an *in vitro* proof of principle study. Adv Med Sci 56:138–144

Wessels JG (1997) Hydrophobins: proteins that change the nature of the fungal surface. Adv Microb Physiol 38:1–45

Whiteford JR, Spanu PD (2001) The hydrophobin HCf-1 of *Cladosporium fulvum* is required for efficient water-mediated dispersal of conidia. Fungal Genet Biol 32:159–168

Wohlleben W, Subkowski T, Bollschweiler C, von Vacano B, Liu Y, Schrepp W, Baus U (2010) Recombinantly produced hydrophobins from fungal analogues as highly surface-active performance proteins. Eur Biophys J 39:457–468

Wösten HAB (2001) Hydrophobins: multipurpose proteins. Annu Rev Microbiol 55:625–646

Wösten HAB, Scholtmeijer K (2015) Applications of hydrophobins: current state and perspectives. Appl Microbiol Biotechnol 99:1587–1597

Wösten H, Wessels J (1997) Hydrophobins, from molecular structure to multiple functions in fungal development. Mycoscience 38:363–374

Wosten HA, Asgeirsdottir SA, Krook JH, Drenth JH, Wessels JG (1994) The fungal hydrophobin Sc3p self-assembles at the surface of aerial hyphae as a protein membrane constituting the hydrophobic rodlet layer. Eur J Cell Biol 63:122–129

Yaguchi M, Pusztai-Carey M, Roy C, Surewicz WK, Carey PR, Stevenson KJ, Richards WC, Takai S (1993) Cellular and molecular approaches. In: Sticklen MB, Sherald JL (eds) Dutch elm disease research. Springer, New York, pp 152–170

Zhang M, Wang Z, Wang Z, Feng S, Xu H, Zhao Q, Wang S, Fang J, Qiao M, Kong D (2011) Immobilization of anti-CD31 antibody on electrospun poly(ε-caprolactone) scaffolds through hydrophobins for specific adhesion of endothelial cells. Colloids Surf B Biointerfaces 85:32–39

Zhao Z-X, Qiao M-Q, Yin F, Shao B, Wu B-Y, Wang Y-Y, Wang X-S, Qin X, Li S, Yu L, Chen Q (2007) Amperometric glucose biosensor based on self-assembly hydrophobin with high efficiency of enzyme utilization. Biosens Bioelectron 22:3021–3027

Zhao Z-X, Wang H-C, Qin X, Wang X-S, Qiao M-Q, Anzai J, Chen Q (2009) Self-assembled film of hydrophobins on gold surfaces and its application to electrochemical biosensing. Colloids Surf B Biointerfaces 71:102–106

Zhao L, Ma S, Pan Y, Zhang Q, Wang K, Song D, Wang X, Feng G, Liu R, Xu H, Zhang J, Qiao M, Kong D (2016a) Functional modification of fibrous PCL scaffolds with fusion protein VEGF-HGFI enhanced cellularization and vascularization. Adv Healthc Mater 5(18):2376–2385

Zhao L, Xu H, Li Y, Wnag X, Qiao M, Gong M (2016b) Novel application of hydrophobin in medical science: a drug carrier for improving serum stability. Sci Rep 6:26461

Fungal Exopolymeric Substances and Their Applications

Murat Topal and E. Işıl Arslan Topal

Abstract Exopolymeric substances that are also known as extracellular polymeric substances, extracellular polysaccharides, exopolymers, and exopolysaccharides are produced by various microorganisms. They primarily include polysaccharides, lipids, nucleic acids, and proteins. Exopolymeric substances protect microorganisms from various environmental stresses. Exopolymeric substances have unique characteristics. Some of the functional properties of them include adhesion, aggregation, binding activity, energy and nutrient source, water retention, and sorption. One of the exopolymeric substances is fungal exopolymeric substances. The fungal exopolysaccharides are pullulan, scleroglucan, fungal β-glucans, botryosphaeran, and others. The fungal exopolymeric substances are valuable products because of their different application areas such as industries of medical, cosmetic, food, wastewater treatment and agriculture.

Keywords Exopolymeric substances · Fungal · Industry · Agriculture · Wastewater

1 Introduction

The production of exopolymeric substances by microorganisms was firstly reported by Pasteur in the year 1861 as viscous fermentation. Compared with the cell walls and intracellular polysaccharides, exopolysaccharides have various advantages including easy isolation, extremely high production in a little while, and easy purification (Mahapatra and Banerjee 2013). The microbial exopolymeric substances are a class of high-value biopolymers (Hwang et al. 2004). Exopolymeric substances are also named as extracellular polymeric substances, extracellular

M. Topal (✉)
Department of Chemistry and Chemical Processing Technologies, Tunceli Vocation School, Munzur University, Tunceli, Turkey
e-mail: murattopal@munzur.edu.tr

E. I. Arslan Topal
Department of Environmental Engineering, Faculty of Engineering, Fırat University, Elazığ, Turkey

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_4

polysaccharides, exopolymers, and exopolysaccharides. The various strains of microbes (bacteria, cyanobacteria, microalgae, yeasts, fungi, and protists) biosynthesize exopolymeric substances (Pavlova and Grigorova 1999; Hwang et al. 2004; Jain et al. 2005; Parikh and Madamwar 2006; Elisashvili et al. 2009; Boonchai et al. 2014; Lee Chang et al. 2014; Costa et al. 2018). The extracellular polymeric substances are secondary metabolites (Nookongbut et al. 2020). Exopolymeric substances are heat stable, non-branched carbohydrate polymers (Giovannangelo et al. 2007). The exopolymeric substances having microbial origins include protein, polysaccharides, lipids, and nucleic acid (Baumert et al. 2021). Extracellular polymeric substances mainly include polysaccharides. However, protein is an important functional component (Flemming et al. 2007; Redmile-Gordon et al. 2020). The results of the researches on exopolymeric substances released by various strains of microbes proved that polysaccharide in extracellular polymeric substances varies highly in structure and composition. The polysaccharides can consist of by $\geq$1 structural units that arrangement of them is special for per extracellular polymeric substances (Roca et al. 2015; Costa et al. 2018).

There are two types of extracellular polymeric substances that may be separated when centrifugation is used. The extracellular polymeric substances that remain in the supernatant are soluble. The extracellular polymeric substances that form bio pellets are bound to extracellular polymeric substances (Yu et al. 2009; Liu et al. 2010; Guo et al. 2016). Bound extracellular polymeric substances can be defined by two layer model (Nielsen and Jahn 1999). Bound exopolymeric substances consist of tightly-bound exopolymeric substances and loosely bound exopolymeric substances. The tightly-bound exopolymeric substances are an inner layers that has a definite shape and are bound tightly and stably with the surface of the cell while the loosely-bound exopolymeric substances are the outer layers that are loose and dispersible, without evident edge (Guo et al. 2016).

Exopolymeric substances are fundamental for the life of microorganisms. They supply an ideal environment for microorganisms. They protect the microorganisms from environmental stresses. Exopolymeric substances production is triggered primarily by various environmental signals (Costa et al. 2018). Exopolymeric substances create appropriate outer coating allowing microbes for protection against drying by the accumulation of water. In addition, because of the anionic nature of extracellular polymeric substances, suitable nutrients from the medium can be captured. The extracellular polymeric substances protect microorganisms against unwanted metals (Donot et al. 2012; Bancerz et al. 2018).

There is an urgent need for sustainable resources to meet the requirements of industrial applications. Fungal exopolymeric substances that have been recognized as valuable bio macromolecules for recent years are one of that resources. The further development on the commercialization of fungal exopolymeric substances will support sustainable solutions for environmental protection.

2 Functions of Extracellular Polymeric Substances

The exopolymeric substances have various functions, most of them related to protection. Extracellular polymeric substances matrix shields microorganisms against heavy metals and antimicrobials, retain water and protect microorganism against drought (Costa et al. 2018). In the literature, the functions of communication with other plants and microorganisms, antioxidant, C storage, and nutrient capture have been researched (Wingender et al. 1999; Wang et al. 2015; Vardharajula and Ali 2015; Costa et al. 2018). Functional properties of exopolymeric substances are as follows: adhesion, aggregation, binding activity, cohesion, electron acceptor or donor, energy and nutrient source, enzymatic activity, export of cell components, protective barrier, water retention, sorption, and transfer of genetic information (Siddharth et al. 2021). Exopolymeric substances of microbes play a significant influence on the formation of biofilm, transport of bacteria, biomineralization, and accumulation of metals (Chan et al. 2004; Fang et al. 2010; Yu et al. 2018; Bao et al. 2018).

Microbial exopolymeric substances have the heavy metal binding ability. This situation may have several implications (e.g. essential in vertical transport of dissolved ions) (Bhaskar and Bhosle 2005). Extracellular protein plays a significant role to resist heavy metal toxicity and maintain the stability of cell membranes. The extracellular polymeric substances are capable to bind metals (Wang et al. 2021). The exopolymeric substances role on biomineralization is classified to crystal geometric matching, electrostatic interaction and charge accumulation, and complementary structure in space (Andrew et al. 1998; Couchourel et al. 1999; McGrath 2001; Bontognali et al. 2014; Bao et al. 2018). Bao et al. (2018) noticed that fungal exopolymeric substances were important for providing nucleation sites for binding metals and facilitating large mineral aggregates generation. In biologically induced mineralization microorganisms produce extracellular polymeric substances or charge the surface of the cell acting as interaction or adsorption sites for metals and thereby modifying their local micro environments. This situation can result in the nucleation and growth of the new minerals. The extracellular polymeric substances in biomineralization include charge accumulation and electrostatic interactions (McGrath 2001). The fungal extracellular polymeric substances have the ability of chelating harmful metals (e.g. Pb, Zn, and Cu) (Moon et al. 2006; Yin et al. 2011). Planý et al. (2021) reported in their study that Scopulariopsis-Scedosporium-like fungus precipitated gypsum. The fungus tolerated high concentrations of Fe by depositing on fungal structures. In the study of Yu et al. (2019), *T. guizhouense* fungus was able to dissolve hematite and form secondary minerals. Yu et al. (2019) reported that fungal hyphae are redox active. Furthermore, fungal hyphae alter the local redox state of Fe. However, Planý et al. (2021) proved that iron deposition can happen on dead fungal mycelia. The dead and living fungus can act as biosorbents for the metals (Mali et al. 2014; Planý et al. 2021). Wang et al. (2021) studied *Aspergillus fumigatus* assisted algae symbiosis to determine the flocculation and the immobilization by researching adsorption behavior for metals and change in

extracellular polymeric substances. Wang et al. (2021) reported that the findings of their study provided basis to develop industrial stable fungi and algae symbiosis systems. The content of exopolymeric substances was higher than extracellular proteins content. The extracellular polymeric substances were rich in functional groups that were negatively charged. Functional groups that negatively charged (e.g. carboxyl, amine, sulfhydryl, imidazole) provide more binding sites to chelate and ion exchange the heavy metals, thereby reducing migration and biotoxicity of the heavy metal.

The extracellular polymeric substances have significance for the structure of soil (Redmile-Gordon et al. 2020). The microbial exopolymeric substances' functions for soil include drought protection, salt tolerance, protection against low/high temperatures, adhesion, cohesion, symbiosis with plants, genetic exchange, antibiotics protection, signaling, biofilm structure, the trap of nutrients, carbon source, and pathogenicity/virulence factor (Costa et al. 2018). The microbes attaching to the physical habitat or co-habiting in groups called biofilms produce exudates as a biological binding agents (Flemming and Wingender 2010). The exuded matrix includes exopolymeric substances (Redmile-Gordon et al. 2020). The protein rich extracellular polymeric substances play the main role in the adhesion and stabilization of the aggregates with microbial origin (Guo et al. 2016; Redmile-Gordon et al. 2020).

The exopolymeric substances have significance for bioaggregate formation, such as flocs of sludge, in process of activated sludge. Guo et al. (2016) investigated the composition and chemical structure of different fractions of exopolymeric substances released from the culture of the activated sludge. Guo et al. (2016) used the techniques of multi analysis to examine the aggregation ability of the sludge. The authors reported that there were the great proportion of hydrophobic groups in tightly-bound exopolymeric substances. The tightly-bound exopolymeric substances contained high amounts of protein substance (86.7 mg of MLVSS per gram, with 39.7% of total extracellular polymeric substances). The hydrogen bonds were reported as the dominant trigger promoting the aggregation of the sludge and the hydrophobic proteins in tightly-bound exopolymeric substances. Hydrophobic proteins had an important effect on the hydrogen bonds in the formation of sludge (Guo et al. 2016).

3 Applications of Exopolymeric Substances

The microbial products have distinctive conformations and uncommon molecular structures, giving them unique and potentially interesting properties with uses in industries (Mahapatra and Banerjee 2013). Therefore, microbial exopolysaccharides have different applications in various industries (Looijestenijn et al. 1999; Levander and Rådström 2001; Hwang et al. 2004). Exopolymeric substances have individual characteristics including gelling, biocompatibility, and thickening capabilities, with applications in industries (Costa et al. 2018). The polysaccharides have many uses in

different areas because of the diversity of structures that give the polysaccharides individual chemical and physical properties (Mahapatra and Banerjee 2013; Bancerz et al. 2018). Polysaccharides have different applications in the industries of food, cosmetics, agriculture, and medical (Osińska-Jaroszuk et al. 2015; Bancerz et al. 2018).

Applications of exopolymeric substances include dye decolorization and removal of heavy metal from effluents, management of leachate, soil remediation and reclamation, treatment of wastewater and water (e.g. removal of organic matters and turbidity from water sources, sludge conditioning) (Siddharth et al. 2021). The microbial exopolymeric substances are also valid alternatives to products originating from alga and plants (Mahapatra and Banerjee 2013).

Extracellular polymeric substances are particularly relevant to the bioremediation process as they are involved in the flocculation and binding of metals from the aqueous phase (Kachlany et al. 2001). Adsorption of heavy metal by extracellular polymeric substance is addressed to the interaction between functional groups that negatively charged and metal cations (e.g. carboxyl, phosphoric, hydroxyl, and amide) present in extracellular polymeric substances (Kim et al. 1996). The extracellular polymeric substance that is non-living and non-metabolic biosorbent is readily available during the process of treatment, does not require any nutritional support maintaining sorbent biomass, and avoids pathogenicity problems from related organisms (Gavrilescu 2004). The problems with the toxicity of metal for biomass living do not apply to extracellular polymeric substances. The requirement for economic, safe, and effective methods to remove the metals from wastewater has focused attention on extracellular polymeric materials (Yin et al. 2011).

The exopolysaccharides are used for pharmaceutical purposes. Because they have different biological activities (Wasser 2002). The extracellular polymeric substances have versatile applications in pharmaceuticals. The exopolysaccharides have emulsifying properties making them a prominent candidate in applications of pharmaceutical. The different extracellular polymeric substances with important functional potentials, making massive applications in pharma industry as viscosifier, biothickeners, emulsifiers, and stabilizers. Furthermore, properties with the biological origin of the extracellular polymeric substances revealed properties of multiple health promotion that can be explored for further applications in the pharmaceutical sectors (Kavitake et al. 2020). In the literature, exopolysaccharides from various microorganisms are suggested for their usage of in the pharmaceutical industry. Eteshola et al. (1996) proved that polysaccharides of *Porphyridium* sp. had good surface activity property showing the potential usage of it as pharmaceutical material. Kavitake et al. (2019) suggested applying exopolysaccharides obtained from *Rhizobium radiobacter* in products of pharmaceutical. The production of (1,3)-β-D-glucans that attracting the increasing interest of pharmaceutical industry (Zekovic et al. 2005) is determined by Garai-Ibabe et al. (2010). Garai-Ibabe et al. (2010) determined that *Pediococcus parvulus* and *Lactobacillus suebicus* produced (1,3)-β-D-glucans. There are reports describing therapeutic usage and biological activity of (1,3)-β-D-glucans (Sletmoen and Stokke 2008; Garai-Ibabe et al. 2010).

The matrix of the extracellular polymeric substances has the ability to aggregate particles of soil. This ability has importance for fertility, structure, and health of the soil. Application of exopolymeric substances on soil aggregation includes plant inoculation and the addition of pure extracellular polymeric substances to soil (Costa et al. 2018). The exopolymeric substances can act as glue, attaching to clay and ions, holding solid particles of solids together. Because extracellular polymeric substances have ionic charges and slimy texture (Chenu 1995). Microbial extracellular polymeric substances have benefits for plants. Microbial extracellular polymeric substances can maintain the moisture of the environment and trap the nutrients. The extracellular polymeric substances structures are variable. Thus, application efficiencies of extracellular polymeric substances vary in soil accordingly (Costa et al. 2018).

Exopolymeric substances produced by cocultures of fungi, microalgae, and bacteria have different application areas (e.g. bio remediation, wastewater treatment, and dewatering of sludge) (Houghton et al. 2001; Ben Rebah et al. 2018; Rashid et al. 2018; Siddharth et al. 2021). Exopolymeric substances act as a primary constituents to produce various biobased product (e.g. bio flocculants) (Urbain et al. 1993). They are important for biofilm matrix formation (Flemming and Wingender 2010) and biofouling of reverse osmosis membrane (Gutman et al. 2014; Siddharth et al. 2021). In the literature, Siddharth et al. (2021) reviewed extracellular polymeric substance applications (e.g. soil remediation of soil, control of erosion, and removal of metals from effluents of various industries). Moreover, wastewater applications and water applications were consolidated by Siddharth et al. (2021).

Microbial extracellular polymeric substances are good biosorbents to recover valuable metal or to remove toxic heavy metal from different wastewaters. Because, the microbial extracellular polymeric substances have properties of ion exchange, high selectivity for various metals as well as cheap production cost and high range of operating conditions (Geesey and Jang 1990; Marques et al. 1990; Volesky 1990; Moon et al. 2006).

The extracellular polymeric substances are alternative sources to product microbial bio flocculants (Siddharth et al. 2021). The microbial bio flocculants, due to their structure, are bio degradable, harmless, and one of the ecofriendly materials due to their structure. The microbial bio flocculants consist of proteins, glycoproteins, and polysaccharides. The microbial flocculants' intermediates are safe for the environment and human beings. Furthermore, microbial enzymes that provide the degradation of bioflocculant are present in various environments (e.g. wastewater, soil) (Ben Rebah et al. 2018). Exopolymeric substances have flocculation activity to treat effluents of industries and wastewaters (Siddharth et al. 2021). Ben Rebah et al. (2018) investigated the extracellular polymeric substance's performance as bioflocculants for conditioning of sludge as well as wastewater treatment to remove pollutants with the organic origin, heavy metal, and solid (Ben Rebah et al. 2018).

In wastewater treatment, exopolymeric substances in sludge have significance for sludge dewatering and thickening (Zhou et al. 2014). The exopolymeric substance helps biofloc formation in process of activated sludge and contributes to sludge's

structure, surface charge, and settling (Sheng et al. 2010). The increase of exopolymeric substances usually leads poor level of sludge's dewatering. The extracellular polymeric substances generate a steric force which inhibits contact between the cells (Sheng et al. 2010; Zhou et al. 2014). Macromolecules in exopolymeric substances cause retention of water in sludge floc thus increasing interstitial water amount (Yang and Li 2009). The low content of exopolymeric substances is useful to improve sludge dewaterability (Yu et al. 2008; Zhou et al. 2014; Huo et al. 2014). The exopolymeric substances in flocs of sludge may be stratified into slime extracellular polymeric substances (loosely bound extracellular polymeric substances and tightly bound extracellular polymeric substances). The protein content or the protein to polysaccharide ratio (PN/PS) content in the slime layer affects the dewaterability of the sludge (Yu et al. 2008; Liu et al. 2010; Zhou et al. 2014; Wang et al. 2015).

The exopolymeric substances have an important role to form bioaggregate (e.g. floc of sludge) in the activated sludge (Ding et al. 2015). Exopolymeric substances are necessary building blocks of aggregates. The amount of the extracellular polymeric substances accounts for about a percentage of 80 of activated sludge mass (Tian 2008). Thus, deciphering mechanisms of microbial aggregation related to the formation of the exopolymeric substances is very important for development of the strategies that are efficient to overcome bulking of sludge (Guo et al. 2016). The aggregation of sludge is essential for separation of liquid and solid in wastewater treatment systems that are of biological origin (Liu et al. 2010). Aggregation is defined as a physical process wherein microparticles collide and remain stuck to each other to form clumps of particles called aggregates. The binding properties of extracellular polymeric substances influence the aggregation process (Bhaskar and Bhosle 2005). Low aggregation of the sludge increases the turbidity of effluent. The aggregation of sludge depends primarily on the structure of the sludge. The structure of the sludge is generally loose and the exopolymeric substances matrix glues the cells together (Wingender et al. 1999; Long et al. 2009). The cells of sludge in the matrix of floc have a two layered extracellular polymeric substances structure. The extracellular polymeric substances bound loosely diffused from extracellular polymeric substances bound tightly surrounding cells (Wingender et al. 1999; Liu and Fang 2003). Exopolymeric substances have importance to maintaining the structure of floc of sludge and functions (Sobeck and Higgins 2002; Sheng and Yu 2006; Liu et al. 2010).

Exopolymeric substance interacts with the other exopolymeric substances through bio-chemical reactions. This situation forms a compact and dense extracellular matrix. The extracellular matrix provides mechanical strength and architectural structure for aerobic granular sludge (Chen et al. 2007; Felz et al. 2016; Geng et al. 2021). Starved microbes progressively consume the extracellular polymeric substances during long term operation due to the diffusion limitation. This situation weakens the matrix structure of aerobic granular sludge. The metal ions that charged positively are able to enhance the strength of the extracellular matrix by stimulating the production of the extracellular polymeric substances and binding functional groups that are negatively charged (Sajjad and Kim 2015; Yilmaz et al. 2016;

Geng et al. 2021). Exopolymeric substance plays a key role in structural stability of aerobic granular sludge (Felz et al. 2016).

4 Fungal Exopolymeric Substances

Some exopolymeric substances from different fungi and their applications are given in Table 1.

The polysaccharides with fungal origin are one of the groups of biopolymers forming components of the wall of cell. The fungal polysaccharides can accumulate inside the cells as a reserve of energy or secrete outside the cells to protect microorganisms against bad environmental conditions (Giavasis 2014). According to the location in cells, polysaccharides are divided into intracellular extracellular polymeric substances, fungal cell wall polysaccharides, and polysaccharides (Jaroszuk-Ściseł and Kurek 2012; Yan et al. 2014). The polysaccharide is a predominant intracellular building block in the cells. Basic function of extracellular polymeric substances is associated with protection of cell against environment (Donot et al. 2012; Bancerz et al. 2018). Fungi secrete extracellular polymeric substances to form heterogeneous units called biofilms on natural or abiotic surfaces.

Table 1 Some exopolymeric substances from different fungi and their applications

Exopolymeric substances	Fungi	Applications	References
Chitosan	*Cunninghamella elegans*	Wound dressing sorbent for chromatography collecting transition metal ions from solutions	Sathiyaseelan et al. (2017), Kucera (2004), Saad (2006)
	Aspergillus niger		
Pullulan	*Aureobasidium pullulans*	Thickener and viscosity stabilizer, low-calorie food additive with excellent film-forming properties in food industry, biomedical application	Shingel (2004), Mahapatra and Banerjee (2013)
Schizophyllan	*Schizophyllum commune*	Antitumor and immune stimulating action, thickener for cosmetic lotions, petroleum recovery, oxygen-impermeable films for food preservation	Zhang et al. (2013), Osemwegie et al. (2020)
Botryosphaeran	*Botryosphaeria rhodina*	Hypolipidaemic and hypoglycaemic, antioxidant, and anticancer activities	Miranda-Nantes et al. (2011), Giese et al. (2015), Queiroz et al. (2015), Eisele et al. (2019)
Lentinan	*Lentinusedodes*	Treat solid tumors	Zhang et al. (2005), Surenjav et al. (2006), Giese et al. (2015)

The extracellular polymeric substances protect cells from aggressive factors (e.g. immunologic defense systems and nutrient limitations) (Zandi et al. 2018). Filamentous fungus growing in a dry environment produces exopolymeric substances protecting fungus from dehydration and maintaining osmotically favorable micro environment for fungal cells. Extracellular polymeric substance is glycoprotein secreted by fungi to surrounding. Some metal ions such as potassium, copper (Cu), sodium (Na), magnesium (Mg), and phosphate could stimulate the extracellular polymeric substances secretion by fungus (Mahapatra and Banerjee 2013).

Fungal exopolysaccharides include pullulan, scleroglucan, and botryosphaeran (Mahapatra and Banerjee 2013). The pullulan is extracellular α-glucan elaborated by yeast like fungus *Pullularia pullulans* (Catley 1971). Pullulan is a non-ionic exopolysaccharide. Pullulan is soluble in water and generally insoluble in solvents with an organic origin. Pullulan aqueous solution is more stable than the other polysaccharides and has relatively low viscosity (Plucinski et al. 2021). Scleroglucan is a fungal polysaccharide. Depending on biological source, it is also called as schizophyllan or lentinan (Van der Valk et al. 1977; Jong and Donovic 1989; Pretus et al. 1991; Hamed and Belhadri 2009). *Sclerotium rolfsii* secretes scleroglucan that is a water soluble beta glucan. Scleroglucan creates extracellular matrix capsule and thus protects cell from dehydration (Farwick et al. 2009). It is glucose polymer forming triple helical structure and representing basis constituent of cell wall of fungi (Jacobi 1958; Farwick et al. 2009). Polysaccharides β-glucans consist as main component of cell walls (Mantovani et al. 2008). Approximately half of mass of cell wall of fungi consists of β-glucans (Seviour et al. 1992; Klis et al. 2001; McIntosh et al. 2005). Many of the β-glucans are also secreted into growth medium. This property makes β-glucan recovery, purification and chemical characterization simple (Seviour et al. 1992; Schmid et al. 2001; Chen and Seviour 2007).

The production of exopolymeric substances from fungi was investigated by different researchers. Various extracellular polymeric substances production by enthomopathogenic fungi, basidiomycete, and fungi (*A. blazi*, *G. lucidum*, *Cordyceps* sp., *G. frondosa,* and *L. edodes*) were also studied (Bae et al. 2000, 2001; Kim et al. 2002a, b, 2003a; Hwang et al. 2003, 2004; Yang and He 2008; Mahapatra and Banerjee 2013). Fungal strains isolated from soil (e.g. *Cladosporium tricoides*, *Ceratocystis stenoceras*, *Talaromyces* sp., and *F. pinicola*) and with nutrient supplements (e.g. NH_4Cl, glucose) as C and N sources exhibited potential to extract extracellular polymeric substances (Graber et al. 1988; Choi et al. 2007; Ben Rebah et al. 2018; Siddharth et al. 2021).

5 Applications of Fungal Exopolymeric Substances

The recognition that fungal extracellular polymeric substances are high-value bio macromolecules has been made for the last two decades (Mahapatra and Banerjee 2013). The fungal exopolymeric substances have various application areas. Application areas of the fungal exopolymeric substances can be listed as follows medical

applications (e.g. antioxidative and antimicrobial activities, immunomodulatory activities and wound management, hypoglycemic, hypolipidemic, and hepatoprotective activities), potential environmental and agricultural applications, soil applications (e.g. as potential biofertilizers for enhancement of mineral solubilization), bioremediation applications, and plant applications (Zhang et al. 2002, 2007; Chen and Seviour 2007; Liu et al. 2009; Osińska-Jaroszuk et al. 2015).

Fungal exopolysaccharides (e.g. pullulan, botryosphaeran, and scleroglucan) have different application options in various industries, pharmaceuticals, medicine, and food (Mahapatra and Banerjee 2013). Pullulan has been widely used for different aims. Because, pullulan is nontoxic, nonmutagenic, noncarcinogenic, bio compatible, and bio degradable (Silva et al. 2018; Plucinski et al. 2021). Scleroglucan has immunostimulatory properties (Hamed and Belhadri 2009). This polymer is an exopolysaccharide that has usefulness in applications including improved recovery of oil, food industry and pharmaceutical industry (Sandford 1979; Hamed and Belhadri 2009). The high water binding capacity and various biological activities of it present scleroglucan as active ingredient for cosmeceutical and dermatological applications (Farwick et al. 2009). β-glucans are found in all fungi (Chen and Seviour 2007). Because of the polysaccharides β-glucans, mushrooms have economic importance. The mushrooms contain high β-glucan amounts. The immune system is stimulated by β-glucans stimulation. Thus, they have useful effect to fight various infections (e.g. parasitic, bacterial, fungal, and viral). They exhibit anticoagulant and hypocholesterolemic properties. β-glucans are anti-cytotoxic, anti-mutagenic and anti-tumorogenic. These properties make β-glucans promising candidate as pharmacological promoters of health (Mantovani et al. 2008). Chen and Seviour (2007) discussed potential of fungal β-glucans in medicine in their review. Fungal β-glucans have antiviral activity, antibacterial activity, and antifungal activity (Chen and Seviour 2007). They have a role in wound healing and hypercholesterolaemia, high blood pressure, and diabetes treatment (Chen and Seviour 2007). Certain fungal β-glucans have remarkable effects of on various tumors (Vetvicka and Yvin 2004). Currently, lentinan and schizophyllan are approved in Japan for the aim of clinic usage in treatment of cancer for humans (Mizuno et al. 1999; Chen and Seviour 2007). Some fungal β-glucans usefully influence the promotion and progression of cancer (Takaku et al. 2001; Nilsson et al. 2004; Nam et al. 2005; Chen and Seviour 2007). They also have a synergistic effect with monoclonal antibodies used for treatment of the cancer (Chen and Seviour 2007).

The marine fungus is a rich and promising source of novel anti-cancer, antibacterial, anti plasmodial, antiinflammatory and antiviral agent, and bioactive compounds isolated from marine fungi have been investigated (Bugni and Ireland 2004; Bhadury et al. 2006; Saleem et al. 2007; Blunt et al. 2016). The metabolites that are also named secondary metabolites, compounds with organic origin are not directly involved in organism reproduction, growth, and development. However, they provide long term improvement of organism's survival prospects. They are bioactive and have low molecular weight. Secondary metabolites in fungi are produced by a few biosynthetic strategies based on variations and combinations of

a small number of fundamental path ways (Keller et al. 2005; Reich and Labes 2017). Fungal ribotoxins are extracellular and highly specific ribonucleases. Fungal ribotoxin behaves as inhibitor of biosynthesis of protein by being able to inactivate ribosomes from almost any organism (Gasset et al. 1994; Martínez-Ruiz et al. 2001; Kao et al. 2001; Lacadena et al. 2007). Fungal ribotoxins are extracellular ribonucleases with antitumoral properties (Olombrada et al. 2013). Many types of exopolysaccharides secreted by mushrooms are caused for purposes of pharmaceutical because of their various biological activities (Chihara et al. 1970; Kuo et al. 1996; Wasser 2002; Kim et al. 2003a). Thus, various researchers have been investigated influence of culture conditions to improve production of medicinal exopolysaccharides from some mushrooms or enthomopathogenic fungi (Bae et al. 2000; Kim et al. 2002a, b, 2003b, c; Fang and Zhong 2002; Xu et al. 2006). The exopolymeric substances secreted by and *Basidiomycota* and *Ascomycota* fungal cultures have properties of antitumor, antimicrobial, and antioxidant (Osińska-Jaroszuk et al. 2015). From the family of Polyporaceae, Basidiomycete *Phellinus* sp. has been used for purposes of medicinal since diversified physiological activities especially antitumour activity of *Phellinus* sp. (Chung et al. 1993; Song et al. 1995; Lee et al. 1996). There are researches on pharmacological activities of exopolymeric substances from species of *Phellinus* (Chi et al. 1996; Han et al. 1999; Hwang et al. 2004). *Basidiomycota* and *Ascomycota* fungi are used for biotechnological derivation of extracellular polymeric substances in conditions of laboratory using equal acidic potential of hydrogen of medium, techniques, culture conditions, carbon, nitrogen and etc. sources, and period of culture (Shu and Lung 2004; Mahapatra and Banerjee 2013; Osińska-Jaroszuk et al. 2015). Goyzueta et al. (2020) evaluated exopolysaccharide biological activities (e.g. antitumor properties and antioxidant effect) from the filamentous fungus *Mortierella alpina*. Goyzueta et al. (2020) optimized chitin like exopolysaccharide production through experimental design by evaluating influence of the phosphate, urea, and glucose. In their study, the authors reported 1.51 g chitin-like exopolysaccharide/liter production under optimized conditions. The authors evaluated the physicochemical characteristics of chitin-like exopolysaccharides by chromatography techniques. The homogeneous exopolysaccharide composed of chitin, a linear polymer of β-(1 → 4)-linked *N*-acetyl-D-glucosamine residues were reported. Authors finally reported production of a new alternative of extracellular chitin like polysaccharides with promising bioactive properties from fungus.

Fungal exopolymeric substances have an application area for wastewater treatment (Ben Rebah et al. 2018). Pu et al. (2014) prepared biopolymer by *Rhizopus* sp. M9 and M17. The prepared biopolymer was reported to allow many advantages (e.g. working at low dose, without control of potential of hydrogen, low preparation cost, and high removal percentages for chemical oxygen demand and turbidity) during the treatment of wastewater of the potato starch. The wastewater parameters were reported as follows: chemical oxygen demand = 7965 mg/L, turbidity = 712 NTU, and potential of hydrogen = 6. Pu et al. (2014) noticed that for removal of organic matter and turbidity the removal percentages were 92.11 and 54.09 for turbidity and chemical oxygen demand, respectively. Fang and Shi (2016) studied

the treatment of swine wastewater by microbial flocculants (strain of *Talaromyces trachyspermus* OU5) with flocculant dosage of percentage of 5. Fang and Shi (2016) reported the parameters of the swine wastewater as follows: chemical oxygen demand = 6746 mg/L, $NH4^{+}$-N = 785 mg/L, total Kjeldahl nitrogen = 1158 mg/L, total phosphorus = 153 mg/L, and turbidity = 35,742 NTU. Fang and Shi (2016) obtained that the removal percentages were 52.1, 39.7, 18.6, 21.5, 75 for chemical oxygen demand, total Kjeldahl N, ammonium nitrogen, total phosphorus, and turbidity, respectively. Fungal exopolymeric substances are used as bioflocculants in wastewater treatment for harvesting microalgae. The micro algae harvesting is one of the key challenges for the treatment of wastewaters. Because (a) microalgae have a small volume (about between 2 and 40 μm) (b) microalgae exist in low concentrations (about 0.3–5.0 g/L) (c) density of microalgae is similar to the density of the aqueous solution (Li et al. 2008) (d) the harvesting is hindered by negative surface charge as settling naturally is inhibited by gravity. Major methods for harvesting the micro algae are flotation, filtration, flocculation, and centrifugal sedimentation which have disadvantages (e.g. specialized technical requirements and high cost) (Pragya et al. 2013) that hinder the expansion of relevant commercial applications (Wang et al. 2021). Major methods for harvesting the micro algae are flotation, filtration, flocculation and centrifugal sedimentation, which have disadvantages (e.g., special technical requirements and high cost) that hinder the expansion of relevant commercial applications. Bioflocculation assisted by pellets with fungal origin is a solution for this problem. The pellets with fungal origin are one of the new biological materials that can adsorb pollutants as an adsorbent with biological origin or immobilize pollutants to remove them from wastewaters (Espinosa-Ortiz et al. 2016).

As biomass material, pellet with fungal origin (a) plays a positive role to degrade the pollutants with organic origin by producing enzymes (b) secretes extracellular polymeric substances contributing to cementing substance (Geng et al. 2020). Fungus is used as bioflocculant to harvest the micro algae (Pei et al. 2021). Pei et al. (2021) investigated development of bioflocculation assisted by fungal *Aspergillus niger* pellets to achieve effective harvesting of microalgae *Scenedesmus* sp. The authors investigated effects of some parameters (e.g. temperature, flocculation type, initial pH value, and rotation speed) on the developed method. They reported efficiency of flocculation percentage as 99.4 with pellets inoculated in algae solution at 30 °C, initial potential of hydrogen value of 8.0, and rotation of 160 revolutions per minute. Also, they explored the mechanism of the process by microscopy examination. They determined that interaction of microalgae and fungi was due to surface functional groups of the pellet of fungal *Aspergillus niger*. The cocultivation pellets can secrete more extracellular polymeric substances by the hyphae to more tightly agglomerate the microalgae during the formation process compared with the precultivation mycelium pellets. As a result of the research, the authors reported that carbonyl groups were one of the priorities in spectrum of FTIR. Furthermore, they reported that fungi cell walls possessed a layer of protein (Pei et al. 2021). The groups of carboxyl, amino, phosphate, amides, and hydroxyl play role in algal flocculation assisted by pellets with fungal origin. Different metabolites

(e.g. enzymes, polysaccharides, organic acids, and proteins) are produced by the hyphae of fungi (Bizukojc and Ledakowicz 2010; Pei et al. 2021). Therefore, fungal growth associated metabolite secretion and surface functional groups are the basis impact key of micro algal flocculation by fungi hyphae (Bosso et al. 2015; Pei et al. 2021). Pei et al. (2021) also reported that extracellular polymeric substance of *Aspergillus niger* contained glycoprotein being source of groups of N–H (Pei et al. 2021). The carbon peak was seen in extracellular polymeric substances from granules with aerobic origin (Adav and Lee 2008; Pan et al. 2010; Pei et al. 2021). The results of three dimensional EE matrix fluorescence spectrum proved that basis components of extracellular polymeric substances with fungal origin were tryptophan like, tyrosine like, and tyrosine content was lightly high (Pei et al. 2021). In the literature, Kobayashi (2015) reported the isoelectric point of tyrosine as 5.66 and the isoelectric point of tryptophan as 5.89. Pei et al. (2021) reported that and components of tryptophan and tyrosine in extracellular polymeric substances were basis factors that affect fungal pellet electrification.

The fungal mycelium pellets based bioreactor has perfect adaptation ability. It is environmentally friendly, and easy of filtering. Therefore, these valuable properties encourage the use of these reactors by various researchers (Hanafiah et al. 2019). Zheng et al. (2021) cultivated the fungal pellets immobilized denitrifying *P. stutzeri* sp. GF3 for establishing bio reactor for the efficient removal of nitrate at low C to N ratio wastewaters. The pellets aforementioned were compact and densely packed form of filamentous region of hyphae of fungi. The pellets had porous and inter twined structure with rich functional groups, large specific surface area, and high capacity of adsorption (Espinosa-Ortiz et al. 2016; Yu et al. 2020). Application of the pellets with fungi origin results in granules with a high MLVSS/MLSS ratio (Geng et al. 2020). For this reason, it was reported that application of pellets with fungi origin was probable for enhancement of aerobic granular sludge stability. Therefore, application of pellets with fungi origin is done for accelerating aerobic granular sludge formation in aerobic granular sludge system (Geng et al. 2020; Wang et al. 2014).

Fungal exopolymeric substances are efficient biosorbents as reported by some researchers. In the study of Yin et al. (2011), the researchers used fungus *A. fumigatus* for extraction of the exopolymeric substances. *A. fumigatus* fungus is a good sorbent with properties of efficiency and low cost (Cheng and Hu 2004; Tsekova et al. 2010; Yin et al. 2011; Javanbakht et al. 2011). Yin et al. (2011) researched copper and cadmium sorption onto exopolymeric substances secreted by *A. fumigatus*. They changed initial potential of hydrogen value of solution, extracellular polymeric substances concentration, time of contact, sodium chloride concentration, the initial metal concentrations and existence of other ions in solution. Equilibrium isotherms were reported. The existence of functional groups in extracellular polymeric substances that play role in process of sorption was approved by the analysis of the FTIR. They used the analysis of EDX to confirm heavy metal mechanism sorption by extracellular polymeric substances. They reported that the value of the potential of hydrogen, concentrations of extracellular polymeric substances, the initial concentrations of the metals, concentrations of sodium chloride

and co ions significantly affected the adsorption of ions of metals. Increased potential of hydrogen value and increased initial concentrations of ions of metals increased the copper and cadmium sorption. However, increased concentrations of sodium chloride decreased the copper and cadmium sorption. Pestan is a fungal extracellular polysaccharide of *Pestalotiopsis* sp. KCTC 8637P. Pestan haves a perfect activity to flocculate solid particles (Kwon et al. 1996; Moon et al. 1998, 2006). Moon et al. (2006) investigated potential applicability of pestan to treat wastewaters as a sorbent with a biological origin for lead and zinc sorption.

Fungal exopolymeric substances have an application area for the production of bioflocculants. In the literature, there are a few studies on fungal flocculants with biological origin have been reported (Ben Rebah et al. 2018). Liu and Cheng (2010) reported that *Penicillium* strain HHE-P7 produced a bioflocculant with flocculating activity (percentage of 93–96) with use of extract of yeast and glucose as nutrient sources. Dongchen et al. (2013) reported that fungal strain *Phanerochaete chrysosporium* produced a bioflocculant (an acidic polysaccharide) with high flocculating activity (percentage of 93.5) of coal slurry with use of glucose potatoes as a nutrient source. Aljuboori et al. (2013) reported that isolated fungal strain *Aspergillus flavus* produced a bioflocculant composed mainly of protein (percentage of 28.5) and polysaccharide (percentage of 69.7), with a good flocculating activity (over percentage of 90). The addition of peptone and sucrose led to optimum production of flocculant with biological origin. In the study of Aljuboori et al. (2014), *Aspergillus niger* produced bioflocculant composed mainly of protein (percentage of 31.4) and polysaccharide (percentage of 66.8), with flocculating activity (percentage of 81) with use of palm oil mill effluent and glutamic acid as nutrient sources. Pu et al. (2014) detected that *Rhizopus* sp. secreted bio flocculant with flocculating activity (percentage of 90.2) with use of potato starch wastewater and urea as nutrient sources. Abraham et al. (2015) reported that 4 fungal isolates produced bio flocculant with the activity of flocculation (percentage of 80) with use of glucose and NH_4Cl_2 as nutrient sources. Aljuboori et al. (2015) reported that *Aspergillus flavus* produced a bioflocculant with flocculating activity (percentage of 97.4) with use of sucrose and peptone as nutrient sources. The produced flocculant with biological origin had ability of flocculating industrial wastewaters and flocculant was thermostable. Jebun et al. (2015) reported that filamentous fungal strain produced a bioflocculant with flocculating activity (percentage of 59.34–99.18) with use of potato dextrose agar as nutrient source. Fang and Shi (2016) reported that extracellular polymeric substances strain of *Talaromyces trachyspermus* OU5 produced proteoglycan bioflocculant (composed of 15.2% protein and 84.6% polysaccharide) while use of glucose and urea as nutrient sources. The authors reported that 20 mg of proteoglycan per liter led flocculating activity of more than percentage of 92.5 in swine wastewater with a flocculant dosage of 5% v/v. The turbidity with an initial concentration of 35,742 NTU was reported to be 8935 NTU (removal percentage of 75). Xu et al. (2006) investigated intensity of agitation for exopolysaccharides and biomass of mycelium (*Paecilomyces tenuipes*) in reactors of airlift and stirred tank. The authors reported that notable differences between performance of them do not exist. Besides, intensity of agitation and hydrodynamic reactors affected quality,

chemical composition, and molecular structure of exopolysaccharides. Authors suggested monitoring of combination of the medium and the environmental conditions for obtaining enough exopolysaccharide for good mycelium and thus development of the bio composites (Rafiee et al. 2021).

Bancerz et al. (2018) investigated polysaccharides with fungi origin as material of water adsorber to product the esters by using lipase from *R. variabilis*. They studied effect of two exopolysaccharides extracted from *A. biennis* mycelium, *G. applanatum* mycelium, and two endopolysaccharides isolated from fruiting body of *Laetiporus sulphurous* and *P. betulinus*. Petersen et al. (1990) reported that yeast-like fungus (*Rhodotorula glutinis, Cryptococcus laurentii*, *Candida boidinii*, *Hansenula capsulata*, *Rhinocladellia elatior*, and *Lipomyces starkeyii*) and yeast strains produced drag reducing exopolysaccharides. Yang et al. (2001) investigated effect of exopolymer from *G. lucidum* on mice's swimming endurance capacity. The administration of the exopolymer reduced glycogen exhaustion of liver and muscle and increased swimming endurance capacity. Decrease of percentage of 50.7 in serum lactate accumulation was reported under exopolymer influence. Exopolymer was reported to be glycoprotein having molecular weight of 780 kDa and reported to include percentage of 17.2 protein and percentage of 82.8 carbohydrates.

6 Conclusions and Future Prospects

In the present chapter, we have evaluated the exopolymeric substances (also known as extracellular polymeric substances, extracellular polysaccharides, exopolymers, and exopolysaccharides) followed by the discussion of the importance of exopolymeric substances and summarized the functions of the exopolymeric substances. Furthermore, we have emphasized the applications for exopolymeric substances followed by fungal exopolymeric substances. Then, we reported the various applications reported in the literature for fungal exopolymeric substances. We have given a special focus to the medical and environmental applications of fungal exopolymeric substances.

The ever-increasing requirement of resources for industrial developments has been a global crisis. There is an urgent need for sustainable resources to meet these requirements in the various applications in different sectors. Natural resources can meet these needs due to their sustainable characteristics. Fungal exopolymeric substances that have unique functions and have been recognized as valuable bio macromolecules for recent years are one of that natural resources. The further development on the commercialization of these valuable substances will support the usage of sustainable resources for industrial productions and a sustainable solution for environmental protection. Furthermore, the future prospect will also involve the new applications of fungal exopolymeric substances.

References

Abraham J, Singh N, Janapal Y, Kumar NU (2015) Optimized bioflocculant production from fungi using response surface methodology. Int J Pharm Tech Res 8:230–235

Adav SS, Lee DJ (2008) Extraction of extracellular polymeric substances from aerobic granule with compact interior structure. J Hazard Mater 154:1120–1126

Aljuboori AHR, Idris A, Abdullah N, Mohamad R (2013) Production and characterization of a bioflocculant produced by *Aspergillus flavus*. Bioresour Technol 127:489–493

Aljuboori AHR, Uemura Y, Osman NB, Yusup S (2014) Production of a bioflocculant from *Aspergillus niger* using palm oil mill effluent as carbon source. Bioresour Technol 171:66–70

Aljuboori AHR, Idris A, Al-joubory HHR, Uemura Y, Ibn Abubakar BSU (2015) Flocculation behavior and mechanism of bioflocculant produced by *Aspergillus flavus*. J Environ Manag 150: 466–471

Andrew CAW, Khor E, Hastings GW (1998) The influence of anionic chitin derivatives on calcium phosphate crystallization. Biomaterials 19:1309–1316

Bae JT, Park JP, Song CH, Yu CB, Park MK, Yun JW (2001) Effect of carbon sources on the mycelial growth and exo-biopolymer production by submerged culture of Paecilomyces japonica. J Biosci Bioeng 91(5):522–524

Bae JT, Sinha J, Park JP, Song CH, Yun JW (2000) Optimization of submerged culture conditions for exo-biopolymer production by Paecilomyces japonica. J Microbiol Biotechnol 10:482–487

Bancerz R, Osińska-Jaroszuk M, Jaszek M, Sulej J, Wiater A, Matuszewska A, Rogalski J (2018) Fungal polysaccharides as a water-adsorbing material in esters production with the use of lipase from *Rhizomucor variabilis*. Int J Biol Macromol 118(A):957–964

Bao P, Xia M, Liu A, Wang M, Shen L, Yu R, Liu Y, Li J, Wu X, Fang C, Chen M, Qiu G (2018) Extracellular polymeric substances (EPS) secreted by *Purpureocillium lilacinum* strain Y3 promote biosynthesis of jarosite. RSC Adv 8:22635–22642

Baumert VL, Forstner FJ, Zethof JHT, Vogel C, Heitkötter J, SchulzfIngri S, Carsten K-K, Muellerah W (2021) Root-induced fungal growth triggers macroaggregation in forest subsoils. Soil Biol Biochem 157:108244

Ben Rebah F, Mnif W, Siddeeg MS (2018) Microbial flocculants as an alternative to synthetic polymers for wastewater treatment: a review. Symmetry 10(11):556

Bhadury P, Mohammad BT, Wright PC (2006) The current status of natural products from marine fungi and their potential as anti-infective agents. J Ind Microbiol Biotechnol 33:325–337

Bhaskar PV, Bhosle NB (2005) Microbial extracellular polymeric substances in marine biogeochemical processes. Curr Sci 88(1):45–53

Bizukojc M, Ledakowicz S (2010) The morphological and physiological evolution of *Aspergillus terreus* mycelium in the submerged culture and its relation to the formation of secondary metabolites. World J Microbiol Biotechnol 26:41–54

Blunt JW, Copp BR, Keyzers RA, Munro MHG, Prinsep MR (2016) Marine natural products. Nat Prod Rep 33:382–431

Bontognali TRR, Mckenzie JA, Warthmann RJ, Vasconcelos C (2014) Microbially influenced formation of Mg-calcite and Ca-dolomite in the presence of exopolymeric substances produced by sulphate-reducing bacteria. Terra Nova 26:72–77

Boonchai R, Kaewsuk J, Seo G (2014) Effect of nutrient starvation on nutrient uptake and extracellular polymeric substance for microalgae cultivation and separation. Desalin Water Treat 55:360–367

Bosso L, Lacatena F, Cristinzio G, Cea M, Diez MC, Rubilar O (2015) Biosorption of pentachlorophenol by Anthracophyllum discolor in the form of live fungal pellets. New Biotechnol 32: 21–25

Bugni TS, Ireland CM (2004) Marine-derived fungi: a chemically and biologically diverse group of microorganisms. Nat Prod Rep 21:143–163

Catley BJ (1971) Role of pH and nitrogen limitation in the elaboration of the extracellular polysaccharide pullulan by Pullularia pullulans. Appl Microbiol 22(4):650–654

Chan CS, Stasio GD, Welch SA, Girasole M, Frazer BH, Nesterova MV, Fakra S, Banfield JF (2004) Microbial polysaccharides template assembly of nanocrystal fibers. Science 303:1656–1658

Chen J, Seviour R (2007) Medicinal importance of fungal β-(1-3), (1-6)-glucans. Mycol Res 111: 635–652

Chen MY, Lee DJ, Tay JH (2007) Distribution of extracellular polymeric substances in aerobic granules. Appl Microbiol Biotechnol 73(6):1463–1469

Cheng W, Hu YY (2004) Adsorptive decolorization of azo dyes by *Aspergillus pellets* HX. Chin J Appl Environ Biol 10:489–492

Chenu C (1995) Extracellular polysaccharides: an interface between microorganisms and soil constituents. In: Huang PM, Berthelin J, Bollag JM, Mcgill WB, Page AL (eds) Environmental impact of soil component interactions. Natural and anthropogenic organics. CRC Lewis Publishers, Boca Raton

Chi JH, Ha TM, Kim YH, Rho YD (1996) Studies on the main factors affecting the mycelial growth of *Phellinus linteus*. Korean J Mycol 24:214–222

Chihara G, Himuri J, Maeda YY, Arai Y, Fukuoka F (1970) Fractionation and purification of the polysaccharides with marked antitumor activity, especially, lentinan, from Lentinus edodes (Berk) SING. Cancer Res 30:2776–2781

Choi D, Maeng J, Ding J, Cha W (2007) Exopolysaccharide production and mycelial growth in an air-lift bioreactor using Fomitopsis pinicola. J Microbiol Biotechnol 17:1369

Chung KS, Kim HS, Kim KY, Han MW, Kim KH (1993) Effect of Kp, an antitumor protein polysaccharide from mycelial culture of Phellinus linteus on the humeral immune response of tumor bearing ICR mice to sheep red blood cells. Arch Pharm Res 16:336–338

Costa OYA, Raaijmakers JM, Kuramae EE (2018) Microbial extracellular polymeric substances: ecological function and impact on soil aggregation. Front Microbiol 9:1636

Couchourel D, Escoffier C, Rohanizadeh R, Bohic S, Daculsi G, Fortun Y, Padrines M (1999) Effects of fibronectin on hydroxyapatite formation. J Inorg Biochem 73:129–136

Ding ZJ, Bourven I, Guibaud G, van Hullebusch ED, Panico A, Pirozzi F, Esposito G (2015) Role of extracellular polymeric substances (EPS) production in bioaggregation: application to wastewater treatment. Appl Microbiol Biotechnol 99:9883–9905

Dongchen Z, Zhixiang H, Zhiyong L, Tao W (2013) Experimental research on Phanerochaete chrysosporium as coal microbial flocculant. Int J Min Sci Technol 23:521–524

Donot F, Fontana A, Baccou JC, Schorr-Galindo S (2012) Microbial exopolysaccharides: main examples of synthesis, extraction genetics and extraction. Carbohydr Polym 87:951–962

Eisele APP, Valezi CF, Mazziero T, Dekker RFH, Barbosa-Dekker AM, Sartori ER (2019) Layering of a film of carboxymethyl-botryosphaeran onto carbon black as a novel sensitive electrochemical platform on glassy carbon electrodes for the improvement in the simultaneous determination of phenolic compounds. Sensors Actuators B Chem 287:18–26

Elisashvili VI, Kachlishvili ET, Wasser SP (2009) Carbon and nitrogen source effects on basidiomycetes exopolysaccharide production. Appl Biochem Microbiol 45:531–535

Espinosa-Ortiz EJ, Rene ER, Pakshirajan K, van Hullebusch ED, Lens PN (2016) Fungal pelleted reactors in wastewater treatment: applications and perspectives. Chem Eng J 283:553–571

Eteshola E, Gottlieb M, Arada SM (1996) Dilute solution viscosity of red microalga exopolysaccharide. Chem Eng Sci 51:1487–1494

Fang D, Shi C (2016) Characterization and flocculability of a novel proteoglycan produced by *Talaromyces trachyspermus* OU5. J Biosci Bioeng 121:52–56

Fang QH, Zhong JJ (2002) Submerged fermentation of higher fungus *Ganoderma lucidum* for production of valuable metabolites-ganoderic acid and polysaccharide. Biochem Eng J 10:61–65

Fang L, Huang Q, Wei X, Liang W, Rong X, Chen W, Cai P (2010) Microcalorimetric and potentiometric titration studies on the adsorption of copper by extracellular polymeric substances (EPS), minerals and their composites. Bioresour Technol 101:5774–5779

Farwick M, Lersch P, Schmitz G, Müllner S, Wattenberg A (2009) Skin-omics: use of genomics, proteomics and lipidomics to assess effects of low molecular weight scleroglucan. Cos Sci Technol E Indus:100–105

Felz S, Al-Zuhairy S, Aarstad OA, Van Loosdrecht MCM, Lin YM (2016) Extraction of structural extracellular polymeric substances from aerobic granular sludge. J Vis Exp Jove 115:e54534

Flemming HC, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8:623–633

Flemming HC, Neu TR, Wozniak DJ (2007) The EPS matrix: the "house of biofilm cells". J Bacteriol 189:7945–7947

Garai-Ibabe G, Dueñas MT, Irastorza A, Sierra-Filardi E, Werning ML, López P, Corbí AL, Fernández de Palencia P (2010) Naturally occurring 2-substituted (1,3)-β-d-glucan producing *Lactobacillus suebicus* and *Pediococcus parvulus* strains with potential utility in the production of functional foods. Bioresour Technol 101:9254–9263

Gasset M, Mancheño JM, Lacadena J, Turnay J, Olmo N, Lizarbe MA, Martínez-del-Pozo A, Oñaderra M, Gavilanes JG (1994) α-Sarcin, a ribosome-inactivating protein that translocates across the membrane of phospholipid vesicles. Curr Top Pept Protein Res 1:99–104

Gavrilescu M (2004) Removal of heavy metals from the environment by biosorption. Eng Life Sci 4:219–232

Geesey GG, Jang L (1990) Extracellular polymers for metal binding. In: Ehrlich HL, Brierley CL (eds) Microbial mineral recovery. McGraw-Hill, New York, pp 223–247

Geng M, Ma F, Guo H, Su D (2020) Enhanced aerobic sludge granulation in a sequencing batch reactor (SBR) by applying mycelial pellets. J Clean Prod 274:123037

Geng M, You S, Guo H, Ma F, Xiao X, Zhang J (2021) Impact of fungal pellets dosage on long-term stability of aerobic granular sludge. Bioresour Technol 332:125106

Giavasis I (2014) Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. Curr Opin Biotechnol 26:162–173

Giese EC, Gascon J, Anzelmo G, Barbosa AM, Cunha MAA, Dekker RFH (2015) Free-radical scavenging properties and antioxidant activities of botryosphaeran and some other β-D-glucans. Int J Biol Macromol 72:125–130

Giovannangelo ME, Gehring U, Nordling E, Oldenwening M, van Rijswijk K, de Wind S, Heinrich HG, Bellander T, Brunekreef B (2007) Levels and determinants of β(1→3)-glucans and fungal extracellular polysaccharides in house dust of (pre) school children in three European countries. Environ Int 33(1):9–16

Goyzueta MLD, Noseda MD, Bonatto SJR, Freitas RA, de Carvalho JC, Soccol CR (2020) Production, characterization, and biological activity of a chitin-like EPS produced by Mortierella alpina under submerged fermentation. Carbohydr Polym 247:116716

Graber M, Morin A, Duchiron F, Monsan P (1988) Microbial polysaccharides containing 6-deoxysugars. Enzym Microb Technol 10:198–206

Guo X, Wang X, Liu J (2016) Composition analysis of fractions of extracellular polymeric substances from an activated sludge culture and identification of dominant forces affecting microbial aggregation. Sci Rep 6:28391

Gutman J, Herzberg M, Walker SL (2014) Biofouling of reverse osmosis membranes: positively contributing factors of Sphingomonas. Environ Sci Technol 48:13941–13950

Hamed SB, Belhadri M (2009) Rheological properties of biopolymers drilling fluids. J Pet Sci Eng 67(3–4):84–90

Han SB, Lee CW, Jeon YJ, Hong ND, Yoo ID, Yang KH, Kim HM (1999) The inhibitory effect of polysaccharides isolated from *Phellinus linteus* on tumor growth and metastasis. Immunopharmacology 41:157–164

Hanafiah ZM, Wan Mohtar WHM, Abu Hasan H, Jensen HS, Klaus A, Wan-Mohtar W (2019) Performance of wild-Serbian Ganoderma lucidum mycelium in treating synthetic sewage loading using batch bioreactor. Sci Rep 9(1):16109

Houghton JJ, Quarmby J, Stephenson T (2001) Municipal wastewater sludge dewaterability and the presence of microbial extracellular polymer. Water Sci Technol 44:373–379

Huo M, Zheng G, Zhou L (2014) Enhancement of the dewaterability of sludge during bioleaching mainly controlled by microbial quantity change and the decrease of slime extracellular polymeric substances content. Bioresour Technol 168:190–197

Hwang HJ, Kim SW, Xu CP, Choi JW, Yun JW (2003) Production and molecular characteristics of four groups of exopolysaccharides from submerged culture of *Phellinus gilvus*. J Appl Microbiol 94:708–719

Hwang HJ, Kim SW, Xu CP, Choi JW, Yun JW (2004) Morphological and rheological properties of the three different species of basidiomycetes *Phellinus* in submerged cultures. J Appl Microbiol 96:1296–1305

Jacobi O (1958) Water and water vapor absorption of the stratum corneum of the living human skin. J Appl Physiol 12:403–407

Jain R, Raghukumar S, Tharanathan R, Bhosle NB (2005) Extracellular polysaccharide production by *Thraustochytrid* protists. Mar Biotechnol 7:184–192

Jaroszuk-Ściseł J, Kurek E (2012) Hydrolysis of fungal and plant cell walls by enzymatic complexes from cultures of *Fusarium* isolates with different aggressiveness to rye (Secale cereale). Arch Microbiol 194:653–665

Javanbakht V, Zilouei H, Karimi K (2011) Lead biosorption by different morphologies of fungus *Mucor indicus*. Int Biodeterior Biodegradation 65:294–300

Jebun N, Al-Mamun A, Alam MZ, Abdul Karim MI, Raus RA (2015) Evaluation of entrapment potentiality and turbidity removal efficiency of fungi. J Technol 77:23–28

Jong SC, Donovic R (1989) Antitumor and antiviral substances from fungi. Adv Appl Microbiol 34:183–262

Kachlany SC, Levery SB, Kim JS, Reuhs BL, Lion LW, Ghiorse WC (2001) Structure and carbohydrate analysis of the exopolysaccharide capsule of Pseudomonas putida G7. Environ Microbiol 3:774–784

Kao R, Martínez-Ruiz A, Martínez-del-Pozo A, Crameri R, Davies J (2001) Mitogillin and related fungal ribotoxins. Methods Enzymol 341:324–335

Kavitake D, Marchawala ZF, Delattre C, Shetty PH, Pathak H, Andhare P (2019) Biotechnological potential of exopolysaccharide as a bioemulsifier produced by *Rhizobium radiobacter* CAS isolated from curd. Bioact Carbohydr Diet Fibre 20:100202

Kavitake D, Devi PB, Shetty PH (2020) Overview of exopolysaccharides produced by Weissella genus—a review. Int J Biol Macromol 164:2964–2973

Keller NP, Turner G, Bennett JW (2005) Fungal secondary metabolism—from biochemistry to genomics. Nat Rev Microbiol 3:937–947

Kim SY, Kim JH, Kim CI, Oh O (1996) Metal adsorption of the polysaccharide produced from *Methylobacterium organophilum*. Biotechnol Lett 18:1161–1164

Kim SW, Hwang HJ, Xu CP, Na YS, Song SK, Yun JW (2002a) Influence of nutritional conditions on the mycelial growth and exo-biopolymer production in Paecilomyces sinclairii. Lett Appl Microbiol 34:389–393

Kim SW, Hwang HJ, Park JP, Cho YJ, Song CH, Yun JW (2002b) Mycelial growth and exo-biopolymer production by submerged culture of various edible mushrooms under different media. Lett Appl Microbiol 34:56–61

Kim SW, Hwang HJ, Xu CP, Sung JM, Choi JW, Yun JW (2003a) Optimization of submerged culture process for the production of mycelial biomass and exo-polysaccharides by Cordyceps militaris C738. J Appl Microbiol 94:120–126

Kim SW, Xu CP, Hwang HJ, Choi JW, Kim CW, Yun JW (2003b) Production and characterization of exopolysaccharides from an enthomopathogenic fungus *Cordyceps militaris* NG3. Biotechnol Prog 19:428–435

Kim SW, Hwang HJ, Xu CP, Choi JW, Yun JW (2003c) Effect of aeration and agitation on the production of mycelial biomass and exopolysaccharides in an enthomopathogenic fungus *Paecilomyces sinclairii*. Lett Appl Microbiol 36:321–326

Klis FM, de Groot P, Hellingwerf K (2001) Molecular organization of the cell wall of *Candida albicans*. Med Mycol J 39:1–8

Kobayashi K (2015) Tryptophan. In: Gargaud M, Irvine WM, Amils R, Cleaves HJ, Pinti DL, Quintanilla JC, Rouan D, Spohn T, Tirard S, Viso M (eds) Encyclopedia of astrobiology. Springer, Berlin

Kucera J (2004) Fungal mycelium—the source of chitosan for chromatography. J Chromatogr B 808:69–73

Kuo YC, Tasi WJ, Shiao MS, Chen CF, Lin CY (1996) Cordyceps sinensis as an immunomodulatory agent. Am J Chin Med 24:111–125

Kwon GS, Moon SH, Hong SD, Lee HM, Kim HS, Oh HM, Yoon BD (1996) A novel flocculant biopolymer produced by *Pestalotiopsis* sp KCTC 8637P. Biotechnol Lett 18:1459–1464

Lacadena J, Álvarez-García E, Carreras-Sangrà N, Herrero-Galán E, Alegre-Cebollada J, García-Ortega L, Oñaderra M, Gavilanes JG, Martínez-del-Pozo A (2007) Fungal ribotoxins: molecular dissection of a family of natural killers. FEMS Microbiol Rev 31:212–237

Lee Chang KJ, Nichols CM, Blackburn SI, Dunstan GA, Koutoulis A, Nichols PD (2014) Comparison of thraustochytrids *Aurantiochytrium* sp., *Schizochytrium* sp., *Thraustochytrium* sp., and *Ulkenia* sp. for production of biodiesel, long-chain omega-3 oils, and exopolysaccharide. Mar Biotechnol 16:396–411

Lee JH, Cho SM, Kim HM, Hong ND, Yoo ID (1996) Immunostimulating activity of polysaccharides from mycelia of *Phellinus linteus* grown under different culture conditions. J Microbiol Biotechnol 6:52–55

Levander F, Rådström P (2001) Requirement for phosphoglucomutase in exopolysaccharide biosynthesis in glucose- and lactose-utilizing Streptococcus thermophilus. Appl Environ Microbiol 67:2734–2738

Li Y, Horsman M, Wu N, Lan C, Dubois-Calero N (2008) Biofuels from microalgae. Biotechnol Prog 24:815–820

Liu L, Cheng W (2010) Characteristics and culture conditions of a bioflocculant produced by *Penicillium* sp. Biomed Environ Sci 23:213–218

Liu Y, Fang HHP (2003) Influence of extracellular polymeric substances (EPS) on aggregation, settling, and dewatering of activated sludge. Crit Rev Environ Sci Technol 33:237–273

Liu J, Gunn L, Hansen R, Yan J (2009) Combined yeast-derived β-glucan with anti-tumor monoclonal antibody for cancer immunotherapy. Exp Mol Pathol 86:208–214

Liu XM, Sheng GP, Luo HW, Zhang F, Yuan SJ, Xu J, Zeng RJ, Wu JG, Yu HG (2010) Contribution of extracellular polymeric substances (EPS) to the sludge aggregation. Environ Sci Technol 44:4355–4360

Long GY, Zhu PT, Shen Y, Tong MP (2009) Influence of extracellular polymeric substances (EPS) on deposition kinetics of bacteria. Environ Sci Technol 43:2308–2314

Looijestenijn PJ, Boels IC, Kleerebezem M, Hugenholtz J (1999) Regulation of exopolysaccharide production by Lactococcus lactis subsp. cremoris by the sugar source. Appl Environ Microbiol 65:5003–5008

Mahapatra S, Banerjee D (2013) Fungal exopolisaccharide: production, composition and applications. Microbiol Insights 6:1–16

Mali A, Pandit V, Majumder D (2014) Biosorption of heavy metals by dead fungal biomass. Int J Civ Struct Environ Infrastruct Eng Res Dev 4:11–20

Mantovani MS, Bellini MF, Angeli JPF, Oliveira RJ, Silva AF, Ribeiro LR (2008) B-glucans in promoting health: prevention against mutation and cancer. Mutat Res 658(3):154–161

Marques AM, Bonet R, Simon-Pujo MD, Fuste MC, Congregado F (1990) Removal of uranium by an exopolysaccharide from *Pseudomonas* sp. Appl Microbiol Biotechnol 34:429–431

Martínez-Ruiz A, García-Ortega L, Kao R, Lacadena J, Oñaderra M, Mancheño JM, Davies J, Martínez-del-Pozo A, Gavilanes JG (2001) RNase U2 and α-sarcin: a study of relationships. Methods Enzymol 341:335–351

McGrath KM (2001) Probing material formation in the presence of organic and biological molecules. Adv Mater 13:989–992

McIntosh M, Stone BA, Stanisich VA (2005) Curdlan and other bacterial (1→3)-β-D-glucans. Appl Microbiol Biotechnol 68:163–173

Miranda-Nantes CCBO, Fonseca EAI, Zaia CTBV, Dekker RFH, Khaper N, Castro LA, Barbosa AM (2011) Hypoglycemic and hypocholesterolemic effects of Botryosphaeran from Botryosphaeria rhodina MAMB-05 in diabetes-induced and hyperlipidemia conditions in rats. Mycobiology 39(3):187–193

Mizuno M, Minato K, Ito H, Kawade M, Terai H, Tsuchida H (1999) Anti-tumor polysaccharide from the mycelium of liquid-cultured Agaricus blazei mill. Int J Biochem Mol Biol 47:707–714

Moon SH, Hong SD, Kwon GS, Suh HH, Kim HS, An KH, Oh HM, Mheen TI, Yoon BD (1998) Determination of medium components in the flocculating activity and production of Pestan produced by Pestalotiopsis sp. by using the Plackett–Burman design. J Microbiol Biotechnol 8: 341–346

Moon SH, Park CS, Kim YJ, Park YI (2006) Biosorption isotherms of Pb (II) and Zn (II) on Pestan, an extracellular polysaccharide of *Pestalotiopsis* sp. KCTC 8637P. Process Biochem 41(2): 312–316

Nam SW, Park JY, Ramasamy A, Shevade S, Islam A, Long PM, Park CK, Park SE, Kim SY, Lee SH, Park WS, Yoo NJ, Liu ET, Miller LD, Lee JY (2005) Molecular changes from dysplastic nodule to hepatocellular carcinoma through gene expression profiling. Hepatology 42:809–818

Nielsen PH, Jahn A (1999) In: Wingender J, Neu TR, Flemming HC (eds) Microbial extracellular polymeric substances: characterization, structure and function (Chapter 3). Springer-Verlag, Berlin, pp 49–72

Nilsson S, Norlen BJ, Widmark A (2004) A systematic overview of radiation therapy effects in prostate cancer. Acta Oncol 43:316–381

Nookongbut P, Kantachote D, Khuong NQ, Tantirungkij M (2020) The biocontrol potential of acid-resistant Rhodopseudomonas palustris KTSSR54 and its exopolymeric substances against rice fungal pathogens to enhance rice growth and yield. Biol Control 150:104354

Olombrada M, Herrero-Galán E, Tello D, Oñaderra M, Gavilanes JG, Martínez-del-Pozo A, García-Ortega L (2013) Fungal extracellular ribotoxins as insecticidal agents. Insect Biochem Mol Biol 43(1):39–46

Osemwegie OO, Adetunji CO, Ayeni EA, Adejobi OI, Arise RO, Nwonuma CO, Oghenekaro AO (2020) Exopolysaccharides from bacteria and fungi: current status and perspectives in Africa. Heliyon 6(6):e04205

Osińska-Jaroszuk M, Jarosz-Wilkołazka A, Jaroszuk-Ściseł J, Szałapata K, Nowak A, Jaszek M, Ozimek E, Majewska M (2015) Extracellular polysaccharides from Ascomycota and Basidiomycota: production conditions, biochemical characteristics, and biological properties. World J Microbiol Biotechnol 31(12):1823–1844

Pan XL, Liu J, Zhang DY, Chen X, Li LH, Song WJ, Yang JY (2010) A comparison of five extraction methods for extracellular polymeric substances (EPS) from biofilm by using three-dimensional excitation-emission matrix (3DEEM) fluorescence spectroscopy. Water SA 36: 111–116

Parikh A, Madamwar D (2006) Partial characterization of extracellular polysaccharides from cyanobacteria. Bioresour Technol 97:1822–1827

Pavlova K, Grigorova D (1999) Production and properties of exopolysaccharide by *Rhodotorula acheniorum* MC. Food Res Int 32:473–477

Pei XY, Ren HY, Liu BF (2021) Flocculation performance and mechanism of fungal pellets on harvesting of microalgal biomass. Bioresour Technol 321:124463

Petersen GR, Schubert WW, Richards GF, Nelson GA (1990) Yeasts producing exopolysaccharides with drag-reducing activity. Enzyme Microb Technol 12(4):255–259

Planý M, Flavi P, Šoltys K, Kraková L, Cornis L, Pangallo D, Jungblut AD, Little B (2021) Fungal-induced atmospheric iron corrosion in an indoor environment. Int Biodeterior Biodegrad 159: 105204

Plucinski A, Lyu Z, Bernhard VKJ, Schmidt BVKJ (2021) Polysaccharide nanoparticles: from fabrication to applications. J Mater Chem B 9:7030–7062

Pragya N, Pandey KK, Sahoo PK (2013) A review on harvesting, oil extraction and biofuels production technologies from microalgae. Renew Sust Energ Rev 24:159–171

Pretus HA, Ensley HE, McNamee RB, Jones EL, Browder IW, Williams DL (1991) Isolation, physicochemical characterization and preclinical efficacy evaluation of soluble scleroglucan. J Pharmacol Exp Ther 257:500–510

Pu S, Qin L, Che J, Zhang B, Xu M (2014) Preparation and application of a novel bioflocculant by two strains of Rhizopus sp. using potato starch wastewater as nutrilite. Bioresour Technol 162: 184–191

Queiroz EAIF, Fortes ZB, Cunha MAA, Barbosa AM, Khaper N, Dekker RFH (2015) Antiproliferative and pro-apoptotic effects of three fungal exocellular β-glucans in MCF-7 breast cancer cells is mediated by oxidative stress, AMP-activated protein kinase (AMPK) and the forkhead transcription factor, FOXO3a. Int J Biochem Cell Biol 67:14–24

Rafiee K, Kaur G, Brar SK (2021) Fungal biocomposites: how process engineering affects composition and properties? Bioresour Technol Rep 14:100692

Rashid N, Park WK, Selvaratnam T (2018) Binary culture of microalgae as an integrated approach for enhanced biomass and metabolites productivity, wastewater treatment, and bioflocculation. Chemosphere 194:67–75

Redmile-Gordon M, Gregory AS, White RP, Watts CW (2020) Soil organic carbon, extracellular polymeric substances (EPS), and soil structural stability as affected by previous and current land-use. Geoderma 363:114143

Reich M, Labes A (2017) How to boost marine fungal research: a first step towards a multidisciplinary approach by combining molecular fungal ecology and natural products chemistry. Mar Genomics 36:57–75

Roca C, Alves VD, Freitas F, Reis MA (2015) Exopolysaccharides enriched in rare sugars: bacterial sources, production, and applications. Front Microbiol 6:288

Saad MM (2006) Chelating ability of chitosan glucan complex from *Aspergillus niger* NRRL595 biomass recycling in citric acid production. Res J Agric Biol Sci 2:132–136

Sajjad M, Kim KS (2015) Studies on the interactions of Ca2+ and Mg2+ with EPS and their role in determining the physicochemical characteristics of granular sludges in SBR system. Process Biochem 50(6):966–972

Saleem M, Ali MS, Hussain S, Jabbar A, Ashraf M, Lee YS (2007) Marine natural products of fungal origin. Nat Prod Rep 24:1142–1152

Sandford PA (1979) Exocellular microbial polysaccharides. Adv Carbohydr Chem Biochem 36: 265–312

Sathiyaseelan A, Shajahan A, Kalaichelvan PT, Kaviyarasan V (2017) Fungal chitosan based nanocomposites sponges—an alternative medicine for wound dressing. Int J Biol Macromol 104:1905–1915

Schmid F, Stone BA, McDougall BM, Bacic A, Martin KL, Brownlee RT, Chai E, Seviour RJ (2001) Structure of epiglucan, a highly side-chain/branched (1→3;1→6)-β-glucan from the micro fungus *Epicoccum nigrum* Ehrenb. ex Schlecht. Carbohydr Res 331:163–171

Seviour RJ, Stasinopoulos SJ, Auer DPF (1992) Production of pullulan and other exopolysaccharides by filamentous fungi. Crit Rev Biotechnol 12:279–298

Sheng GP, Yu HQ (2006) Chemical-equilibrium-based model for describing the strength of sludge: taking hydrogen-producing sludge as an example. Environ Sci Technol 40:1280–1285

Sheng GP, Yu HQ, Li XY (2010) Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. Biotechol Adv 28(6):882–894

Shingel KI (2004) Current knowledge on biosynthesis, biological activity, and chemical modification of the exopolysaccharide, pullulan. Carbohydr Res 339(3):447–460

Shu CH, Lung M (2004) Effect of pH on the production and molecular weight distribution of exopolysaccharide by *Antrodia camphorata* in batch cultures. Process Biochem 39:931–937

Siddharth T, Sridhara P, Vinilaa V, Tyagib RD (2021) Environmental applications of microbial extracellular polymeric substance (EPS): a review. J Environ Manag 287:112307

Silva NH, Vilela C, Almeida A, Marrucho IM, Freire CS (2018) Pullulan-based nanocomposite films for functional food packaging: lysozyme nanofibers as antibacterial and antioxidant reinforcing addtives. Food Hydrocoll 77:921–930

Sletmoen M, Stokke BT (2008) Higher order structure of (1, 3)-d-glucans and its influence on their biological activities and complexation abilities. Biopolymers 89:310–321
Sobeck DC, Higgins MJ (2002) Examination of three theories for mechanisms of cation-induced bioaggregation. Water Res 36:527–538
Song KS, Cho SM, Lee JH, Kim HM, Han SB, Ko KS, Yoo ID (1995) B-lymphocyte-stimulating polysaccharide from mushroom *Phellinus linteus*. Chem Pharm Bull 43:2105–2108
Surenjav U, Zhang L, Xu X, Zhang X, Zeng F (2006) Effects of molecular structure on antitumor activitiesof (1/3)-b-D-glucans from different Lentinus edodes. Carbohydr Polym 63:97–104
Takaku T, Kimura Y, Okuda H (2001) Isolation of an antitumor compound from Agaricus blazei Murill and its mechanism of action. Nutr J 131:1409–1413
Tian Y (2008) Behaviour of bacterial extracellular polymeric substances from activated sludge: a review. Int J Environ Pollut 32:78–89
Tsekova K, Todorova D, Ganeva S (2010) Removal of heavy metals from industrial wastewater by free and immobilized cells of *Aspergillus niger*. Int Biodeterior Biodegradation 64:447–451
Urbain V, Block JC, Manem J (1993) Bioflocculation in activated sludge: an analytic approach. Water Res 27:829–838
Van der Valk P, Marchant R, Wessels JGH (1977) Ultrastructural localization of polysaccharides in the wall and septum of the basidiomycete Schizophyllum commune. Exp Mycol 1:69–82
Vardharajula S, Ali SZ (2015) The production of exopolysaccharide by Pseudomonas putida GAP-P45 under various abiotic stress conditions and its role in soil aggregation. Microbiology 84:512–519
Vetvicka V, Yvin JC (2004) Effects of marine β-1,3 glucan on immune reactions. Int Immunopharmacol 4:721–730
Volesky B (1990) Removal and recovery of heavy metals by biosorption. In: Volesky B (ed) Biosorption of heavy metals. CRC Press, Boca Raton, pp 7–44
Wang H, Li P, Jin Q, Qin G (2014) Specific aerobic granules can be developed in a completely mixed tank reactor by bioaugmentation using micro-mycelial pellets of *Phanerochaete chrysosporium*. Appl Microbiol Biotechnol 98(6):2687–2697
Wang X, Sharp CE, Jones GM, Grasby SE, Brady AL, Dunfield PF (2015) Stable-isotope probing identifies uncultured planctomycetes as primary degraders of a complex heteropolysaccharide in soil. Appl Environ Microbiol 81:4607–4615
Wang J, Chen R, Fan L, Cui L, Zhang Y, Cheng J, Wu X, Zeng W, Tian Q, Shen L (2021) Construction of fungi-microalgae symbiotic system and adsorption study of heavy metal ions. Sep Purif Technol 268:118689
Wasser SP (2002) Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol 60(3):258–274
Wingender J, Neu TR, Flemming HC (1999) What are bacterial extracellular polymeric substances? In: Wingender J, Neu TR, Flemming H-C (eds) Microbial extracellular polymeric substances: characterization, structure and function. Springer, Berlin, pp 1–19
Xu CP, Kim SW, Hwang HJ, Yun JW (2006) Production of exopolysaccharides by submerged culture of an enthomopathogenic fungus, *Paecilomyces tenuipes* C240 in stirred-tank and airlift reactors. Bioresour Technol 97(5):770–777
Yan YK, Wang WQ, Wu JY (2014) Recent advances in Cordyceps sinensis polysaccharides: mycelial fermentation, isolation, structure, and bioactivities: a review. J Funct Foods 6:33–47
Yang H, He G (2008) Influence of nutritional conditions on exopolysaccharide production by submerged cultivation of the medicinal fungus *Shiraia bambusicola*. World J Microbiol Biotechnol 24(12):2903–2907
Yang SF, Li XY (2009) Influences of extracellular polymeric substances (EPS) on the characteristics of activated sludge under non-steady-state conditions. Process Biochem 44:91–96
Yang BK, Jeong SC, Park JB (2001) Swimming endurance capacity of mice after administration of exo-polymer produced from submerged mycelial culture of *Ganoderma lucidum*. J Microbiol Biotechnol 11(5):902–905

Yilmaz G, Bozkurt U, Magden KA (2016) Effect of iron ions (Fe2+, Fe3+) on the formation and structure of aerobic granular sludge. Biodegradation 28(1):1–16

Yin Y, Hu Y, Xiong F (2011) Sorption of Cu(II) and Cd(II) by extracellular polymeric substances (EPS) from *Aspergillus fumigatus*. Int Biodeterior Biodegradation 65(7):1012–1018

Yu GH, He PJ, Shao LM, Zhu YS (2008) Extracellular proteins, polysaccharides and enzymes impact on sludge aerobic digestion after ultrasonic pretreatment. Water Res 42(8–9):1925–1934

Yu GH, He PJ, Shao LM (2009) Characteristics of extracellular polymeric substances (EPS) fractions from excess sludges and their effects on bioflocculability. Bioresour Technol 100: 3193–3198

Yu R, Hou C, Liu A, Peng T, Xia M, Wu X, Shen L, Liu Y, Li J, Yang F, Qiu G, Chen M, Zeng W (2018) Extracellular DNA enhances the adsorption of *Sulfobacillus thermosulfidooxidans* strain ST on chalcopyrite surface. Hydrometallurgy 176:97–103

Yu GH, Chi ZL, Teng HH, Dong HL, Kappler A, Gillings MR, Polizzotto ML, Liu CQ, Zhuj YG (2019) Fungus-initiated catalytic reactions at hyphal-mineral interfaces drive iron redox cycling and biomineralisation. Geochem Cosmochim Acta 260:192–203

Yu T, Wang L, Ma F, Wang Y, Bai S (2020) A bio-functions integration microcosm: self-immobilized biochar-pellets combined with two strains of bacteria to remove atrazine in water and mechanisms. J Hazard Mater 384:121326

Zandi G, Lotfipour F, Ghanbarzadeh S, Medghalchi M, Hamishehkar H (2018) A comparative study on the potentials of nanoliposomes and nanoethosomes for fluconazole delivery. J Drug Deliv Sci Technol 44:264–269

Zekovic DB, Kwlatkowski S, Vrvic MM, Jakovijevic D, Moran CA (2005) Natural and modified (1→3)-β-d-glucans in health promotion and disease alleviation. Crit Rev Biotechnol 25:205–230

Zhang GL, Wang YH, Ni W, Teng HL, Lin ZB (2002) Hepatoprotective role of Ganoderma lucidium polysaccharide against BCG-induced immune liver injury in mice. World J Gastroenterol 15(8):728–733

Zhang L, Li X, Xu X, Zeng F (2005) Correlation between antitumor activity, molecular weight, and conformation of lentinan. Carbohydr Res 340:1515–1521

Zhang M, Cui SW, Cheung PCK, Wang Q (2007) Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. Trends Food Sci Technol 18:4–19

Zhang Y, Kong H, Fang Y, Nishinari K, Phillips GO (2013) Schizophyllan: a review on its structure, properties, bioactivities and recent developments. Bioactive Carbohydr Diet Fibre 1: 53–71

Zheng Z, Ali A, Su J, Huang T, Wang Y, Zhang S (2021) Fungal pellets immobilized bacterial bioreactor for efficient nitrate removal at low C/N wastewater. Bioresour Technol 332:125113

Zhou J, Zheng G, Zhang X, Zhou L (2014) Influences of extracellular polymeric substances on the dewaterability of sewage sludge during bioleaching. PLoS One 9(7):e102688

Production and Application of Nanofibres from Pullulan

N. Vigneshwaran and Komal Saraf

Abstract Pullulan, an extracellular water-soluble polysaccharide, is mainly produced by the fungus, *Aureobasidium pullulans*. Pullulan consists of maltotriose units and helps the fungus to resist desiccation and predation. This also helps the fungus in transport of molecules into the fungal cell wall. This polymer is commercially used in the food industry as low-calorie food additive providing the bulk and texture. Due to the oxygen barrier and anti-fungal properties, pullulan is an excellent candidate in food preservation industries. But, due to various constraints in large-scale production of pullulan, wide spread commercial use of pullulan is limited. To make the best use of available pullulan, nanotechnology offers huge scope by enhancing its performance characteristics. Electrospinning is a well demonstrated process employed for the production of nanofibres from pullulan. This process uses very high voltage (20 to 75 kV) on the polymer solution to extrude nanofibres on the opposite charged collector. Thus produced nanofibres form a non-woven mat of pullulan fibres in the form of a film that can be directly used for any applications. In this chapter, chemistry of pullulan, biosynthesis, production of nanofibres by electrospinning and their applications in diversified fields are discussed.

Keywords *Aureobasidium pullulans* · Electrospinning · Fungus · Nanofibres · Nanotechnology · Polysaccharide · Pullulan

1 Introduction

Many microorganisms have been reported to produce the exopolysaccharide pullulan, such as *Aspergillus japonicas, Aureobasidium pullulans, Cryphonectria parasitica, Cytaria darwinii, C. harioti, Rhodototula bacarum, Rhosossporidium paludigenum, Teloschistes flavicans,* and *Tremella mesenterica.* Amongst all the different organisms, the main producer of pullulan with the highest yield is

N. Vigneshwaran (✉) · K. Saraf
ICAR-Central Institute for Research on Cotton Technology, Mumbai, India
e-mail: Vigneshwaran.N@icar.gov.in

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_5

A. pullulans. But not all the strains of this fungus are capable of producing pullulan. Some of the high yielding strains of this fungus include RG-5 (37.1 g/L), LDT-1 (31.25 g/L), P56 which is a melanin deficient strain (31.3 g/L), SK 1002 (30.28 g/L), CJ001 (26.13 g/L) and SZU1001 (25.6 g/L). Strain improvement for increasing the yield of high molecular weight pullulan and reduced pigment can be carried out by using mutagenic agents like ethidium bromide, ultra violet rays and γ rays (Mishra et al. 2018, Viveka et al. 2020).

Aureobasidium pullulans (also known as *Pullularia pullulans*) is a yeast-like fungus belonging to the sub-kingdom, Eumycota with septate mycelium under the division of Ascomycota which are described as sac fungi. It shows saprotrophic mode of nutrition that feeds on dead and decaying organic matter. Even though this fungus was identified more than hundred years ago, still it is the fungus of choice due to its varied biochemical properties shown by different strains, their diverse habitats and ability to survive various environmental conditions. Wide spectrum of habitats of this fungus includes fresh and saline waters, epiphytes on the surfaces of leaves and flowers of different plants, artificially created surfaces like bathroom tiles, natural surfaces of rocks and soils. Environmental conditions like extreme temperatures seen in cold and temperate areas of central Europe, Antarctica and Alaska to the warmer and dry areas of Egypt and Pakistan can also be inhabited by this fungus. Based on the availability of sufficient moisture content, it is also seen in humid tropical regions of India, Malaysia, Jamaica and Thailand (Prasongsuk et al. 2018).

2 Pullulan: Structure and Properties

Bauer in 1938 was the first person to discover that pullulan was produced by *A. pullulans*. Wallenfels, Bender, Keilich and Bechtler, together provided the basic structure of pullulan and also coined the term 'pullulan' (Farris et al. 2014). Pullulan, which is a poly-α-1,6-maltotriose homo-polysaccharide was first studied in detail in the year 1958. The maltotriose units are connected by α (1 → 4) glycosidic bond whereas the consecutive maltotriose units are inter-linked with α (1 → 6) glycosidic bond. This alternating combination of the two bonds enables it to attain the property of structural flexibility like fibre and film forming ability and hydrophilicity that enhances its adhesive nature. Figure 1 shows the chemical structure of pullulan. It is a highly soluble substance both in aqueous (cold and hot) and organic solvents, dilute alkali but insoluble in alcohol. The structural appearance of pullulan is odorless, colourless, tasteless, white, amorphous or crystalline, non-hygroscopic and dry powder. Its molecular mass can range from 5000–9000 kDa. It can have chemical modifications including esterification, carboxymethylation and sulfation, for further applications. Other properties of pullulan include anti-oxidant capacity, anti-sticking, stabilizer, low viscosity filler, gas barrier property, non-mutagenic, non-carcinogenic, non-toxic, non-ionic, non-immunogenic, inert nature, stability over wide range of pH and biocompatibility. Exceptional properties of high gloss and transparency, easily blending with other biopolymers and heat sealability make

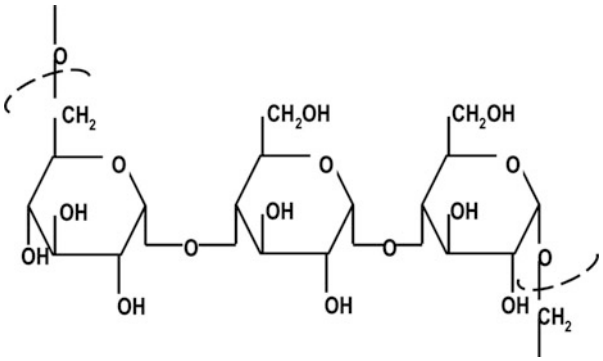


Fig. 1 Chemical structure of pullulan

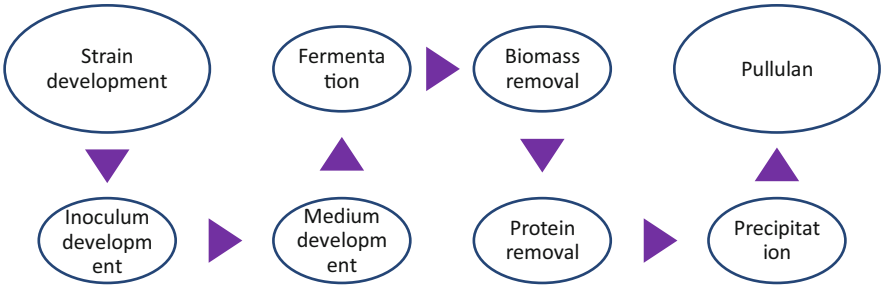


Fig. 2 Upstream and downstream processing sequence for pullulan production

it an ideal candidate for potential applications in packaging industry (Farris et al. 2014, Mishra et al. 2018, Prasongsuk et al. 2018, Khan et al. 2020, Li et al. 2021,).

3 Biosynthesis of Pullulan

Since a long time, tremendous efforts are being made to increase the yield and to get better quality of pullulan. Industrial large scale production is carried out by fermentation of liquefied starch or sucrose by *A. pullulans* (Prasongsuk et al. 2018). The upstream processing includes selection of appropriate strain, inoculum and medium development, studying the growth kinetics and its optimization. Downstream processing enables us to purify the product and improve its quality from the fermentation broth, which is the most expensive part of the entire production system. First, centrifugation is carried out to separate the biomass from culture broth. This is followed by treatment with decolourizing agents like potassium chloride, activated charcoal or hydrogen peroxide for removal of proteins and melanin pigment. Finally, precipitation of pullulan has to be carried out using a suitable organic solvent like isopropyl alcohol or ethanol (Mishra et al. 2018). The upstream and downstream processes of pullulan are given in Fig. 2.

In order to make the pullulan production cost effective, agro-industrial wastes have been used as the raw material, which is an ecologically and economically sound idea. Some examples of high yielding wastes include corn steep liquor (88.59 g/L), de-oiled *Jatropha* seed cake (83.98 g/L), potato starch (79.4 g/L), coconut milk (58 g/L), jaggery which contains 75–85% sucrose (51.9 g/L) and beet molasses (49 g/L) and others. The varying yield of pullulan can depend on the type of organism used, conditions maintained, kind of fermentation and composition of the medium. In the search of cheap and reliable carbon and nitrogen sources for pullulan production, sweet potato is the most favourable substrate with little sugar and large amount of active β-amylase in submerged fermentation. When wheat bran, rice bran, coconut kernels and palm kernels were compared, palm kernels are found to be superior carbon source with a yield of 16 g/L. Further optimization with Response Surface Methodology (RSM), this yield could be increased to 30.4 g/L. Soyabean pomace with its high nutritional value and high salt content is considered a good source of nitrogen for pullulan production. Other examples of wastes are brewery waste, jackfruit seeds, cassava bagasse and rice hull hydrolysate (Mishra et al. 2018).

Cassava contributes around one fifth of the world-wide agricultural crop production that makes its waste easily available. It has a very high residual starch content of more than 50% that can be rightfully put to use by converting to pullulan by solid state fermentation. The low ash and cellulose content makes it a better candidate as compared to other biomass residues from rice and maize. While comparing the organic (yeast extract) and inorganic (ammonium sulphate) nitrogen sources, use of organic source results in higher yield of pullulan (6.45 g/L). Other important elements needed for cell growth and exopolysaccharide production are potassium biphosphate and magnesium in optimal concentrations. Thus the use of inexpensive sources of carbon and nitrogen would greatly reduce the production cost, which contributes to nearly one third of the cost. To further increase the yield of pullulan, combination of carbon sources and mutated strains of *A. pullulans* need to be used (Viveka et al. 2020).

Cell immobilization with diatomaceous earth and chitosan have been also evaluated for the production of pullulan (Prasongsuk et al. 2018). The biosynthetic pathway of pullulan production is very complex and intricate process, wherein the pullulan is known to be produced intracellularly but it is later excreted out as a mucus layer (Viveka et al. 2020). Metabolic engineering has been performed to evaluate the relation between pullulan production and metabolic products and it was found that there is a direct relationship between pullulan production and ATP concentration of the cell, which indicates that it is an energy consuming process. Genetic engineering of *Aureobasidium melanogenum* P16 led to the production of pullulan by converting inulin directly to pullulan that would save a lot of time and cut shorts the biosynthetic pathway for pullulan production (Mishra et al. 2018).

4 Production of pullulan based nanofibres

Some of the exceptional properties of nanofibres are attributed to their large surface area to volume ratio, high porosity, improved mechanical strength, permeability, stability and functional flexibility with surface modifications. Nanofibres can be prepared by a simple but very promising non-conventional technique called electrospinning. They find wide scale applications in large number of fields such as biomedical, textile, filter media, gene and drug delivery, tissue engineering, catalysis, food technology, smart packaging and sensors (Diab et al. 2001).

The beauty of the technique of electrospinning lies in its simplicity, reproducibility, continuity of production and scalability for large scale production of nanofibres (Fig. 3). As compared to other nanofibre fabrication techniques, it is quite economical and flexible with regulating the morphology of the nanofibres in terms of diameter, surface area and pore volume. There are three stages in this process namely, jet initiation, elongation and solidification of the nanofibres. The principle of electrospinning technique is based on electrostatic force of attraction between opposite charges where the polymer solution fed in the syringe with spinneret connected to the positive terminal of power supply and aluminum or copper coated collector is connected to grounded negative terminal. When the syringe pump allows the polymer to extrude out at a controlled flow rate (0.1–1 mL/h), it forms a droplet at the tip of the spinneret due to its own surface tension. A high voltage (5–50 kV) is applied to overcome this surface tension and making the droplet highly electrified forming a cone shaped structure called Taylor cone. As we increase the voltage gradually, electrostatic force beats the surface tension causing the cone to elongate and form a viscoelastic jet attracted towards the collector which is placed at an appropriate distance (5–20 cm). There is simultaneous

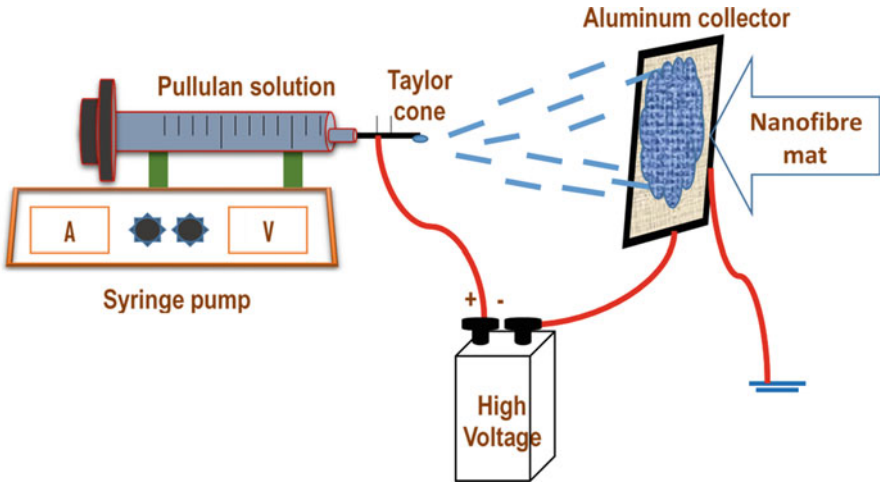


Fig. 3 Schematic of the electrospinning setup for production of pullulan nanofibres

evaporation of the solvent from the polymer solution on the way towards the collector forming a non-woven mat of nanofibres. Apart from flow rate, applied voltage and tip to collector, other important parameters that affect the process of electrospinning are solution concentration, viscosity, conductivity, surface tension, solvent vapour pressure, solvent content, ionic character of polymer, and environmental factors like temperature, airflow and humidity (Diab et al. 2001, Kumar et al. 2019).

When polymer solution concentration is low, beads are formed on nanofibres that are not suitable for most of the applications. Thus to get uniform bead free fibres, optimal solution concentration is required. Low viscosity of the solution also creates the issue of discontinuity in the fibre formation and if the viscosity is too high then polymer extrusion from the spinneret becomes very difficult. Thus optimization of viscosity is also required for successful electrospinning to occur. If the molecular weight of the polymer is high then it increases the chain entanglement seen in the polymer solution, which results in larger fibres. In case of low molecular weight, beaded nanofibres are formed. If we reduce the surface tension of polymer solution then there are higher chances of getting beadless fibres. If the electrical conductivity of the solution is more, then the diameter of nanofibres will be smaller; but, if the conductivity is low then elongated jet forms beaded nanofibres. If the flow rate of the solution is faster, then there will be very less time for evaporation of the solvent leading to improper drying of the nanofibre mat. Tip to collector distance also should be sufficient to allow complete evaporation of the solvent and bead development is seen if it is not optimized properly. Temperature affects the viscosity by reducing it making the diameters of nanofibres narrower. Humidity makes it difficult to allow complete evaporation of solvent whereas low humidity can lead to very fast evaporation of solvent not allowing the jet to reach the collector. So we need optimize the three basic parameters of electrospinning in such a manner to get uniform bead-free nanofibres (Diab et al. 2001).

Pullulan has been known to give uniform nanofibres when dissolved in distilled water to a concentration of 22% with applied voltage of 31 kV, TCD of 20 cm and flow rate of 0.5 mL/h (Diab et al. 2001). Addition of pullulan in the polymer solution increases the molecular entanglement making it easier for nanofibre production of starch based films. The successful electrospinning of 12%, 15% and 20% (w/v) aqueous high molecular weight starch dispersions required a minimum addition of 6%, 5%, and 3% (w/v) of pullulan, respectively (Li et al. 2021). Various solvents other than water like formic acid and dimethyl sulfoxide (DMSO) have been used to make electrospun nanofibre mats of pullulan. But there are restrictions in use of food packaging due to its low water resistance and it is not possible to encapsulate thermo-sensitive bioactives due to its poor thermal stability. Thus blending of pullulan with other biopolymers like ethyl cellulose (EC) helps to attain better film properties than pullulan alone. EC is a low molecular weight polymer and its addition to high molecular weight pullulan decreases the viscosity and conductivity, resulting in decreased average diameter of the composite nanofibres. Even gelatin with EC gave rise to low diameter nanofibres due to similar reason of decrease in viscosity and conductivity. In the blended composite films of pullulan and EC, there

is hydrogen bonding between them that increases the thermal stability and hydrophobicity of the film. Even the melting point of the composite film of pullulan/chitosan increased due to similar interactions at the molecular level (Yang et al. 2020).

Apart from electrospinning, rotary jet spinning method is also being used as one of the most efficient techniques for micro/nanofibres production from pullulan. In a latest report, the influence of the pullulan/PVA blend solution composition, the solvent volatility and spinneret-collector distance was evaluated on the pullulan nanofibre's diameter (Souza et al. 2021). It was reported a strong dependence of the fibre diameter on the blend composition and DMSO content, which could be explained mainly by the rheological properties of the polymer solution. The Response Surface Methodology analysis showed a complex influence of the independent variables on the fibre's diameter that could be fitted well by an empirical model.

5 Applications of Pullulan

In pharmaceutical field, it can be used for controlled drug delivery in the form of nanocarriers for antiviral drugs; and, they are known to increase their concentration in the liver due to the hydrophilic nature of pullulan. Specific interferons can also be delivered to the target liver cells due to the binding capacity of pullulan. Same binding property can further be used to deliver the anticancer drugs to destroy the tumours (Prasongsuk et al. 2018). Other pharmaceutical applications include capsule coating, oral care products and plasma substituent (Mishra et al. 2018). It can also be used as a bulking agent (Viveka et al. 2020). The advantages of pullulan nanofibres in the biomedical field are biocompatibility, load efficiency and extracellular matrix mimicking. Recently quick dissolving oral aspirin films have been produced using pullulan/chitosan blend. Thus nanofibre mats find potential application in oral mucosal drug release too (Diab et al. 2001). Pullulan can be a good substitute for gelatin to form soft and hard capsules, which makes it safe for the consumption of restricted diet people, vegetarians and diabetics (Farris et al. 2014).

The fast-dissolving drug delivery systems are developed as nanofibers using food-grade water-soluble hydrophilic biopolymers like pullulan that can disintegrate fast in the oral cavity and deliver drugs. In an earlier work, Jelly fig polysaccharide and pullulan blend was to prepare fast-dissolving nanofiber by electrospinning process. The nanofibers loaded with hydrophobic model drugs like ampicillin and dexamethasone were rapidly dissolved in water within 60 s and released the encapsulants into the surrounding. Hence, such a nanofiber mat could be a promising carrier to encapsulate hydrophobic drugs for fast-dissolving/disintegrating delivery applications (Ponrasu et al. 2021).

In the field of tissue engineering, it can be used as blood-plasma expander since it degrades much faster in blood serum as compared to other polysaccharides like dextran. Other biomedical applications include wound dressing, formation of

molecular chaperons, quantum dots, and artificial organs and for gene delivery (Mishra et al. 2018). Diagnostic applications where pullulan can be used are vascular compartment imaging and perfusion, receptor, and lymph node target specific imaging (Farris et al. 2014). Though pullulan in extensively used as such in drug and gene deliveries (Singh et al. 2021), its conversion to nanofibres is bound to enhance its efficacy.

There is increasing demand of bio-based packaging material to enhance shelf life and quality of all types of food products whether they are fresh or frozen or formulated because of the factors like safe disposal of synthetic packaging material, demand to increase organic food products directly from farms with minimal processing and expansion of distribution network to reach out to more and more consumers. The biopolymers used for making edible coatings should not only fulfill the mechanical and physiochemical requirements of the packaging material but also act as good carriers of flavour, colour, and nutrition retention with inexpensive raw materials and simple technology like electrospinning (Diab et al. 2001).

The Food and Drug Administration has declared pullulan to be a safe food additive (Viveka et al. 2020). The United States has given 'GRAS' status to pullulan, which means 'Generally Regarded As Safe' (GRAS) (Farris et al. 2014). Since pullulan is a safe and biodegradable substance with negligible health risks, it can used to make films, which form edible active food packaging coatings. Earlier, it was demonstrated by incorporating lysozyme enzyme into pullulan films to impart antibacterial property (Prasongsuk et al. 2018). Antibacterial property can also be imparted to pullulan films with the help of silver nanoparticles and essential oils that make them active packaging material especially for increasing the shelf life of products like meat, eggs and fruits. Such films show slow and consistent release of these active antibacterial agents for continuous and long lasting effect with the impact of killing deadly organisms like *Listeria monocytogenes*, *Clostridium perfringens, Campylobacter jejuni, S. typhimurium, E. coli, S. aureus* etc. Another bioactive agent incorporated in pullulan was the extract of meadowsweet flower that significantly restricted the growth of *Rhizopus arrhizus* mold on the apples. Also the spoilage causing organisms are unable to utilize pullulan as a carbon source limiting the deterioration of the food. Its oxygen barrier property paves a way to prevent oxidative rancidity of perishable food products. In comparison to other edible polysaccharides like chitosan and seaweeds, pullulan is preferred due to its homogeneous distribution, translucent appearance and improved thermal stability (4 to 25 °C) with increased tensile strength of the edible active packaging films. Thus they can retain the delicacy and original taste of the product and provide protection against physical and mechanical damages. Due to the hydrophilic nature, it shows good amount of resistance to oil making it an ideal material for packaging of fat soluble substances. All these amazing properties make pullulan as one of the most promising emerging food preservation candidate of the future in terms of customer safety (Khan et al. 2020, Farris et al. 2014).

Owing to its gas barrier property of pullulan nanofibre coated packaging films, there is accumulation of ethylene, growth regulator of plants that promotes ripening of the fruit, enables us to get organic form of fruit in much lesser period of time.

There is also a reduction in the rate of respiration of fruits that enhances the shelf life and quality of fruits like strawberry and kiwi (Diab et al. 2001). Firmness of strawberry was maintained by coating with pullulan blended with tea polyphenols, which reduced the titratable acidity. Physical barrier property of pullulan prevents the loss of moisture and slows the softening process from fruits like apple that maintains the weight. Edible pullulan nano-coating also helps in retention of quality and weight of white asparagus spears and prevents purple colour formations on the surface of the tips. In the recent years, there is an increasing demand for smart packaging with faster reacting biosensors allowing reduction in response time with high specificity and sensitivity, possible with the help of nanofibres. Nanosensors have been incorporated to monitor and trace the condition of the food. It helps the consumers in identifying the quality and safety levels of consumption of food without opening it. The pH sensitive dyes can be used along with nanosensors making optical visualization very easy provided with colour code for specific pH values. Such optical indicator of spoilage food with smart packaging makes it safe and convenient for the consumers to understand the freshness of the packaged food. Further, these dyes also increase the thermal and storage stabilities of the products (Farris et al. 2014; Kumar et al. 2019).

Since pullulan has the properties of anti-sticking and low viscosity, it has been used as a stabilizer in the products like mayonnaise (Prasongsuk et al. 2018). As pullulan is resistant to the breakdown by mammalian amylases and slow digesting macromolecule, its intake would incur lesser amount of calories thus making a good alternative as dietary fibre. In the past, study also shows its potential as a *prebiotic* promoting the growth of good bacteria like *Bifidobacterium*. It can be used as a texturizer and glazing agent in chewing gum and foaming agents in milk-based desserts. Lastly it can also be used in making biodegradable flocculating agent (Mishra et al. 2018, Farris et al. 2014). The pullulan nanofibrous web can also be used to encapsulate essential oil like eugenol (having very good antioxidant property) for potential application in in both food and pharmaceutical areas (Celebioglu and Uyar, 2021).

Other major application is in cosmetic industry, where the pullulan is used already in the products like shampoos, lotion and facial masks in cosmetic range, toothpaste and denture adhesive in oral care products. In chromatographic applications, it can be either used as stationary phase or as a molecular weight standard for the separation technique (Prasongsuk et al. 2018). It has also found its use in paper coating, photographic, lithographic, optic and electronic applications and enzyme immobilization (Farris et al. 2014; Mishra et al. 2018). The major applications of pullulan nanofibres are given in Fig. 4.

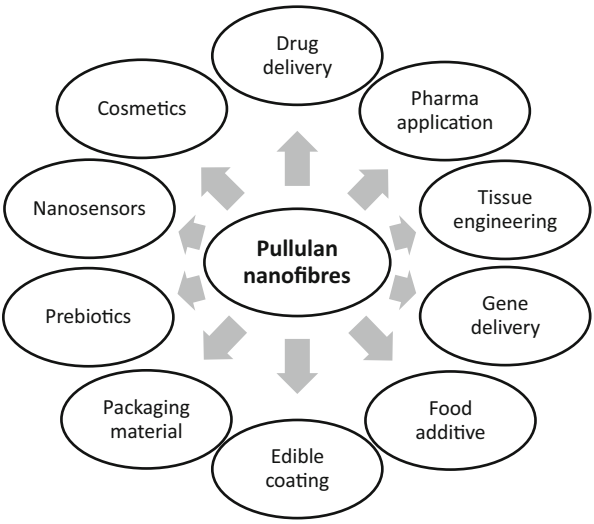


Fig. 4 Major applications of pullulan nanofibres

6 Conclusion

A major hindrance in the usage of pullulan for different applications is its high affinity towards water. Another major drawback on the use of pullulan is its higher cost of production, which is almost three times more than the other commercial polysaccharides. Detailed understanding at the molecular level of pullulan and bio-engineering for efficient production are the need of the hour to overcome the existing hindrances. Recent research work focus on the utilization of agro-industrial wastes as carbon and nitrogen sources for pullulan production. Electrospinning of pullulan for production of nanofibres enhances its value by utilizing it in high end applications. Thus produced nanofibres form a non-woven mat of pullulan fibres in the form of a film that can be directly used for diversified applications. Transforming the success of electrospinning from laboratory scale to industrial scale is the need of the hour to take the electrospun pullulan mat to the market.

Acknowledgements The authors are grateful to Dr. Sujata Saxena, Director, ICAR-Central Institute for Research on Cotton Technology, Mumbai for technical support in the project work.

References

Celebioglu A, Uyar T (2021) Electrohydrodynamic encapsulation of eugenol-cyclodextrin complexes in pullulan nanofibers. Food Hydrocoll 111:106264

Diab T, Biliaderis CG, Gerasopoulos D, Sfakiotakis E (2001) Physicochemical properties and application of pullulan edible films and coatings in fruit preservation. J Sci Food Agric 81(10): 988–1000

Farris S, Unalan IU, Introzzi L, Fuentes-Alventosa JM, Cozzolino CA (2014) Pullulan-based films and coatings for food packaging: present applications, emerging opportunities, and future challenges. J Appl Polym Sci 131(13). https://doi.org/10.1002/app.40539

Khan MJ, Kumari S, Selamat J, Shameli K, Sazili AQ (2020) Reducing meat perishability through pullulan active packaging. J Food Qual 1-2:8880977. https://doi.org/10.1155/2020/8880977

Kumar TSM, Kumar KS, Rajini N, Siengchin S, Ayrilmis N, Rajulu AV (2019) A comprehensive review of electrospun nanofibers: Food and packaging perspective. Compos Part B Eng 175: 107074. https://doi.org/10.1016/j.compositesb.2019.107074

Li S, Kong L, Ziegler GR (2021) Electrospinning of Octenylsuccinylated Starch-Pullulan Nanofibers from Aqueous Dispersions. Carbohydr Polym 258:116933. https://doi.org/10.1016/j.carbpol.2020.116933

Mishra B, Zamare D, Manikanta A (2018) Selection and utilization of agro-industrial waste for biosynthesis and hyper-Production of pullulan: A Review. In: Varjani S, Parameswaran B, Kumar S, Khare S (eds) Biosynthetic technology and environmental challenges. Springer Nature Singapore Pte Ltd, Springer Singapore, pp 89–103

Ponrasu T, Chen BH, Chou TH, Wu JJ, Cheng YS (2021) Fast Dissolving Electrospun Nanofibers Fabricated from Jelly Fig Polysaccharide/Pullulan for Drug Delivery Applications. Polymers 13(2):241

Prasongsuk S, Lotrakul P, Ali I, Bankeeree W, Punnapayak H (2018) The current status of *Aureobasidium pullulans* in biotechnology. Folia microbial 63(2):129–140

Singh RS, Kaur N, Hassan M, Kennedy JF (2021) Pullulan in biomedical research and development–A review. Int J Biol Macromol 166:694–706

de Souza L, Alavarse AC, da Vinci MA, Bonvent JJ (2021) The Synergistic Effect of Polymer Composition, Solvent Volatility, and Collector Distance on Pullulan and PVA Fiber Production by Rotary Jet Spinning. Fibers Polym 22:942–956

Viveka R, Varjani S, Ekambaram N (2020) Valorization of Cassava Waste for Pullulan Production by *Aureobasidium pullulans* MTCC 1991. Energy & Environ. https://doi.org/10.1177/0958305x20908065

Yufan Y, Xie B, Liu Q, Kong B, Wang H (2020) Fabrication and Characterization of a Novel Polysaccharide Based Composite Nanofiber Films with Tunable Physical Properties. Carbohydr Polym 236:116054

Using Fungal Biopolymers for Enhanced Oil Recovery

S. H. Al-Araimi, S. N. Al-Bahry, and Y. M. Al-Wahaibi

Abstract Fungal biopolymers have attracted the attention of the scientific community because of their superior biological and physicochemical properties. They have been exploited for use in different applications for decades, such asin food and agricultural applications. However, unlike chemical polymers, little has been reported about the potential applications of fungal biopolymers in enhanced oil recovery (EOR). Nonetheless, it has been found that three fungal biopolymers meet the technical requirements for EOR. Details on their structures, properties, and EOR performance have been summarized based on successful laboratory experimentsandfield applications. This chapter addresses the literature gap by discussing these threebiopolymers: scleroglucan, produced by *Sclerotium* spp.; schizophyllan, produced by *Schizophyllum commune*; and pullulan, produced by *Aureobasidium* spp. In particular, their potential use in oil recovery as eco-friendly alternatives is discussed as they have rigid structures that make them less sensitive to high temperatures, salinities, and mechanical shear than synthetic polymers.

Keywords Biopolymers · Enhanced Oil Recovery · Scleroglucan · Schizophyllan · Pullulan

1 Introduction

Despite recent efforts to find new renewable energy resources, the global energy demands for the traditional resource crude oil have continually increased. Moreover, conventional oil recovery methods have obtained only about 30–40% of the crude oil in reservoirs, while the rest has remained trapped in the tiny pores of the

S. H. Al-Araimi (✉) · S. N. Al-Bahry
Department of Biology, College of Science, Sultan Qaboos University, Muscat, Sultanate of Oman

Y. M. Al-Wahaibi
A'Sharqiyah University, Ibra, Sultanate of Oman

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_6

reservoirs (Shibulal et al. 2018). Retrieving this trapped crude oil is the third(tertiary) step in enhanced oil recovery (EOR).

Polymer flooding is a successful EOR approach used widely in many oil fields worldwide. Polymers have the ability to improve one's sweep efficiency by lowering a field's water to oil mobility ratio and thus facilitating trapped oil mobilization (Rellegadla et al. 2017). Chemically synthesized polymers, such as hydrolyzed polyacrylamides (HPAMs), are used the most extensively in EOR due to their availability at relatively low costs (Pu et al. 2018). However, from an environmental and safety perspective, HPAMs cause pollution and serious health effects as they contain acrylamide monomers, which are neurotoxic and carcinogenic agents (Al-Azkawi et al. 2013; Al-Moqbali et al. 2018). Hence, biopolymers of fungal origins should be considered as alternatives as they are safer and eco-friendly. In particular, fungal biopolymers with high tolerance levels for harsh conditions and performance levels superior to those of chemically synthesized polymers and should be considered.

2 Enhanced Oil Recovery: An Overview

The global demand for energy has increased with population growth, the industrial revolution, and overall life development at arate of approximately 1.5% per year (El-hoshoudy et al. 2017). Most of this demand has been met by fossil fuels. Indeed, fossil fuels have provided 80–90% of the energy supply; about 60% of this supply has come from oil and gas reserves (Shibulal et al. 2014). It is therefore important to increase the production of petroleum reserves, and this can be achieved by improving the production of mature reservoirs or discovering new reservoirs (Firozjaii and Saghafi 2020).

Currently, the majority of the supply comes from mature crude oil reservoirs as new discoveries of reservoirs have steadily declined for decades (Ali et al. 2018; Manrique and Alvarado 2010). Crude oil is a fossil fuel that is produced naturally by the remains of dead organisms buried under the ground, and it exists as liquid petroleum in underground reservoirs of tiny pores within rocks. It is composed of a mixture of hydrocarbons, or hydrogen and carbon atoms. It can be classified as light or heavy based on its physical characteristics and chemical composition. Light crude oil has a lower viscosity and more light hydrocarbons than heavy crude oil. The latter is highly viscous and contains a high amount of long-chain hydrocarbons. Consequently, the recovery of light crude oil is easier and less expensive than the recovery of heavy crude oil (Shibulal et al. 2014).

The oil recovery process has three main phases that use different mechanical, physical, and chemical approaches (Al-Sulaimani et al. 2011a). After drilling a wellbore, crude oil is initially recovered using natural reservoir pressure; this is called primary oil recovery. At this stage, crude oil is driven out of a production well because of a differential pressure between the reservoir and the wellbore (Rellegadla et al. 2017). Primary recovery usually obtains around 25% of the original oil in place

(OOIP) for light oils and only around 5% of the OOIP for heavy oils (Thomas 2008). Once the natural pressure depletes, an external fluid, such as water, is injected to maintain the reservoir's pressure and sweep more oil toward the wellbore (Al-Sulaimani et al. 2011a). This stage is called secondary recovery. At this stage, water physically sweeps oil toward a wellbore, as aforementioned (Rellegadla et al. 2017), to produce around 30% of the OOIP for light oil and around 5% of the OOIP for heavy oils (Thomas 2008).

Two-thirds of the crude oil is still remaining in the ground even after primary and secondary recovery due to the oil trapped in the reservoir pores by capillary forces, which referred to as residual oil (Gbadamosi et al. 2019). Globally, it was estimated that around 2 trillion barrels of light oils and 5 trillion barrels of heavy oils are left behind after primary and secondary recovery collectively (Al-Sulaimani et al. 2011a). Therefore, attention has been focused on finding cheap and efficient technologies to increase crude oil production and extend the productive life of the oil reservoirs (Shibulal et al. 2014). Researchers developed enhanced oil recovery (EOR) or tertiary recovery methods and were consequently adopted by the oil industries to improve oil flow and sweep efficiency in the reservoir (Rellegadla et al. 2017).

Enhanced oil recovery focuses mainly on the retained oils in the light reservoirs and immobile highly viscose oils in the heavy oil reservoirs. The EOR target differs between different types of hydrocarbons. For light oils, EOR technologies targeted around 45% of OOIP. Whereas heavy oils respond poorly to the conventional oil recovery, and thus EOR methods targeted the bulk of the production, nearly 90% of OOIP (Thomas 2008). EOR technologies are classified into four main groups; chemical (CEOR), thermal, miscible flooding, and microbial (MEOR) (Firozjaii and Saghafi 2020). There are many economic as well as technological considerations that play the role in selecting the best EOR method for each reservoir in each country (Thomas 2008). In fact, starting any EOR project depends mainly on the readiness and willingness of the investors to manage EOR risks and economic exposure and the availability of more attractive investment options, so the economics are the key factor; promotor or deterrent the commercialization of the above mentioned EOR technologies (Manrique and Alvarado 2010; Al-Sulaimani et al. 2011a).

2.1 Thermal EOR

Thermal EOR, as the name implies, is a process that mainly depends on the use of thermal energy to raise the temperature of a reservoir, which in turn reduces the viscosity of heavy crude oil and facilitates its flow toward a wellbore (Mokheimer et al. 2019). The process has been one of the most common methods of global oil production since the 1950s (Thomas 2008). It is suitable for reservoirs in high-porosity sandstone formations and for dense, thick, highly viscous oils (Al-Sulaimani et al. 2011a; Mokheimer et al. 2019). The method has two categories: the hot fluid injection technique, involving, for example, steam or hot water, and the

in-situ combustion technique, involving combustion (Al-Sulaimani et al. 2011a). While these techniques have been highly effective in Canada, the United States of America, Venezuela, Indonesia, and other countries (Thomas 2008), they have not been applicable to all reservoirs. That is, the methods have been less effective at extracting oil from reservoirs at great depths and with thin pay zones (Gbadamosi et al. 2019). Therefore, there is a significant need to find new technologies suitable for extracting oil trapped in such reservoirs.

2.2 Miscible Enhanced Oil Recovery

Miscible flooding is one of the important EOR schemes, by definition miscible is a term describing the relationship between fluids or gases that are capable of being mixed in all proportions. This technology uses a displacing fluid that is miscible with the reservoir crude oil either at first contact or after multiple contacts, where they develop a narrow mixing zone between the displacing fluid and the crude oil inducing oil displacement (Thomas 2008). Because the miscible solvents having less density compared to crude oil, the tapped immobile oil in the tiny pores of the rocks become mobile enough to flow toward the wellbore. Miscible flooding includes mainly carbon dioxide (CO_2) miscible gas injection, nitrogen (N_2) miscible injection, and others (Al-Sulaimani et al. 2011a). Miscible flooding is mostly suitable for light, condensate, and volatile oil reservoirs (Manrique and Alvarado 2010). CO_2 miscible gas injection is the most commonly used for medium and light oils especially in sandstone reservoirs due to the availability of CO_2 from natural sources at a low cost (Manrique and Alvarado 2010). In fact, the miscible gases used in miscible flooding are toxic and difficult to be separated from the crude oil that will eventually lead to higher flow rates (Shibulal et al. 2014) which limits the use of this technology.

2.3 Chemical EOR

Among the approaches used for EOR, chemical EOR is the most promising because it has a higher effectiveness, technical efficiency, and economic feasibility than the other methods (Gbadamosi et al. 2019). The process became popular in the 1980s, when an increase in oil prices and advances in technological sciences enabled the discovery and understanding of different approaches involving the process (Gbadamosi et al. 2019; Thomas 2008). The method injects water mixed with chemicals into a reservoir to change the characteristics of the reservoir's fluids and to thus improve the oil recovery (Al-Sulaimani et al. 2011a). The injected chemicals displace trapped residual crude oil into a wellbore by altering the fluid–fluid and/or fluid–rock interactions in the reservoir. The alterations are achieved using different approaches, such as lowering the interfacial tension between the displacing fluid and

the oil; affecting wettability; and improving the mobility ratio and overall sweep efficiency (Ali et al. 2018; Gbadamosi et al. 2019). The three main components of this process are polymers, surfactants, and alkalines (Al-Sulaimani et al. 2011a). These chemicals can be injected individually or in combination with each other, such as in surfactant-polymer and alkaline-surfactant-polymer mixtures (Ali et al. 2018).

A polymer, which is composed of repeating units of monomers, is produced naturally or synthesized through chemical reactions. Because of their chemical and physical properties, polymers play an essential roles in everyday life. Polymers have been used extensively for EOR applications due to their ability to lower the water to oil mobility ratio (Rellegadla et al. 2017). In fact, the difference in viscosity between the water and crude oil makes them flowing at different rates in the secondary oil recovery. Hence, for this reason, polymers are used to increase the viscosity of the displacement water, therefore reduce the mobility ratio of water relative to crude oil that would facilitate the oil mobilization toward the wellbore (Al-Araimi et al. 2021). There are two types of polymers used for EOR applications; synthetic polymers such as polyacrylamide and biologically produced polymer (biopolymer) such as polysaccharides (Rellegadla et al. 2017).

Surfactants, as from the name, are surface-acting agents having amphiphilic nature composed of hydrophilic (water-soluble) and hydrophobic (oil-soluble) functional groups (Gbadamosi et al. 2019). Surfactant flooding uses the same principle of chemical cleaning, in which it is improving the solubility of the crude oil by utilizing the hydrophilic group of the surfactant and the water phase of the crude oil (Hui et al. 2020). In fact, displacing the trapped oils in the pores through water flooding is practically impossible due to the action of capillary forces in trapping the oil (Gbadamosi et al. 2019). Thus, addition of the surfactant to the water enabled reduction in IFT between water and oil lowering the capillary forces in porous media withholding the trapped oil (Ali et al. 2018). The hydrophilic heads of the surfactant interact with water and the hydrophobic tails interact with the crude oil allow the trapped oil in the pores of the rocks to flow with ease toward the wellbore (Gbadamosi et al. 2019). One of the drawbacks limiting the use of surfactant flooding in EOR is the adsorption of the surfactant onto the rock surface. There is an inverse relationship between the adsorption and the efficiency of crude oil recovery. Besides, the adsorption result in the loss of costly surfactant thus increase the overall cost of surfactant-based EOR (Ali et al. 2018). The different types of surfactants have different adsorption behaviour towards the rock formations. Based on the nature of the hydrophilic heads, they are classified into cationic surfactants, anionic surfactants, non-ionic surfactants, and zwitterionic surfactants (Gbadamosi et al. 2019). Among them, anionic surfactants are the most commonly used in surfactant-based EOR because of their low adsorption behavior on sandstone formations (Ali et al. 2018).

Alkaline flooding is another EOR technology uses similar mechanism used in surfactant-based EOR with a different injectants (Gbadamosi et al. 2019). The injectant used in this technology is basically a chemical agent, with high pH value, that have the ability to react with the crude oil downhole to form in-situ surfactant (Fakher et al. 2019). The generated surfactants in turn reducing the IFT between

water and oil and thus increase the recovery efficiency (Thomas 2008). The injectant, namely alkali, are mainly basic compound, ionic salt of an alkali metal or alkaline earth metal (Gbadamosi et al. 2019). In fact, the reaction between alkali and the crude oil depends on two factors; the ability of the alkali to elevate the pH significantly and availability of acidic components in the crude oil (Fakher et al. 2019). As a byproduct of this reaction, heat is generated which in turn reduces the viscosity of the crude oil, thus increase the oil mobility as an extra advantage to this technology in general (Fakher et al. 2019).

Although conventional chemical EOR showed success in terms of enhancing oil recovery, they have some drawbacks limiting their use. For instance, polymers that improve mobility ratio by increasing the viscosity of the displacing fluid showed viscosity loss in the reservoirs with high temperature and salinity. On the other hand, surfactants and alkali lose their effectiveness because of adsorption during their flow in the rock formations (Gbadamosi et al. 2019). To overcome these limitations, combining the functions of these chemical methods was suggested and ended in the creation of new chemical EOR strategies referred to as alkali–surfactant (AS), surfactant–polymer (SP), alkaline–polymer (AP), and alkaline–surfactant–polymer (ASP) (Ali et al. 2018). The synergy of the combined methods showed higher efficiency in the crude oil recovery compared to the conventional chemical injections (Gbadamosi et al. 2019). In ASP approach, surfactant and polymer collectively enhance the mobility of the trapped oil by reducing IFT and mobility ratio between oil and water whereas alkali work as a protective agent reducing the adsorption of the surfactant to the porous media (Ali et al. 2018).

It was found that the previously mentioned EOR strategies have an adverse impacts to the environment. For instance, spills of oil, brine or the injected chemicals caused pollution of land and surface water, loss of biota, contamination of groundwater with toxic chemicals used in chemical EOR, and excessive air emissions from thermal EOR (Millemann et al. 1982). From this perspective, microbial enhanced oil recovery (MEOR) has been introduced and developed in an environmentally acceptable manner (Al-Wahaibi et al. 2014).

2.4 Microbial EOR (MEOR)

MEOR is becoming one of the most popular, quickly developing, and cost-effective methods used in tertiary oil recovery (Shibulal et al. 2018). The process improves the oil mobility of a reservoir by utilizing both microorganisms that are indigenous or exogenous to a reservoir and the microorganisms 'bioproducts (Al-Bahry et al. 2013a; Al-Sulaimani et al. 2010). The method is considered cheaper than other EOR processes as it can be implemented at significantly lower operating costs that are not directly affected by crude oil prices (Al-Sayegh et al. 2015; Al-Sulaimani et al. 2011a). Moreover, the method is better for the environment than other processes as it does not involve any toxic chemicals and the bioproducts that are

produced by the microorganisms are biodegradable and environmentally friendly (Al-Bahry et al. 2013b; Al-Sulaimani et al. 2011b).

However, the mechanism was not a novel discovery; it was developed to use a process similar to that of chemical EOR but to replace the synthetic chemicals, such as surfactants and polymers, with biologically produced products, such as biosurfactants and biopolymers (Al-Ghailani et al. 2021). In MEOR, microorganisms synthesize desired products by fermenting low-cost substrates or raw materials (Shibulal et al. 2014). This trend of shifting from chemically synthesized to biologically produced materials has attracted global attention mainly because of the environmental issues associated with the non-biodegradability of chemical compounds, which makes it difficult to dispose of the compounds safely (Souayeh et al. 2014a).

With MEOR, the microbes in an oil reservoir interact with various substrates and react to microbes that are either endogenously presented or exogenously injected into the reservoir. Al-Bahry et al. (2013c) studied the microbial consortia of two different oil reservoirs in Oman and found 33 genera and 58 species, some of which were the first to be reported as being in oil fields. Most of the identified microbes were anaerobic, halophilic, and thermophilic and produced biogases, bio-solvents, biopolymers, and biosurfactants as by-products, which made them good candidates for MEOR (Al-Bahry et al. 2013c). Moreover, some of the microbes were efficient at utilizing crude oil as a carbon source in a process called biodegradation or bio-transformation; this made them good candidates for solving the problem of oil spill pollution. For instance, Shibulal et al. (2017) isolated *Paenibacillus ehimensis* BS1 from soil contaminated with heavy oils and found the potential to transform heavy crude oils to lighter oils. Similarly, Shibulal et al. (2018) found that *Bacillus halodurans* and *Bacillus firmus* had biotransformation efficiencies of 81.9% and 81.4%, respectively; the microbes utilized the aromatic components in heavy crude oil to convert the components to aliphatic species.

Based on the place of bioproducts production, MEOR can be classified into two processes namely; in-situ and ex-situ. In the in-situ process, the desired bioproduct is produced inside the reservoirs either by stimulating the indigenous microbes or introducing exogenous ones and directed toward enhancing oil recovery. On the other hand, the production of the target bioproducts in the ex-situ process takes place outside the reservoirs in a controlled bioreactor, followed by extracting and injecting the final product into the reservoir (Al-Sulaimani et al. 2011a). In fact, in-situ processes are cheaper but uncontrollable compared to ex-situ in which microbial growth, productivity, and yield are not guaranteed. Whereas ex-situ processes are completely opposite in which the productivity and yield can be controlled, quantified, and optimized (Souayeh et al. 2014b; Al-Wahaibi et al. 2014). Thus, the ex-situ process is the most promising approach implemented in the field scale with a higher success rate and better reservoir condition adaptability (Niu et al. 2020).

Evaluating the feasibility and potential of MEOR needs a deep understanding of the mechanisms used in MEOR. In principle, MEOR technologies improve sweep efficiency by altering the chemical and physical properties of the reservoir rocks, or water, or crude oil (Souayeh et al. 2014a). At the physical level, this can be achieved

by reducing the oil-water interfacial tension, emulsifying the crude oil, or injecting high viscosity displacing fluid relative to the viscosity of the oil, facilitating the mobility of the trapped crude oil (Niu et al. 2020; Al-Ghailani et al. 2021). Whereas at the chemical level, the target of reducing crude oil viscosity is achieved by enzymes or gases or biodegradation and biotransformation of long-chain saturated hydrocarbons (Niu et al. 2020; Shibulal et al. 2018). Amongst the different bioproducts evaluated, biosurfactant and biopolymers are the key players in MEOR (Al-Bahry et al. 2013b).

Biosurfactant, which work in a similar way of chemical surfactant, has the ability to lower the IFT between water and oil thus facilitate the mobilization of entrapped oil (Al-Sulaimani et al. 2010). Isolating biosurfactant producers and maximizing its production have attracted a great attention in the last few years. Al-Sulaimani et al. (2010), found that *Bacillus licheniformis* has the ability to produce biosurfactant which is effectively reduce IFT from 46.6 to 3.28 mN/m in 16 hours, recovering 10% of the residual oil. In addition, Al-Sulaimani et al. (2011b, 2012), Al-Bahry et al. (2013a, b), Souayeh et al. (2014a, b) and Al-Wahaibi et al. (2014, 2016), reported biosurfactant production by *Bacillus subtillus*, that showed high potential for MEOR. This biosurfactant was found to recover 23% of residual oil (Al-Sulaimani et al. 2011b), stable over wide range of pH, salinity and temperature (Al-Wahaibi et al. 2014). One of the biosurfactants can maintain an extra recovery of 14% of residual oil even with 20 times dilution (Souayeh et al. 2014b). The performance of biosurfactant was significantly maximized by mixing it with chemical surfactant, producing 50% of the residual oil in place (Al-Sulaimani et al. 2012; Souayeh et al. 2014b; Al-Wahaibi et al. 2016). Al-Ghailani et al. (2021), used ASP strategy of mixing alkaline, biosurfactant produced by *B. subtillus* and biopolymer produced by fungus *Schizophyllam commune* that collectively showed better performance than using each alone.

3 Principle and Mechanisms of Polymer Flooding for EOR

Polymer flooding is considered one of the most efficient methods of EOR that has been used in the last two decades, especially as there have been many successful implementations of large-scale polymer flooding projects in oilfields around the world (Ali et al. 2018). The main purpose of polymer flooding is to improve a reservoir's mobility ratio (i.e., the mobility of a displacing fluid, such as water, divided by the mobility of a displaced fluid, such as crude oil; Firozjaii and Saghafi 2020). Differences in the mobility of water and the mobility of crude oil are the main reasons for poor sweep efficiency, in which water moves rapidly toward a wellbore, leaving behind a huge quantity of unswept crude oil. This phenomenon is called the fingering effect, in which a calculated mobility ratio is greater than 1 (Fig. 1a); El-hoshoudy et al. 2017; Rellegadla et al. 2017).

Adding polymer to injected water can increase the water's viscosity and can thus eventually decrease the water's mobility relative to the oil's mobility. This can result

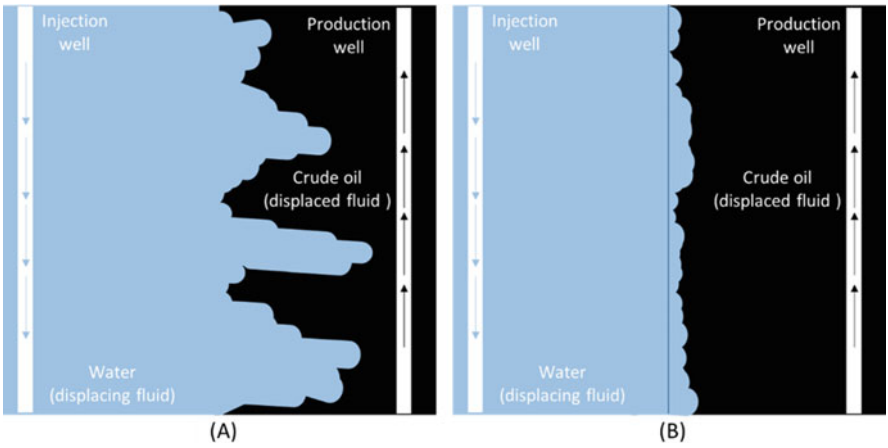


Fig. 1 (**a**) Fingering phenomena promoted by the unfavorable mobility ratio of water and oil ($M > 1$), (**b**) uniform displacing of the crude oil facilitated by the use of polymer flooding ($M \leq 1.0$)

in an overall mobility ratio of less than 1, which is favorable for the uniform displacement of crude oil (Fig. 1b); Rellegadla et al. 2017).

The most extensively used polymers in many such flooding projects worldwide are HPAMs (Al-Araimi et al. 2021). An HPAM is a copolymer made up of a polyacrylamide and polyacrylic acid, and it is synthesized either through the copolymerization of sodium acrylate with an acrylamide or through the partial hydrolysis of a polyacrylamide (Gbadamosi et al. 2019). It is available in large quantities with specific customized properties at relatively low costs, making it attractive to oil industries (Pu et al. 2018).

Despite these favorable characteristics, it does not meet all the requirements for EOR because it experiences severe degradation and hydrolysis when it is injected into high temperature and/or high salinity reservoirs. Specifically, unfavorable reservoir conditions result in an increased hydrolysis rate for amide functionalities, creating an increase in acrylic acid in the polymer backbone and making the polymer highly sensitive to brine and macroscopically flocculate (Pu et al. 2018). Moreover, from an environmental point of view, the hydrolysis of HPAMs leads to a release of acrylamide monomers that are considered toxic environmental pollutants; they can filter into groundwater during an injection or remain in the water produced after separating crude oil (Al-Araimi et al. 2021). Daily exposure to acrylamide monomers is associated with neurotoxicity, genotoxicity, and carcinogenicity (Al-Azkawi et al. 2013; Raju et al. 2015). These effects drastically reduce the safety and viscosity of the polymer and limit its use (Ali et al. 2018).

To solve this issue, efforts were made in the last decades to discover and develop suitable polymers for EOR applications that meet the technical and environmental needs of the oil industries. Thus, biopolymers have received global attention and become a topic of interest because of their environmental compatibility and outstanding chemical stability (Pu et al. 2018). Basically, living organisms such as

bacteria, fungi, and plants produce biopolymers to protect themselves from dehydration and predation, as well as to aid in adherence to surfaces (Al-Sulaimani et al. 2011a). To meet these purposes, the released biopolymers have double or triple helical structures with superior rigidity and charge-free chains, which makes them withstand harsh environmental conditions with high stability (Pu et al. 2018). Considering these characteristics, besides their eco-friendliness, biopolymers are the ideal option for EOR applications. Xanthan gum produced by the bacterium *Xanthomonas campestris* was the first biopolymer proposed and used in EOR and was successfully implemented at a pilot scale in ShengLiGudong oilfield in China with a high oil production rate (Gbadamosi et al. 2019). Compare to HPAM, xanthan gum owned a higher rigid structure, make it less sensitive to high temperatures, salinities, and mechanical shear. However, xanthan is not without technical limitations, the major drawback of xanthan flooding is the poor injectivity because of some impurities present in the polymer solution (Jensen et al. 2018). Thus, screening and finding tough biopolymers for EOR is still a topic of interest among researchers and decision-makers in oil companies.

Below the three main fungal biopolymers will be discusses that showed the potential for EOR applications. These are scleroglucan produced by *Sclerotium* spp., schizophyllan produced by *Schizophyllum commune*, and pullulan produced by *Aureobasidium* spp.

4 Fungal Biopolymers for EOR

4.1 Scleroglucan

Scleroglucan is a fungal biopolymer produced during the fermentation of fungi belonging to the genus *Sclerotium*, a plant pathogenic fungi (Rivenq et al. 1992). The polymer is a non-ionic exopolysaccharide with a high viscosifying efficiency and a favorable stability in regards to temperature and salinity; due to these characteristics, it has been the subject of many studies regarding EOR (Fournier et al. 2018; Kozlowicz et al. 2020). Indeed, in the early 1980s, it was found to be effective at EOR involving polymer flooding in harsh conditions (Jensen et al. 2018). It has since been considered one of the best environmentally friendly alternatives to chemically synthesized HPAMs (Pu et al. 2018) as it has a better resistance to mechanical degradation, which is provided by a triple helical structure that displays a rigid rod-like conformation in a solution (Fournier et al. 2018). The backbone of scleroglucan is mainly a tetrasaccharide repeating unit composed of linearly linked β (1–3) glucose residues, with one occasional branched β (1–6) glucose side chain attached to every third main chain residue in the backbone (Pu et al. 2018; Rivenq et al. 1992; Seright et al. 2021). The biopolymer has a high molecular weight and chain rigidity that are produced by the triple-helical structure of the polymer backbone; this structure gives it a very high viscosifying power at minimal concentrations (Pu et al. 2018; Rivenq et al. 1992).

In addition to the high viscosifying power, the rigid triple-helical structure and the non-ionic nature of the biopolymer give it favorable solution properties, such as thermal stability, charge neutrality, salt tolerance, pH insensitivity, and shear resistance. Indeed, Rivenq et al. (1992) found that the biopolymer remains stable at high temperatures (90–100 °C) in the absence of oxygen for at least 3 months. Further, scleroglucan tolerates temperatures of up to 130 °C, while HPAM degrades significantly at temperatures above 70–80 °C.Scleroglucan's tolerance is due to an absence of easily degradable groups, such as acrylamides, in its backbone (Fournier et al. 2018). Moreover, in regards to its long-term thermal stability, the biopolymer maintains about 95% of its initial viscosity at 100–105 °C over 2 years (Fournier et al. 2018; Kozlowicz et al. 2020).

Fariña et al. (2001) found that the viscosity of scleroglucan remains stable when shifting a pH to either highly acidic or moderately alkaline. However, although the polymer retains its original viscosity at a low pH value, it starts losing its viscosity at a pH greater than13, which is likely due to a change from the triple-helical arrangement to a single-stranded conformation in the process of denaturation (Fariña et al. 2001). Nonetheless, because of its non-ionic nature, the biopolymer's viscosity is insensitive to high ionic strengths, maintaining constant values of viscosity at a wide range of salinity values (Fournier et al. 2018; Rivenq et al. 1992). Scleroglucan can maintain stability in seawater for 1 year of storage, and in synthetic seawater, it has insignificant viscosity loss, proving that the biopolymer is well suited to harsh reservoir environments, particularly offshore applications (Jensen et al. 2018; Seright et al. 2021). Finally, scleroglucan has a favorable injectivity in regards to low permeability cores, even at high concentrations, and its rod-like chains are oriented in the direction of flow during polymer flooding (Fournier et al. 2018).

Different researchers studied the performance of scleroglucan in EOR applications and compared it with other well-known polymers for this purpose at the same running conditions. For instance, Kalpakci et al. (1990) found that scleroglucan has superior thermal stability compared to xanthan; in which scleroglucan maintained its initial viscosity at 100 °C for more than 2 years whereas xanthan lost 70–80% of its initial viscosity at 100 °C within the same period of time. Liang et al. (2019), compared scleroglucan with xanthan and HPAM and found that both scleroglucan and xanthan were less sensitive to salinity, while HPAM showed a decrease in viscosity with the increase in salinity. Thermal stability of the three polymers within 90 days at 85 °C showed that the viscosity of scleroglucan remained stable; on the contrary, the viscosity of xanthan and HPAM drops drastically. Polymer flooding tests showed excellent mobility control by all of the three polymers with 13, 11, and 10% of oil recovery obtained by scleroglucan, xanthan, and HPAM respectively, underpin the potential of scleroglucan to be used in the EOR applications (Liang et al. 2019).

Furthermore, 30 years ago, scleroglucan biopolymer was injected at field scale in North Sea Reservoir. Nonetheless, there are still no pilot studies of scleroglucan polymer flooding so far (Pu et al. 2018). In fact, the major drawback limiting the use of scleroglucan in EOR applications is the plugging tendency of the polymer caused by its poor filterability. The reason behind this behavior is the fermentation broth

residues and polymer aggregates present in the polymer solution injected in the reservoir (Fournier et al. 2018). Therefore, to overcome this limitation researchers tried to improve filterability by modifications in the biosynthesis process and in post purification and treatments. Rivenq et al. (1992), noted an improvement in the scleroglucan filterability when using ultrafiltration along with heating at 90 °C for at least two days, eliminating all of the suspended aggregates. Recently, a new EOR-grade scleroglucan with high purity was developed to meet the requirements for EOR applications. Physical and chemical testing of the developed EOR-grade scleroglucan indicated it has superior viscosifying efficiency, good filterability and injectivity, and tolerating high salinity and temperature, making it well-suited for the proposed use in the harsh reservoirs (Jensen et al. 2018; Fournier et al. 2018; Kozlowicz et al. 2020).

4.2 Schizophyllan

Schizophyllan is a non-ionic water soluble homoglucan polysaccharide that is produced extracellularly by the fungus *Schizophyllum commune*, a white-rot ubiquitous mushroom that grows in the forests of Germany (Joshi et al. 2016; Pu et al. 2018; Quadri et al. 2015). The polymer is biodegradable, environmentally compatible, non-toxic, and highly stable; consequently, it has been used widely for various health and food applications. For instance, schizophyllanhas been used as an anti-aging and healing agent for skin as it helps with skin cell proliferation, reduces skin irritation, and assists with the recovery of burnt skin after direct sunlight exposure. Schizophyllan has also been used as an immunestimulating agent in many vaccines and as an immunotherapeutic agent for cancer treatment since the late 1980s. Clinical studies of patients with uterine, lung, and gastric cancers have shown that schizophyllan injections have the potential to prolong the lives of cancer patients (Gao 2016). However, its use in petroleum industries has not been well investigated (Ogezi et al. 2014), although it has recently received attention from both scientists and engineers in this regard. It has been shown to have features highly desirable in oil industries as it possesses an excellent viscosifying efficiency and a high resistance against harsh reservoir conditions (Mukherjee et al. 2018).

Schizophyllan is produced by fermentation by which glucose monomers in the feedstock are polymerized to the schizophyllan, which is in turn being secreted to the fermentation broth and separated completely from the cellular debris (Leonhardt et al. 2014). The chemical structure of schizophyllan is identical to the previously described polymer; scleroglucan (Gao 2016). To be more precise, the backbone chain consists of linearly linked β-1,3 D-glucose residues with one β-1,6 glucose side chain at approximately every third glucose molecule within the chain (Mukherjee et al. 2018; Pu et al. 2018). In aqueous solutions, schizophyllan adopts a triple-helical structure stabilized by interchain hydrogen bonding, this conformation in fact is the basis for its attractive properties such as its superior viscosifying efficiency and thermal stability (Quadri et al. 2015).

This triple helical overstructure and intermolecular interactions of this biopolymer resulting in high thermal stability as well as high mechanical stability (Leonhardt et al. 2014). Quadri et al. (2015), found that schizophyllan had a positive thermal stability and salt tolerance, where no viscosity loss was observed in a study conducted over 8 months at temperature of 120 °C and salinity of 220 g/L under anaerobic conditions. Similarly, Joshi et al. (2016), found that schizophyllan is isothermal over a wide range of temperatures (25–60 °C). Long term thermal stability studies of schizophyllan at 130 °F (54.4 °C), 195 °F (90.5 °C) and between 230 °F (110 °C) and 265 °F (129.4 °C) have shown half-life values of several years, exceeding 10 years (Leonhardt et al. 2014). Besides thermal stability, loss of viscosity due to salinity has not been observed at all at any salt concentrations (Leonhardt et al. 2014). The absence of charged functional groups in the structure of schizophyllan is the one of the main reasons for its high resistance to the very high salinity level reaching up to 250,000 ppm TDS (Mukherjee et al. 2018). In terms of rheological properties, schizophyllan has a pseudoplastic properties with shear thinning behavior, highly depends on the concentration. To illustrate, at low concentration schizophyllan showed viscoelastic properties, whereas at high concentration it showed a solid-like state conformation (Pu et al. 2018).

On the top of the good stability performance, schizophyllan provides superior viscofying efficiency and injectable into the tight cores (Wazir and Manap 2020). The success of any polymer in a polymer flooding project is mainly depends on maintaining the mobility control while propagating deeply in the reservoir. Schizophyllan have shown good mobility control with no plugging tendency or injectivity problems (Quadri et al. 2015; Wazir and Manap 2020). Owing semi-rigid rod-like structure, schizophyllan molecules might oriented themselves to pass through the core, in a similar way it passed through a membrane filter when such constant pressure methods were applied (Mukherjee et al. 2018). Approximately 28% additional heavy crude oil was recovered from Berea sandstone core plugs over residual oil saturation, after schizophyllan injection (Joshi et al. 2016). In a similar way, after 65% of oil recovery achieved by five pore volume water flooding, the percentage of oil recovery reached 75% by four pore volumes schizophyllan flooding, proving that this biopolymer is not only improving the oil recovery but also speeding up the recovery process (Gao 2016). Hence, from all the previous characteristics, schizophyllan have shown that it meet the technical requirements needed for EOR polymer flooding. Apart from its technical abilities, the fact that this polymer is classified as environmentally friendly due to its biodegradability and low eco-toxicity make it an ideal option for EOR applications (Wazir and Manap 2020).

In comparison with other commercial polymers used in EOR applications such as HPAM and xanthan that have a single chain and double helix structures, respectively, schizophyllan with its tertiary structure showed superior performance in terms of viscosifying efficiency and mechanical stability (Leonhardt et al. 2014; Mukherjee et al. 2018). Gao (2016) compared the stability of HPAM, Xanthan and schizophyllan at the same running conditions and found that all polymers performed well at low temperatures. However, when the temperature increased up to 90 °C, the viscosity of schizophyllan declined mildly, maintaining 90% of its

original viscosity, whereas HPAM and xanthan degraded much more severely, losing 32 and 50% of their original viscosity, respectively.

All these attractive properties of schizophyllan promoted testing this novel biopolymer at the field pilot scale for the first time in a mature onshore oil field in Northern Germany in early 2014 (Leonhardt et al. 2014). By the end of 2015, more than 283,000 barrels of schizophyllan were injected, resulting in an increase in the oil production rate by over 20% compared with the previous rate under waterflooding (Pu et al. 2018). The field pilot results have been very positive so far proven good injectivity and improved oil production (Gao 2016), highlighting the potential of schizophyllan as an ideal eco-friendly alternative to the commercial polymers used in EOR at the current time.

4.3 Pullulan

Pullulan is one of the most popular commercially emerging biopolymers produced extracellularly by species belonging to the genus *Aureobasidium* (Singh et al. 2008). The biopolymer was firstly observed by Bauer (1938) as amorphous slime matter secreted by the polymorphic fungus *A. pullulans*. Later, Bernier (1958) successfully isolated and identified the polymer, and Bender et al. (1959) gave it the name pullulan. Bender and Wallenfels (1961) characterized its chemical structure as maltotriose repeating units connected internally by α-1,4 glucosidic linkages; between each adjacent maltotrioseare α-1,6 glucosidic linkages (Al-Araimi et al. 2021; Cheng et al. 2011).

Owing distinctive glucosidic bonds in the polymer backbone, pullulan gained an attractive physiochemical properties including structural flexibility, enhanced solubility along with adhesive properties and ability to form fibers and biodegradable films (Cheng et al. 2011). Pullulan is white to off-white tasteless, odorless powder, forming a viscous solution when dissolving in water (Prajapati et al. 2013). It is stable up to 250 °C where it starts to be decomposed, with no significant change in viscosity with heat, pH, and salinity (Prajapati et al. 2013). Pullulan has generally regarded as safe (GRAS) status in US for a wide range of applications because its non-toxicity, biodegradability, non-carcinogenicity and non-immunogenicity (Singh et al. 2008; Prajapati et al. 2013; Al-Araimi et al. 2021).

Numerous microbes were found to produce pullulan, such as *Aureobasidium* spp., *Tremella mesenterica*, *Cytaria* spp., *Cryphonectria parasitica*, *Teloschistes flavicans*, and *Rhodototula bacarum* (Singh et al. 2008). However, the species belong to *Aureobasidium* are still the most popular producers of pullulan biopolymer due to their high production yield.

Aureobasidium is a fungal genus distributed worldwide. It is found on dead plants and has been isolated from soil, air, water, plant leaves, food materials, textile, painted walls, wood and bathroom surfaces (Cheng et al. 2011; Moubasher et al. 2012). *Aureobasidium* species are known for their biotechnological and commercial significance. They have ability to produce an extracellular polysaccharide (EPS),

commercial hydrolytic enzymes, antimicrobial compounds and biosurfactant (van Nieuwenhuijzen et al. 2016).

Taxonomically, the genus *Aureobasidium* is classified under the family *Saccotheciaceae* of the order Dothideales (Wijayawardene et al. 2014). In fact, with the development of the different taxonomical tools and molecular techniques, different species have been included and excluded from this genus, so far five main species have been confirmed to this genus; *A. pullulans*, *A. melanogenum*, *A. subglaciale*, *A. namibiae*, and *A. mangrovei* (Zalar et al. 2008; Gostinčar et al. 2014; Nasr et al. 2018). *A. mangrovei* was the latest discovered species that was firstly reported from leaves of mangrove trees in Iran (Nasr et al. 2018), and then reported from plant debris in Sultanate of Oman (Al-Araimi et al. 2019).

Pullulan has high commercial significance and has long been exploited for many industrial applications such as cosmetic and manufacturing industries. Due to its edibility, pullulan has been used by food industries as a stabilizer, binder, intensifier, beverage filler, dietary fiber, thickener, texture improver and food packaging. Moreover, it has been utilized as a novel molecule in biomedical applications in drug delivery, gene delivery, medical imaging, corneal wound healing, and tissue engineering (Singh et al. 2019; Cheng et al. 2011). In fact, pullulan got the acceptance for use as in pharmaceutical tablets and is listed in the Japanese Standards for Ingredients for drugs (Prajapati et al. 2013). All these applications highlighting the fact that pullulan is one of the most important polymer used recently worldwide.

The potential of pullulan for EOR applications has been investigated recently by Al-Araimi et al. (2021). The newly discovered strain *Aureobasidium mangrovei* SARA-138H was used for the pullulan production, which shows the high potential of this strain as a pullulan producer. The fermentation conditions were optimized and among different tested biotic and abiotic factors, sucrose as the carbon source in the medium, a pH of 9, incubation at 25 °C, and 250 rpm agitation were preferable to produce a maximum of 10 g/L of pullulan. Rheological analysis of the polymer solution indicated that pullulan has a shear thinning behavior with the maximum viscosity of 318 cP achieved under the optimized conditions. Core flooding experiments showed that the recovery percentage of heavy oil was 36.7% for the pullulan solution (10 g/L) and 20.23% for the diluted pullulan in brine at the ration (1:1) at concentration 5 g/L. A 20-day injectivity test revealed that pullulan chains are orienting themselves to pass smoothly through the core, having no tendency to cause a blockage. All these properties concluding the high potential of pullulan biopolymer as an excellent candidate for polymer flooding with a high percentage of oil recovery comparable with polymers used in oil filed around the world (Al-Araimi et al. 2021).

5 Summary and Outlook

Fungi have been used to produce considerable quantities of different compounds, including biopolymers, for thousands of years. They either accumulate the biopolymers intracellularly

or secrete them extracellularly in response to various biotic and abiotic stresses as protective defense mechanisms. These naturally produced polymers have been exploited in many fields and have been used to support and/or improve the lives of human beings in different ways.

Among these different fungal biopolymers are three that have been shown to have potential for use in EOR: scleroglucan, produced by *Sclerotium* spp.; schizophyllan, produced by *Schizophyllum commune*; and pullulan, produced by *Aureobasidium* spp. The structural conformations and chemical compositions of the polymers provide stabilities, mechanical strengths, and rheological properties that allow the biopolymers to tolerate reservoirs with harsh conditions, such as high temperatures and high salinities. Consequently, all three meet the technical requirements for polymer flooding and can replace commercially used synthetic polymers. In particular, schizophyllan was successfully introduced to the field on a pilot scale ata mature onshore oil field in Northern Germany; the polymer increased the field's oil production rate by over 20%. However, both scleroglucan and pullulan have produced favorable lab results and are ready to be field tested to evaluate their abilities to enhance oil recovery on a large scale as well.

References

Al-Araimi SH, Al-Hatmi AMS, Elshafie AE, Al-Bahry SN, Al-Wahaibi YM, Al-Bimani AS, De HS (2019) New record of *Aureobasidium mangrovei* from plant debris in the Sultanate of Oman. Czech Mycol 71(2):219–229

Al-Araimi SH, Elshafie A, Al-Bahry SN, Al-Wahaibi YM, Al-Bemani AS (2021) Biopolymer production by *Aureobasidium mangrovei* SARA-138H and its potential for oil recovery enhancement. Appl Microbiol Biotechnol 105(1):105–117

Al-Azkawi A, Al-Bahry S, Mahmoud I, Barry M (2013) Effect of acrylamide on liver proteins expression in mice. J Food Res 2:132–142

Al-Bahry SN, Al-Wahaibi YM, Elshafie AE, Al-Bemani AS, Joshi SJ, Al-Makhmari HS, Al-Sulaimani HS (2013a) Biosurfactant production by Bacillus subtilis B20 using date molasses and its possible application in enhanced oil recovery. Int Biodeterior Biodegradation 81:141–146

Al-Bahry SN, Elshafie AE, Al-Wahaibi YM, Al-Bemani AS, Joshi SJ, Al-Lawati A (2013b) isolation and characterization of biosurfactant/biopolymer producing spore forming bacteria from oil contaminated sites and oil field of Oman. APCBEE Procedia 5:242–246

Al-Bahry SN, Elsahfie AE, Al-Wahaibi YM, Al-Bimani AS, Joshi SJ, Al-Maaini RA, Al-Alawai WJ, Sugai Y, Al-Mandhari M (2013c) Microbial consortia in Oman oil fields: A possible use in enhanced oil recovery. J Microbiol Biotechnol 23(1):106–117

Al-Ghailani T, Al-Wahaibi YM, Joshi SJ, Al-Bahry SN, Elshafie AE, Al-Bemani AS (2021) Application of a new bio-ASP for enhancement of oil recovery: Mechanism study and core displacement test. Fuel 287:119432

Ali JA, Kolo K, Manshad AK, Mohammadi AH (2018) Recent advances in application of nanotechnology in chemical enhanced oil recovery: Effects of nanoparticles on wettability alteration, interfacial tension reduction, and flooding. Egypt J Pet 27(4):1371–1383

Al-Moqbali W, Joshi SJ, Al-Bahry SN, Al-Wahaibi YM, Elshafie AE, Al-Bemani AS, Al-Hashmi A, Soundra Pandian SB (2018) Biodegradation of partially hydrolyzed polyacrylamide HPAM using bacteria isolated from Omani oil fields. In: SPE EOR Conference, oil and gas West Asia. society of petroleum engineer

Al-Sayegh A, Al-Wahaibi Y, Al-Bahry S, Elshafie A, Al-Bemani A, Joshi S (2015) Microbial enhanced heavy crude oil recovery through biodegradation using bacterial isolates from an Omani oil field. Microb Cell Factories 14(1):1–11

Al-Sulaimani H, Al-Wahaibi Y, Ai-Bahry S, Elshafie A, Al-Bemani A, Joshi S, Ayatollahi S (2012) Residual-oil recovery through injection of biosurfactant, chemical surfactant, and mixtures of both under reservoir temperatures: Induced-wettability and interfacial-tension effects. SPE Reserv Evaluation Eng 15(2):210–217

Al-Sulaimani H, Al-Wahaibi Y, Al-Bahry S, Elshafie A, Al-Bemani A, Joshi S, Zargari S (2010) Experimental investigation of biosurfactants produced by Bacillus species and their potential for MEOR in Omani oil field. SPE EOR Conference at Oil and Gas West Asia 2010, OGWA - EOR Challenges, Experiences and Opportunities in the Middle East, 378–386

Al-Sulaimani H, Al-Wahaibi Y, Al-Bahry S, Elshafle A, Al-Bemani A, Joshi S, Zargari S (2011b) Optimization and partial characterization of biosurfactants produced by bacillus species and their potential for ex-situ enhanced oil recovery. SPE J 16(3):672–682

Al-Sulaimani H, Joshi S, Al-Wahaibi Y, Al-Bahry S, Elshafie A, Al-Bemani A (2011a) Microbial biotechnology for enhancing oil recovery: current developments and future prospects. Biotechnol Bioinformatics Bioeng 1(2):147–158

Al-Wahaibi Y, Al-Hadrami H, Al-Bahry S, Elshafie A, Al-Bemani A, Joshi S (2016) Injection of biosurfactant and chemical surfactant following hot water injection to enhance heavy oil recovery. Pet Sci 13(1):100–109

Al-Wahaibi Y, Joshi S, Al-Bahry S, Elshafie A, Al-Bemani A, Shibulal B (2014) Biosurfactant production by Bacillus subtilis B30 and its application in enhancing oil recovery. Colloids Surf B: Biointerfaces 114:324–333

Bauer R (1938) Physiology of dematium pullulans de Bary. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt 98:133–167

Bender H, Lehmann J, Wallenfels K (1959) Pullulan, an extracellular glucan from Pullularia pullulans. Biochim Biophys Acta 36:309–316

Bender H, Wallenfels K (1961) Investigations on pullulan. II. Specific degradation by means of a bacterial enzyme. Biochem Z 334:79–95

Bernier B (1958) The production ofpolysaccharide by fungi active in the decomposition of wood and forest litter. Can J Microbiol 4:195–204

Cheng KC, Demirci A, Catchmark JM (2011) Pullulan: Biosynthesis, production, and applications. Appl Microbiol Biotechnol 92(1):29–44

El-Hoshoudy AN, Desouky SEM, Elkady MY, Al-Sabagh AM, Betiha MA, Mahmoud S (2017) Hydrophobically associated polymers for wettability alteration and enhanced oil recovery–Article review. Egypt J Pet 26(3):757–762

Fakher S, Abdelaal H, Elgahawy Y, Imqam A (2019) A characterization of different alkali chemical agents for alkaline flooding enhanced oil recovery operations: an experimental investigation. SN Appl Sci 1(12):1–11

Fariña JI, Siñeriz F, Molina OE, Perotti NI (2001) Isolation and physicochemical characterization of soluble scleroglucan from *Sclerotium rolfsii*. Rheological properties, molecular weight and conformational characteristics. Carbohydr Polym 44(1):41–50

Firozjaii AM, Saghafi HR (2020) Review on chemical enhanced oil recovery using polymer flooding: Fundamentals, experimental and numerical simulation. Petroleum 6(2):115–122

Fournier R, Tiehi JE, Zaitoun A (2018) Laboratory study of a new EOR-Grade Scleroglucan. In: SPE EOR Conference at Oil and Gas West Asia 2018 Mar 26, OnePetro

Gao C (2016) Application of a novel biopolymer to enhance oil recovery. J Pet Explor Prod Technol 6(4):749–753

Gbadamosi AO, Junin R, Manan MA, Agi A, Yusuff AS (2019) An overview of chemical enhanced oil recovery: recent advances and prospects. Int Nano Lett 9(3):171–202

Gostinčar C, Ohm R, Kogej T, Sonjak S, Turk M, Zajc J, Zalar P, Grube M, Sun H, Han J, Sharma A, Chiniquy J, Ngan C, Lipzen A, Barry K, Grigoriev I, Gunde-cimerman N (2014)

Genome sequencing of four Aureobasidium pullulans varieties: biotechnological potential, stress tolerance, and description of new species. BMC Genomics 15(1):549
Hui K, Tang J, Lu H, Xi B, Qu C, Li J (2020) Status and prospect of oil recovery from oily sludge: A review. Arab J Chem 13(8):6523–6543
Jensen T, Kadhum M, Kozlowicz B, Sumner ES, Malsam J, Muhammed F, Ravikiran R (2018) Chemical EOR under harsh conditions: Scleroglucan as a viable commercial solution. In: SPE Improved Oil Recovery Conference. Society of petroleum engineers
Joshi SJ, Al-Wahaibi YM, Al-Bahry S, Elshafie A, Al-Bemani AS, Al-Hashmi A, Samuel P, Sassi M, Al-Farsi H, Al-Mandhari MS (2016) Production and application of schizophyllan in microbial enhanced heavy oil recovery. Society of Petroleum Engineers–SPE EOR Conference at Oil and Gas West Asia, OGWA 2016: 1–15
Kalpakci B, Jeans YT, Magri NF, Padolewski JP (1990) Thermal stability of scleroglucan at realistic reservoir conditions. In: SPE/DOE Enhanced oil recovery symposium. OnePetro
Kozlowicz B, Jensen T, Khambete M, Kadhum MJ, Garshol FK, Vik EA, Ray C (2020) Scleroglucan polymer stability: thermal, chemical, and microbial. In: SPE Improved oil recovery Conference. Society of petroleum engineers
Leonhardt B, Ernst B, Reimann S, Steigerwald A, Lehr F (2014) Field testing the Polysaccharide schizophyllan: results of the first year. SPE–DOE improved oil recovery symposium proceedings 1:57–72
Liang K, Han P, Chen Q, Su X, Feng Y (2019) Comparative study on enhancing oil recovery under high temperature and high salinity: Polysaccharides versus synthetic polymer. ACS omega 4(6): 10620–10628
Manrique E, Alvarado V (2010) Enhanced oil recovery: an update review. Energies 3:1529–1575
Millemann RE, Haynes RJ, Boggs TA, Hildebrand SG (1982) Enhanced oil recovery: environmental issues and state regulatory programs. Environ Int 7(3):165–177
Mokheimer E, Hamdy M, Abubakar Z, Shakeel MR, Habib MA, Mahmoud M (2019) A comprehensive review of thermal enhanced oil recovery: techniques evaluation. J Energy Resour Technol 141(3)
Moubasher H, Wahsh S, El-kassem N (2012) Isolation of *Aureobasidium pullulans* and the effect of different conditions for pullulanase and pullulan production. Microbiology 82(2):155–161
Mukherjee S, Garrido GI, Prasad D, Behr A, Reimann S, Ernst B (2018) Injectivity, propagation and retention of biopolymer schizophyllan in porous media. Society of Petroleum Engineers–SPE EOR Conference at oil and gas West Asia 2018, 2018-March, 1–18
Nasr S, Mohammadimehr M, Vaghei M, Amoozegar M, Fazeli S (2018) *Aureobasidium mangrovei* sp. nov., an ascomycetous species recovered from Hara protected forests in the Persian Gulf, Iran. Antonie Van Leeuwenhoek 111(9):1697–1705
Niu J, Liu Q, Lv J, Peng B (2020) Review on microbial enhanced oil recovery: mechanisms, modeling and field trials. J Pet Sci Eng 192:107350
Ogezi O, Strobel J, Egbuniwe D, Leonhardt B (2014) Operational aspects of a biopolymer flood in a Mature Oilfield. SPE–DOE improved oil recovery symposium proceedings 3:1735–1751
Prajapati VD, Jani GK, Khanda SM (2013) Pullulan: an exopolysaccharide and its various applications. Carbohydr Polym 95(1):540–549
Pu W, Shen C, Wei B, Yang Y, Li Y (2018) A comprehensive review of polysaccharide biopolymers for enhanced oil recovery (EOR) from flask to field. J Ind Eng Chem 61:1–11
Quadri SMR, Shoaib M, AlSumaiti AM, Alhassan SM (2015) Screening of polymers for EOR in high temperature, high salinity and carbonate reservoir conditions
Raju J, Roberts J, Taylor M, Patry D, Chomyshyn E, Caldwell D, Cooke G, Mehta R (2015) Toxicological effects of short-term dietary ac- rylamide exposure in male F344 rats. Environ Toxicol Pharmacol 39(1):85–92
Rellegadla S, Prajapat G, Agrawal A (2017) Polymers for enhanced oil recovery: fundamentals and selection criteria. Appl Microbiol Biotechnol 101(11):4387–4402
Rivenq RC, Donche A, Nolk C (1992) Improved scleroglucan for polymer flooding under harsh reservoir conditions. SPE Reserv Eng 7(01):15–20

Seright RS, Wavrik KE, Zhang G, AlSofi AM (2021) Stability and behavior in carbonate cores for new Enhanced-oil-recovery polymers at elevated temperatures in hard saline brines. SPE Reserv. Evaluation Eng. 24(01):1–18

Shibulal B, Al-Bahry SN, Al-Wahaibi YM, Elshafie AE, Al-Bemani AS, Joshi SJ (2014) Microbial enhanced heavy oil recovery by the aid of inhabitant spore-forming bacteria: an insight review. Sci World J 2014

Shibulal B, Al-Bahry SN, Al-Wahaibi YM, Elshafie AE, Al-Bemani AS, Joshi SJ (2018) Microbial-enhanced heavy oil recovery under laboratory conditions by bacillus firmus bg4 and bacillus halodurans bg5 isolated from heavy oil fields. Colloids and Interfaces 2(1):1–18

Shibulal B, Al-Bahry SN, Al-Wahaibi YM, Elshafie AE, Al-Bemani AS, Joshi SJ (2017) The potential of indigenous Paenibacillus ehimensis BS1 for recovering heavy crude oil by bio-transformation to light fractions. PLoS One 12(2):1–18

Singh RS, Kaur N, Kennedy JF (2019) Pullulan production from agro-industrial waste and its applications in food industry: A review. Carbohydr Polym 217:46–57

Singh RS, Saini GK, Kennedy JF (2008) Pullulan: microbial sources, production and applications. Carbohydr Polym 73(4):515–531

Souayeh M, Al-Wahaibi Y, Al-Bahry S, Elshafie A, Al-Bemani A, Joshi S, Al-Hashmi A, Al-Mandhari M (2014a) Optimization of a low-concentration Bacillus subtilis strain biosurfactant toward microbial enhanced oil recovery. Energy and Fuels 28(9):5606–5611

Souayeh M, Al-Wahaibi Y, Al-Bahry S, Elshafie A, Al-Bemani A, Joshi S, Al-Hashmi A, Al-Mandhari M (2014b) Microbial enhanced oil recovery at high salinities using biosurfactant at lower concentrations. Society of Petroleum Engineers–SPE EOR Conference at Oil and Gas West Asia 2014: Driving Integrated and Innovative EOR, 207–213

Thomas S (2008) Enhanced oil recovery-an overview. Oil & Gas Science and Technology-Revue de l'IFP 63(1):9–19

van Nieuwenhuijzen E, Houbraken J, Meijer M, Adan O, Samson R (2016) *Aureobasidium melanogenum*: a native of dark biofinishes on oil treated wood. Antonie Van Leeuwenhoek 109(5):661–683

Wazir NA, Manap AAA. (2020) Schizophyllan as potential environmental friendly eor polymer for a selected Malaysian field. Offshore Technology Conference Asia 2020, OTCA 2020

Wijayawardene NN, Crous PW, Kirk PM, Hawksworth DL, Boonmee S, Braun U, Dai DQ, D'souza MJ, Diederich P, Dissanayake A, Doilom M (2014) Naming and outline of Dothideomycetes–2014 including proposals for the protection or suppression of generic names. Fungal Divers 69(1):1–55

Zalar P, Gostinčar C, de Hoog S, Uršič V, Sudhadham M, Gunde-cimerman N (2008) Redefinition of *Aureobasidium pullulans* and its varieties. Stud Mycol 61:21–38

Fungal Polysaccharides as Biosurfactants and Bioemulsifiers

Luciana Luft

Abstract Fungi play a substantial role in tackling major challenges in the world. Applications of fungal processes and materials is directly related to the increase in sustainability by more efficient usage of natural resources. Filamentous fungi are useful in several industrial fermentation like production of scleroglucan, an extracellular polysaccharide formed by species of the genus *Sclerotium*. The polysaccharides have been reported to be used as surface-active compounds, that is, the fungal polysaccharides exhibit bioemulsifier and biosurfactant properties, giving them promising characteristics for biomedical, pharmaceutical, agricultural, food and other industries field. Due to these eco-sustainable characteristics, price as well as standardized production, fungal polysaccharides signify an alternative to replace their synthetic counterparts, making their production economically competitive and attracting the attention of the global market. On this context, this chapter focuses production of polysaccharides from different filamentous fungi.

Keywords Amphipathic complex · Bioactivity · Biopolymers · Eco-friendly properties · Exopolysaccharides · Microbial biotechnology · Multifunctional biomolecules · Polysaccharide-protein-lipid complexes

1 Introduction

Over the years, the production of polymers by petroleum extraction and refining processes have increased a lot and causing several environmental impacts. This overgrowth has raised serious concerns about environmental and socioeconomic aspects. Both aspects, as the possible scarcity of this nonrenewable source and high cost, are driving new routes in the production of polymers. As society has increased its environmental awareness, increases the concern of sustainable economic systems dependent on renewable sources. Currently, the biotechnology possibilities the

L. Luft (✉)
Department of Chemical Engineering, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_7

production of biologically derived polymers, called biopolymers, that is, the polymers production is not restricted only to fossil resources, and can be derived or extracted from microorganisms (Quintana-Quirino et al. 2019), plants (Kora et al. 2012), animals (Ranganathan et al. 2019), renewable raw materials (Joye 2019), or synthesized chemically from basic biological building (Chanchal et al. 2014).

A class of biopolymers, designated as multifunctional biomolecules of twenty-first century, which has received considerable attention due to their functional abilities and eco-friendly properties like specificity, biodegradability, low toxicity, stable under different environmental conditions (e.g. temperature and pH), and relative ease of preparation are the surface-active compounds (SAC) produced by filamentous fungi (Table 1). According their physicochemical properties, molecular weight and mode of action, these compounds are classified as biosurfactants or bioemulsifiers. Generally, these terms have been used interchangeably to describe SAC. Although amphiphilic, both classes present individual functions in nature, based on their physiological roles and physicochemical properties (Uzoigwe et al. 2015). The biosurfactants are low molecular weight compounds such as fatty acids, glycolipids, lipopeptides, phospholipids, and polymeric polysaccharide-protein complexes (Kopsahelis et al. 2018), and their main function is lowering the interfacial tension (Sałek and Euston 2019). The bioemulsifiers are high molecular weight compounds like heteropolysaccharides, lipopolysaccharides, proteins, lipoproteins or complexes of these components (Kourmentza et al. 2019), which adsorb fairly slowly to the surface of droplet and confer long-term stability (Sałek and Euston 2019).

Biopolymers consisting of polysaccharides, polysaccharides bound to proteins; or polysaccharides bound to lipids have been labeled as efficient SAC (Amargo-De-Morais et al. 2003; Luna-Velasco et al. 2007; Silva et al. 2014). Calvo et al. (2009) suggested that addition of hydrophobic substance to culture media influences directly the quantity of proteins and carbohydrates of the biopolymer. Besides the amount, the nature and ratio of the nitrogen and carbon sources, the physical parameters and nutritional limitations influence the types of polymer produced. Thus, they play an essential role on the structure of microbial SAC as well as yield, while change of substrate usually influence the structure of the product and consequently leads to changes in the properties.

Different properties of SAC compared to chemically synthesized materials and the abundance of substrate make them appropriate for commercial applications. Surfactants and emulsifiers are used in combination to prospect their desirable properties in consumer product formulations (Sałek and Euston 2019). They have wide application as emulsifiers and demulsifiers, functional food ingredients, foaming, wetting and spreading agents, detergents, and can be used in various industries including foods, beverages, pharmaceuticals, organic chemicals, cosmetics, agrochemicals, fertilizers, pulp and paper processing and paint industries, petroleum, petrochemicals, mining, metallurgy, and others (Vijayakumar and Saravanan 2015). In addition to these applications, there is a crescent number of reports on their diverse biological activities. The main bioactive properties such as antimicrobial and antioxidant activities demonstrate great potential for medicinal

Table 1 Surface-active compounds: fungal species, type, emulsifying index (E_{24}), surface tension (SF) and production yield

Fungal species	Type	E_{24}	SF (mN/m)	Yield	Reference
Aspergillus niger	Chitin-glucan complex	–	–	0.085 g/g	Mislovičová et al. (2000)
Curvularia lunata	Polysaccharide-protein complex	95% (kerosene)	–	2.6 g/L	Paraszkiewicz et al. (2002)
Penicillium citrinum	Polysaccharide-lipid-protein complex	60%	–	0.54 g/L	Camargo-De-Morais et al. (2003)
Penicillium sp.	Polysaccharide-lipid-protein complex	93% (kerosene)	–	2 g/L	Luna-Velasco et al. (2007)
Penicillium chrysogenum	Lipopeptides	–	–	–	Konishi et al. (2008)
Aspergillus fumigatus	–	11.17 (EU)/g (diesel oil)	–	–	Castiglioni et al. (2009)
Fusarium fujikuroi	Trehalolipids		20	–	Morita et al. (2011)
Fusarium sp.	Lipopeptides		32	5.25 g/L	Qazi et al. (2013)
Cunninghamella echinulata	Polysaccharide-lipid-protein complex	80% (burnt motor oil)	36	–	Silva et al. (2014)
Fusarium sp.	Lipopeptides	70% (kerosene)	32	1.21 g/L	Qazi et al. (2014)
Aspergillus flavus	Glycosides	–	20	13.6 g/L	Ishaq et al. (2015)
Ustilago scitaminea	Glycolipids	–	25.2	25.1 g/L	Lourenço et al. (2017)
Aspergillus brasiliensis	Polysaccharide-protein complex	–	–	1.6 g/g	Sánchez-Vázquez et al. (2018)
Rhizopus arrhizus	–	91.7% (burnt motor oil)	31.8	–	Pele et al. (2018)

applications (Osińska-Jaroszuk et al. 2015). The current chapter identifies the possible applications of some promising SAC produced from filamentous fungi.

2 Fungal Polysaccharides

Fungal polysaccharides are hydrophilic substances that increase the viscosity of a system after to disperse or dissolve in water (Barreto et al. 2011). Some of them are derived from the edible mushrooms or the organisms GRAS (Generally Recognized

Table 2 Summary of some polysaccharide bioactivities from filamentous fungi

Bioactivity	Fungal species	Reference
Antioxidant	*Lasiodiplodia* sp.	Kumar et al. (2018)
	Aspergillus fumigatus	Jin and Zhao (2014)
	Botryosphaeria rhodina	Giese et al. (2015)
Antimicrobial	*Lasiodiplodia* sp.	Kumar et al. (2018)
	Botryosphaeria rhodina	Sacchelli et al. (2019)
Antitumor	*Aspergillus terreus*	Li et al. (2016)
	Aspergillus fumigatus	Jin and Zhao (2014)
	Aspergillus fumigatus	Jin and Ning (2013)
	Botryosphaeria rhodina	Geraldelli et al. (2020)
	Diaporthe sp.	Orlandelli et al. (2017)
Immunomodulatory	*Lasiodiplodia* sp.	Kumar et al. (2018)
	Botryosphaeria rhodina	Weng et al. (2011)

as Safe), or even from filamentous fungi. They comprehend a broad assembly of biopolymers those are form many intracellular inclusions or part of the cell wall (intrapolysaccharides, IPS) and serve as energy reserve, or are excreted extracellularly (exopolysaccharides, EPS) when the microorganism is under stress conditions or after the growth phase, provide a mechanism for cell protection or by attachment to other surfaces (Giavasis 2014).

When polysaccharide chains is attached to protein or lipid residues the ability to stabilize oil-in-water emulsions increases and can be defined as polymeric surfactants (Vijayakumar and Saravanan 2015). In a reversible form, the protein or lipid (hydrophobic part) binds the hydrocarbons, while the polysaccharide binds to the protein producing a stable oil-in-water emulsion (Evans et al. 2013). Liposan as an extracellular water-soluble emulsifier consists of carbohydrate (83%) and protein (17%), synthesized by the *Candida lipolytica*, is one of the examples of polysaccharide-protein complex (Cirigliano and Carman 1985). Emulsan is an example of polysaccharide–lipid complex, an extracellular heteropolysaccharide emulsifier of *Acinetobacter calcoaceticus* (Shoham et al. 1983).

There are increasing number of reports have been developed on bioactive fungal polysaccharides produced by different filamentous species (Table 2). There are evidences that the bioactivities of some polysaccharides depend on their chemical structural characteristics, including molecular weight, composition of monosaccharide, the nature and configuration of the glycosidic linkages, degree and size of the branch points, conformation, solubility and charge on the polymer (Synytsya and Novák 2013). The knowledge of the structures and biological properties, as well as the fermentation process of microbial polysaccharides is crucial for understanding their physiological activities and possible industrial applications (Kumar et al. 2018).

The process of biotechnological production of polysaccharides is submerged fermentation. This has some limitations and its main challenge is to obtain purified biopolymers (Akila 2014). Ensuring high productivity and polysaccharide yields is not possible, as there are no general conditions for cultivation suitable for all microbial producers. Cultivation times for fungi are generally longer (2 to 32 days) than for bacteria (0.5 to 7 days), while in some cases results in lower volumetric productivity. Although, fungi like *Aureobasidium pullulans* and *Sclerotium rolfsii* can attain high productivity values (up to 18 and 13 g/L.day of pullulan and scleroglucan, respectively) (Freitas et al. 2017). Pullulan, scleroglucan, emulsan, alasan, liposan, lipomanan and other complexes of polysaccharide protein are the widely studied and relevant polymeric bioemulsifiers/biosurfactants (Luft et al. 2020; Santos et al. 2016). However, most of these are produced by yeast and bacteria (Adetunji and Olaniran 2021). The surface-active polymers produced by filamentous fungi have not been adequately explored, and only some of them have been produced on an industrial scale.

2.1 Pullulan

Pullulan is a water soluble polysaccharide consists of α 1,4 and α 1,6 bound glucose units. It is an edible polymer, tasteless, odorless, non-mutagenic, nontoxic and non-hygroscopic. Due to its distinct properties, that include excellent transparent film-forming ability, moisture absorptivity, and adhesivity, it is widely used in various industrial applications (Singh et al. 2021; Sugumaran and Ponnusami 2017). Pullulan is known as a valuable tool in basic research as a well-known model substance due its strictly linear structure (Singh and Saini 2012). A great number of studies referring to this polysaccharide produced by the black yeast *Aureobasidium pullulans*, even though it is not specificity of this species (Ruiz-Herrera and Ortiz-Castellanos 2019). The filamentous fungi *Cryphonectria parasitica* has been used for pullulan production (Corsaro et al. 1998; Delben et al. 2006; Forabosco et al. 2006). As pullulan produced by *Aureobasidium pullulans* is costly, the discovery that *C. parasitica* produces high quantity of pullulan stimulated the use of these strains for the purpose of industrial applications (Corsaro et al. 1998). The cost of pullulan is three-fold higher than other polysaccharides like xanthan and dextran (Sugumaran and Ponnusami 2017). The commercially available pullulan is over 90% pure, with mono-, di- and oligosaccharides being the main impurities (Park and Khan 2009). Pullulan has been exploited by its principle producer, Hayashibara Company (Okayama, Japan), since 1976, as an edible coating and a thickening agent (Freitas et al. 2017). A worldwide marketing license for film-based products of oral care contain pullulan has been granted to Pfizer by Hayashibara Biochemical Labs (HBL). In Canada, the product is marketed under the brand name Listerine (Singh and Saini 2012).

2.2 Scleroglucan

The scleroglucan is a branched neutral homopolysaccharide consists of β-glucan secreted by Sclerotium sp., which has attracted much attention from industry and academia since its first description was as early as 1960s (Schmid et al. 2011). Different biotechnological and biomedical applications of scleroglucan are developed due to its water solubility, hydrolysis resistance, viscosification, electrolyte and temperature properties. Since the 1970s, it has been marketed and is presently available under different trademarks for applications like enhanced oil recovery in the food, pharmaceutical and cosmetic fields (Castillo et al. 2015; Freitas et al. 2017). Scleroglucan as a commercial product also known with other product names based on the company which produces the polysaccharide (e.g. Actigum and Clearogel) (Coviello et al. 2005). Owing to its capability to form a very low energy dispersion, the scleroglucan is stable oil-in-water emulsions. Perhaps, the combination of its ability to prevent coalescence and suspending effect causes the stabilizing effect. Its properties may be governed by the molecular mass as well as the recovery methods (Survase et al. 2007).

2.3 Nigeran

Nigeran is a linear α-glucan with alternate α-1,3- and α-1,4-glycosidic linkages, obtained from the mycelia of *Aspergillus niger* and other filamentous genera such as *Aspergillus* and *Penicillium* (Synytsya and Novák 2013). The synthesis of this cold-water insoluble polysaccharide improved by depletion of the nitrogen and carbon sources in the media and metal ions (e.g. copper) increases its accumulation (Gupta and Mukerji 1982). Its easiness of extraction from the hyphal wall with boiling water and its outstanding solubility properties make its convenient identification possible. The pure form of nigeran can be obtained from cold water (Bobbitt and Nordin 1978).

2.4 Pseudonigeran

Pseudonigeran is a linear (1 → 3)-α-d-glucans, which are abundant polysaccharides in the cell walls of many fungi. However, these polysaccharides are specific for the Dikarya, and therefore they are not present in fungi belonging to the lower phyla (Ruiz-Herrera and Ortiz-Castellanos 2019). Horisberger et al. (1972) isolated this polysaccharide from mycelium of filamentous fungi *Aspergillus niger*. Later, similar glucans were isolated from the mycelium of *Penicillium chrysogenum* (Wang et al. 2007).

2.5 *Elsinan*

The water-soluble extracellular polysaccharide elsinan is isolated from *Elsinoe leucospila* (Tsumuraya et al. 1978), *Elsinoe fawcetti* and *Elsinoe ampelina*. It is a glucan essentially linear, consisting both (1 → 4)-α- and (1 → 3)-α-linkages and is mainly composed of (1 → 3)-α-linked residues of maltotriose (Synytsya and Novák 2013). When these fungi grown in an appropriate medium (28–30 g/L from 5% sucrose), considerable amount of elsinan are obtained. Due to its unique structure (non-ionic nature), it possesses interesting rheological properties, such as gelation at a high concentration, high viscosity at a low concentration, forming resilient and oxygen-impervious films (Misaki et al. 2000). Its aqueous solution exhibits high viscosity. There are several applications of elsinan like various types of film in pharmaceutical, cosmetics, food, forestry, agriculture, fisheries, and stock raising (Park and Khan 2009).

2.6 *Botryosphaeran*

The botryosphaeran is a (1 → 3)(1 → 6)-β-D-glucan produced by the fungus *Botryosphaeria rhodina*. It is an exopolysaccharide soluble in water with high molecular mass, it consists of a main chain of (1 → 3)-β-d-glucan with about 22% of side chains at O-6 consisted of β-d-Glcp and gentiobiosyl residues (Barbosa et al. 2003). Many studies demonstrated the biological properties of botryosphaeran and its value in health applications to develop new products of pharmaceuticals and foods (Silva-Sena et al. 2018). These bioactivities included immunomodulatory (Weng et al. 2011), antitumor (Malini et al. 2015), strong anticlastogenic (Miranda et al. 2008), hypoglycemic, hypocholesterolemic, hyperlipidemic (Miranda-Nantes et al. 2011), and antioxidant activities (Giese et al. 2011). Botryosphaeran produced by mild sulfonylation that exhibited the property of anticoagulation (Mendes et al. 2009) and antiviral activity (Sacchelli et al. 2019).

3 Application of Fungal Polysaccharides

3.1 *Biomedical and Pharmaceutical*

The polysaccharides have a wide variety of pharmacologic activities (e.g. antitumor, antiviral, immune regulation, hypoglycemic, antiaging, and blood lipid activities, especially in antitumor immunity) (Deng et al. 2020). The capacity of natural polysaccharides in stimulation of innate immunity by the activating immune cells with minimal adverse reactions have been studied (Li et al. 2021). These polysaccharides immunotherapeutics promising a future in cancer therapy and are classified as

biological response modifiers (BRMs). β-glucans are the most relevant BRMs among the carbohydrate BRMs (Survase et al. 2007). In addition, the polysaccharides are used in wound dressing compounds and have been useful in tissue engineering as films, scaffolds, and hydrogels (Agrawal et al. 2014).

3.1.1 Antimicrobial Activity

Scleroglucan, in addition to having higher immune stimulator and antineoplastic activity, also has greater antimicrobial activity than any other β-glucan (Park and Khan 2009). The exopolysaccharide, designated as Lasiosan, synthesized by *Lasiodiplodia* sp. (strain B2; MTCC 6000) displayed broad-range antimicrobial (antibacterial and antifungal) activities against many pathogenic bacteria (*Chromobacterium violaceum, Enterococcus faecalis, Escherichia coli*, *Klebsiella planticola, Micrococcus luteus*, *Staphylococcus aureus*) and yeast *Candida* (Kumar et al. 2018). Scleroglucan also has antiviral effect. The attachment and entry of the virus into the cell may be prevented or reduced by its binding on the host cell membrane. There are two explanations for antiviral activity. The first is the binding of scleroglucan with cell membrane glycoproteins, which prevents the interaction between the host cell plasma and the virus. The second can be that scleroglucan is internalized after the virus enters the cell, encapsulating and inhibiting the virus activity. However, for the case of scleroglucan, a polysaccharide with high molecular mass, the host cell penetration is unlikely (Survase et al. 2007). The chitosan extracted from *Aspergillus brasiliensis* proved its powerful antimicrobial action against meat contaminants. The sensory and microbiological quality of minced meat was increased after its application as a biopreservative (Tayel et al. 2014). The chitosan extracted from *Cunninghamella elegans* also have been presented antimicrobial activity. De Oliveira et al. (2014) applied chitosan obtained from *C. elegans* as a coating on table grapes, which inhibited the post-harvest pathogenic fungi such as *Botrytis cinerea* and *Penicillium expansum*. Moreover, the tested chitosan did not affect the quality (physical, physicochemical or sensory characteristics) of the grapes during the storage period.

3.1.2 Antioxidant Activity

Antioxidants of natural origin are of great importance to the human body, protecting it from free radicals, slowing the progress of many diseases and aging. The purified exopolysaccharide Lasiosan, produced by ascomycete fungi *Lasiodiplodia* sp., showed potential antioxidant activity, reducing and eliminating DPPH free radicals, lipid peroxyl radicals and superoxide anions (Kumar et al. 2018). Botryosphaeran, a β-(1 → 3; 1 → 6)-D-glucan produced by endophytic fungus *Botryosphaeria rhodina* (MAMB-05), known to exhibit *in vitro* antioxidant activity (Giese et al. 2015). *Lasiodiplodia theobromae* strain (MMPI) produced the lasiodiplodan, which is an exocellular β-(1 → 6)-D-glucan exist in a triple-helix conformation and its

carboxymethylation exhibited an increase in the EPS antioxidant capacity (Theis et al. 2017).

3.1.3 Immunomodulatory Activity

Even though the mechanisms involved in the antitumor action of natural polysaccharides are partially known, the study of them on tumor immunotherapy is of utmost importance. Many of polysaccharides are employed as adjuvants in for diverse types of tumors (Pires et al. 2013). They are BRMs, which result in immune enhancement mainly by activation the host immune system. Polysaccharides are known to regulate the host immune system as well as activate the antitumor activity of immune cells in the microenvironment of a tumor (Li et al. 2021). Regarding antitumor activity, scleroglucan is more efficient than other exopolysaccharides (Survase et al. 2007). According Pires et al. (2013), polymers of high molecular weight seem to show better antitumor action compared to smaller ones. Kumar et al. (2018) investigated the immunostimulatory functions of Lasiosan by examining its effects on the generation of ROS, NO and cytokines by the macrophages. The *in vitro* models demonstrated that Lasiosan was quite effective.

3.1.4 Wound Dressing

Although the traditional dressings made up of biocompatible animal materials, which may be rejected due human's immune reactions. Incorporation of synthetic polymers into granulation tissue remain in the body once the wound is healed. Therefore, dressings must contain a perfect balance of features, facilitating the rapid, assured wound healing, safe and biocompatible (Kofuji et al. 2010). Chitosan could be easily processed to form gels, nanofibers, membranes, beads, scaffolds, nanoparticles, and sponge forms those are useful in wound healing purposes (Archana et al. 2013). Due to the nontoxicity, inertness, antibacterial function, healing potential, gel-formation, extraordinary affinity to proteins, anesthetic effect and haemostasis, chitosan has attracted high attention in biomedical material inventions as a potential component of wound dressing (Agrawal et al. 2014; Anbazhagan and Thangavelu 2018; Kurakula and Raghavendra 2020). Chitosan-glucan complex is an advanced wound dressing, which have many benefits as solid form, antibacterial function, healing potential and drug carrying capacity compared to the traditional dressing materials like gauze and cotton wool will not active in wound healing process (Abdel-Mohsen et al. 2016). Pullulan can be used as drug carriers, and also can be employed as medical adhesives (U.S.Congress 1993), acting as a plasma extender with no undesired side effects, completely excreted after metabolic turnover.

3.2 Fungal Polysaccharides in Food and Cosmetics

In the food and cosmetic industries, polysaccharides have useful qualities such as emulsifiers (Banat et al. 2000), anti-adhesive agents and antimicrobials (Singh and Cameotra 2004). Due to their functional properties, these compounds are an indispensable part of food products that require sensory superiority, stability and longer shelf life (Nitschke et al. 2009). Chitosan have shown great promise for use in food industry. Owing to its high antimicrobial function against several microorganisms, chitosan can serve as preservative. It also serves as potential natural antioxidant by chelation of metal ions and is useful to stabilize lipid-containing food stuffs to prolong the shelf life (Aranaz et al. 2009). The high moisture holding and film-forming properties of chitosan resulted in a number of used in the cosmetics and personal care products (U.S.Congress 1993). Emulsification plays a vital role in the phase dispersion, solubilization of aromas, and in development of consistency and texture. Surface-active compounds find application in bakery, products derived from meat (Nitschke and Pastore 2002) and milk, from which the addition of emulsifiers enhances the texture and creaminess, influencing the rheological properties of flour and the emulsification of fats (Araujo et al. 2013). Scleroglucan applications may be useful in various skin care products and in the formulations for hair sprays (Survase et al. 2007).

3.2.1 Anti-Adhesive or Biofilm Inhibition Activity

The anti-adhesive activity is desirable to avoid the formation of biofilms on surfaces to which the food will come into contact. It is possible to reduce the adhesion of pathogens on surfaces commonly used in food industries by conditioning them with biosurfactants (Araujo et al. 2013). The most common means of bacterial growth in the environment is biofilm formation. Mixed populations of *Candida albicans* and many bacteria coexist to produce biofilms, which cause infections related medical device as well as nosocomial infections (Kumar et al. 2018). Pre-conditioning of surfaces with biosurfactants can considerably decrease microbial contamination of materials and inhibit or decrease the subsequent formation of biofilms (Araujo et al. 2013). Lasiosan obtained from *Lasiodiplodia* sp. strain B2 showed effectiveness in inhibition of biofilm (80–90%) against several pathogens (*Chromobacterium violaceu, Klebsiella planticola*, *Micrococcus luteus*, *Staphylococcus aureus*, and *S. aureus* MLS16), and moderate biofilm inhibition (70–80%) against other bacteria (*Bacillus subtilis, Escherichia coli*, and *Pseudomonas aeruginosa*). Lasiosan is the first report showed anti-biofilm activity, while such activity was not reported by other fungal EPS (Kumar et al. 2018).

3.2.2 Enzyme Immobilization

Chitosan is widely employed as a support to immobilize enzymes and whole cells, since it has considerable advantages such as biodegradability, form versatility, cost, nontoxicity, and high affinity towards proteins. Chitosan isolated from *Syncephalastrum racemosum* was used as a film support for immobilization of lipase (Amorim et al. 2003). The results showed that this filamentous fungi is a relevant producer of chitosan with application capacity as an immobilization support. Gels of pullulan have been employed for enzyme immobilization. The hydrophilic pullulan gel possessing a three-dimensionally reticulated structure, acquired by the reaction between pullulan and a bifunctional compound, is used as a carrier (Singh and Saini 2012).

3.3 Agricultural and Environmental Applications

Polysaccharides have a wide range of applications in agriculture. Among them, the main applications in plants are induction of the defense, improvement in physiological properties, and the increase in the shelf life of post-harvest products. Furthermore, application to the soil as a nutrient is highly effective in combination with other fertilizers. In addition to being useful in reducing fertilizer losses due to the coating capacity of polysaccharides, which is important to keep the pollution of environmental under control (Sharif et al. 2018). In mineral solubilization, sorption of heavy metals and removal of hydrocarbon and eliciting plant resistance, the activities of fungal EPS offered several possibilities of potential environmental and agricultural applications (e.g. plant bioprotection, soil/water bioremediation, and biofertilization) (Osińska-Jaroszuk et al. 2015). Scleroglucan is considered as a great antisettling agent for phytosanitary products. It helps in preparation of spraying mixtures and increases the contact of the droplets sprayed on the leaves. It could also be used in seed coatings, pesticides, and defoliant sprays (Survase et al. 2007).

3.3.1 Biocontrol Agent

Chitin/chitosan are biocontrol agents against many pathogenic microorganisms and have been used as a promising replacement to chemical pesticides. Wu et al. (2005) compared the bioactivities of fungal chitinous materials isolated from *Aspergillus niger* and *Mucor rouxii* with commercial chitin and chitosan. The crude fungal chitin and chitosan have the same or higher efficiency than the commercially purified chitosan from crustacean exoskeleton. The fungal chitinous materials presented antibacterial activity against *Salmonella typhimurium* by significantly reducing the lesions on apples caused by plant pathogens (*Botrytis cinerea* and *Penicillium expansum*). In addition, use of fungal EPS as potential biofertilizers as well as

elicitors of the systemic resistance in plants, recent studies have shown their ability to enhance the bioherbicidal activity (Osińska-Jaroszuk et al. 2015). Crude broth from *Phoma* sp. increased the bioherbicidal activity due to the concentration of EPS in the medium, indicating that these compounds might enhance the toxicity (Luft et al. 2021). The same effect was seen for EPS roduced in the fermentative broth by *Fusarium fujikuroi* (Todero et al. 2020). The antimicrobial activity of EPS from *Hirsutella* sp. was attributed with the biopesticidal potential (Li et al. 2010).

3.3.2 Growth Promoter and Elicitor of Plant Resistance

Fungal chitosan is a relevant candidate useful as growth stimulator for plant tissue culture. Its unique combination of properties (growth and protection promoter, environmental friendly, and biodegradable) makes fungal chitosan an appropriate biocontrol agent with a large perspective in the field of horticulture. Effect of fungal chitosan on the growth and development of meristemic tissue of orchids in solid and in liquid culture medium (Nge et al. 2006). The authors concluded that chitosan possesses the ability to induce the differentiation of orchid tissue and the fungal chitosan is effective on protocorm propagation in liquid medium. Restanto et al. (2016) investigated impact of fungal chitosan on the growth and development of seeds in solid medium. The growth of explants of seed into protocorm-like bodies was enhanced up to 4-folds in the presence of fungal chitosan. Application of pullulan to control the release of nitrogen in the fertilizer prevents the burning of crops, so this polysaccharide is considered a good agent for solid fertilizers (U.-S. Congress 1993).

The exopolysaccharides (EPS) can act as elicitors of systemic plant resistance, but their ability to induce resistance have remained to be more studied (Osińska-Jaroszuk et al. 2015). An oligosaccharide obtained from *Fusarium oxysporum* Dzf17 is responsible to an enhance the activity of defense-related enzymes in *Dioscorea zingiberensis* suspension cells as well as seedling cultures (Li et al. 2014). The EPS of *Botrytis cinerea* served as suppressor of the jasmonic acid signaling pathway, induced accumulation of salicylic acid in tomato, and enhanced resistance against the bacterial pathogen *Pseudomonas syringae* (El-Oirdi et al. 2011). Chitin and chitosan also possess the ability to control plant disease through eliciting the plant defense system. Recently, Zhou et al. (2020) studied the impacts of nanochitin on the biosynthesis of key enzymes as well as molecular mechanism leading to the induction of the plant defense *Phytophthora*.

3.3.3 Bioremediation

The bioremediation has emerged as a sustainable and recognized innovative technology to enhance the quality os soil contaminated by heavy metals and hydrocarbon. The polysaccharides can be widely used to enhanced the biodegradation of pollutants, and indirectly promote plant growth due their antimicrobial activity.

Surface-active compounds accelerate the desorption of hydrophobic pollutants those strongly bound to soil particles (Sachdev and Cameotra 2013). Chitosan and scleroglucan have been assessed for their use in bioremediation of toxic phenolic compounds and oil recovery, respectively (Survase et al. 2007). Jia et al. (2011) investigated degradation of pyrene in contaminated soils in the presence of EPS isolated from the culture *Aspergillus niger*. The authors observed that the introduction of EPS into soils increased the reduction of pyrene, indicating that the application of EPS is relevant for the bioremediation of soil contamination by polycyclic aromatic hydrocarbons. *Aspergillus fumigatus* also produced a polysaccharide with sorption ability for two heavy metals: copper and lead (Yin et al. 2011). Besides that, these surface-active polysaccharides can also increase the degradation of certain chemical pesticides present in the soil. The bioremediation of these contaminants can be sucessfully used as a biotechnological tool to reduce the environmental impacts caused by pesticides in agricultural regions located mainly around watersheds (de Lima et al. 2018).

3.3.4 Soil Treatment

Recovering the strength of the soil by increasing its cohesion is fundamental as a counter measure to desertification. But, it is not possible to complement the sands of all arid or semi-arid regions with fine cohesive soils. Chang et al. (2015) presented a new concept to enrichment of soil cohesion using β-glucan. Polysaccharides treatments are a promising alternative in prevention desertification due to their unique hydrogel characteristics, soil erosion resistance, retention of soil moisture, and promotion of cultivation. Others studies have shown the chances of using polysaccharides to reduce the erosion and to strengthen the soil in agricultural and geotechnical practices (Chang and Cho 2012; Orts et al. 2000). Chang and Cho (2012) used a commercial glucan (Polycan™) produced from *A. pullulans* towards improvement of the strength of Korean residual soil. In practice, this polysaccharide enhanced the compressive strength of soil over 200%, becoming a competitive option for aspect economic to replace the use of less environment friendly cement in terms of engineering performance, cost effectiveness, and environmental degradation.

3.3.5 Water and Wastewater Treatment

The polysaccharides are potential flocculants due to their biosorption, biocoagulation, and bioflocculation capabilities (More et al. 2014). Chitinaceous materials have been used to eliminate a great variety of water pollutants. Integrating these natural polymers into an existing system improves the effectiveness towards water treatment and reduces or eliminates the synthetic chemicals (Kaur and Dhillon 2014). Flocculation technique is one of the most commonly methods in wastewater treatment to remove the suspended particles and to enhance the effluent quality,

while biosorption is a potential technology for the treatment of textile industrial effluents. Extracellular polymeric substances are very important in these processes. Their interactions with cells have a significant impact on microbial flocculability. Biopolymer flocculants aggregate cells and particles through bridging and mechanism of charge neutralization. Thus, the biopolymer is capable to absorb the particles to form flocs. In biosorption, the extracellular polymeric substances capacity is related to the polysaccharides, lipid and proteins contents. The functional groups induce attractive forces between the cations and biomass (More et al. 2014). In treatment of aquaculture wastewater, chitosan serve as an adsorbent, coagulant and bactericide (Aranaz et al. 2009). Pullulan can be used as an aggregating or flocculating agent responsible for precipitation of potash and uranium clays, and ferric hydroxide from the slurries used in mineral beneficiation (U.S. Congress 1993).

Extracellular polymeric substances are also very important in the wastewater sludge thickening and dewatering processes. Two types of binding mechanisms between these compounds and water molecules are involved during thickening and dewatering, electrostatic interactions and hydrogen bonds (More et al. 2014). Different species of filamentous fungi have been investigated for application in treatment processes, such as dewatering, sedimentation and degradation of organic compounds. An example is the filamentous fungi *Penicillium expansum* (BS30) used to simultaneously for improvement of sludge settling as well as dewaterability (Subramanian et al. 2008). The degradation of polysaccharides produced by *Mucor* sp. (GY-1) (Wang et al. 2015) and *Talaromyces flavus* S1 (Liu et al. 2017) improved the sludge dewaterability.

4 Patents of Fungal Polysaccharides

Filamentous fungi are vital in production platforms of the biotechnological industries (Chambergo and Valencia 2016), although its potential in the production of biopolymers is still considered an unexplored market (Pessôa et al. 2019). The patents on producing surface-active polysaccharides by fungi for different purposes at an industrial level have been increased, including wound healing, wastewater treatment, biofertilization, and bioprotection, among others. Some of these patents are summarized in Table 3.

Recent patents developed for agriculture applications, such as the patent CN112680360A, which discloses that the filamentou fungi *Aspergillus sydowii* is a plant growth promoting agent, has strong inhibiting effect on bacteria and fungi, can promote the germination of plant seeds, and can achieve the growth promoting effect by regulating the content of endogenous hormones in plants (Yang et al. 2021). The Brazilian patent number BR102013003043A2 refers to fungal chitosan abilities as biofertilizer and bioprotector. The product was produced mixing potassium and phosphate rocks, along with sulfur and the inoculum of bacteria *Acidithiobacillus*, organic matter inoculated with diazotrophs free bacteria, and chitin and chitosan producing fungi (*Cunninghamella elegans*, *Mucor circinelloides*,

Table 3 Summary of some patents based on fungal polysaccharides during the last decade

Title	Patent number	Reference
Method for producing a polymer product from multidimensional aggregated components as barrier or carriers of living microbial cells and biological barriers in plastic and textile	WO2012145803-A2	Dierickx et al. (2012)
A method for the production of a compound of interest	WO2012001169-A1	Peij et al. (2012)
A medicinal fusidic acid cream made using sodium fusidate and incorporating a biopolymer, mometasone as a corticosteroid and clotrimazol as antifungal agent, and a process to make it	WO2012049542-A1	Vanangamudi et al. (2012)
Biofertilizer and bioprotector with fungal chitosan produced from mixed biofertilizers with phosphate and potassic rocks inoculated with bacteria acidithium and dyratic bethycatine butterials	BR102013003043A2	Stamford et al. (2015)
Gas-core microvesicles composed of phospholipids and biopolymers	BR102014028264-A2	Pontarolo et al. (2014)
Process for the fermentation of fungal strains	WO2016091892-A1	Briechle et al. (2016)
Method for preparing an aqueous solution of beta-glucan	WO2016087521-A1	Rollie et al. (2016)
A kind of glucan-Glucomannan compound	CN108938455-A	Yuan (2018)
A kind of Exopolysaccharide Production From The Fermentation waste liquid and sophorolipid complex biological emulsifier and preparation method and application	CN109876731-A	Yin et al. (2019)
A kind of method that solid state fermentation prepares high immunological activity *Dendrobium candidum* endogenetic fungus polysaccharide	CN109234332-A	Lou et al. (2019)
Method for improving application effect of *Trichoderma* in orchard	CN111955485A	Li et al. (2020)
Aspergillus galactomannan detect reagent box	CN212533004U	Yu and Zhou (2021)
Aspergillus sydowii and application thereof in promoting plant growth and preventing and treating plant diseases	CN112680360A	Yang et al. (2021)
Optimized culture medium and culture method for producing extracellular polymer by *Aspergillus niger*	CN112708566A	Liu et al. (2021)
Aureobasidium pullulans with improved beta-glucan yield and application thereof	CN112175843A	Wei et al. (2021)

M. javanica and *M. rouxii*). This production yielded a product purely biological based with a high fertilizer and protection against phytopathogenic microorganisms exist in the soil (Stamford et al. 2015).

Chinese patent number CN112708566A provided an invention for producing extracellular polymers by *Aspergillus niger*, disclosing an optimized culture medium for possible environmental applications. The process comprises the selection of

Aspergillus niger strain with high polysaccharide yield, the spore suspension preparation, the carrying out strain fermentation culture, followed by extraction, purification and chemical components quantification and structures of polysaccharide generated in different growth stages, and finally exploring the complexation and mechanism of the polysaccharide and heavy metal ions. In a second phase, the invention provides important reference for further understanding the migration and transformation process of heavy metals in water environment and further disclosing the mechanism of heavy metal adsorption of the biological membrane (Liu et al. 2021).

The patent number WO2016087521-A1 refers to a method of producing an aqueous solution comprising at least one β-glucan from a filamentous fungus. According inventors, these aqueous solutions of β-glucans have advantageous physicochemical properties, so that they are particularly suitable for polymer flooding (Rollie et al. 2016). Vanangamudi et al. (2012) disclose an invention (WO2012049542A1), which is useful for treating skin inflammations, fungal or bacterial skin infections and also other skin sores including skin burns. The lotion is composed by chitosan and others active ingredients. In summary, the production of fungal surface-active compounds and its several applications have driven the bio-industrial sector for its commercial-scale production, however there are still several challenges for biotechnology to attain a relevant standard.

5 Epilogue

A wide range of opportunities for applying fungal polysaccharides in various biotechnological fields has attracted the attention of many researchers. The major objective of this chapter was to furnish a review of the main surface-active compounds (SAC) produced from filamentous fungi. Some topics covered in this chapter were the roles of SAC as wound dressings, enzyme immobilization, soil and wastewater treatment. The findings presented indicate that these surface-active polysaccharides produced by different species of filamentous fungi beyond presented potential as bioemulsifiers and biosurfactants, also have bioactive properties. However, the majority of compounds in these categories are relatively at the research and patents stage, except pullulan and scleroglucan which have a strong global market. This approach to the surface-active compounds produced by filamentous fungi represents an attractive alternative for fields of industry.

References

Abdel-Mohsen AM, Jancar J, Massoud D, Fohlerova Z, Elhadidy H, Spotz Z, Hebeish A (2016) Novel chitin/chitosan-glucan wound dressing: Isolation, characterization, antibacterial activity and wound healing properties. Int J Pharm 510:86–99

Adetunji AI, Olaniran AO (2021) Production and potential biotechnological applications of microbial surfactants: an overview. Saudi J Biol Sci 28:669–679

Agrawal P, Soni S, Mittal G, Bhatnagar A (2014) Role of polymeric biomaterials as wound healing agents. Int J Low Extrem Wounds 13:180–190

Akila RM (2014) Fermentative production of fungal Chitosan, a versatile biopolymer (perspectives and its applications). Adv Appl Sci Res 5:157–170

Amorim RVS, Melo ES, Carneiro-Da-Cunha MG, Ledingham WM, Campos-Takaki GM (2003) Chitosan from *Syncephalastrum racemosum* used as a film support for lipase immobilization. Bioresour Technol 89:35–39

Anbazhagan S, Thangavelu KP (2018) Application of tetracycline hydrochloride loaded-fungal chitosan and *Aloe vera* extract based composite sponges for wound dressing. J Adv Res 14:63–71

Aranaz I, Mengíbar M, Harris R, Paños I, Miralles B, Acosta N, Galed G, Heras Á (2009) Functional characterization of chitin and chitosan. Curr Chem Biol 3:203–230

Araujo LVD, Freire DMG, Nitschke M (2013) Biosurfactants: anticorrosive, antibiofilm and antimicrobial properties. Quim Nova 36:848–858

Archana D, Dutta J, Dutta PK (2013) Evaluation of chitosan nano dressing for wound healing: characterization, *in vitro* and *in vivo* studies. Int J Biol Macromol 57:193–203

Banat IM, Makkar RS, Cameotra SS (2000) Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol 53:495–508

Barbosa AM, Steluti RM, Dekker RFH, Cardoso MS, Corradi Da Silva ML (2003) Structural characterization of Botryosphaeran: A (1→3;1→6)-β-D-glucan produced by the ascomyceteous fungus, *Botryosphaeria* sp. Carbohydr Res 338:1691–1698

Barreto M, Figueiredo MVB, Burity HA, Silva MLRB, Lima-Filho JL (2011) Production and rheological behavior of biopolymers produced by rhizobia and genetic characterization. Rev Bras Agrociência 17:221–227

Bobbitt TF, Nordin JH (1978) Hyphal nigeran as a potential phylogenetic marker for *Aspergillus* and *Penicillium* species. Mycologia 70:1201–1211

Briechle S, Hollmann R, Kaeppler T, Lehr F, Schmidt JK, Freyer S (2016) Process for the fermentation of fungal strains. Patent WO2016091892-A1

Calvo C, Manzanera M, Silva-Castro GA, Uad I, González-López J (2009) Application of bioemulsifiers in soil oil bioremediation processes. Future prospects. Sci Total Environ 407: 3634–3640

Camargo-De-Morais MM, Ramos SAF, Pimentel MCB, De Morais MA, Lima Filho JL (2003) Production of an extracellular polysaccharide with emulsifier properties by *Penicillium citrinum*. World J Microbiol Biotechnol 19:191–194

Castiglioni GL, Bertolin TE, Costa JAV (2009) Solid-state biosurfactant production by *Aspergillus fumigatus* using agricultural residues as substrate. Quim Nova 32:292–295

Castillo NA, Valdez AL, Fariña JI (2015) Microbial production of scleroglucan and downstream processing. Front Microbiol 6:1–19

Chambergo FS, Valencia EY (2016) Fungal biodiversity to biotechnology. Appl Microbiol Biotechnol 100:2567–2577

Chanchal A, Vohra R, Elesela S, Bhushan L, Kumar S, Kumar S, Ahmad S, Pandey RP (2014) Gelatin biopolymer: a journey from micro to nano. J Pharm Res 8:1387–1397

Chang I, Cho GC (2012) Strengthening of Korean residual soil with β-1,3/1,6-glucan biopolymer. Constr Build Mater 30:30–35

Chang I, Kharis A, Im J, Shin H, Cho G (2015) Geoderma Soil treatment using microbial biopolymers for anti-desertification purposes. Geoderma 253–254:39–47

Cirigliano MC, Carman GM (1985) Purification and characterization of liposan, a bioemulsifier from *Candida lipolytica*. Appl Environ Microbiol 50:846–850

Corsaro MM, De Castro C, Evidente A, Lanzetta R, Molinaro A, Parrilli M, Sparapano L (1998) Phytotoxic extracellular polysaccharide fractions from *Cryphonectria parasitica* (Murr.) Barr strains. Carbohydr Polym 37:167–172

Coviello T, Palleschi A, Grassi M, Matricardi P, Bocchinfuso G, Alhaique F (2005) Scleroglucan: a versatile polysaccharide for modified drug delivery. Molecules 10:6–33

de Lima DP, dos Santos E dos A, Marques MR, Giannesi GC, Beatriz A, Yonekawa MKA, Montanholi A dos S (2018) Fungal bioremediation of pollutant aromatic amines. Curr Opin Green Sustain Chem 11: 34–44

de Oliveira CEV, Magnani M, de Sales CV, de Souza Pontes AL, Campos-Takaki GM, Stamford TCM, de Souza EL (2014) Effects of chitosan from *Cunninghamella elegans* on virulence of post-harvest pathogenic fungi in table grapes (*Vitis labrusca* L.). Int J Food Microbiol 171:54–61

Delben F, Forabosco A, Guerrini M, Liut G, Torri G (2006) Pullulans produced by strains of *Cryphonectria parasitica*–II. Nuclear magnetic resonance evidence. Carbohydr Polym 63:545–554

Deng LJ, Qi M, Li N, Lei YH, Zhang DM, Chen JX (2020) Natural products and their derivatives: Promising modulators of tumor immunotherapy. J Leukoc Biol 108:493–508

Dierickx W, De Cuyper D, Gunde-Cimerman N, Ulrih NP (2012) Method for producing a polymer product from multidimensional aggregated components as barrier or carriers of living microbial cells and biological barriers in plastic and textile. Patent WO2012145803-A2

El-Oirdi M, El-Rahman TA, Rigano L, El-Hadrami A, Rodriguez MC, Daayf F, Vojnov A, Bouarab K (2011) *Botrytis cinerea* manipulates the antagonistic effects between immune pathways to promote disease development in Tomato. Plant Cell 23:2405–2421

Evans M, Ratcliffe I, Williams PA (2013) Emulsion stabilisation using polysaccharide – protein complexes. Curr Opin Colloid Interface Sci 18(4):272–282

Forabosco A, Bruno G, Sparapano L, Liut G, Marino D, Delben F (2006) Pullulans produced by strains of *Cryphonectria parasitica* - I. Production and characterisation of the exopolysaccharides. Carbohydr Polym 63:535–544

Freitas F, Torres CAV, Reis MAM (2017) Engineering aspects of microbial exopolysaccharide production. Bioresour Technol 245:1674–1683

Geraldelli D, Ribeiro MC, Medeiros TC, Comiran PK, Martins KO, Oliveira MF, Oliveira GA, Dekker RFH, Barbosa-Dekker AM, Alegranci P, Queiroz EAIF (2020) Botryosphaeran, a (1 → 3)(1 → 6)-β-D-glucan, reduces tumor development and cachexia syndrome in obese male rats by increasing insulin sensitivity and FOXO3a activity. Int J Biol Macromol 165:985–994

Giavasis I (2014) Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. Curr Opin Biotechnol 26:162–173

Giese EC, Dekker RFH, Scarminio IS, Barbosa AM, da Silva R (2011) Comparison of β-1,3-glucanase production by *Botryosphaeria rhodina* MAMB-05 and *Trichoderma harzianum* Rifai and its optimization using a statistical mixture-design. Biochem Eng J 53: 239–243

Giese EC, Gascon J, Anzelmo G, Barbosa AM, da Cunha MAA, Dekker RFH (2015) Free-radical scavenging properties and antioxidant activities of botryosphaeran and some other β-D-glucans. Int J Biol Macromol 72:125–130

Gupta R, Mukerji KG (1982) Nigeran production in some *Aspergillus* and *Penicillium* species. Folia Microbiol (Praha) 27:38–42

Horisberger M, Lewis BA, Smith F (1972) Structure of a (1→3)-α-d-glucan (pseudonigeran) of Aspergillus niger NNRL 326 cell wall. Carbohydr Res 23:183–188

Ishaq U, Akram MS, Iqbal Z, Rafiq M, Akrem A, Nadeem M, Shafi F, Shafiq Z, Mahmood S, Baig MA (2015) Production and characterization of novel self-assembling biosurfactants from *Aspergillus flavus*. J Appl Microbiol 119:1035–1045

Jia C, Li P, Li X, Tai P, Liu W, Gong Z (2011) Degradation of pyrene in soils by extracellular polymeric substances (EPS) extracted from liquid cultures. Process Biochem 46:1627–1631

Jin X, Ning Y (2013) Extraction optimization and bioactivity of polysaccharides from *Aspergillus fumigatus* AF1. Carbohydr Polym 96:411–416

Jin X, Zhao S (2014) Extraction optimization and bioactivities of an extracellular polysaccharide produced by *Aspergillus fumigatus*. Int J Biol Macromol 68:13–17

Joye IJ (2019) Cereal biopolymers for nano- and microtechnology: A myriad of opportunities for novel (functional) food applications. Trends Food Sci Technol 83:1–11

Kaur S, Dhillon GS (2014) The versatile biopolymer chitosan: potential sources, evaluation of extraction methods and applications. Crit Rev Microbiol 40:155–175

Kofuji K, Huang Y, Tsubaki K, Kokido F, Nishikawa K, Isobe T, Murata Y (2010) Preparation and evaluation of a novel wound dressing sheet comprised of β-glucan-chitosan complex. React Funct Polym 70:784–789

Konishi M, Fukuoka T, Morita T, Imura T, Kitamoto D (2008) Production of new types of sophorolipids by *Candida batistae*. J Oleo Sci 57:359–369

Kopsahelis A, Kourmentza C, Zafiri C, Kornaros M (2018) Gate-to-gate life cycle assessment of biosurfactants and bioplasticizers production via biotechnological exploitation of fats and waste oils. J Chem Technol Biotechnol 93:2833–2841

Kora AJ, Sashidhar RB, Arunachalam J (2012) Aqueous extract of gum olibanum (*Boswellia serrata*): a reductant and stabilizer for the biosynthesis of antibacterial silver nanoparticles. Process Biochem 47:1516–1520

Kourmentza C, Araujo D, Sevrin C, Roma-Rodriques C, Lia Ferreira J, Freitas F, Dionisio M, Baptista PV, Fernandes AR, Grandfils C, Reis MAM (2019) Occurrence of non-toxic bioemulsifiers during polyhydroxyalkanoate production by *Pseudomonas* strains valorizing crude glycerol by-product. Bioresour Technol 281:31–40

Kumar CG, Mongolla P, Pombala S (2018) Lasiosan, a new exopolysaccharide from *Lasiodiplodia* sp. strain B2 (MTCC 6000): Structural characterization and biological evaluation. Process Biochem 72:162–169

Kurakula M, Raghavendra NN (2020) Prospection of recent chitosan biomedical trends: Evidence from patent analysis (2009–2020). Int J Biol Macromol 165:1924–1938

Li H, Gao T, Wang J, Tian S, Yuan X, Zhu H (2016) Structural identification and antitumor activity of the extracellular polysaccharide from *Aspergillus terreus*. Process Biochem 51:1714–1720

Li P, Luo H, Meng J, Sun W, Wang X, Lu S, Peng Y, Zhou L (2014) Effects of oligosaccharides from endophytic *Fusarium oxysporum* Dzf17 on activities of defense-related enzymes in dioscorea zingiberensis suspension cell and seedling cultures. Electron J Biotechnol 17:156–161

Li R, Jiang X, Guan H (2010) Optimization of mycelium biomass and exopolysaccharides production by *Hirsutella* sp. in submerged fermentation and evaluation of exopolysaccharides antibacterial activity. Afr J Biotechnol 9:195–202

Li W, Huang Q, Xie S, Chen G, Cao M, Zhang J, Xie L (2020) Method for improving application effect of *Trichoderma* in orchard. Patent CN111955485A

Li Y, Wang X, Ma X, Liu C, Wu J, Sun C (2021) Natural polysaccharides and their derivates: A promising natural adjuvant for tumor immunotherapy. Front Pharmacol 12:621813

Liu H, Shi J, Xu X, Zhan X, Fu B, Li Y (2017) Enhancement of sludge dewaterability with filamentous fungi *Talaromyces flavus* S1 by depletion of extracellular polymeric substances or mycelium entrapment. Bioresour Technol 245:977–983

Liu X, Li L, Xu Q, Luo Z, Zhao Y, Fang D (2021) Optimized culture medium and culture method for producing extracellular polymer by *Aspergillus niger*. Patent CN112708566A

Lou W, Zeng Y, Zong M, Yang J, Yang H, Cheng J (2019) A kind of method that solid state fermentation prepares high immunological activity *Dendrobium candidum* endogenetic fungus polysaccharide. Patent CN109234332-A

Lourenço LA, Alberton Magina MD, Tavares LBB, Guelli Ulson de Souza SM. A, García Román, M, Altmajer Vaz D (2017) Biosurfactant production by *Trametes versicolor* grown on two-phase olive mill waste in solid-state fermentation. Environ Technol (United Kingdom) 3330: 1–11

Luft L, Confortin TC, Todero I, Zabot GL, Mazutti MA (2020) An overview of fungal biopolymers: bioemulsifiers and biosurfactants compounds production. Crit Rev Biotechnol 40:1059–1080

Luft L, Confortin TC, Todero I, Roberto J, Neto C, Tonato D, Felimberti PZ, Zabot GL, Mazutti MA (2021) Different techniques for concentration of extracellular biopolymers with herbicidal activity produced by *Phoma* sp. Environ Technol 42:1392–1401

Luna-Velasco MA, Esparza-García F, Cañízares-Villanueva RO, Rodríguez-Vázquez R (2007) Production and properties of a bioemulsifier synthesized by phenanthrene-degrading *Penicillium* sp. Process Biochem 42:310–314

Malini M, de Souza MF, de Oliveira MT, Antunes LMG, de Figueiredo SG, Barbosa AM, Dekker RFH, Cólus IM de S (2015) Modulation of gene expression and cell cycle by botryosphaeran, a (1→3)(1→6)-β-d-glucan in human lymphocytes. Int J Biol Macromol 77: 214–221

Mendes SF, dos Santos O, Barbosa AM, Vasconcelos AF, Aranda-Selverio G, Monteiro NK, Dekker RF, Sá Pereira M, Tovar AM, Mourão PA, da Silva ML (2009) Sulfonation and anticoagulant activity of botryosphaeran from *Botryosphaeria rhodina* MAMB-05 grown on fructose. Int J Biol Macromol 45:305–309

Miranda-Nantes CCBO, Fonseca EAI, Zaia CTBV, Dekker RFH, Khaper N, Castro IA, Barbosa AM (2011) Hypoglycemic and hypocholesterolemic effects of botryosphaeran from *Botryosphaeria rhodina* MAMB-05 in diabetes-induced and hyperlipidemia conditions in rats. Mycobiology 39:187–193

Miranda CCBO, Dekker RFH, Serpeloni JM, Fonseca EAI, Cólus IMS, Barbosa AM (2008) Anticlastogenic activity exhibited by botryosphaeran, a new exopolysaccharide produced by *Botryosphaeria rhodina* MAMB-05. Int J Biol Macromol 42:172–177

Misaki A, Tsumuraya Y, Kakuta M (2000) Elsinan, a potential food hydrocolloid produced by *elsinoe* species; properties and enzymatic degradation. Hydrocolloids 1:205–214

Mislovičová D, Masárová J, Bendžálová K, Šoltés L, MacHová E (2000) Sonication of chitin-glucan, preparation of water-soluble fractions and characterization by HPLC. Ultrason Sonochem 7:63–68

More TT, Yadav JSS, Yan S, Tyagi RD, Surampalli RY (2014) Extracellular polymeric substances of bacteria and their potential environmental applications. J Environ Manag 144:1–25

Morita T, Ishibashi Y, Hirose N, Wada K, Takahashi M, Fukuoka T, Imura T, Sakai H, Abe M, Kitamoto D (2011) Production and characterization of a glycolipid biosurfactant, mannosylerythritol lipid B, from sugarcane juice by *Ustilago scitaminea* NBRC 32730. Biosci Biotechnol Biochem 75:1371–1376

Nge KL, Nwe N, Chandrkrachang S, Stevens WF (2006) Chitosan as a growth stimulator in orchid tissue culture. Plant Sci 170:1185–1190

Nitschke M, Araújo LV, Costa SGVAO, Pires RC, Zeraik AE, Fernandes ACLB, Freire DMG, Contiero J (2009) Surfactin reduces the adhesion of food-borne pathogenic bacteria to solid surfaces. Lett Appl Microbiol 49:241–247

Nitschke M, Pastore GM (2002) Biossurfactantes: propriedades e aplicações. Quim Nova 25:772–776

Orlandelli RC, Corradi da Silva M de L, Vasconcelos AFD, Almeida IV, Vicentini VEP, Prieto A, Hernandez MDD, Azevedo JL, Pamphile JA (2017) β-(1→3,1→6)-D-glucans produced by *Diaporthe* sp. endophytes: purification, chemical characterization and antiproliferative activity against MCF-7 and HepG2-C3A cells. Int J Biol Macromol 94: 431–437

Orts WJ, Sojka RE, Glenn GM (2000) Biopolymer additives to reduce erosion-induced soil losses during irrigation. Ind Crop Prod 11:19–29

Osińska-Jaroszuk M, Jarosz-Wilkołazka A, Jaroszuk-Ściseł J, Szałapata K, Nowak A, Jaszek M, Ozimek E, Majewska M (2015) Extracellular polysaccharides from Ascomycota and Basidiomycota: production conditions, biochemical characteristics, and biological properties. World J Microbiol Biotechnol 31:1823–1844

Paraszkiewicz K, Kanwal A, Dlugoński J (2002) Emulsifier production by steroid transforming filamentous fungus *Curvularia lunata.* Growth and product characterization. J Biotechnol 92: 287–294

Park JK, Khan T (2009) Other microbial polysaccharides: Pullulan, scleroglucan, elsinan, levan, alternant, dextran. In: Phillips GO, Williams PA (eds) Handbook of hydrocolloids, vol 21, 2nd edn, pp 592–614

Peij VNNM, Hans M, Beishuizen M, Schipper D, Hoeven VDRAM, Schouten OL (2012) A method for the production of a compound of interest. Patent WO2012001169-A1

Pele MA, Montero-Rodriguez D, Rubio-Ribeaux D, Souza AF, Luna MAC, Santiago MF, Andrade RFS., Lima e Silva TA S, Santiago ALCMA, Campos-Takaki M (2018) Development and improved selected markers to biosurfactant and bioemulsifier production by *Rhizopus* strains isolated from Caatinga soil. African J Biotechnol 17:150–157

Pessôa MG, Vespermann KAC, Paulino BN, Barcelos MCS, Pastore GM, Molina G (2019) Newly isolated microorganisms with potential application in biotechnology. Biotechnol Adv 37:319–339

Pires A do RA, Ruthes AC, Cadena SMSC, Acco A, Gorin PAJ, Iacomini M (2013) Cytotoxic effect of *Agaricus bisporus* and *Lactarius rufus* β-d-glucans on HepG2 cells. Int J Biol Macromol 58: 95–103

Pontarolo R, Picheth GF, Camarozano AC, de Freitas RA, Sierakowski MR, Kaminski GAT, Pirich CL, dos Santos LA (2014) Gas-core microvesicles composed of phospholipids and biopolymers. Patent BR102014028264- A2

Qazi MA, Kanwal T, Jadoon M, Ahmed S, Fatima N (2014) Isolation and characterization of a biosurfactant-producing *Fusarium* sp. BS-8 from oil contaminated soil. Biotechnol Prog 30: 1065–1075

Qazi MA, Subhan M, Fatima N, Ali MI, Ahmed S (2013) Role of biosurfactant produced by *Fusarium* sp. BS-8 in enhanced oil recovery (EOR) through sand pack column. Int J Biosci Biochem Bioinforma 3:598–604

Quintana-Quirino M, Morales-Osorio C, Vigueras Ramírez G, Vázquez-Torres H, Shirai K (2019) Bacterial cellulose grows with a honeycomb geometry in a solid-state culture of *Gluconacetobacter xylinus* using polyurethane foam support. Process Biochem 82:1–9

Ranganathan S, Balagangadharan K, Selvamurugan N (2019) Chitosan and gelatin-based electrospun fibers for bone tissue engineering. Int J Biol Macromol 133:354–364

Restanto DP, Santoso B, Kriswanto B, Supardjono S (2016) The application of chitosan for protocorm like bodies (PLB) induction of orchid (*Dendrobium* sp) *in vitro*. Agric Agric Sci Procedia 9:462–468

Rollie S, Kaeppler T, Schalb C, Lehr F, Hlubek A, Therre J, Fleck C, Letzelter T, Freyer S, Herold A, Rettenmaier H, Leonhardt B (2016) Method for preparing an aqueous solution of beta-glucan. Patent WO2016087521-A1

Ruiz-Herrera J, Ortiz-Castellanos L (2019) Cell wall glucans of fungi. A review. Cell Surf 5:100022

Sacchelli BAL, Faccin-Galhardi LC, Ito VY, Lopes JL, Dekker RFH, Barbosa-Dekker AM, Orsato A (2019) Botryosphaeran and sulfonated derivatives as novel antiviral agents for herpes simplex and dengue fever. Int J Biol Macromol 138:334–339

Sachdev DP, Cameotra SS (2013) Biosurfactants in agriculture. Appl Microbiol Biotechnol 97: 1005–1016

Sałek K, Euston SR (2019) Sustainable microbial biosurfactants and bioemulsifiers for commercial exploitation. Process Biochem 85:143–155

Sánchez-Vázquez V, Shirai K, González I, Gutiérrez-Rojas M (2018) Polycyclic aromatic hydrocarbon-emulsifier protein produced by *Aspergillus brasiliensis* (niger) in an airlift bioreactor following an electrochemical pretreatment. Bioresour Technol 256:408–413

Santos DKF, Rufino RD, Luna JM, Santos VA, Sarubbo LA (2016) Biosurfactants: Multifunctional biomolecules of the 21st century. Int J Mol Sci 17:1–31

Schmid J, Meyer V, Sieber V (2011) Scleroglucan: biosynthesis, production and application of a versatile hydrocolloid. Appl Microbiol Biotechnol 91:937–947

Sharif R, Mujtaba M, Rahman MU, Shalmani A, Ahmad H, Anwar T, Tianchan D, Wang X (2018) The multifunctional role of chitosan in horticultural crops; a review. Molecules 23:1–20

Shoham Y, Rosenberg M, Rosenberg E (1983) Bacterial degradation of emulsan. Appl Environ Microbiol 46:573–579

Silva-Sena GG, Malini M, Delarmelina JM, Dutra JCV, Gervásio SV, Leal MAS, Costa Pereira T de M, Barbosa-Dekker AM, Dekker RFH, de Paula F, Batitucci M do CP (2018) *In vivo* antimutagenic and antiatherogenic effects of the (1 → 3)(1 → 6)-β-D- glucan botryosphaeran. Mutat Res - Genet Toxicol Environ Mutagen 826: 6–14

Silva NRA, Luna MAC, Santiago ALCMA, Franco LO, Silva GKB, de Souza PM, Okada K, Albuquerque CDC, da Silva CAA, Campos-Takaki GM (2014) Biosurfactant-and-bioemulsifier produced by a promising *Cunninghamella echinulata* isolated from caatinga soil in the North-east of Brazil. Int J Mol Sci 15:15377–15395

Singh P, Cameotra SS (2004) Potential applications of microbial surfactants in biomedical sciences. Trends Biotechnol 22:142–146

Singh RS, Saini GK (2012) Biosynthesis of pullulan and its applications in food and pharmaceutical industry. In: Satyanarayana T, Johri BN, Prakash A (eds) Microorganisms in sustainable agriculture and biotechnology, vol 24, pp 509–553

Singh RS, Saini GK, Kennedy JF (2021) Pullulan production in stirred tank reactor by a colour-variant strain of *Aureobasidium pullulans* FB-1. Carbohydr Polym Technol Appl 2:100086

Stamford NP, Santos CERS, Stamford TCM, Franco LC, Arnaud TMS, Berger LRR (2015) Biofertilizer and bioprotector with fungal chitosan produced from mixed biofertilizers with phosphate and potassic rocks inoculated with bacteria acidithium and dyratic bethycatine butterials. Patent BR1020130030430

Subramanian SB, Yan S, Tyagi RD, Surampalli RY (2008) A new, pellet-forming fungal: its isolation, molecular identification, andp erformance for simultaneous sludge-solids reduction, flocculation, and dewatering. Water Environ Res 80:840–852

Sugumaran KR, Ponnusami V (2017) Review on production, downstream processing and characterization of microbial pullulan. Carbohydr Polym 173:573–591

Survase SA, Saudagar PS, Bajaj IB, Singhal RS (2007) Gellan gum: Fermentative production, downstream processing and applications. Food Technol Biotechnol 45:107–118

Synytsya A, Novák M (2013) Structural diversity of fungal glucans. Carbohydr Polym 92:792–809

Tayel AA, Ibrahim SIA, Al-Saman MA, Moussa SH (2014) Production of fungal chitosan from date wastes and its application as a biopreservative for minced meat. Int J Biol Macromol 69:471–475

Theis TV, Calegari GC, Santos VAQ, Zorel Junior HE, Barbosa AM, Dekker RFH (2017) Exocellular (1→6)-β-D-glucan (lasiodiplodan): carboxymethylation, thermal behavior, antioxidant and antimicrobial activity. Am J Immunol 13:19–33

Todero I, Confortin TC, Luft L, Seibel J, Kuhn RC, Tres MV, Zabot GL, Mazutti MA (2020) Concentration of exopolysaccharides produced by *Fusarium fujikuroi* and application of bioproduct as an effective bioherbicide. Environ Technol 41:2742–2749

Tsumuraya Y, Misaki A, Takaya S, Torii M (1978) A New Fungal α-D-glucan, elsinan, elaborated by Elsinoe leucospila. Carbohydr Res 66:53–65

U.S. Congress, Office of Technology Assessment (1993) Biopolymers: Making Materials Nature's Way-Background Paper, OTA-BP-E-102 (Washington, DC: U.S. Government Printing Office, September 1993)

Uzoigwe C, Burgess JG, Ennis CJ, Rahman PKSM (2015) Bioemulsifiers are not biosurfactants and require different screening approaches. Front Microbiol 6:1–6

Vanangamudi SS, Srinivasan M, Chulliel NN, Selvaraj B (2012) A medicinal fusidic acid cream made using sodium fusidate and incorporating a biopolymer, mometasone as a corticosteroid and clotrimazol as antifungal agent, and a process to make it. Patent WO2012049542-A1

Vijayakumar S, Saravanan V (2015) Biosurfactants-types, sources and applications. Int J Microbiol Res 10:181–192

Wang T, Deng L, Li S, Tan T (2007) Structural characterization of a water-insoluble (1→3)-α-d-glucan isolated from the *Penicillium chrysogenum*. Carbohydr Polym 67:133–137

Wang Z, Zheng G, Zhou L (2015) Degradation of slime extracellular polymeric substances and inhibited sludge flocs destruction contribute to sludge dewaterability enhancement during fungal treatment of sludge using filamentous fungus *Mucor* sp. GY-1. Bioresour Technol 192: 514–521

Wei G, Chen X, Wang D, Wang C (2021) *Aureobasidium pullulans* with improved beta-glucan yield and application thereof. Patent CN112175843A

Weng BBC, Lin YC, Hu CW, Kao MY, Wang SH, Lo DY, Lai TY, Kan LS, Chiou RYY (2011) Toxicological and immunomodulatory assessments of botryosphaeran (β-glucan) produced by *Botryosphaeria rhodina* RCYU 30101. Food Chem Toxicol 49:910–916

Wu T, Zivanovic S, Draughon FA, Conway WS, Sams CE (2005) Physicochemical properties and bioactivity of fungal chitin and chitosan. J Agric Food Chem 53:3888–3894

Yang T, Qi H, Wang Z, Ma Y, Yang H, Xie H, Ma Y, Zhao Z, Zhao J, Fang Y (2021) *Aspergillus sydowii* and application thereof in promoting plant growth and preventing and treating plant diseases. Patent CN112680360A

Yin S, Xie P, Qu H (2019) A kind of exopolysaccharide production from the fermentation waste liquid and sophorolipid complex biological emulsifier and preparation method and application. Patent CN109876731A

Yin Y, Hu Y, Xiong F (2011) Sorption of Cu(II) and Cd(II) by extracellular polymeric substances (EPS) from *Aspergillus fumigatus*. Int Biodeterior Biodegradation 65:1012–1018

Yu X, Zhou CP (2021) *Aspergillus* galactomannan detect reagent box. Patent CN212533004U

Yuan, T, 2018. A kind of glucan-Glucomannan compound. Patent CN108938455A

Zhou Y, Jing M, Levy A, Wang H, Jiang S, Dou D (2020) Molecular mechanism of nanochitin whisker elicits plant resistance against *Phytophthora* and the receptors in plants. Int J Biol Macromol 165:2660–2667

Part II
Bio-composites

Challenges and Advantages of Building with Mycelium-Based Composites: A Review of Growth Factors that Affect the Material Properties

Ali Ghazvinian and Benay Gürsoy

Abstract Scholars and industries are studying the use of fungi-based materials as sustainable alternatives for materials in the several industries. Fungi are the decomposers of nature. They secrete enzymes through their vegetative root that is called mycelium and break down biopolymers of organic matter to simpler structures of carbon-based nutrients. Mycelium-based composites (MBC) are the most widely used form of fungi-based materials. These are foam-like, light-weight, and biodegradable composite materials. Since MBC do not depend on fossil fuels during production, are renewable, and create no waste throughout their life cycle, their use in architectural applications are being increasingly explored. In this chapter, we review the ongoing efforts to explore and enhance material properties of MBC to render them more suitable for the architecture, engineering, and construction (AEC) industry. In the AEC industry, MBC are currently used as insulation panels, load-bearing masonry components, and cores for sandwich structures. In this chapter, we review the methods used to enhance the material properties of MBC. Since material properties of MBC depend on various cultivation and post-processing factors, the effect of the growth factors on the final material outcome are reviewed from scholarly papers written and published from 2012 to 2021 related to MBCs and their use in design, architecture, and construction industry.

Keywords Biomaterials · Mycelium · Bio-design · Bio-fabrication · Material exploration

1 Introduction

Mycelium-based composites (MBC) are fungal biomaterials that gained a lot of interest in the last decade among the design fields. Fungi kingdom includes organisms such as mushrooms, molds, yeasts, truffles, and toadstools. Although they share

A. Ghazvinian (✉) · B. Gürsoy
Department of Architecture, Penn State University, University Park, PA, USA
e-mail: axg1370@psu.edu

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_8

similar features with both animals and plants, fungi are neither considered animal nor plant. Instead, these are unicellular or multicellular organisms, made of carbon with cell walls of chitin that cannot photosynthesize (McGaw 2018). The most significant difference of the fungal kingdom from animals and plants is their filamentous structure.

In contrast to animals and plants, which have organs and tissues, fungi are made of long and branching cellular structures called *hyphae*. Mycelium is the root part of a fungal body that has a hyphal structure. Through mycelium, fungi decompose organic substrates: Hyphae secrete enzymes and break down the biopolymers of organic matter into simpler structures of carbon-based nutrients, making these ready for digestion by fungi. The result of this process is a layer of bound organic matter, fluffy or compact, that covers the rest of the organic matter. Appels et al. (2019) call this layer "fungal skin." The characteristics of fungal skin depend on several factors such as fungi type, substrate, and environmental conditions during cultivation. Researchers have been exploring the decomposition process of mycelium to generate novel *bio-based materials.* These are grouped under two major categories: pure mycelium and mycelium-based composites (MBC). The complete degradation of organic matter results in the pure mycelium. Pure mycelium is a soft, paper-like material used for synthetic leathers and biodegradable papers (Jones et al. 2021). MBC, on the other hand, are foam-like and lightweight materials generated by stopping the decomposition process of the fungi. This stop can be done by either drying or heating hyphae, which hibernates or kills the fungal mycelium respectively. Different grades of MBC can be produced by changing the parameters influencing the cultivation process. These parameters are related to the various steps of cultivating MBC, discussed in the following sections. This diversity of products enables the use of this material in multiple applications such as insulators (Dias et al. 2021; Schritt et al. 2021), structural and semi-structural components (Moser et al. 2017; Ghazvinian 2021), furniture (Karana et al. 2018), packaging (Abhijit et al. 2018; Mojumdar et al. 2021), and electronic boards (Vasquez and Vega 2019a, b).

The primary motivations behind using this bio-composite in different industries are reducing the reliance on fossil fuels, embodied energy, and waste. In addition, compared to conventional materials of use, MBC produce considerably less carbon footprint (Elsacker et al. 2020) and is entirely biodegradable (Zimele et al. 2020). Another advantage of using MBC is the possibility of cultivating this material on a wide range of agricultural waste, such as wood stems, sawdust, husks, hulls, peels, and straws (Elsacker et al. 2021). This shift enables the re-entry of waste materials into the circular economy (Vallas and Courard 2017). Despite these advantages, there are particular challenges regarding the industrialization of MBC, especially for large-scale production (Derme et al. 2016).

This chapter reviews the ongoing efforts to explore and enhance material properties of MBC to render them more suitable for the AEC industry applications, following the literature review method proposed by Pickering and Byrne (2014).

2 Background on MBC Cultivation and the Parameters that Influence the Material Properties

Several growth factors affect the material properties of MBC. The production of MBC has different stages depending on the design intentions. In most cases, it follows a three-stage process as inoculation, growth, and ceasing. In the first inoculation stage, the substrate is mixed, sterilized, and fungal spawns are added. Based on the literature, during the inoculation stage, fungal species used for mycelium growth, substrates on which mycelium grows, and supplements used have the most significant effects on material properties of MBC (Elsacker et al. 2020). The second stage is growth. It is the stage in which mycelium grows in sterilized containers. Changing the environmental conditions during the growth process (i.e., humidity, temperature, light) can change the material properties of MBC (Appels et al. 2019). The last stage is ceasing. It is the stage in which mycelium growth is paused or stopped by drying or heating. Some characteristics may be enhanced by hot or cold-pressing (Liu et al. 2020). Figure 1 illustrates these different stages of MBC production, along with the factors influencing each stage. Each of these factors has a different effect on the material outcome. In the following section, these factors are reviewed.

2.1 Inoculation Factors

2.1.1 Fungal Species

MBC are made of two main parts, the binding agent and the filler. Mycelium is the binding agent of MBC. Most fungal species used in composite production are *white-rot fungi* and *brown-rot fungi*, belonging to the *saprophytic fungi* group. These fungi species convert dead organic matter into *mycelial mass*. *White rot fungi* decompose all compounds in wood, including lignin, and the outcome is white. *Brown rot fungi* decompose cellulose and hemicellulose, and the outcome is brown (Lundell et al. 2014).

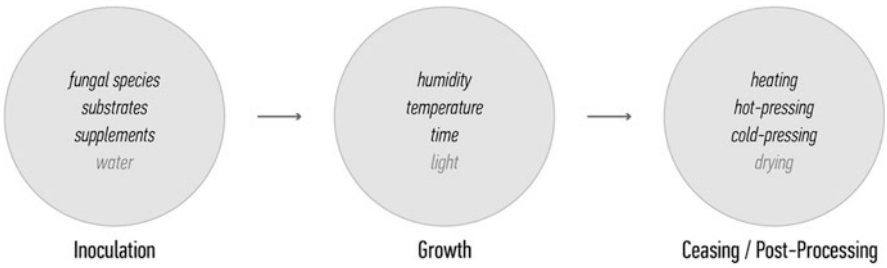


Fig. 1 Stages of MBC production and the factors affecting each stage

Most scholars who use MBC to design objects and architectural structures do not reveal the fungal species used in their designs to keep their technology and processes protected (Vasquez and Vega 2019b). Patented, readily inoculated mixtures developed by the company Ecovative are being extensively used in design explorations (Holt et al. 2012). *Pleurotus*, *Trametes* and *Ganoderma* genera typically stand out due to their faster and more efficient growth rates. While *Pleurotus ostreatus* (Grey Oyster mushroom) is the most used species of the *Pleurotus* genus; *Pleurotus albidus*, *Pleurotus sanguineus*, *Pleurotus pulmonarius* (Indian oyster), *Pleurotus salmoneostramineus* (Pink oyster), and *Pleurotus eryngii* (King oyster) species are also commonly used in MBC cultivation. Among the *Trametes* genus, *Trametes versicolor* (Turkey Tail) and *Trametes multicolor* are frequently used for cultivating composites. Abundance, lignin digestion, and the ability to grow on several natural waste products render *Ganoderma lucidim* (Reishi) and *Ganoderma* appalantum (Artist's bracket) species suitable for MBC cultivation.

Besides these three genera, there are other genera that the researchers have studied for MBC cultivation. To begin with, *Polyporus brumalis*, *Lentinula edudes* (Shiitake), and *Ceriporia lacerate* are more known species (Jones et al. 2019a and de Lima et al. 2020). *Ceriporia lacerate* is suitable to be used with non-sterilized substrates. The purpose of the research by Shao et al. (2016) is to reduce the energy and cost needed for the sterilization process and ease and enable large-scale production of MBC. Jones et al. (2019b) studied *Agaricus bisporus* (White Button), *Allomyces arbuscula*, *Mucor genevesis,* and *Trametes versicolor*. They compared the effect of each of these fungal species on hyphal branching during the production of mycelium-based matter, specifically nanopapers. Wimmers et al. (2019) used uncommon species of brown rot fungi. They compared these with well-known white rot fungi species for insulation purposes. *Fomitopsis pinicola*, *Gloeophyllum sepiarium*, *Laetiporus sulphureus*, *Phaeolus schweinitzii,* and *Piptoporus betulinus* are brown rot fungi studied in this research. They also looked at *Trichaptum abietinum*, which is a white-rot fungus. The results show that white-rot fungi grow faster, and their products are denser than brown rot fungi. Some scholars present novel strategies for inoculation and their influence on the material properties of mycelium-based materials. Zhang et al. (Zhang et al. 2019) optimized the "inoculum quantity" to assess how much spawn is needed to yield a better growth and mechanical behavior for *Pleurotus ostreatus* cultivated with corn straw as substrate. Appels et al. (2018), compared the mechanical properties of MBCs cultivated by wild and genetically modified versions of *Schizophyllum commune*. They reported that mechanical behavior of MBCs cultivated with wild species was close to natural materials, while with modified fungi, MBCs presented thermoplastic behavior.

2.1.2 Substrates and Supplements

The second part of the composite is the substrate, which is considered the filler of MBC. Depending on the growth ability of fungi on dead organic matter, scholars have studied different waste materials for producing MBC. The most commonly

used substrates in the literature are straw, woodchips (in smaller particles: sawdust), and hemp. Substrates have two prominent roles in composite production. They simultaneously act as fillers and as nutrients for mycelium growth. One of the reasons why researchers experiment with various substrates is to find mixtures in which substrates can fulfill both purposes. Woodchips seem more appropriate for filler: Wood is denser and more rigid than other waste materials. In literature, woodchips and sawdust from different trees have been used, such as eucalyptus, oak, pine, apple, vine (Attias et al. 2017), birch, aspen, spruce, pine, and pine fir (Wimmers et al. 2019). In order to be used as nutrients for mycelium, simpler and softer substrates have also been studied. Wheat, rice, corn and soybean straw, hemp hurd, and fiber are among the other common substrates used to produce fungal composites. Beyond the two main goals outlined above, researchers also explored recycling waste or byproducts of agriculture as substrates. Some studies used rice hulls, flax waste, coconut, cotton burs, switchgrass, sorghum stalks, kenaf, sugarcane bagasse, blackstrap molasses, cotton stalk, cotton ginning waste, and plant husks.

The results of these studies show that mixing different substrates work better for MBC cultivation, as this way, substrates can function both as fillers and nutrients. However, some researchers report that the size of the substrates and how they are processed have a more significant effect on the physicochemical and mechanical behavior of MBC than the type of substrates used. For example, Elsacker et al. (2019) have found that powder-like substrates are not suitable to sustain mycelium growth since they do not provide enough air and nutrients. On the contrary, chopped fibers yield more coherent and smoother fungal skins with better mechanical properties. It is also shown that the particle size affects the growth rate (Shao et al. 2016). For instance, when the straw is chopped to less than 2 mm particles, it failed to fully colonize in 5 days with *Ceriporia lacerate*, while the same mixture with straw chopped to 3–8 mm particles has fully cultivated in the same time frame (Shao et al. 2016). However, existing research also demonstrates that loosely packed substrates yielded lower mechanical properties (Appels et al. 2019).

Fungi need carbon and nitrogen as essential nutrients. When there are not enough nutrients in the fillers, supplements can be added. Wheat bran (Sisti et al. 2021; Ghazvinian et al. 2019; Bruscato et al. 2019; Teixeira et al. 2018) and wheat grain (Jones et al. 2017a; 2018a, b) are among the common supplements used for this purpose. Supplements can also enhance the mechanical properties of MBC; Glass fines (Jones et al. 2018a), fiber fabrics (Ziegler et al. 2016), and nanofibers (Sabantina et al. 2019) are supplements that can be used to improve the load-bearing capacity of MBC.

2.2 Growth Factors

As previously mentioned, the way the MBC are cultivated and processed considerably affects their material performance (Nashiruddin et al. 2021). Mycology research

identifies some primary factors during the cultivation phase: humidity, temperature, and light/darkness. Several researchers explored *time* as a cultivation parameter to study its effect on growth, compressive strength (Teixeira et al. 2018), thermal conductivity (Yang et al. 2017), and fire resistance of MBC (Jones et al. 2018b). These various explorations present the optimized time to grow each species, resulting in more coherent and uniform composites with thicker fungal skins. However, different fungal species require other environmental conditions for optimal growth. From this perspective, fungi are classified as *neutrophilic*, *mesophilic,* and *extremophilic*. While *neutrophilic* and *mesophilic* fungi thrive better in normal conditions, *extremophilic* fungi need extreme temperature, humidity, or acidity for growth (Jones et al. 2017b). Along these lines, (Appels et al. (2018) tested the growth of fungi in different environmental conditions with changes in light and amount of carbon dioxide. They found that in the light, increasing the CO_2 content enhances the mechanical strength. In contrast, in the dark, the results were the opposite.

2.3 Ceasing/Post-Processing Factors

The way that the growth process ends and the post-processing of the product can also influence the outcome of the cultivation. *Pressing* is among the factors that influence the material performance of MBC the most after the cultivation process is ceased. Pressing can be done at high and low temperatures. Researchers who studied the role of pressing on the mechanical behavior of MBC report that hot-pressed MBC samples have better mechanical properties than cold-pressed ones. Hot-pressing shifts the performance of materials from "foam-like to wood-like" by enhancing their homogeneity and stiffness (Appels et al. 2019). In order to find the optimum temperature for hot-pressing the composites, Liu et al. (2019) tested pressing the material with different temperatures and have found that 200 °C is the optimum temperature for pressing *Ganoderma lucidim* grown on the cotton stalk. They report that the composites burn and deform beyond this temperature, and lower temperatures do not yield workable results. Besides the factors outlined above, there is research on ways to suppress fruiting body formation. This way, ceasing is not necessary. Providing a biochemical solution, Chang et al. (2019) studied treatments to eliminate the heat-killing stage of the cultivation process in order to decrease the cost and energy needed for composite production and using the potential self-healing ability of the material.

3 Assessing the Material Properties of Mycelium-Based Composites

There is no consensus on the method to test and assess the material properties of MBC. Scholars performed tests using standards related to different materials such as sand, concrete, and foams. Experiments realized to assess the physicochemical and mechanical characteristics of MBC can be divided into two groups. One group of scholars used standards for particle-based materials to test these characteristics. Another group used standards related to rigid bodies, such as concrete and polystyrene. According to the literature, MBC are not strong enough to bear tensile loads. The characteristics reported show that MBC work better under compression (Attias et al. 2020). The load-bearing capacity of most MBC is comparable to expanded polystyrene (EPS) (Girometta et al. 2019). The results of tensile and flexural strength tests indicate that the non-compressive properties of MBC are not reliable for construction purposes. In studies related to thermal insulation agency and fire reaction of mycelium-based materials, scholars have used tests developed for similar materials, such as polystyrene and EPS, to compare this biodegradable material with its most similar, petroleum-based competitors. Results in most of the studies show that MBC can be used as alternatives for their petroleum-based correspondents if mass production can be achieved (Jones et al. 2019a). For acoustic purposes, standard ISO10534 has been used. Results indicate that OSB and MDF tiles can be replaced with mycelium-based tiles of either pressed or non-pressed composites (Pelletier et al. 2013; Pelletier et al. 2017).

From another perspective, one crucial stage in working with novel materials is to simulate their behavior using computational tools to have a broader sense of their response to different conditions. For example, Islam et al. (2018) developed a computational model that can predict the behavior of mycelium-based materials in parametric regimes that are not easily accessible by experimenting. There are also studies to predict the behavior of mycelium in hybrid use as a core for sandwich structures (Jiang et al. 2019; Wong et al. 2019). Jiang et al. (2019) also created a cost model for sandwich structures built with MBC to calculate the equivalent annual cost of composite manufacturing.

4 The Use of Mycelium-Based Composites in Design and Architecture

Over the past years, designers have started to employ MBC in their designs for various purposes, including electronic boards made out of mycelium (Vasquez and Vega 2019a, b), industrial packaging (Jones et al. 2020), parts for the aerospace industry (Travaglini et al. 2019), shoe soles, vegan leather, and other functions. The initial integration of MBC to architectural design was through molded bricks and blocks, wall and ceiling panels cultivated for insulation purposes (Jones et al.

Fig. 2 **(a)** HyFi Tower (image credits: Kris Graves), **(b)** MycoTree (image credits: Carlina Teteris), **(c)** El Monolito Micelio (image credits: Jonathan Dessi-Olive), **(d)** Circular Garden (image credits: Carlo Ratti), **(e)** The Growing Pavilion (image credits: Oscar Vinck)

2017b). Recently, researchers started to explore the structural aspects of MBC to be used in architectural skeletons. There are a few recent projects built with MBC on an architectural scale. Since MBC performs better under compression (Heisel et al. 2017), architects and designers mostly explored using MBC to build funicular structures: From simpler compression-based structures such as arches and vaults to more complex funicular trees (Heisel and Hebel 2019). Construction methods for MBC are mainly based on the aggregation of discrete masonry elements, such as MBC bricks or blocks (Ongpeng et al. 2020). There are recent efforts to build monolithic structures with MBC as well (Dessi-Olive 2019). In terms of fabrication techniques, molding is the most well-known technique, although recent studies explore additive manufacturing or 3D printing using MBC (Bhardwaj et al. 2020; Soh et al. 2020; Jauk et al. 2021; Colmo and Ayres 2020; Goidea et al. 2020) and subtractive manufacturing with abrasive wire cutters (Elsacker et al. 2021). Figure 2 illustrates some of these architectural projects employing MBC.

The first architectural prototype built in 2009 with MBC is the *Mycotecture Alpha* by Phil Ross. This was a barrel vault structure made of mycelium-based bricks (*Ganoderma lucidim)*, and has been exhibited in a gallery. After the end of the exhibition, the bricks were brewed into herbal tea. Following this effort, David Benjamin of The Living studio, in collaboration with the companies Arup and Ecovative, designed and built the 13-meter-high *HyFi Tower* using more than 10,000 MBC bricks in 2014 (Fig. 2a). The design of this tower, as well as the MBC bricks used, had more complex forms than the *Mycotecture Alpha*. The tower

has been demolished and the MBC bricks have been composted at the end of the three-month exhibition as part of the MoMA PS1 Young Architects Program. Yassin-Areddia and Beetles 3.3 designed and fabricated a shell-like structure with a triangular timber grid, covered with organic matter and coconut pith inoculated with mycelium in 2016. Following these efforts, the Block Research Group at ETH designed and built a funicular structure, *MycoTree* in 2017, employing 3D graphic statics method for form-finding (Fig. 2b). This method enables the generation of forms in which parts are only subject to compressive forces. *MycoTree* was a hybrid structure made of MBC and bamboo, and the researchers have designed and built a similar follow-up structure in 2019 called the *Mycotree II*. Jonathan Dessi-Olive explored the possibilities of building monolithic structures with MBC. In 2018, he designed and built *MycoArch*, *Thick and Thin*, and *El Monolito Micelio*, the latter being the largest monolithic structure built with MBC so far (Fig. 2c). It was a groin vault built with the help of a complex system of formworks and falseworks, which has been composted after demolition. In Milan Design Week 2019, Carlo Ratti cultivated more than a kilometer-long MBC in pipe-like formworks to build the *Circular Garden* (Fig. 2d). In a more recent use of MBC in architecture, Pascal Leboucq and Erik Klarenbeek designed and built the *Growing Pavilion* in 2019 (Fig. 2e). This pavilion is made of a wooden frame, covered with MBC panels as claddings. All these architectural structures are currently experimental in nature and inform each other about the possibilities, challenges and advantages of using MBC in the architectural context. The architectural projects built with MBC are reviewed in detail in an upcoming publication (Ghazvinian and Gursoy 2022).

5 Conclusions

In this section, we are revisiting the main questions that this review aims to address.

5.1 Environmental Benefits of Employing MBC in Architecture and Design

Regarding the environmental benefits of using MBC in design and architecture, MBC can address the main requirements of *circular economy* and *sustainable development*. The production process of MBC does not rely on fossil fuels. This material results in fewer greenhouse gas emissions and a considerably lower impact on global warming. On the one hand, compared to its competitors, less distance is needed to transfer the ingredients, as MBC can be cultivated with different species and on several substrates found in various cultivation sites. On the other hand, while conventional masonry materials often require very hot temperatures, there is no such need to heat the material in the case of MBC production, except for heating to cease

the mycelium growth and for hot pressure curing, both of which require low temperatures.

Moreover, the inputs of mycelium cultivation can come from the byproducts or wastes of other industries. After completing its lifetime, this bio-based and compostable material can be biodegraded and used as fertilizers if it is not mixed with chemicals or pesticides during cultivation. This way, MBC can contribute to the circular economy: Instead of ending in landfills, MBC can have another lifecycle. In addition, because of their high thermal insulation capabilities, MBC, when used in construction, can improve the building insulation systems passively, resulting in less usage of gas and electricity for air-conditioning.

5.2 *Constraints and Affordances of MBC for Architecture and Design*

Although MBC offers numerous environmental advantages, to employ MBC in the industry as an alternative to conventional material, better material properties in mechanical and environmental aspects are needed. As mentioned, the mechanical properties of MBC are limited. In terms of environmental loads, humidity, rain, and severe temperature fluctuations influence the material performance of MBC negatively. In specific built examples, the MBC structures have broken down because of the environmental effects and not because of mechanical failure (Dessi-Olive 2019; Gruber and Imhof 2017). Another bottleneck of using MBC in the building industry is the limitations of cultivation size due to the need for sterilization and pasteurization. In addition, specific fabrication techniques used in MBC production are controversial to the environmental benefits of employing MBC in construction. For instance, formworks used in MBC production become environmental wastes. An alternative solution can be to design and create novel formworks, which can serve as nutrients for the mycelium or be taken apart to be reused. Another option can be to adapt 3D-printing technologies as an alternative to molding (Bhardwaj et al. 2020; Goidea et al. 2020).

5.3 *Strategies to Enhance Material Properties of MBC for Architecture and Design*

Several parameters are affecting the material properties of MBC. Cultivation factors can significantly alter the outcomes. Considering that material properties of biomaterials are not always predictable and that the construction industry primarily relies on standardization, it is necessary to explore the effects of cultivation factors on the material outcomes of MBC to be able to use them in large-scale and large quantity applications in architecture and design. The mechanical performance of

MBC is in direct correlation with the substrate mixture and the post-cultivation procedure. Therefore it is necessary to understand and control these parameters to be able to use MBC in the industry. Post-processing, on the other hand, is challenging for large or complex elements. For such products, novel methods for post-processing need to be developed. Another strategy to integrate MBC in architecture and construction is to explore structural forms that can compensate for the weaknesses of MBC's mechanical performances. This is where advanced computational design technologies that enable form-finding, simulation, and optimization of structures by considering the material properties of MBC can come into play.

To conclude, to take full advantage of MBC in design and architecture, it is necessary to go back and forth between bottom-up material explorations and top-down design explorations that involve form-finding and fabrication. These are not only causally linked but also strictly interdependent. Therefore, when designing with MBC, architects and designers should either have complete control over the material properties of MBC and manipulate these according to their needs or design by considering the material properties of MBC that they cultivate.

6 Future Studies

This review paper is part of an on-going research to explore the structural aspects of MBC in architecture for temporary and/or low-rise constructions. The focus is on enhancing the material properties of MBC by investigating the factors that affect the nature and growth of the cultivated materials. Based on the constraints and affordances of MBC, structural systems that work under compression are developed using computational form-finding techniques, generative design and optimization methods. With this respect, several large-scale architectural structures are being designed and built, and the implementation of advanced digital fabrication methods to MBC cultivation are being explored. These digital fabrication methods will expand the possibilities that MBC offer as an architectural element by providing autonomy, flexibility, and repeatability to its manufacturing process.

References

Abhijith R, Ashok A, Rejeesh CR (2018) Sustainable packaging applications from mycelium to substitute polystyrene: a review. Mater Today Proc 5(1):2139–2145

Appels FVW, Camere S, Montalti M, Karana E, Jansen KMB, Dijksterhuis J, Krijgsheld P, Wösten HAB (2019) Fabrication factors influencing mechanical, moisture- and water-related properties of mycelium-based composites. Mater Des 161:64–71

Appels FVW, Dijksterhuis J, Lukasiewicz CE, Jansen KMB, Wösten HAB, Krijgsheld P (2018) Hydrophobin gene deletion and environmental growth conditions impact mechanical properties of mycelium by affecting the density of the material. Sci Rep 8:1–7

Attias N, Danai O, Abitbol T, Tarazi E, Ezov N, Pereman I, Grobman YJ (2020) Mycelium bio-composites in industrial design and architecture: comparative review and experimental analysis. J Clean Prod 246:119037

Attias N, Danai O, Ezov N, Tarazi E, Grobman YJ (2017) Developing novel applications of mycelium based bio-composite materials for design and architecture. Proceedings of Building with Biobased Materials: Best practice and Performance Specification, 6th–7th September (2017), 76–77

Bhardwaj A, Vasselli J, Lucht M, Pei Z, Shaw B, Grasley Z, Wei X, Zou N (2020) 3D printing of biomass-fungi composite material: a preliminary study. Manuf Lett 24:96–99

Bruscato C, Malvessi E, Brandalise RN, Camassola M (2019) High performance of macrofungi in the production of mycelium-based biofoams using sawdust - sustainable technology for waste reduction. J Clean Prod 234:225–232

Chang J, Chan PL, Xie Y, Ma KL, Cheung MK, Kwan HS (2019) Modified recipe to inhibit fruiting body formation for living fungal biomaterial manufacture. PLoS One 14(5):e0209812

Colmo C, Ayres P (2020) 3d Printed Bio-hybrid Structures Investigating the architectural potentials of mycoremediation. (2020)

de Lima GG, Schoenherr ZCP, Magalhães WLE, Tavares LBB, Helm CV (2020) Enzymatic activities and analysis of a mycelium-based composite formation using peach palm (Bactris gasipaes) residues on *Lentinula edodes*. Bioresour Bioprocess 7:1–17

Derme T, Mitterberger D, Di Tanna U (2016) Growth based fabrication techniques for bacterial cellulose: three-dimensional grown membranes and Scaffolding design for biological polymers. In: ACADIA // 2016: POSTHUMAN FRONTIERS: Data, Designers, and Cognitive Machines

Dessi-Olive J (2019) Monolithic mycelium: growing vault structures. Proc. 18th International Conference on Non-Conventional Materials and Technologies "Construction Materials & Technologies for Sustainability" (18 th NOCMAT 2019) 24th–26th July 2019 Nairobi, Kenya

Dias PP, Jayasinghe LB, Waldmann D (2021) Investigation of mycelium-Miscanthus composites as building insulation material. Results Mater 10:100189

Elsacker E, Søndergaard A, Van Wylick A, Peeters E, De Laet L (2021) Growing living and multifunctional mycelium composites for large-scale formwork applications using robotic abrasive wire-cutting. Constr Build Mater:283

Elsacker E, Vandelook S, Brancart J, Peeters E, De Laet L (2019) Mechanical, physical and chemical characterisation of mycelium-based composites with different types of lignocellulosic substrates. PLoS One 14:e0213954

Elsacker E, Vandelook S, Van Wylick A, Ruytinx J, De Laet L, Peeters E (2020) A comprehensive framework for the production of mycelium-based lignocellulosic composites. Sci Total Environ 725:138431

Ghazvinian A (2021) A sustainable alternative to architectural materials: mycelium-based bio-composites. In: Proceedings of the ConCave Ph.D. Symposium: Divergence in Architectural Research. pp. 159–167

Ghazvinian A, Gursoy B (2022) Basics of building with mycelium-based bio-composites: a review of built projects and related material research. J Green Building 17(1):37–69

Ghazvinian A, Farrokhsiar P, Vieira F, Pecchia J, Gursoy B (2019) Mycelium-based bio-composites for architecture: assessing the effects of cultivation factors on compressive strength. Res Innov 2:505–514

Girometta C, Picco AM, Baiguera RM, Dondi D, Babbini S, Cartabia M, Pellegrini M, Savino E (2019) Physico-mechanical and thermodynamic properties of mycelium-based biocomposites: a review. Sustainability 11(1):281

Goidea A, Floudas D, Andréen D (2020) Pulp faction: 3d printed material assemblies through microbial biotransformation. In: Fabricate. UCL Press, pp 42–49

Gruber P, Imhof B (2017) Patterns of growth-biomimetics and architectural design. Buildings 7:32. https://doi.org/10.3390/buildings7020032

Heisel F, Hebel DE (2019) Pioneering construction materials through prototypological research. Biomimetics 4:56

Heisel F, Lee J, Schlesier K, Rippmann M, Saeidi N, Javadian A, Nugroho AR, Van MT, Block P, Hebel DE (2017) Design, cultivation and application of load-bearing mycelium components: the MycoTree at the 2017 Seoul biennale of architecture and urbanism. Int J Sustain Energy Dev 6: 296–303

Holt GA, McIntyre G, Flagg D, Bayer E, Wanjura JD, Pelletier MG (2012) Fungal mycelium and cotton plant materials in the manufacture of biodegradable molded packaging material: evaluation study of select blends of cotton byproducts. J Biobaased Mater Bioenergy 6:431–439

Islam MR, Tudryn G, Bucinell R, Schadler L, Picu RC (2018) Stochastic continuum model for mycelium-based bio-foam. Mater Des 160:549–556

Jauk J, Vašatko H, Gosch L, Christian I, Klaus A, Stavric M (2021) Digital Fabrication Of Growth Combining digital manufacturing of clay with natural growth of mycelium. Proceedings of the 26th International Conference of the Association for Computer-Aided Architectural Design Research in Asia (CAADRIA) 2021: 1, 753–762

Jiang L, Walczyk D, McIntyre G, Bucinell R, Li B (2019) Bioresin infused then cured mycelium-based sandwich-structure biocomposites: resin transfer molding (RTM) process, flexural properties, and simulation. J Clean Prod 207:123–135

Jones M, Bhat T, Huynh T, Kandare E, Yuen R, Wang CH, John S (2018a) Waste-derived low-cost mycelium composite construction materials with improved fire safety. Fire Mater 42:816–825

Jones M, Bhat T, Kandare E, Thomas A, Joseph P, Dekiwadia C, Yuen R, John S, Ma J, Wang CH (2018b) Thermal degradation and fire properties of fungal mycelium and mycelium—biomass composite materials. Sci Rep 8:1–10

Jones M, Gandia A, John S, Bismarck A (2021) Leather-like material biofabrication using fungi. Nat Sustain 4:9–16

Jones M, Huynh T, Dekiwadia C, Daver F, John S (2017b) Mycelium composites: a review of engineering characteristics and growth kinetics. J Bionanosci 11(4):241–257

Jones M, Mautner A, Luenco S, Bismarck A, John S (2020) Engineered mycelium composite construction materials from fungal biorefineries: a critical review. Mater Des 187:108397. https://doi.org/10.1016/j.matdes.2019.108397

Jones M, Wang CH, Moinuddin K, Jones M, Bhat T, Wang CH, John S (2017a) Thermal degradation and fire reaction properties of mycelium composites. In *Proceedings of the 21st International Conference on Composite Materials, Xi'an, China* (pp. 20–25)

Jones M, Lawrie AC, Huynh TT, Morrison PD, Mautner A, Bismarck A, John S (2019a) Agricultural by-product suitability for the production of chitinous composites and nanofibers utilising *Trametes versicolor* and *Polyporus brumalis* mycelial growth. Process Biochem 80:95–102

Jones M, Weiland K, Kujundzic M, Theiner J, Kählig H, Kontturi E, John S, Bismarck A, Mautner A (2019b) Waste-derived low-cost mycelium nanopapers with tunable mechanical and surface properties. Biomacromolecules 20:3513–3523

Karana E, Blauwhoff D, Hultink EJ, Camere S (2018) When the material grows: a case study on designing (with) mycelium-based materials. Int J Des 12:119–136

Liu R, Li X, Long L, Sheng Y, Xu J, Wang Y (2020) Improvement of mechanical properties of mycelium/cotton stalk composites by water immersion. Compos Interfaces 27:953–966

Liu R, Long L, Sheng Y, Xu J, Qiu H, Li X, Wang Y, Wu H (2019) Preparation of a kind of novel sustainable mycelium/cotton stalk composites and effects of pressing temperature on the properties. Ind Crop Prod 141:111732

Lundell TK, Mäkelä MR, de Vries RP, Hildén KS (2014) Genomics, lifestyles and future prospects of wood-decay and litter-decomposing basidiomycota. In: Advances in botanical research. Academic Press Inc., pp 329–370

McGaw J (2018) Dark matter. Archit Theory Rev 22:120–139

Mojumdar A, Behera HT, Ray L (2021) Mushroom mycelia-based material: an environmental friendly alternative to synthetic packaging. In: Microbial polymers. Springer, Singapore, pp 131–141

Moser F, Trautz M, Beger AL, Löwer M, Jacobs G, Hillringhaus F, Wormit A, Usadel B, Reimer J (2017) Fungal mycelium as a building material. In *Proceedings of IASS Annual Symposia* (Vol. 2017, No. 1, pp. 1–7). International Association for Shell and Spatial Structures (IASS)

Nashiruddin NI, Chua KS, Mansor AF, Rahman RA, Lai JC, Azelee NIW, El Enshasy H (2021) Effect of growth factors on the production of mycelium-based biofoam. Clean Techn Environ Policy 1:1–11

Ongpeng JMC, Inciong E, Sendo V, Soliman C, Siggaoat A (2020) Using waste in producing bio-composite mycelium bricks. Appl Sci 10:5303. https://doi.org/10.3390/APP10155303

Pelletier MG, Holt GA, Wanjura JD, Bayer E, McIntyre G (2013) An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates. Ind Crop Prod 51:480–485

Pelletier MG, Holt GA, Wanjura JD, Lara AJ, Tapia-Carillo A, McIntyre G, Bayer E (2017) An evaluation study of pressure-compressed acoustic absorbers grown on agricultural by-products. Ind Crop Prod 95:342–347

Pickering C, Byrne J (2014) The benefits of publishing systematic quantitative literature reviews for PhD candidates and other early-career researchers. High Educ Res Dev 33:534–548

Sabantina L, Kinzel F, Hauser T, Többer A, Klöcker M, Döpke C, Böttjer R, Wehlage D, Rattenholl A, Ehrmann A (2019) Comparative study of Pleurotus ostreatus mushroom grown on modified PAN nanofiber mats. Nano 9:475

Schritt H, Vidi S, Pleissner D (2021) Spent mushroom substrate and sawdust to produce mycelium-based thermal insulation composites. J Clean Prod 313:127910

Shao GB, Yang P, Jiang WX (2016) Research and preparation of mycelium-soybean straw composite materials. In 2nd Annual International Conference on Advanced Material Engineering (AME 2016) (pp. 9–15). Atlantis Press

Sisti L, Gioia C, Totaro G, Verstichel S, Cartabia M, Camere S, Celli A (2021) Valorization of wheat bran agro-industrial byproduct as an upgrading filler for mycelium-based composite materials. Ind Crop Prod 170:113742

Soh E, Chew ZY, Saeidi N, Javadian A, Hebel D, Le Ferrand H (2020) Development of an extrudable paste to build mycelium-bound composites. Mater Des 195:109058

Teixeira JL, Matos MP, Nascimento BL, Griza S, Holanda FSR, Marino RH (2018) Production and mechanical evaluation of biodegradable composites by white rot fungi. Cienc e Agrotecnologia 42:676–684

Travaglini S, Parlevliet PP, Dharan CKH (2019) Bio-based mycelium materials for aerospace applications. In: Proceedings of the American Society for Composites—34th Technical Conference, ASC 2019

Vallas T, Courard L (2017) Using nature in architecture: building a living house with mycelium and trees. Front Archit Res 6:318–328

Vasquez ESL, Vega K (2019a) Demo: from plastic to biomaterials: prototyping DIY electronics with mycelium. In: UbiComp/ISWC 2019—Adjunct Proceedings of the 2019 ACM International Joint Conference on Pervasive and Ubiquitous Computing and Proceedings of the 2019 ACM International Symposium on Wearable Computers. pp. 308–311. ACM (2019)

Vasquez, ESL, Vega K (2019b) Myco-accessories: sustainable wearables with biodegradable materials. In: Proceedings—International Symposium on Wearable Computers, ISWC. pp. 306–311. ACM (2019)

Wimmers G, Klick J, Tackaberry L, Zwiesigk C, Egger K, Massicotte H (2019) Fundamental studies for designing insulation panels from wood shavings and filamentous fungi. Bioresources 14:5506–5520

Wong YJ, Arumugasamy SK, Mustapha KB (2019) Development of a computational predictive model for the nonlinear in-plane compressive response of sandwich panels with bio-foam. Compos Struct 212:423–433

Yang Z(J), Zhang F, Still B, White M, Amstislavski P (2017) Physical and mechanical properties of fungal mycelium-based biofoam. J Mater Civ Eng 29:04017030

Zhang J, He P, Lin Y, Song H, Dong H, Zhu X, Zhang J (2019) The cushion performance of mycelium-Cornstraw biofoams. J Biobaased Mater Bioenergy 13:484–489

Ziegler AR, Bajwa SG, Holt GA, McIntyre G, Bajwa DS (2016) Evaluation of physico-mechanical properties of mycelium reinforced green biocomposites made from cellulosic fibers. Appl Eng Agric 32:931–938

Zimele Z, Irbe I, Grinins J, Bikovens O, Verovkins A, Bajare D (2020) Novel mycelium-based biocomposites (Mbb) as building materials. J Renew Mater 8:1067–1076

Applications of Fungal Mycelium-Based Functional Biomaterials

Sehrish Manan, Omer Muhammad Atta, Ajmal Shahzad, Mazhar Ul-Islam, Muhammad Wajid Ullah, and Guang Yang

Abstract The utilization of biological systems has been receiving considerable attention in the past couple of decades in the development of bio-based functional materials. This has been largely inspired by the use of green, biodegradable, and environmentally sustainable materials for the development of new functional biomaterials. The utilization of renewable resources for the production of materials introduces fast-growing and biodegradable fungal mycelium-derived materials for various applications. Mycelium secretes enzymes and decomposes the substrate to take nutrients for growth and make an interwoven three-dimensional network. The elastic, porous, stiff, and dense mycelia are rich in antioxidants, antiviral, and anti-inflammatory compounds. The properties of mycelium-derived materials are greatly dependent upon the feeding substrate, fungus type, and processing conditions. Both pure mycelial materials and their composites secure an important position in the race of utilization of renewable resources for material synthesis. This chapter summarizes the utilization of mycelium-based materials for numerous applications like cosmetics, medicine, textile, construction, packaging, and the food industry. It also describes the potential of mycelial-derived materials as an alternative to the traditional insulators, packaging materials, and bovine leather. It further explains the importance of mycelium-based functional foods, cosmetics, and medicines.

S. Manan · M. W. Ullah (✉)
Biofuels Institute, School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang, People's Republic of China

Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan, People's Republic of China
e-mail: wajid_kundi@hust.edu.cn

O. M. Atta · A. Shahzad · G. Yang
Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan, People's Republic of China

M. Ul-Islam
Department of Chemical Engineering, College of Engineering, Dhofar University, Salalah, Sultanate of Oman

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_9

Keywords Mycelium-derived materials · Medicine · Functional food · Packaging · Textile · Construction · Cosmetics

1 Introduction

Fungi are eukaryotes having diverse habitats and morphologies and capable of building large colonial zones (Ibrar et al. 2020). The fungi are known to colonize their feeding substrate by invading the elongated thread-like structures known as hypha. The hypha grows and penetrates the substrate to make a three-dimensional intermingled network called mycelium. The mycelium can produce enzymes for substrate breakdown into simpler components for accessible nutrients uptake by the fungal cell. Fungi use these simple nutrient components and increase their biomass through hypha branching (de Ulzurrun et al. 2017). Most of the filamentous mushroom-forming fungi are famous for breakdown the agricultural waste comprised of lignocellulose such as sawdust, rice husk, and wheat straw (Hawksworth and Lücking 2017). Some fungi penetrate into the substrate as well as grow on the surface, while some grow out of the substrate or on the surface of liquid culture medium and form a compact fluffy skin-like material known as "fungal skin".

In the past, mushrooms were mainly used for edible or therapeutic uses owing to their high nutritional and medicinal value (Poucheret et al. 2006). Among the different parts of fungi, mycelium is a rapid and self-growing vegetative part that can be a source of green bio-based materials due to its growth on biological/agricultural waste. The mycelium-based materials, both alone and in combination with other materials, find applications in different areas. The synthesis procedure for pure and composite mycelium–derived material is different. The production of pure materials involves complete degradation of the substrate, while composites are obtained by heating or drying the substrate during colonization (Pelletier et al. 2013; Islam et al. 2018). The fungal type, substrate, synthesis method, environmental conditions, post-synthesis treatments, and type of reinforcing material used for composite synthesis define the final structural and physico-chemical properties of the materials (Jones et al. 2017; Appels et al. 2019). The manufacturing process of mycelial material is considered environment-friendly owing to its ability to recycle waste materials, hence this process does not cause any damage to the eco-network, such as *via* excavation of natural resources. Besides, the natural adaptability of fungi to diverse environments poses an advantage to its sustainable growth and ability to grow and decompose a variety of substances. Moreover, the mycelium-based materials are biocompatible and biodegradable (Chambergo and Valencia 2016).

The filamentous network, along with the bioactive compounds like polysaccharides, phenols, polyphenols, carotenoids, etc., in edible/medicinal fungal species (Zhang et al. 2021), are ideal properties to develop mycelium-based films for medical applications. In the last decade, several researchers have developed mycelium-based films and scaffolds for their applications in wound healing, drug delivery, tissue engineering, and several other areas (Khamrai et al. 2018; Narayanan et al. 2020). Mushrooms also possess anti-inflammatory, antioxidant, and good

moisture retention properties. Currently, mushrooms are part of most beauty products. Several mushroom-based lotions, serums, ointments, creams, and facial packs are used as moisturizers, whitening agents, or antiaging products (Mohorčič et al. 2007; Stanikunaite et al. 2009; Wu et al. 2016a). Mycelium-based leather is also a good substitute for bovine leather having similar features to bovine leather in terms of stiffness, strength, and moisture resistance (Meyer et al. 2021). The mycelium-based textiles also serve as biosensors for various medical conditions, such as electromyography signals and biophotonic sensing (Adamatzky et al. 2021). Moreover, mycelium smart wearables are capable of self-grow and self-heal compared to electronic wearables, which can be a major breakthrough in the industry of smart wearables. Mycelium can adopt the shape of the mold, grow fast into a dense material, and decompose naturally in a few weeks. Mycelium-based material is an excellent choice to replace conventional packaging materials such as polystyrene and polypropylene-based materials, especially when we consider eco-friendliness, sustainability, density, and strength (Abhijith et al. 2018).

This chapter summarizes the applications of mycelium-based biomaterials in various fields, including medicine, food, textile, packaging, cosmetics, and construction engineering (Fig. 1). It further describes the usage of fungal extracts as a functional food for therapeutic purposes and their use in various antiaging and skin whitening cosmetics products. It also highlights the issues with smart electronic wearables and discusses the importance of mycelium materials in the field of self-repairing intelligent wearables and conventional leather substitutes in the textile industry. The chapter further explains the acoustic and thermal absorbance of mycelium-based panels and their fire resistance capacity to develop acoustic and thermal insulators in the construction industry.

2 Applications

2.1 Food

Mushrooms are known for their nutraceutical compounds like polysaccharides, antioxidants, biologically active proteins, and terpenes (Ma et al. 2018; Mingyi et al. 2019). The mushroom-based diet is not just only proteinaceous but also provides vitamins, minerals, and high fiber content. Currently, mushroom-based functional foods are in high demand, especially the foods prepared from mushroom fruit bodies and mycelia (Rathore et al. 2017). Several studies reported that mycelia are capable of solubilizing and accumulating almost all kinds of minerals. An antioxidant compound, ergothioneine, is present in large amounts in fungal mycelium compared to the fruiting bodies of *Phellinus linteus* (Lee et al. 2008). Another study reported a high content of volatile compounds in mycelia rather than the mature fruiting bodies of *Pleurotus* species (Kabbaj et al. 2002). In a study, the powder obtained from mycelia of different mushrooms was used in the development of fortified bread which did not affect the rheology and texture of the final product

Fig. 1 Illustration of potential uses of various mycelium-based biomaterials in different areas

(Ulziijargal et al. 2013). Bread also confined enough amounts of ergothioneine and γ-aminobutyric acid even after baking at high temperatures. Meat analogs with *A. bisporus* mycelium possess enhanced texture, better springiness, high hardness, and good umami properties compared to soy-protein-derived meat analogs (Kim et al. 2011). Scleroglucan gained by fermentation of *S. rolfsii* fungus has been utilized as a texture stabilizer in sausages and desserts (Ioannis et al. 2005). Clinical data showed that mycelia as a drug have the potential to intervene and prevent various aggressive diseases. Several pharmaceutical marketing brands are trading mycelium-based nutraceuticals from medicinal mushrooms in the form of powders and capsules. Mycelium extracts of *Cordyceps,* besides inhibiting the progression of liver cirrhosis, also helped in healing the already developed liver cirrhosis in experimental rats. Moreover, their positive effect on coronary and cerebral blood circulation are revealed by researchers. They are involved in lowering blood pressure either directly through dilatory effects of or by regulating M-cholinergic receptors

Table 1 List of some mycelium-based commercial products available in the market. Table adapted from Rathore et al. (2019)

Product Name	Ingredient	Dose	Benefits
Mycelium Pro Ultimate Immune AMFM capsules	Mixture of Beta glucan derived from *G. lucidum, C. sinesis, A. blazie, H. erinaceus, Flamulina velutipes, L. edodes* and *Coriolus versicolor*	2 capsules/day	Helps balance the body's natural defense to maintain wellness year-round
Cordyceps	*Cordyceps* mushroom extract and mycelial biomass	2 capsules/day	Beneficial in respiratory and cardiovascular health
Power potion (Body health booster)	Mixture of 8 mushroom extracts Inonotus obliquus, *G. frondsa, C. militaris, P. ostreatus, H. erinaceus, L. edodes, T. versicolor* and *G. lucidum*	1 g	Potent anti-inflammatory adaptogens deliver huge health enhancing properties. Boost brain function, improve gut health and support adrenal & nervous system
Host Defense – MyCommunity Extract	17 mushroom mycelium blend includes *G. lucidum, T. versicolor, C. sinesis,* etc	1 mL	Helps in immune strengthening
Lion 's Mane mushroom powder	*H. erinaceus* mycelial biomass and fruit bodypowder	I teaspoon	Help support immunity, sports performance, recovery, cognitive health, blood sugar balance and overall health and wellness
Seek Doctor's advice before use			

(Tuli et al. 2014). According to the findings of a study, the mycelial extract of *L. edodes* prevented the *in vitro* growth of human breast cancer cells and showed immunostimulatory property by demonstrating mitogenic and comitogenic activities (Israilides et al. 2008). Bioactive component ergosterol, a precursor for vitamin D synthesis is present in mycelia of *A. bisporus, G. lucidum*, and *L. edodes.* It has been found that the extended exposure of mushrooms to UV-B light can effectively increase their potential to produce vitamin D_2 (Sławińska et al. 2016). For example, the consumption of button mushrooms grown under UV-B light enhanced the vitamin D content in humans, which was similar to the commercially available vitamin D supplements (Urbain et al. 2011). The ergosterol-rich mushrooms can also treat tumors besides preventing osteoporosis. After 20-days oral supplement of ergosterol extracted from *A. blazie* markedly treat prostate cancer in experimental models (Yu et al. 2009). A few mushroom-based nutraceuticals are listed in Table 1.

Fungus–based foods are in wide consumer demand around the globe due to their distinct and umami flavor. The presence of different volatile compounds like oct-1-en-3-ol, octan-3-ol, octan-3-on, and oct-1-en-3-on in mushrooms are responsible for their unique flavor (Moliszewska 2014). Beside these volatile compounds, some other molecules such as guanosine-5 ′-monophosphate and adenosine-5-′-monophosphate also contribute to the flavor-fullness of mushrooms by stimulating the flavors of several amino acids, mainly the glutamic acid (Mau 2005). The *P. eryngii, L. edodes*, and *A. aegerita,* mycelia contain an adequate amount of free

amino acids that can serve as a source for extraction of free amino acids to enhance flavors of various food products. Mycelia from *C. militaris* are commercially used as functional foods and nutraceuticals in Taiwan (Rathore et al. 2019). A mushroom-based meat analog containing corn starch, wheat gluten, and soy protein is recently developed by a group of Korean researchers. The texture, chewiness, springiness, hardness, cutting strength, and cohesiveness of the mean analog enhanced with the increment of mushroom content, whereas a decline in water-holding capacity and solubility of nitrogen was noticed. This meat analog also has enhanced antioxidant levels (Ahirwar et al. 2015; Cho and Ryu 2020).

2.2 Medicine

The demand for sustainable and eco-friendly biopolymers is increasing because of global pollution and large urbanization. The use of renewable sources and living systems is increasing in the field of material science and nanotechnology to develop renewable and biodegradable polymers. Presently, the main focus is to use materials and polymers that are naturally available, cost-effective, sustainable, biodegradable, and possess good physio-chemical properties. Researchers have developed and investigated several natural polymers, including cellulose (Di et al. 2017; Farooq et al. 2020; Khan et al. 2021; Mao et al. 2021; Xu et al. 2021), pectin (Munarin et al. 2012), lignin (Thakur et al. 2014), chitosan (Luo et al. 2015; Ul-Islam et al. 2019), gelatin (Khan et al. 2016, 2018), etc., to produce polymer-based biomaterials. Biopolymers are promising candidates for applications in tissue engineering and in the development of regenerative medicines because of their great similarity with the extracellular matrix, biocompatibility, biodegradability, and tuned structural properties. Many fungal species have been part of traditional medicine since ancient times because of their therapeutic products. Mushrooms fruiting body and mycelia contain antiviral, anti-inflammatory, antioxidant, and antimicrobial compounds (Chen and Seviour 2007; Gunawardena et al. 2014). Several fungal-based antiviral polysaccharides are extracted and investigated for their activities against lethal viruses (He et al. 2020). To date, the China Food and Drug Administration (CFDA) has permitted the use of some fungal-based patent drugs in the form of capsules, tablets, liquids, and injections against chronic hepatitis to enhance immunity with negligible chemotherapy effects (http://www.sda.gov.cn/WS01/CL0001/). The *Poria cocos*-derived polysaccharides are capable of producing anti-influenza (H1N1) and anti-HBsAg antibodies at fairly high concentrations, which induce stable and long-lasting immunity (Wu et al. 2016b). The sulfated poly-L-lysine from the fruit bodies of *Pleurotus abalonus* successfully inhibited the expression of HIV-1 reverse transcriptase (Wang et al. 2011). The polysaccharide also showed hypoglycemic function in diabetic mice as well as demonstrated antioxidant properties, mainly in scavenging hydroxyl and 1,1-diphenyl-2-picryl-hydrazyl radicals. The mycelial extract from *Agaricus brasiliensis* showed inhibitory action against

several strains of herpes simplex virus (HSV) such as KOS and 29R strains of HSV-1 and 333 strain of HSV2 (Cardozo et al. 2013).

The interwoven 3D structure of mycelia provides strength and facilitates interaction with host components. The high mechanical strength and porous nature, as well as the medicinal properties of various fungi, make them attractive biomaterials for medical applications like tissue engineering, drug delivery, and wound healing. Curcumin, having antimicrobial, antioxidant, and healing properties (Sajjad et al. 2020), was loaded into the mycelium patch to treat burn wounds (Khamrai et al. 2018). The developed film was capable of sustained release of drug at the wound site, and provided better healing due to continuous drug supply (Mohanty et al. 2012). It is proposed that the therapeutic efficacy of a drug-loaded material is directly related to the drug release kinetics and concentration at the site of infection. A sponge-like wound dressing called rhizochitin was developed using the mycelial matrix of *Rhizopus stolonifera*. An *in vivo* study showed healing of 40% more area in the treated group than control in mice model after five days of the wound covering with rhizochitin dressing (Chien et al. 2015). Recently, a novel biomimetic fungal scaffold has been developed through the mycelia of *Aspergillu*s sp. The chitin-glucan polysaccharides are crosslinked with fungal-scaffold (F-scaffold) and tested *in vitro* for skin tissue engineering. The 3D filamentous F-scaffolds showed good biocompatibility and hemocompatibility with human keratinocytes for skin tissue engineering applications (Narayanan et al. 2020).

2.3 Cosmetics

Cosmetics refers to the products used to beautify the skin, hairs, and nails, also known as personal care products (Millikan 2001). In general cosmetics industry deals with two types of products: cosmeceuticals for topical use and nutricosmetics for oral administration. In addition to the positive effects on a person's appearance in terms of skin whitening, wrinkle, and acne-free cosmetics products should be non-toxic, having no side effects on consumer's health. Considering this point, many fungal species have been explored and valued as the natural sources of various bioactive materials for a long period (Hyde et al. 2010). Explicitly, mushrooms extracts contain several compounds such as phenols, polyphenols, omega 3, 6, and 9 fatty acids, vitamins, ceramides, terpenoids, lentinan, etc., which are beneficial for skin and hair (Poucheret et al. 2006; Ahmad et al. 2013; Camassola 2013; Wu et al. 2016a). Such chemical compounds possess antimicrobial, antioxidant, anti-aging, anti-wrinkle, and skin whitening effects (El Enshasy and Hatti-Kaul 2013; Kalač 2013). The anti-inflammatory and regenerative function and the nutritional value of different mushrooms make them beneficial in developing cosmeceuticals and nutricosmetics.

The extracts of various mushrooms are used in moisturizing, antiaging, skin whitening, anti-acne facial products, and hair serums or creams. The lipids and amount of water held in the stratum corneum define the physical appearance and

function of the skin. The application of moisturizer limits the skin humidity loss and appearance of fine lines. A typical moisturizer either contains hyaluronic acid, pantothenic acid, or 6-palmitoyl-L-ascorbic acid as the active moisturizing ingredient (Sator et al. 2003). A fungal-based moisturizer improves the quality and texture of the skin and balances the water content of skin cells. The moisture retention capability of carboxymethylated polysaccharides acquired from *Tremella* was 65.7% after 96 h; hence it could be used in developing moisturizing products (Wang et al. 2015). In another study, the addition of only 0.05% *Tremella*-derived polysaccharides to a cosmetic product enhanced the moisture-holding capability in comparison with 0.02% hyaluronic acid (Liu and He 2012). *Fomes officinalis* mushroom powder was patented in the USA to treat the shiny look of skin and to improve skin appearance (Marie et al. 2003). Aging is a slow and steady process that results in the dysfunction of all body organs. Particularly in the skin aging process, the collapse of skin elastin and collagen takes place. The antiaging products are meant to prevent, slow, or reverse the aging process, and antioxidants effectively play their role by not only maintaining the body's repair system but further boosting it (Lupo and Cole 2007; Ocampo et al. 2016). Mushrooms like *L. edodes* and *V. volvacea* are rich sources of antioxidants that can pave the way in several the development of various cosmetic products (Dubost et al. 2006; Keleş et al. 2011). *L. edodes* are capable of preventing skin oxidative damage by inducing two antioxidant enzymes known as superoxidase dismutase and glutathione peroxidase (Cheung et al. 2003).

In an experiment on mice with atopic dermatitis, the application of extract from *P. cornucopiae* fungal species significantly treated atopic dermatitis in the experimental group (Tomiyama et al. 2008). The extract of *P. nebrodensis* has been approved as effective skin whitening agent, hence it can be used as a bioactive product to treat pigmentation and melasma of skin (Dangre et al. 2012). Likewise, bioactive compounds isolated from the mycelium of *G. fromosanum* reduced the skin discoloration via tyrosinase inhibition, an enzyme involved in skin pigmentation (Hsu et al. 2016). In addition, several marine fungi also showed inhibitory features against tyrosine, therefore capable of becoming the potential ingredients of skin-whitening products (Li et al. 2003). Furthermore, *Shiitake* were used as a skin exfoliator, which effectively regenerated skin and enhanced the elasticity as well as brought glow to the skin (Jikai 2002). More extracellular melanin production by *Gliocephalo trichum simplex* showed proficient UV absorbance, demonstrating its potential use in the manufacturing of sunscreens (Jalmi et al. 2012). Recently *Penicillium decumbens* fermented *Scutellaria baicalensis*, a common Chinese medicine, showed enhanced anti-acne potential than the non-fermented counterpart (Zhu et al. 2020). A list of commercially available mushroom-based cosmetic products and their function is provided in Table 2.

Table 2 Cosmetic products containing mushrooms and their ingredients. Table adopted from (Wu et al. 2016a) under the Creative Commons Attribution (CC-BY) license

Product	Fungal specie	Function
Aveeno Positively Ageless Daily Exfoliating Cleanser, U. S	*Lentinula edodes*	Lift away dirt, oil and makeup and fight signs of aging
One Love Organics Vitamin D Moisture Mist, U.K.	*Lentinula edodes*	Part lightweight moisturizer and part toner
Osmia Organics Luz Facial Brightening Serum, U.S.	*Lentinula edodes*	Skin looking bright and luminous
CV Skinlabs Body Repair Lotion, U.S	*Ganoderma lucidum*	Wound-healing and anti-inflammatory
Dr. Andrew Weil for Origins Mega-Mushroom Skin Relief Face Mask, U.S.	*Ganoderma lucidum*	Anti-inflammatory properties
Four Sigma Foods Instant Reishi Herbal Mushroom Tea, U.K	*Ganoderma lucidum*	Immunity boost
Kat Burki Form Control Marine Collagen Gel, U.K.	*Ganoderma lucidum*	Boost collagen, improve elasticity and provide hydration
Menard Embellir Refresh Massage, France	*Ganoderma lucidum*	Skin antiaging
Moon Juice Spirit Dust, U.S.	*Ganoderma lucidum*	Immune system
Tela Beauty Organics Encore Styling cream, U.K.	*Ganoderma lucidum*	Provide hair with sun protection and prevent color fading
Yves Saint Laurent Temps Majeur Elixir De Nuit, France	*Ganoderma lucidum*	Antiaging
Vitamega Facial Moisturizing Mask, Brazil	*Agaricus subrufescens* (also known as *A. brasiliensis*)	Renew and revitalize skin
Kose Sekkisei cream, Japan	*Cordyceps sinensis*	Moisturizer and suppress melanin production
Root Science RS Reborn Organic Face Mask, U.S.	*Inonotus obliquus*	Anti-inflammatory to help soothe irritated skin
Alqvimia Eternal Youth cream Facial Máxima Regeneración, Spain	*Schizophyllum commune*	Antiaging and lifting
Sulwhasoo Hydroaid, Korea	*Schizophyllum commune* extract	Hydrating cream promoting clear, radiant skin
La Prairie Advanced Marine Biology Night Solution, Switzerland	*Tremella fuciformis*	Moisturizer which nourishes, revitalizes and hydrates skin
BeautyDiy aqua circulation Hydrating Gel, Taiwan	*Tremella* polysaccharide	Moisturizing gel
Surkran Grape Seed Lift Eye Mask, U.S.	*Tremella* polysaccharide	Improve skin around eyes
Hankook Sansim Firming cream (Tan Ryuk SANG), Korea	*Ganoderma lucidum* and *Pleurotus ostreatus*	Make skin tight and vitalized

(continued)

Table 2 (continued)

Product	Fungal specie	Function
La Bella Figura Gentle Enzyme Cleanser, Italia	*Ganoderma lucidum* and *Lentinula edodes* extracts	Antioxidants and vitamin D
Pureology NanoWorks Shineluxe, France	*Ganoderma lucidum*, *Lentinula edodes*, and *Mucor miehe*	Anti-age and anti-fade
Snowberry Bright Defense Day cream No. 1, New Zealand	Mushroom extract	Hydrate and illuminate dull skin, along with anti-bacterial properties to help prevent acne
Murad Invisiblur Perfecting Shield, U.S.	Mushroom peptides	Diminish fine lines and wrinkles by aiding regulation of collagen and elastin

2.4 Packaging

Currently, industrial globalization and consumer demand urge more supply of packaging material. For instance, petroleum-derived expanded polystyrene (EPS) is used for a wide range of packaging applications. Several important characteristics of EPS, like lightweight, moisture resistance, and moldability, make it a suitable packaging material. EPS remains solid at room temperature, melts at high temperatures, and re-solidifies when the temperature falls down thus it can find various potential packaging applications. Although EPS has a good shock absorber acoustic properties and is suitable for transporting and storing delicate and fragile stuff, its production is an energy-consumable process and involves the emission of greenhouse gases (Abhijith et al. 2018). Additionally, the EPS is non-biodegradable and non-recyclable, thus hazardous to the environment. The strategy to develop biocomposites derived from pure bio-based resources could be a possible substitute to conventional petroleum-derived packaging materials for various applications (Ziegler et al. 2016). The unique features of mycelium-based materials like lightweight, durable, low cost, high strength, biodegradable and non-toxic nature presents it as an ideal material for a variety of applications such as packaging material for fragile items, food items, and electronics (Chambergo and Valencia 2016; Yang et al. 2017). The ability of mycelium to cast the shape of a mold during growth is an attractive feature to develop mycelium-based materials for fancy and decorative packaging for special items. A customized mycelium material can also be produced by providing a specific substrate, temperature, and fungal strain during growth (Yang et al. 2017; Manan et al. 2021). The characteristics of mycelial material primarily depend on the fungal strain, feeding substrate, growth conditions, manufacturing method, and post-synthesis treatments (Yang et al. 2017; Appels et al. 2019). A mycelium-patent by a Shenzhen-based Chinese company was developed by growing a fungal strain on wheat straw. The produced material was flexible, biodegradable, and lightweight. Another naturally dyed orange-red pigmented packaging material was produced using fungus *P. cinnabarinus*. The

synthesized material possesses high buoyancy, hence can be scaled to manufacture sea buoys (Cerimi et al. 2019). A green bio-block based on mycelium composites with different agricultural waste was synthesized. *P. ostreatus* was grown on sawdust, sugarcane bagasse, wheat bran, and a mixture of these materials to make mycelium composites. The composites were hydrophobic with exceptional thermal insulation and mechanical stability, thus paving the way as packaging materials, paneling material, and filtration membrane for the exclusion of lethal dyes and pollutants (Joshi et al. 2020). Recently, a novel humidity-resistant mycelium composite was developed by growing *Agaricus bisporus*, and *Trichoderma asperellum* on oat and rapeseed husk (Tacer-Caba et al. 2020). The developed composites are strong, hydrophobic, and not affected by a change in temperature or moisture content of the environment. The composite grown on rapeseed cake is more water-resistant comparable to oat cake.

2.5 Construction

In the last few decades, the rapid expansion and construction of urban sprawl are exerting extensive pressure on the construction industry for a constant availability of building stuff like cement, bricks, acoustic absorbers, thermal panels, etc. The manufacturing of traditional building materials requires more energy and emits greenhouse gases that cause environmental pollution (Madurwar et al. 2013). The exhaustion of natural resources, like stones and sand, is creating an alarming situation because the present pace of their consumption in the building industry is not viable. The worldwide sustainable development policy is targeted towards replacing non-renewable resources with renewable counterparts. The bio-based materials are capable of effectively diverting the traditional linear economy model to the viable bio-economy (Butu et al. 2020). The organic salvaging capacity of fungi is drawing attention in the development of the bio-economy. The growth of mycelium on low-cost agricultural waste, high strength, hydrophobicity, more thermal resistance, and nonflammable properties suggest it as an ideal material in the construction industry (Manan et al. 2021). Moreover, tunable and controllable characteristics during growth without the involvement of complex and expensive procedures are another attraction to use mycelium-materials in construction (Haneef et al. 2017).

To develop a mycelium-composite, fungal strain is grown on a substrate such as rice husk, sugarcane brass, wheat straw, sawdust, or a liquid medium. The mycelium secretes enzymes to break down the substrate and start their growth and take the mold shape. The foam-like chemical and physical characteristics of mycelial-based composites are appropriate for their use in non-structural construction, such as the door cores and insulation panels (MycoComposite™—Ecovative Design n.d.). The soaked mycelium composite in the soy-derived resin can be used for semi-structural construction applications like flooring, paneling, closets, and other decorative purposes (Jiang et al. 2019). The low density and thermal conductivity of mycelium

materials provide better insulation features. Mycelium composites with straw and hemp fibers have low density and thermal conductivity, thus making it an exceptional insulating material. The mycelium-based composite could be a better substitute to replace the traditional insulators such as sheep wool, glass wool, EPS, and kenaf (Jones et al. 2020). A study proved that agricultural stock-fed mycelium composites could absorb 70–75% of the generated sounds waves and provide good acoustic absorbance (Pelletier et al. 2013). Material porosity also exhibits a strong influence on its sound absorbance. A study claimed that less porous material aids better acoustic absorption compared to material with large pores (Samsudin et al. 2016). Additionally, mycelium materials give excellent termite resistance via using natural termicides and possess good fire resistance compared to conventional construction materials (Jones et al. 2020). Economic and sustainable fire-resistant and acoustic absorber panels are developed by growing mycelium on Miscantus (Dias et al. 2021). Miscantus is known for its fire resistance properties, and thin fibers of mycelium provide better resistance to sound waves upon vibration and provides better sound absorbance.

2.6 Textile

2.6.1 Mycelium Leather

The leather industry has a long history due to the easy availability and non-toxic and biodegradable nature of leather. Since ancient times it has been considered a durable and strong material and used for a broad range of applications. In the current era, alarming global warming and the sustainability of industrial products raise demand for renewable fossil-based raw materials for every type of industry (Ashby 2013; Karana et al. 2013). Despite its bio-based origin and renewability, the conventional development of leather-based material is facing serious concerns owing to the emission of greenhouse gases of cattle breeding. Moreover, questions have been raised over the sustainability of the leather industry and animal welfare (Meyer et al. 2021). Likewise, consumers are demanding meat-free products or product of pure animal origin is not preferable. These concerns raise challenges in cattle farming for leather production (Ashby 2013).

As an extremely flexible material, fungal mycelium can be potentially used to in the development of leather-like materials (Fig. 2). Presently, the development of leather-like materials from fungus is one of the main targets of the fast growing global commercialization efforts. Mycelium-derived leather is comprised of nonwoven mycelium mats harvested from solid or liquid state substrate-fermentation, which is further treated chemically or physically to mimic the original leather. The mechanical properties are tuned via several micro or nanoscale treatments, including acetylation, denaturation of proteins, chitin crosslinking, material densification, and dyeing of mats (Jones et al. 2021). Despite a thousand-year history of crafting felt-like materials, known as ‘amadou’ from mushroom fruiting bodies, only a few

Fig. 2 Fungi-derived leather substitutes. (**a**) Traditional Corund-style amadou crafted hats and handbags, (**b**) Stella McCartney mini-pouch made from Mylo™, and (**c**) backpack made from PURA Flex™. Images reproduced, with permission, from Grado Zero Innovation (Firenze, Italy), Bolt Threads Inc. (Emeryville, USA), and MOGU SRL (Inarzo, Italy). Figure reproduced from (Gandia et al. 2021)

products are commercialized, such as Amadou Leather™. The internal fleshy part of the basidiocarp is manually pulled out from the soaked or fresh species, cut into pieces, and then extensively beaten with a wooden hammer to slacken off the fibers to make it stretchable during crafting. To mold hats, 20–30 piece is stretched on a wooden mold, whereas small pieces are used to make bags, belts, scarves or table clothes (Pegler 2001; Papp et al. 2017). Naturally brown colored nonwoven amadou mat is light weighted and eco-friendly vegan leather. The brown color is due to the

high melanin content in basidiocarp (Kalitukha and Sari 2019). Although the material is soft, breathable, and capable of absorbing the moisture that makes it an ideal material for making shoe soles and watches straps but highly fragile and vulnerable to break when wet (Meyer et al. 2021). Moreover, the material is also antibacterial and antifungal in nature that makes it suitable for the fabrication of dressings and bandages (Kolundžić et al. 2016). Soft-felt-like material, generally known as the German-felt, was produced using fungus *Fomes fomentarius*. German-felt is used to make gloves, bandages, hoods, hats, and even trousers (Schmidt 2006; Seniuk et al. 2011).

Mylea™ (MycTech, Indonesia), a mycelium-derived leather alternative, has improved tensile strength and durability compared to Amadou Leather™ and is more similar to bovine and synthetic polyurethane leather substitutes in terms of mechanical properties (Florentina and Cristina 2010). The elongation break of mycelia leather can be improved by adding a glycerol-like plasticizer to gain rubber-like stretching properties (Appels et al. 2020). The mechanical properties can also be increased by combining mycelium with various polymers to form composite materials. Reshi™ (MycoWork, USA) nonwoven mycelium mats with reinforcement materials like polystyrene and polylactic acid surface coating have a smooth surface and better mechanical properties (Meyer et al. 2021). In addition, Reshi™ resistance to color fading on exposure to UV light, perspiration, water spotting resembles bovine leather (Gandia et al. 2021).

2.6.2 Smart Wearables

Smart wearables are the devices that enhance the function of gadgets or clothes. These wearables serve as an interface between environmental stimuli and the wearer. The integrated high level of technology aids easy detection of stimulus and operates accordingly. Smart wearables are responsive to the wearer and broadly categorized into three subgroups. The first group is comprised of passive smart wearables which can sense the user and environment. The second group contains the reactive smart wearables, which are capable of sensing the user and environment and perform some actions in response. The third group contains some advanced smart wearables which can sense, react, and adapt their behavior to given signals. Textile-embedded sensing systems have been produced and commercialized for various biomedical and safety applications. Smart wearables are used to record temperature, electromyography signals, electrocardiography signals, biophotonic sensing, oxygen, moisture, and salinity content (Sibinski et al. 2010; Adamatzky et al. 2021). Generally, the intelligent wearables should be soft, able to bend/wrap, amenable to extension, and tough (Rajan et al. 2017). A lack of self-growth and self-healing abilities limit the application of electronic wearables in the field of self-growing and soft robotics. However, the development of slime molds on the body of robots/clothes can sense biochemical, mechanical, and optical signals but is highly fragile and dependent on environmental conditions.

Fungi could be a better alternative to slime mold. The filamentous mycelium-based materials are emerging as reliable, economical, and eco-friendly replacements for high cost, hazardous conventional packaging, construction materials, and fabric (Adamatzky et al. 2021; Jones et al. 2021). Fungi can sense chemicals, light, gases, electric fields, and gravity. Several studies proved that fungi show a clear response towards toxic metals, pH change, CO_2 accumulation, mechanical stimulation, and stress inducers (Howitz and Sinclair 2008; Adamatzky 2013; Beasley et al. 2020). Thus, the wearables with mycelium patches/incorporated mycelium fabric act as a sensorial network for a variety of applications. Fungi respond to various physical or chemical stimuli *via* modification in their electrical properties (Adamatzky 2013; Beasley et al. 2020). Such a feature would permit the mycelium-based materials to serve as a new smart wearable. Moreover, fungal materials provide sustainable infrastructure in terms of extension and interconnectivity. In addition, mycelium is able to sense the environmental signals and also respond according to the stimulus, which is an extraordinary feature that can be used to convey and translate the fungal responses into the Boolean circuits, and consequently used in the manufacturing of fungal wearables with biological processing system (Beasley et al. 2020; Adamatzky et al. 2021). A recent study reported the development of fungal wearables with a hemp fabric colonized with the mycelium of oyster fungus *Pleurotus ostreatus*. The stretching capacity and response towards attractants and repellents were studied on the basis of changes in the electric field activity. The research opens the gate to developing fungal-based intelligent wearables for several other applications, such as biomedical applications (Beasley et al. 2020; Adamatzky et al. 2021). An experimental setup for the development of fungal wearable incorporated into the real cloth and connected with electrodes is shown in Fig. 3.

3 Conclusion and Future Prospect

The mycelium materials are derived from mycelial biomass grown on liquid or solid media. In addition to the chemically defined media, such mycelia biomass is also produced by utilizing various agricultural wastes. This process not only recycles the environmental wastes but converts the agricultural and forestry residues into useful and environmentally compatible, multipurpose, and cost-effective materials. Currently, mycelium-based materials are emerging alternatives to conventional packaging, insulators, and leather. High porosity, more sound absorbance, low thermal conductivity, and less density of mycelium-derived panels are more appropriate for thermal and sound insulators instead of synthetic foam or wood fibers. In the current era where the production of quality food is gaining significant importance, it is of utmost importance to explore alternative resources, like mycelium, for the production of functional foods. Their nutritional and therapeutic potential could be further enhanced by combining with other bioactive materials of known functionalities, thus could greatly contribute to human health. To date, the potential of only a few fungi to produce various functional materials has been explored. Further studies to explore

Fig. 3 (**a**) Overall view of the experimental setup. (**b**) Close up of the fungal wearable incorporated into real cloth. (**c**) Exemplar locations of electrodes. Figure reproduced from (Adamatzky et al. 2021)

mycelia pharmaceutical, nutraceuticals, and nutricosmetics properties provide a base for sustainable mycelium materials for food, medicine, and cosmetic applications. Moreover, a combination of interdisciplinary studies with the latest genomics and metabolomics techniques helps to tune the properties of mycelium material at the molecular level. Molecular regulation of properties of mycelium materials paves the pathway for more fungal species in the arena of material science.

Acknowledgments This work was supported by the BRICS STI Framework Programme third call 2019 (2018YFE0123700), National Natural Science Foundation of China (21774039, 51973076), China Postdoctoral Science Foundation (2016 M602291), and the Fundamental Research Funds for Central Universities, Open Research Fund of State Key Laboratory of Polymer Physics and Chemistry, and Changchun Institute of Applied Chemistry, Chinese Academy of Sciences.

Conflict of Interest All authors declare no competing financial conflict of interest associated with the publication of this work.

References

Abhijith R, Ashok A, Rejeesh CR (2018) Sustainable packaging applications from mycelium to substitute polystyrene: a review. Mater Today: Proc. https://doi.org/10.1016/j.matpr.2017.09.211

Adamatzky A (2013) Towards slime mould colour sensor: recognition of colours by *Physarum polycephalum*. Org Electron 14(12):3355–3361

Adamatzky A, Nikolaidou A, Gandia A, Chiolerio A, Dehshibi MM (2021) Reactive fungal wearable. Biosystems 199:104304

Ahirwar R, Jayathilakan K, Reddy KJ, Pandey M, Batra H (2015) Development of mushroom and wheat gluten based meat analogue by using response surface methodology. IJAR 3(1):923–930

Ahmad MF, Ahmad FA, Azad Z, Ahmad A, Alam MI, Ansari JA, Panda BP (2013) Edible mushrooms as health promoting agent. Adv Sci Focus 1(3):189–196

Appels FV, Camere S, Montalti M, Karana E, Jansen KM, Dijksterhuis J, Krijgsheld P, Wösten HA (2019) Fabrication factors influencing mechanical, moisture-and water-related properties of mycelium-based composites. Mater Des 161:64–71

Appels FV, van den Brandhof JG, Dijksterhuis J, de Kort GW, Wösten HA (2020) Fungal mycelium classified in different material families based on glycerol treatment. Commun Biol 3:1. https://doi.org/10.1038/s42003-020-1064-4

Ashby M (2013) Foreword: materials experience-fundamentals of materials and design', *materials experience: fundamentals of materials and design*. Elsevier Inc., pp. xvii–xxii. doi: https://doi.org/10.1016/B978-0-08-099359-1.06001-X

Beasley AE, Powell AL, Adamatzky A (2020) Fungal photosensors. ArXiv. abs/2003.07825 (2020): n. pag

Butu A, Rodino S, Miu B, Butu M (2020) Mycelium-based materials for the ecodesign of bioeconomy. Dig J Nanomater Biostruct 15(4):1129–1140

Camassola M (2013) Mushrooms—the incredible factory for enzymes and metabolites productions. Ferment Technol 2(1):e117

Cardozo FT, Larsen IV, Carballo EV, Jose G, Stern RA, Brummel RC, Camelini CM, Rossi MJ, Simões CM, Brandt CR (2013) In vivo anti-herpes simplex virus activity of a sulfated derivative of *Agaricus brasiliensis* mycelial polysaccharide. Antimicrob Agents Chemother 57(6):2541–2549. https://doi.org/10.1128/AAC.02250-12

Cerimi K, Akkaya KC, Pohl C, Schmidt B, Neubauer P (2019) Fungi as source for new bio-based materials: a patent review. Fungal Biol Biotechnol. https://doi.org/10.1186/s40694-019-0080-y

Chambergo FS, Valencia EY (2016) Fungal biodiversity to biotechnology. Appl Microbiol Biotechnol. https://doi.org/10.1007/s00253-016-7305-2

Chen J, Seviour R (2007) Medicinal importance of fungal β-(1→3), (1→6)-glucans. Mycol Res 111(6):635–652. https://doi.org/10.1016/j.mycres.2007.02.011

Cheung LM, Cheung PCK, Ooi VEC (2003) Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem 81(2):249–255. https://doi.org/10.1016/S0308-8146(02)00419-3

Chien MY, Chen LC, Chen YC, Sheu MT, Tsai YC, Ho HO, Su CH, Liu DZ (2015) Mycelial mattress from a sporangia formation-delayed mutant of *Rhizopus stolonifer* as wound healing-enhancing biomaterial. PLoS One 10:8. https://doi.org/10.1371/journal.pone.0134090

Cho SY, Ryu GH (2020) Effects of mushroom composition on the quality characteristics of extruded meat analog. Korean J Food Sci Technol 52(4):357–362

Dangre DM, Dafne LP, Bhagat RP, Chandekar CJ (2012) Effect of *Pleurotus nebrodensis* extract on melanin synthesis: a natural alternative for cosmetics. Int J Med Aromat Plants 4(2):579–588

de Ulzurrun GV, Baetens JM, Van den Bulcke J, De Baets B (2017) Modelling three-dimensional fungal growth in response to environmental stimuli. J Theor Biol 414:35–49

Di Z, Shi Z, Ullah MW, Li S, Yang G (2017) A transparent wound dressing based on bacterial cellulose whisker and poly(2-hydroxyethyl methacrylate). Int J Biol Macromol 105:638–644

Dias PP, Jayasinghe LB, Waldmann D (2021) Investigation of mycelium-Miscanthus composites as building insulation material. Results Mater 10:100189. https://doi.org/10.1016/j.rinma.2021.100189

Dubost NJ, Beelman RB, Peterson D, Royse DJ (2006) Identification and quantification of ergothioneine in cultivated mushrooms by liquid chromatography-mass spectroscopy. Int J Med Mushrooms. https://doi.org/10.1615/IntJMedMushr.v8.i3.30

El Enshasy HA, Hatti-Kaul R (2013) Mushroom immunomodulators: unique molecules with unlimited applications. Trends Biotechnol. https://doi.org/10.1016/j.tibtech.2013.09.003

Farooq U, Ullah MW, Yang Q, Aziz A, Xu J, Zhou L, Wang S (2020) High-density phage particles immobilization in surface-modified bacterial cellulose for ultra-sensitive and selective electrochemical detection of Staphylococcus aureus. Biosens Bioelectron 157:112163. https://doi.org/10.1016/j.bios.2020.112163

Florentina H, Cristina S (2010) Researches upon the tensile strength and elongation at break of the leather substitutes, Annals of the Oradea University. Fascicle Manage Technol Eng 9. https://doi.org/10.15660/auofmte.2010-2.1865

Gandia A, van den Brandhof JG, Appels FV, Jones MP (2021) Flexible fungal materials: shaping the future. Trends Biotechnol. https://doi.org/10.1016/j.tibtech.2021.03.002

Gunawardena D, Bennett L, Shanmugam K, King K, Williams R, Zabaras D, Head R, Ooi L, Gyengesi E, Münch G (2014) Anti-inflammatory effects of five commercially available mushroom species determined in lipopolysaccharide and interferon-γ activated murine macrophages. Food Chem. https://doi.org/10.1016/j.foodchem.2013.10.015

Haneef M, Ceseracciu L, Canale C, Bayer IS, Heredia-Guerrero JA, Athanassiou A (2017) Advanced materials from fungal mycelium: fabrication and tuning of physical properties. Sci Rep 7:1. https://doi.org/10.1038/srep41292

Hawksworth DL, Lücking R (2017) Fungal diversity revisited: 2.2 to 3.8 million species. Microbiol Spectr. https://doi.org/10.1128/microbiolspec.funk-0052-2016

He X, Fang J, Guo Q, Wang M, Li Y, Meng Y, Huang L (2020) Advances in antiviral polysaccharides derived from edible and medicinal plants and mushrooms. Carbohydr Polym 229:115548. https://doi.org/10.1016/j.carbpol.2019.115548

Howitz KT, Sinclair DA (2008) Xenohormesis: sensing the chemical cues of other species. Cell 133(3):387–391

Hsu KD, Chen HJ, Wang CS, Lum CC, Wu SP, Lin SP, Cheng KC (2016) Extract of Ganoderma formosanum mycelium as a highly potent tyrosinase inhibitor. Sci Rep 6(1):1–9

Hyde KD, Bahkali AH, Moslem MA (2010) Fungi—An unusual source for cosmetics. Fungal Divers. https://doi.org/10.1007/s13225-010-0043-3

Ibrar M, Ullah MW, Manan S, Farooq U, Rafiq M, Hasan F (2020) Fungi from the extremes of life: An untapped treasure for bioactive compounds. Appl Microbiol Biotechnol 104(7):2777–2801

Ioannis G, Harvey LM, McNeil B (2005) Scleroglucan. In: Biopolymers online. Wiley-VCH Verlag GmbH et Co. KGaA. https://doi.org/10.1002/3527600035.bpol6002

Islam MR, Tudryn G, Bucinell R, Schadler L, Picu RC (2018) Mechanical behavior of mycelium-based particulate composites. J Mater Sci 53(24):16371–16382

Israilides C, Kletsas D, Arapoglou D, Philippoussis A, Pratsinis H, Ebringerová A, Hříbalová V, Harding SE (2008) In vitro cytostatic and immunomodulatory properties of the medicinal mushroom *Lentinula edodes*. Phytomedicine 15(6–7):512–519

Jalmi P, Bodke P, Wahidullah S, Raghukumar S (2012) The fungus *Gliocephalotrichum simplex* as a source of abundant, extracellular melanin for biotechnological applications. World J Microbiol Biotechnol 28(2):505–512

Jiang L, Walczyk D, McIntyre G, Bucinell R, Li B (2019) Bioresin infused then cured mycelium-based sandwich-structure biocomposites: resin transfer molding (RTM) process, flexural properties, and simulation. J Clean Prod 207:123–135

Jikai L (2002) Biologically active substances from mushrooms in Yunnan, China. Heterocycles 57(1):157–167

Jones M, Gandia A, John S, Bismarck A (2021) Leather-like material biofabrication using fungi. Nat Sustainability 4(1):9–16

Jones M, Huynh T, Dekiwadia C, Daver F, John S (2017) Mycelium composites: a review of engineering characteristics and growth kinetics. J Bionanosci 11(4):241–257

Jones M, Mautner A, Luenco S, Bismarck A, John S (2020) Engineered mycelium composite construction materials from fungal biorefineries: a critical review. Mater Des. https://doi.org/10.1016/j.matdes.2019.108397

Joshi K, Meher MK, Poluri KM (2020) Fabrication and characterization of bioblocks from agricultural waste using fungal mycelium for renewable and sustainable applications. ACS Appl Bio Mater 3(4):1884–1892

Kabbaj W, Breheret S, Guimberteau J, Talou T, Olivier JM, Bensoussan M, Sobal M, Roussos S (2002) Comparison of volatile compound production in fruit body and in mycelium of Pleurotus ostreatus identified by submerged and solid-state cultures. Appl Biochem Biotechnol 102(1):463–469

Kalač P (2013) A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. J Sci Food Agric. https://doi.org/10.1002/jsfa.5960

Kalitukha L, Sari M (2019) Fascinating vital mushrooms. Tinder fungus (Fomes fomentarius (L.) Fr.) as a dietary supplement. Int J Res Stud Sci Eng Technol 6:1–9

Karana E, Pedgley O, Rognoli V (2013) 'Introduction to materials experience', materials experience: fundamentals of materials and design. Elsevier Inc, pp xxv–xxxiii. https://doi.org/10.1016/B978-0-08-099359-1.02001-4

Keleş A, Koca I, Gençcelep H (2011) Antioxidant properties of wild edible mushrooms. J Food Process Technol 2(6):2–6

Khamrai M, Banerjee SL, Kundu PP (2018) A sustainable production method of mycelium biomass using an isolated fungal strain *Phanerochaete chrysosporium* (accession no: KY593186): its exploitation in wound healing patch formation. Biocatal Agric Biotechnol 16:548–557

Khan S, Siddique R, Huanfei D, Shereen MA, Nabi G, Bai Q, Manan S, Xue M, Ullah MW, Bowen H (2021) Perspective applications and associated challenges of using nanocellulose in treating bone-related diseases. Front Bioeng Biotechnol 9:350. https://doi.org/10.3389/fbioe.2021.616555

Khan S, Ul-Islam M, Ikram M, Islam SU, Ullah MW, Israr M, Jang JH, Yoon S, Park JK (2018) Preparation and structural characterization of surface modified microporous bacterial cellulose scaffolds: a potential material for skin regeneration applications in vitro and in vivo. Int J Biol Macromol 117:1200–1210. https://doi.org/10.1016/j.ijbiomac.2018.06.044

Khan S, Ul-Islam M, Ikram M, Ullah MW, Israr M, Subhan F, Kim Y, Jang JH, Yoon S, Park JK (2016) Three-dimensionally microporous and highly biocompatible bacterial cellulose–gelatin composite scaffolds for tissue engineering applications. RSC Adv 6(112):110840–110849

Kim K, Choi B, Lee I, Lee H, Kwon S, Oh K, Kim AY (2011) Bioproduction of mushroom mycelium of *Agaricus bisporus* by commercial submerged fermentation for the production of meat analogue. J Sci Food Agric 91(9):1561–1568. https://doi.org/10.1002/jsfa.4348

Kolundžić M, Grozdanić NĐ, Dodevska M, Milenković M, Sisto F, Miani A, Farronato G, Kundaković T (2016) Antibacterial and cytotoxic activities of wild mushroom *Fomes fomentarius* (L.) Fr. Polyporaceae Ind Crops Prod 79:110–115. https://doi.org/10.1016/j.indcrop.2015.10.030

Lee JH, Yoo SB, Kim NY, Cha MJ, Jahng JW (2008) Interleukin-6 and the hypothalamic-pituitary-adrenal activation in a tumor bearing mouse. Int J Neurosci 118(3):355–364. https://doi.org/10.1080/00207450701592915

Li X, Li Y, Jeong JH, Lee KT, Choi HD, Son BW (2003) Screening of tyrosinase inhibiting activity from the marine-derived fungus. Korean J Pharmacogn 34(2):138–141. https://doi.org/10.1034/j.1600-0749.2003.83100.x

Liu H, He L (2012) Comparison of the moisture retention capacity of Tremella polysaccharides and hyaluronic acid. J Anhui Agric Sci 40:13093–13094

Luo Y, Teng Z, Li Y, Wang Q (2015) Solid lipid nanoparticles for oral drug delivery: chitosan coating improves stability, controlled delivery, mucoadhesion and cellular uptake. Carbohydr Polym 122:221–229

Lupo MP, Cole AL (2007) Cosmeceutical peptides. Dermatol Ther 20(5):343–349

Ma G, Yang W, Zhao L, Pei F, Fang D, Hu Q (2018) A critical review on the health promoting effects of mushrooms nutraceuticals. Food Sci Hum Wellness 7(2):125–133

Madurwar MV, Ralegaonkar RV, Mandavgane SA (2013) Application of agro-waste for sustainable construction materials: a review. Constr Build Mater 38:872–878

Manan S, Ullah MW, Ul-Islam M, Atta OM, Yang G (2021) Synthesis and applications of fungal mycelium-based advanced functional materials. J Bioresour Bioprod:15. https://doi.org/10.1016/j.jobab.2021.01.001

Mao L, Wang L, Zhang M, Ullah MW, Liu L, Zhao W, Li Y, Ahmed AA, Cheng H, Shi Z, Yang G (2021) In situ synthesized selenium nanoparticles-decorated bacterial cellulose/Gelatin hydrogel with enhanced antibacterial, antioxidant, and anti-inflammatory capabilities for facilitating skin wound healing. Adv Healthc Mater 29:2100402. https://doi.org/10.1002/adhm.202100402

Marie SI, Gans RJ, Xiaohong ZV (2003) Anhydrous cosmetic compositions containing mushroom extract'. US: US Patent. http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PALL&s1=6645502.PN

Mau J (2005) The umami taste of edible and medicinal mushrooms. Int J Med Mushrooms 7:119–125

Meyer M, Dietrich S, Schulz H, Mondschein A (2021) Comparison of the technical performance of leather, artificial leather, and trendy alternatives. Coatings 11(2):226. https://doi.org/10.3390/coatings11020226

Millikan LE (2001) Cosmetology, cosmetics, cosmeceuticals: definitions and regulations. Clin Dermatol 19(4):371–374

Mingyi Y, Belwal T, Devkota HP, Li L, Luo Z (2019) Trends of utilizing mushroom polysaccharides (MPs) as potent nutraceutical components in food and medicine: a comprehensive review. Trends Food Sci Technol 92:94–110

Mohanty C, Das M, Sahoo SK (2012) Sustained wound healing activity of curcumin loaded oleic acid based polymeric bandage in a rat model. Mol Pharm 9(10):2801–2811

Mohorčič M, Friedrich J, Renimel I, André P, Mandin D, Chaumont JP (2007) Production of melanin bleaching enzyme of fungal origin and its application in cosmetics. Biotechnol Bioprocess Eng 12(3):200–206

Moliszewska E (2014) Mushroom flavour. Folia Biologica et Oecologica 10:80–88

Munarin F, Tanzi MC, Petrini P (2012) Advances in biomedical applications of pectin gels. Int J Biol Macromol 51(4):681–689

MycoComposite™—Ecovative Design (n.d.)

Narayanan KB, Zo SM, Han SS (2020) Novel biomimetic chitin-glucan polysaccharide nano/microfibrous fungal-scaffolds for tissue engineering applications. Int J Biol Macromol 149:724–731

Ocampo A, Reddy P, Belmonte JCI (2016) Anti-aging strategies based on cellular reprogramming. Trends Mol Med. https://doi.org/10.1016/j.molmed.2016.06.005

Papp N, Rudolf K, Bencsik T, Czégényi D (2017) Ethnomycological use of Fomes fomentarius (L.) Fr. and Piptoporus betulinus (Bull.) P. Karst. in Transylvania, Romania. Genet Resour Crop Evol 64(1):101–111

Pegler DN (2001) Useful fungi of the world: Amadou and Chaga. Mycologist 4(15):153–154

Pelletier MG, Holt GA, Wanjura JD, Bayer E, McIntyre G (2013) An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates. Ind Crop Prod 51:480–485. https://doi.org/10.1016/j.indcrop.2013.09.008

Poucheret P, Fons F, Rapior S (2006) Biological and pharmacological activity of higher fungi: 20-year retrospective analysis. Cryptogam Mycol 27(4):311

Rajan K, Bocchini S, Chiappone A, Roppolo I, Perrone D, Castellino M, Bejtka K, Lorusso M, Ricciardi C, Pirri CF, Chiolerio A (2017) WORM and bipolar inkjet printed resistive switching devices based on silver nanocomposites. Flex Print Electron 2(2):024002. https://doi.org/10.1088/2058-8585/aa64be

Rathore H, Prasad S, Kapri M, Tiwari A, Sharma S (2019) Medicinal importance of mushroom mycelium: mechanisms and applications. J Funct Foods 56:182–193

Rathore H, Prasad S, Sharma S (2017) Mushroom nutraceuticals for improved nutrition and better human health: a review. Pharma Nutr 5(2):35–46

Sajjad W, He F, Ullah MW, Ikram M, Shah SM, Khan R, Khan T, Khalid A, Yang G, Wahid F (2020) Fabrication of bacterial cellulose-curcumin nanocomposite as a novel dressing for partial thickness skin burn. Front Bioeng Biotechnol 8. https://doi.org/10.3389/fbioe.2020.553037

Samsudin EM, Ismail LH, Kadir AA (2016) A review on physical factors influencing absorption performance of fibrous sound absorption material from natural fibers. ARPN J Eng Appl Sci 11(6):3703–3711

Sator PG, Schmidt JB, Hönigsmann H (2003) Comparison of epidermal hydration and skin surface lipids in healthy individuals and in patients with atopic dermatitis. J Am Acad Dermatol. https://doi.org/10.1067/mjd.2003.105

Schmidt O (2006) Wood and tree fungi: biology, damage, protection, and use. Springer, Berlin Heidelberg. https://doi.org/10.1007/3-540-32139-X

Seniuk OF, Gorovoj LF, Beketova GV, Savichuk HO, Rytik PG, Kucherov II, Prilutskay AB, Prilutsky AI (2011) Anti-infective properties of the melanin-glucan complex obtained from medicinal tinder bracket mushroom, Fomes fomentarius (L.: Fr.) Fr. (Aphyllophoromycetideae). Int J Med Mushrooms 13(1):7–18

Sibinski M, Jakubowska M, Sloma M (2010) Flexible temperature sensors on fibers. Sensors 10(9):7934–7946

Sławińska A, Fornal E, Radzki W, Skrzypczak K, Zalewska-Korona M, Michalak-Majewska M, Parfieniuk E, Stachniuk A (2016) Study on vitamin D2 stability in dried mushrooms during drying and storage. Food Chem 199:203–209

Stanikunaite R, Khan SI, Trappe JM, Ross SA (2009) Cyclooxygenase-2 inhibitory and antioxidant compounds from the truffle elaphomyces granulatus. Phytother Res 23(4):575–578

Tacer-Caba Z, Varis JJ, Lankinen P, Mikkonen KS (2020) Comparison of novel fungal mycelia strains and sustainable growth substrates to produce humidity-resistant biocomposites. Mater Des 192:108728. https://doi.org/10.1016/j.matdes.2020.108728

Thakur VK, Thakur MK, Raghavan P, Kessler MR (2014) Progress in green polymer composites from lignin for multifunctional applications: a review. ACS Sustain Chem Eng 2(5):1072–1092. https://doi.org/10.1021/sc500087z

Tomiyama T, Kaihou S, Ishida M, Nishikawa H, Yamazaki N, Tsuji K, Mitsutake S, Igarashi Y (2008) The water retention effects and action for atopic dermatitis-like symptoms of ethyl alcohol extract (from Tamogi-take mushroom) on animal model of atopic dermatitis. Nippon Eiyo Shokuryo Gakkaishi. https://doi.org/10.4327/jsnfs.61.21

Tuli HS, Sandhu SS, Sharma AK (2014) Pharmacological and therapeutic potential of Cordyceps with special reference to Cordycepin, 3. Biotech 4(1):1–12. https://doi.org/10.1007/s13205-013-0121-9

Ul-Islam M, Subhan F, Islam SU, Khan S, Shah N, Manan S, Ullah MW, Yang G (2019) Development of three-dimensional bacterial cellulose/chitosan scaffolds: analysis of cell-scaffold interaction for potential application in the diagnosis of ovarian cancer. Int J Biol Macromol 137:1050–1059

Ulziijargal E, Yang JH, Lin LY, Chen CP, Mau JL (2013) Quality of bread supplemented with mushroom mycelia. Food Chem 138(1):70–76

Urbain P, Singler F, Ihorst G, Biesalski HK, Bertz H (2011) Bioavailability of vitamin D 2 from UV-B-irradiated button mushrooms in healthy adults deficient in serum 25-hydroxyvitamin D: a randomized controlled trial. Eur J Clin Nutr 65(8):965–971

Wang CR, Ng TB, Li L, Fang JC, Jiang Y, Wen TY, Qiao WT, Li N, Liu F (2011) Isolation of a polysaccharide with antiproliferative, hypoglycemic, antioxidant and HIV-1 reverse transcriptase inhibitory activities from the fruiting bodies of the abalone mushroom *Pleurotus abalonus*. J Pharm Pharmacol 63(6):825–832

Wang X, Zhang Z, Zhao M (2015) Carboxymethylation of polysaccharides from Tremella fuciformis for antioxidant and moisture-preserving activities. Int J Biol Macromol. https://doi.org/10.1016/j.ijbiomac.2014.08.045

Wu Y, Choi MH, Li J, Yang H, Shin HJ (2016a) Mushroom cosmetics: the present and future. Cosmetics 3(3):22. https://doi.org/10.3390/cosmetics3030022

Wu Y, Li S, Li H, Zhao C, Ma H, Zhao X, Wu J, Liu K, Shan J, Wang Y (2016b) Effect of a polysaccharide from Poria cocos on humoral response in mice immunized by H1N1 influenza and HBsAg vaccines. Int J Biol Macromol 91:248–257. https://doi.org/10.1016/j.ijbiomac.2016.05.046

Xu Y, Liu X, Jiang Q, Yu D, Xu Y, Wang B, Xia W (2021) Development and properties of bacterial cellulose, curcumin, and chitosan composite biodegradable films for active packaging materials. Carbohydr Polym 260:117778. https://doi.org/10.1016/j.carbpol.2021.117778

Yang Z, Zhang F, Still B, White M, Amstislavski P (2017) Physical and mechanical properties of fungal mycelium-based biofoam. J Mater Civ Eng 29(7):04017030. https://doi.org/10.1061/(ASCE)MT.1943-5533.0001866

Yu CH, Kan SF, Shu CH, Lu TJ, Sun-Hwang L, Wang PS (2009) Inhibitory mechanisms of *Agaricus blazei* Murill on the growth of prostate cancer in vitro and in vivo. J Nutr Biochem 20(10):753–764

Zhang Y, Wang D, Chen Y, Liu T, Zhang S, Fan H, Liu H, Li Y (2021) Healthy function and high valued utilization of edible fungi. Food Sci Hum Wellness 10(4):408–420

Zhu X, Mao Y, Guo M, Yu H, Hao L, Hua Q, Lu Z, Hong M, An F (2020) Enhancement of anti-acne effect of Scutellaria baicalensis extract by fermentation with symbiotic fungus *Penicillium decumbens*. J Biosci Bioeng 130(5):457–463. https://doi.org/10.1016/j.jbiosc.2020.06.008

Ziegler AR, Bajwa SG, Holt GA, McIntyre G, Bajwa DS (2016) Evaluation of physico-mechanical properties of mycelium reinforced green biocomposites made from cellulosic fibers. Appl Eng Agric 32(6):931–938. https://doi.org/10.13031/aea.32.11830

Fungal Biopolymers as an Alternative Construction Material

Sabu John

Abstract Mycelium composites are a class biopolymeric composites, consisting of cost-effective and environmentally sustainable materials. Globally, this class of composites is currently experiencing burgeoning research interest. With increasing pressure on cheaper materials with sustainable and 'green' credentials, mycelium composites hold some promise in this space, particularly in the construction industry, where the cost-performance indicator is a critical consideration. This material type uses the biological phenomenon of fungal growth to transition agri-waste materials to low-cost and low energy-embodied construction materials. Mycelium composites are inherently lossy in constitution and hence, have natural thermal and acoustic insulating properties. They have also shown impressive fire-resistant properties. These lossy properties, however, do not attribute good mechanical properties to mycelium composites, which are further compounded by its low hydrophobicity. However, some recent developments in the processing of the mycelium composites using 3D printing technologies by chemical manipulation of its constituents and self-healing mycelium structures, point this class of composites towards more flexural, robust, and strength-based semi-structural applications.

Keywords Mycelium · Mechanical properties · Fire and water resistance · Manufacturing

1 Introduction

Over the past ten years, the building and construction industry has been confronted with increasing demand, due to a burgeoning global population and for the materials used in this industry to have a reduced carbon footprint (Madurwar et al. 2013; Pheng and Hou 2019; UHPC 2020). Manufacture of these conventional building materials can be deleterious to the natural environment (Madurwar et al. 2013). In

S. John (✉)
School of Engineering, RMIT University, Bundoora East Campus, Bundoora 3083, VIC, Australia
e-mail: sabu.john@rmit.edu.au

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_10

nominal living domains, the embodied energy in the use of materials, including its fabrication, transportation to site can be up to 36% (Sartori and Hestnes 2007). Often they consist of energy in the materials used in the construction industry can be significant, especially when concrete and the accompanying steel reinforcements are used. In addition, there is a growing demand from the construction industry, worldwide to employ materials with lower carbon footprints (UHPC 2020).

With the growing world population, especially in Asia, where 4.5+ billion (Roser et al. 2019) people need to produce food, there is a significant production of associated agricultural residues like straw and rice husks. The aggregated organic production of India and South East Asia from these agricultural by-products is up to 1 billion tons per annum (Bhuvaneshwari et al. 2019; Tun et al. 2019; Nawawi et al. 2019b). These by-products are considered to have a low utility value and are usually disposed or burned, which exacerbates the climate change problem (Defonseka 2014).

Mycelium (vegetative part of filamentous fungi) binds organic matter in a natural biological process, which results in value-added products(Holt et al. 2012; Pelletier et al. 2013; Jones et al. 2017; Haneef et al. 2017; Islam et al. 2017) from from agri- and industrial waste materials (Sustainability Victoria 2019; DPCleanTech 2019). The mycelium bonds with the agricultural substrate to form a bio-polymer composite, in a way that is not unlike that of conventional bonding of synthetic polymers and fillers or fibres to form polymeric composite materials (Pelletier et al. 2013; Thakur and Siṅgha 2013).

The virtues of mycelium-derived materials against traditional synthetic materials with their low cost, embodied energy, density and biodegradability (Arifin and Yusuf 2013; Haneef et al. 2017; Abhijith et al. 2018). Using a range of suitable agricultural substrates, fungi species and processing techniques, mycelium composites can be engineered to meet specific physical and mechanical properties, such as strength, heat resistance, thermal and sound insulation (Holt et al. 2012; Pelletier et al. 2013; Jones et al. 2017; Haneef et al. 2017). The paves the way for a biodegradable substitute to synthetic planar resources (e.g., plastic sheets and films) (Haneef et al. 2017), low-density substances (e.g. synthetic plastics and foams) (Holt et al. 2012; Pelletier et al. 2013; Travaglini et al. 2013; López Nava et al. 2016), and semi-structural supplies (e.g. decking,, flooring, furniture and paneling) (Jiang et al. 2016, Jiang et al. 2017, Islam et al. 2017, Abrams 2014). These applications enable green or sustainable practices in the construction industry.

However, mycelium composites currently suffer from a few issues, primarily stemming from their lossy morphological constitution, or structurally sub-par mechanical properties, high hydrophilicity and its shortfalls in large-scale manufacturing. These limitations make the case for further research in the field.

2 Mycelium Composite Processing and Current Mycelium Commercial Products

2.1 Mycelium Composite Processing

Mycelium is the collective name given to the hyphae, which is the vegetative filamentous structure of a fungus. Fungi being natural entities, their products are renewable valuable structural polymers like chitin and chitosan (alternatives to cellulose polymers in plant cell walls) (Fig. 1). Chitin is a linear macromolecule consists of N-acetylglucosamine units, which is the major component of exoskeletons of most of the insects and other arthropods (Rinaudo 2007).

The hyphae tubular cell walls consist principally of chitin. It also has chitosan, glucan, cellulose and relatively small amounts of proteins present (Bartnicki-Garcia 1968; Wessels et al. 1990).

Mycelium composites of fungi are manufactured by low-energy process, schematically shown in Fig. 2. Any carbohydrate-based raw material can be used as the fibrous substrate for the mycelium to feed on (Kavanagh 2005; Jones et al. 2017). The substrate type can however, dictate the eventual mechanical and physical properties of the mycelium composite.

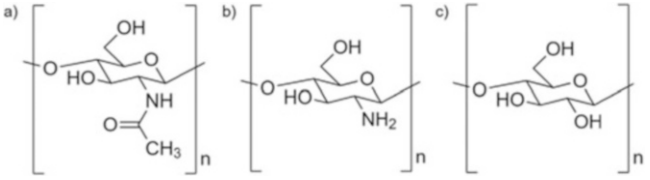


Fig. 1 Structures of chitin (**a**), chitosan (**b**) and cellulose (**c**). (Reproduced from Jones et al. 2020—Under a Creative Commons Attribution 4.0 International License)

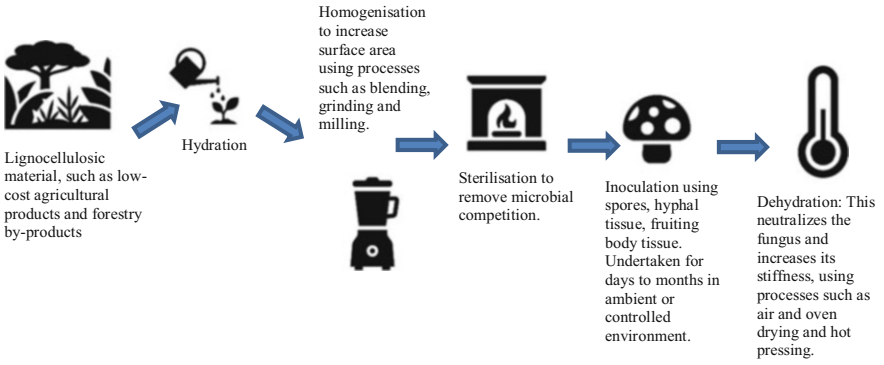


Fig. 2 Mycelium composites fabrication process (Modified from Jones et al. 2020—Under a Creative Commons Attribution 4.0 International License)

Substrates are hydrated in water and the duration of this hydration process is substrate dependent (Elsacker et al. 2019). Other substrates like rice hulls absorb less amount of moisture, thus making the duration of soaking less important compared to the inoculation media (e.g. wheat grains), those are highly swelling and demands long soaking duration (48 h) (Jones et al. 2018a). The soaked raw material is subsequently blend to increase the surface area and sterilized (Elsacker et al. 2019; Jones et al. 2020).

By natural fungal growth, the mycelium composite is formed in a mould, which binds the organic substrate with the growing mycelium sourced from spores in a liquid or a fruit body tissue (Holt et al. 2012; Jones et al. 2017, 2018a, 2020).

On inoculation, moulds can be preserved under ambient conditions or in an environment at ~25–27 °C for a growth period for several days to months based on the fungal species and substrate used to achieve desired degree of bonding (Griffin 1996; Jones et al. 2017).

After the growth period, the mycelium composite is released from the mould and subjected to elevated temperatures and air dried at about 50 °C for 48 h to neutralize or denature the fungal material (Jones et al. 2018a). Hot pressing can further dehydrate and increase the flexural stiffness of the mycelium composite (Jones et al. 2020).

2.2 Current Commercial Products

Current commercial products include materials for insulation and door cores. The composites of mycelium are commercially available for such applications in the USA and Indonesia, however, technical information about these composites is presently not publicly available (Ecovative Design LLC 2019a, b, MycoTech 2019) (Fig. 3a). Similarly, the mycelium-based acoustic insulation foams are available (Ecovative Design LLC 2019b, Mogu 2019) (Fig. 3b). Flexible mycelium structures are increasingly gaining traction in the textile and construction industries (Ecovative Design LLC 2019a) (Fig. 3c). The use of bio-resins with mycelium composites can expand its applications to semi-structural uses such as floorings and cabinets (Mogu 2019; Jiang et al. 2019) (Fig. 3d).

Fungal mycelium foams are used for packaging purposes and the IKEA expressed concern in adopting such materials for packaging (Dell Inc 2016, Gosden 2016). Apart from the aforementioned examples of commercial applications of mycelium composites, the wider uptake, of any significance, for this sustainable bio-polymeric composite, is yet to materialize. However, there is some mycelium-based material activity in the Australia, Austria Belgium, Indonesia, Italy, Netherlands, Switzerland and USA (Huynh and Jones 2018, Ecovative Design LLC 2019a, b, MycoTech 2019, MycoWorks 2019, KrownDesign 2019, Officina Corpuscoli 2019, TUDelft 2019, Zurich ETH 2019, Utrecht University 2019).

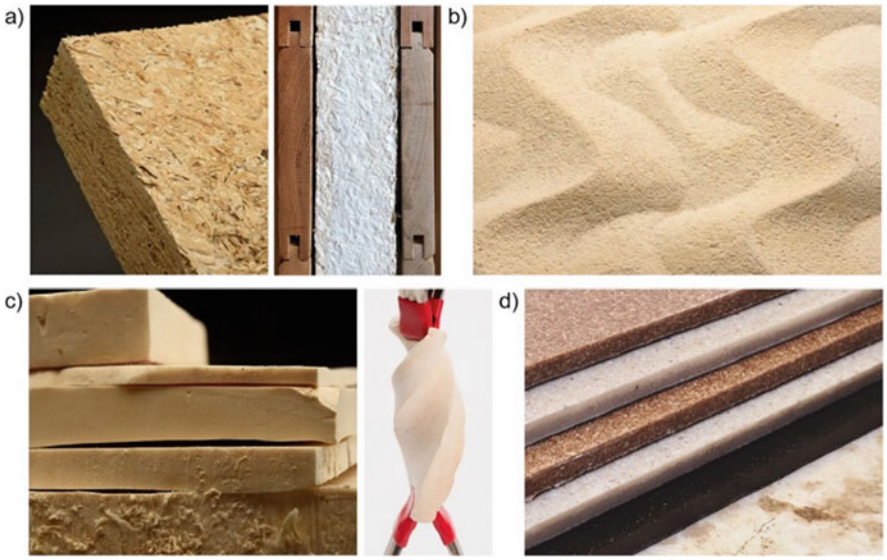


Fig. 3 Fungal mycelium composite construction materials as particleboard replacements for wall paneling and door cores (**a**), acoustic foams (**b**), flexible insulation foams (**c**) and resin-infused laminate flooring (**d**) (Reproduced from Jones et al. 2020—Under a Creative Commons Attribution 4.0 International License)

3 Material Properties of Engineered Mycelium Composites

3.1 Effect of the Mycelium Growth Interactions on Composite Mechanical Performance

Recent investigations on chitin-glucan extracts derived from fungal mycelium have found the mycelium binder to be relatively sturdy (tensile strengths up to 25 MPa (Jones et al. 2019b) and for that of fruit body extract up to 200 MPa (Nawawi et al. 2019a). The fungal mycelium constituent of composites, owing to its density and weak interface with the substrate, is often attributed for its narrow mechanical performance (Travaglini et al. 2013; Jiang et al. 2019). The appears to be a fungi-dependent variation in the substrate-mycelium interface bonding strength (Jones et al. 2018a, b) and this does appear to influence the mechanical properties of the product.

The hyphal architecture, based on its mono-, di and trimitic morphology is also reported to not significantly affect the mechanical properties of the mycelium composite, especially when compared to the influence of the amount of mycelial biomass (binder) in the mix (Jones et al. 2019a, 2020).

3.2 *Influence of the Substrate Filler on Composite Mechanical Performance*

Both physical and mechanical qualities of grown mycelium composites are often reley on the substrate. As such composites typically have a density ranging between 60 and 300 kg/m^3, with composites produced using agricultural byproduct filler phase (e.g. bast fibers or straw), possessing lower densities (60–130 kg/m^3) compared to the composites containing the byproducts of forestry materials (e.g. sawdust: 87–300 kg/m^3) (Table 1).

From Table 1, it shows that sawdust (0.05–0.18 MPa) consists of higher tensile strengths compared to straw substrates (0.01–0.04 MPa). Fungal growth, powered typically by simple sugars (e.g. fructose, glucose and sucrose) appears to be directly

Table 1 Density, Youngs modulus, UTS values of as-grown mycelium composites with fibrous and particulate agri-filler substrates for various loading configurations (Reproduced from Jones et al. 2020—Under a Creative Commons Attribution 4.0 International License)

Loading	Substrate Type	Substrate	Density (kg/m^3)	Youngs Modulus (MPa)	Ultimate Tensile Strength (Mpa)
Tension	Fibrous particulate	Rapseed straw[a], Beech sawdust[a], Red Oak sawdust[b]	115 170 300	3.0 13.0 1.3	0.025 0.05 0.18
Compression	Fibrous	Flax hurd[c] Hemp hurd[c] Wheat straw[d]	99 94 192	0.73 0.64 –	– – 0.17
	Particulate	Pine shavings[c] Red oak sawdust[b] White oak sawdust[d]	87 300 552	0.14 1.0 –	– 0.49 1.1
Flexure	Fibrous	Cotton fibres[a] Rapeseed straw[a]	130 115	1.0 1.5	0.05 0.14
	Particulate	Beech sawdust[a]	170	9.0	0.29

However, scanty data is available on the mechanical properties of mycelium composites for different groups of substrates

[a] Appels et al. (2019)
[b] Travaglini et al. (2013)
[c] Elsacker et al. (2019)
[d] Ghazvinian et al. (2019)

linked to enhanced tensile strength of composites of the mycelium (Travaglini et al. 2013; He et al. 2014; Jones et al. 2019b, 2020).

Fibrous substrates (like sawdust) appear to yield lower compressive strengths compared to particle-based substrates (like straw) (Xia et al. 2013; Ashby et al. 2018). The inverse correlation between increased porosity and compressive strength suggests that the extent of digestion of the fungus will increase the porosity of composite, hence its mechanical performance. (Kavanagh 2005). The compressive features of as-grown fungal composites have also been known to be largely independent on the particle size of the substrate filler phase (Islam et al. 2018).

For flexural strength, substrate morphology appears to be an insignificant factor, even though the expectation of heightened flexural strength is expected, especially when the fibres are aligned in the flexural loading direction (Chand and Fahim 2008). Again, the significance of binder presence, due to adequate nutrition, seems to have a stronger influence on flexural strength than substrate morphology (Jones et al. 2020).

Studies on *G. lucidium* and *P. ostreatus* by Haneef et al. (2017) to explore the effect of nutrition types on the growing mycelia, it was shown that when using pure amorphous cellulose as well as a cellulose potato-dextrose broth (PDB), the presence of PDP resulted in a lower Young's modulus and a higher strain to failure, compared to the mycelium grown with just cellulose.

To summarize, the more fungal growth present, due to appropriate nutrient content in the substrate, the higher the mechanical performance. This performance level is however limited to low-strength foam-based mycelium composites, unless further downstream processes, such as cold or hot pressing, as discussed below, is implemented (Jones et al. 2019b, 2020). In addition, in some studies such as in Haneef et al. 2017, it was shown that the nutrition of the growing mycelium can influence the mechanical qualities of the mycelium composite. This raises for the prospect that certain mycelium species can be engineered to possess desired mechanical properties by tailoring its growth with appropriate nutrition sources.

3.3 Enhancement of the Mechanical Properties of Mycelium Composites

Consolidation using compression by hot or cold pressing improves the mechanical properties of mycelium composites by decreasing its porosity and enhancing its density (Dai et al. 2007). In the mycelium composites produced by *P. ostreatus* developed on rapeseed straw, the cold pressing was linked with a significant improvement in tensile strength (0.01–0.03 MPa) as well as higher elastic modulus (2–9 MPa) (Appels et al. 2019). In addition, significant improvement was seen the flexural properties of the moulds with higher flexural strengths (0.06–0.21 MPa) and moduli (1–15 MPa).

Greater improvements in mechanical performance can be achieved using hot pressing procedures. The tensile properties of hot-pressed *P. ostreatus* and *T. multicolor* composites established on rapeseed straw showed significantly higher compared to as-grown samples (Appels et al. 2019). In addition, the hot pressing improves the flexural strength of *P. ostreatus* and *T. multicolor* composites grown on the rapeseed straw (Appels et al. 2019). Pressing, both hot and cold, results in reduced moisture, which results in lower failure strain (Sombatsompop and Chaochanchaikul 2004).

The inclusion of styrene-butadiene rubber, or natural reinforcements (e.g. cellulose nanofibrils) in mycelium composites has shown some marginal benefits (Jones et al. 2020).

The mycelium composites produced by cotton seed hulls with *P. ostreatus* showed compressive strength up to 177 kPa, it is almost doubled by addition of 5 vol% styrene-butadiene rubber (343 kPa) according to He et al. (2014). Such feature is due to the void volume reduction as well as increased volume density (181–225 kg/m^3) in association with the inclusion of the latex—polymeric microparticles in water (He et al. 2014). A smaller quantities of nanocellulose could be used to increase the mechanical performance with improved in flexural strength (Sun et al. 2019).

Low threshold amounts of nanocellulose was found to produce viable improvements in mechanical performance of the mycelium composites and that the inclusion of latex showed a small improvement in mechanical performance, albeit at increased cost and with reduced environment sustainability benefits (Jones et al. 2020).

3.4 Thermal Conductivity Properties of Mycelium Composites for Insulation Applications

The mycelium composites, incorporating hemp fibres and straw have low densities (57–99 kg/m^3) as well as thermal conductivities (0.04–0.08 W/m·K). These performance values compare well with synthetic insulators (e.g. glass wool, 57 kg/m^3, 0.04 W/m·K; extruded polystyrene insulation, XPS, 34 kg/m^3, 0.03 W/m·K) (Papadopoulos 2005) besides other natural insulators like sheep wool (18 kg/m^3, 0.05 W/ m·K) and kenaf (105 kg/m^3, 0.04 W/m·K) (Asdrubali et al. 2015).

A comprehensive comparison of mycelium composites as a viable low density as well as low thermal conductivity material, in comparison with commercial building insulators is reported in Jones et al. 2020.

3.5 Acoustic Properties of Mycelium and its Composites as Noise Barriers

Mycelium, given its inherent lossy structure, is an excellent acoustic absorber, showing good sound absorption for low (<1 kHz) to mid-frequency range noise, which is the typical frequency range from vehicular-based road noise (Pelletier et al. 2019). Mycelium composites can be used with other suitable substrates to enhance its low frequency sound absorption properties (Pelletier et al. 2013). For higher frequencies, integration with conventional materials like cork or felt is suggested (Pelletier et al. 2019).

For the construction industry, machining and shaping of the acoustic boards can be an important consideration. In one study, it was shown that the depth of mycelium growth does affect the look and machining qualities of the board, with a direct relationship between mycelium growth depth and machinability (Pelletier et al. 2013). In the same study, however, reduced board density and hence, lower machinability, resulted in improved sound absorption. The inverse correlation of sound attenuation with density, however, was not significant when measured across the entire study (Pelletier et al. 2013).

The rice straw is the best individual substrate fillers to achieve the acoustic absorption were (52 dBa), while other substrates like flax shive (53.5 dba), hemp pith (53 dBa), sorghum fiber (54 dBa) and switchgrass (55 dBa) show variable results. Better acoustic absorption could be attained through mixing various fillers (50–50 wt%). The best combinations incluce: rice straw-cotton bur fiber (47 dBa), rice straw-sorghum fiber (45.5 dBa), and sorghum fiber-switchgrass (47 dBa) (Jones et al. 2020).

3.6 Thermal Degradation and Fire Safety Properties of Mycelium and its Composites

The fungal mycelium, on its own, has no prominent or useful fire-retardant qualities, typically exhibit a three-stage thermal degradation, degradation and fire reaction properties are typical for the cellulosic and other biologically-derived substances (Haneef et al. 2017, Jones et al. 2017, 2020). Even though the hyphal constituents (e.g. chitosan and hydrophobins: the cysteine-rich proteins form a hydrophobic layer), have been found to increase the fire retardancy in the fabrics, however, they do not occur in sufficient quantities to offer fire retardancy in the mycelium (El-Tahlawy 2008; Hu et al. 2013; Alongi et al. 2014; Costes et al. 2017; Jones et al. 2018b).

Hydrophobins are reported to promote char formation by allowing dehydration rather than de-polymerisation of polysaccharides (Alongi et al. 2014). However, genetically modified *Schizophyllum commune* biomass of mycelia lacks its hydrophobin gene has been reported to have higher char yields (32 wt% average)

compared to the wild type *S. commune* biomass (27 wt% average) (Appels et al. 2018). About 20–30 wt% carbonaceous char is usually formed at 450–600 °C for mycelial bio-mass on pyrolysis in a nitrogen atmosphere (Haneef et al. 2017; Appels et al. 2018; Jones et al. 2018b).

Hyphal density of the mycelium, as shown with markedly increased hyphal density with growth time between 6 and 18 days, was shown to not cause a significant variation of fire resistance properties (Jones et al. 2018a), implying a low threshold of hyphal density for effective fire resistance in mycelium composites.

Fillers or additives like glass fines, lignin, and rice hulls have been shown to significantly improve improved fire reaction, thermal degradation, and safety properties of mycelium composites (Jones et al. 2018a, 2020). Table 2 compares the fire safety performance of mycelium composites containing various fillers and additives, such as glass fines, rice hulls and wheat grains, with synthetic polystyrene insulation and particle board.

3.7 Water Absorption Properties of Mycelium Composites

Hydrophilicity of mycelium composites is an issue of concern (Jones et al. 2020). The mycelium composites of fungi are typically hydroscopic, which increases the weight by ~40–580 wt% in contact with water for 48–192 h (Holt et al. 2012, López Nava et al. 2016, Appels et al. 2019, Elsacker et al. 2019, Sun et al. 2019). The strong water-absorption capacity of mycelium composites is the result of their cellulosic filler constituents, which contain abundant accessible hydroxyl groups (Zabihzadeh 2010), and the porous hydrophilic mycelium binder and biologically-derived filler phases promote the wicking (Chung et al. 2011; Li et al. 2013; Wei et al. 2015).

In a recent investigation (Appels et al. 2020) of mycelial films using *S. commune* as the fungal species, using glycerol to reduce the water contact angle (WCA), hence increasing its hydrophilicity. In this study, it was demonstrated that by using 32% of glycerol, the highest hydrophilicity of the treated mycelium, with a WCA of $49 \pm 3°$, emerged. The fungal mycelium films with 32% glycerol resulted in the lowest uptake of water, when assessed over a 24 hour period (Appels et al. 2020). However, with 32% glycerol treatment of mycelium, the Young's modulus and strength both dropped, but with an increase to strain at failure.

Further upstream processing can also affect the uptake of water. For example, cold- or hot-pressed mycelial composites experience less than half the water uptake of air-dried composites (~250 wt% compared to ~580 wt%) (Jones et al. 2020). This is probably because pressed materials have lesser void volumes, which hinders capillary action as well as the water uptake (Dai et al. 2007).

Table 2 The cone calorimetry performance and parameters of fire safety. Data from Jones et al. 2018a. (Reproduced from Jones et al. 2020—Under a Creative Commons Attribution 4.0 International License)

Type	Sample	Time		Heat release rate		Gas release		
		Ignition (s)	Flashover t_{fo} (s)	Average RHR_{180} (KW/m^2)	Peak, pHRR (KW/m^2)	Smoke, TSR (m^2/m^2)	CO, COP_{180} (g)	CO_2, CO_2P_{180} (g)
Synthetic	Climafoam® extruded polystyrene insulation foam	9	61	114	503	1184	0.48	15.2
	STRUCTAflor® particleboard	26	173	134	200	64	0.47	30.0
Mycelium composite[a]	75 wt% wheat grains	12	94	107	185	70	0.33	23.8
	75 wt% rice hulls	7	75	85	133	40	0.02	14.6
	25 wt% wheat grains +50 wt% glass fines	12	370	42	79	5	0.39	10.2
	25 wt% rice hulls +50 wt% glass fines	7	311	33	85	0.9	0.91	63

t_{ig} time to ignition, RHR_{180} average heat release rate from ignition to 180 s after ignition, *pHRR* peak heat release rate, t_{fo} estimated time to flashover in room fire test (Babrauskas and Peacock 1992), *TSR* total smoke release, COP_{180} carbon monoxide produced from ignition to 180 s after ignition, CO_2P_{180} carbon dioxide produced from ignition to 180 s after ignition

[a] Inoculated using 25 wt% wheat grain inoculums

3.8 Termite Resistance of Mycelium Composites

The termites are a scourge of the building industry, with termites reported to be able to destroy the wall and timbers of a home within 3 months of construction in Australia (Fumapest Group 2021). The cost of termite infestation globally runs into several billion dollars (Logan and Buckley 1991).

Mycelium composites have no inherent termite resistant properties, comprising completely biological and predominantly lignocellulosic material (Jones et al. 2020). Hemp-based mycelium composites have high termite-resistance, exhibiting high termite mortality rates (directly related to efficacy or repellence by termite treatments) and low mass losses resulting from termite infestation over 4 weeks (16–53 wt%). The mass loss for Kenaf-based or Corn-based composites is more than Hemp-based mycelium composites.

Natural termiticides, the most effective guayule resin (Bultman et al. 1998) and vetiver oil (Zhu et al. 2001). One coat of these oils provides a complete termite mortality as well as associated with mass losses up to 18–28 wt% and 16–27 wt%. Such mass loss is significantly lower than for untreated composites (42–62 wt%) and an untreated southern yellow pine (*Pinus taeda*) used as a reference sample (80 wt%).

3.9 Manufacturing Mycelium Composites at Scale and the Use of Mycelium-Derived Leather in Internally Furnished Structures

3.9.1 Extrusion and Mycelium-Derived Leather

With the aforementioned favourable properties of mycelium composites as a viable a construction material, particularly with enhanced mechanical properties, recent work Soh et al. (2020) has explored the possibilities of 3D printing mycelium composites, by amending its pre-cured consistency, using chitosan, as schematically shown in Fig. 4. By mixing chitosan with bamboo-mycelium to obtain an extrudable mix, albeit with a reduced compression strength (40 kPa) after 20 days, compared to a bamboo-mycelium mix without chitosan (240 kPa). Despite decrease in mechanical properties, such composition has added the virtue of extrudability for complex shaping, those are requirements for direct writing of structures and the additional exploration of mycelium-bound materials for several structural applications. This development might have prospects in architectural forms, especially with low-load-bearing applications for the construction and design industries.

Recently, companies like Ecovative, Mogu and MycoWorks (Ecovative Design LLC 2019b; Mogu 2019; MycoWorks 2019) have developed mycelium-based leather-like materials. These products and processes, involving mycelium-based leather-like products are summarized in Jones et al. 2021. These products can indeed

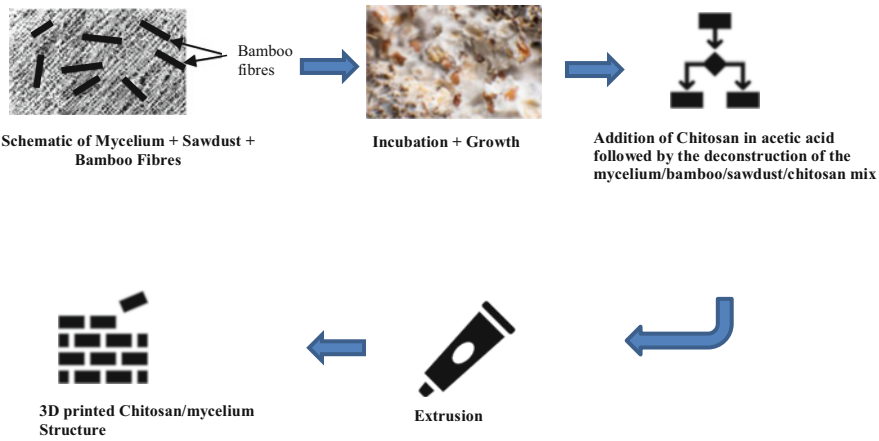


Fig. 4 Schematic of the process for the chitosan/mycelium for possible 3D printing of mycelium (Adapted from Soh et al. 2020)

be used as architectural features or a coverings for internal furniture, particularly for those with animal rights, social and environmental concerns. Some challenges of fungi-based leather include, consistency of mechanical properties, thickness and colour. Of course, flexural strength and hydrophobicity will be critical research directions, regarding the development of fungi-based leather.

3.9.2 Live and Multifunctional Large-Scale Structures

Recently, work on hybridizing live with conventional building materials has been reported (Elsacker et al. 2021). The advantages of this hybridization route include self-healing structures from the live component of this composite ensemble. In Elsacker et al. 2021, large scale living mycelium formwork structures were shaped using an on-site robotic wire cutting technique. The concept of living architecture is not new. Vallas and Courard (2017), indicate that vine bridges found in many parts of the world, such as Japan, Indonesia and India, have been around for centuries and that these structures can be classed as living structures. Vallas and Courard (2017) suggest that with the current trend of aiming for the sustainability of building materials, living architecture can help meet this demand using mycelium-based building structures.

Although the hitherto discussed manufacturing of mycelium composites involves denaturing or killing off the mycelium with heat, ways to keep the mycelium partly alive, merely by supressing the growth of its fruiting bodies is discussed by using a glycogen synthase kinase-3 inhibitor (Chang et al. 2019). The concept of biomineralization using micro-bacterial activity to precipitate calcium carbonate from sand-gelatin scaffolds, is also proposed (Heveran et al. 2020).

Research into optimal substrates, hybrid structures (live mycelium + conventional materials) and growth media, controlled growth features, 3D or 4D (3D + time) printing techniques, post-processing of mycelium tissue and genetic engineering could also allow the desirable mechanical properties of such materials to be realised at scale. Also, by hybridizing mycelium with rubber, applications of fungal-derived flexible materials could be expanded to heavy-duty and possibly semi-structural products, especially when strain resilience is desired.

The concept of building 'at destination' was discussed at NASA, using melanin-rich mycelium to attenuate radiation and other integrated bio-sensors (Rothschild 2018). Even though this concept was planned for future mission to Mars, where payloads on rocket ships to Mars will be at a premium, this concept can be extended to applications on Earth, where construction of buildings in hard-to-reach places, especially with environmental concerns, is needed. This application can also be embedded with multifunctional capabilities, such as biosensors. This 'at-destination' concept can also be extended to mycelium-based internal structures, such as furniture and other non-or semi-structural applications.

3.10 Relative Cost-Performance of Mycelium Composites

The issue of cost is critical, especially in the cost-sensitive construction industry. This topic is understandably not yet widely reported in the literature, given the current stage of development of mycelium composites, globally and restrictions based on propriety considerations. In addition, the cost discussion is dependent on the intended application, be it for insulation, packaging, semi-structural or structural applications.

For example, it is reported that for rice hull- and wheat grain- based mycelium composites containing 50% glass fines are 6- to 12-folds inexpensive than the extruded particleboard and polystyrene based on 2018 prices in Australia (Jones et al. 2018a). This comparison was based on the fire resistance properties of these mycelium composites exceeding that for the extruded polystyrene and particleboard, as seen in Table 2.

The expectation, however, is that when added properties such as strength and water resistance are considered, the costs will increase. In addition, when adaptation of the mycelium processing is made for the large-scale manufacturing and multifunctionality, the cost will initially trend upwards. However, with time, economies of scale and maturing technologies, the unit cost of mycelium composites should begin to drop to levels, which will be cost-performance competitive with existing commercial construction products.

4 Outlook and Future Applications of Mycelium Materials for the Construction Industry

From the above discussion of mycelium composites in this chapter, it is clear that mycelium holds significant promise in the construction industry, especially when its processing costs and at-scale manufacturing impediments are reduced.

From the discussion above, it is clear that mycelium composites have promising prospects in the area of packaging (Holt et al. 2012; Dell Inc 2016; Gosden 2016; Abhijith et al. 2018; Ecovative Design LLC 2019a), thermal insulation (Papadopoulos 2005, Holt et al. 2012, Asdrubali et al. 2015, Yang et al. 2017, Elsacker et al. 2019) and acoustic absorption foams (Pelletier et al. 2013; Mogu 2019; Pelletier et al. 2019) with fire resistant properties (Jones et al. 2017, 2018a, 2018b, 2020), in addition to panelling, flooring and furnishings (Ecovative Design LLC 2019a, b; MycoTech 2019; Mogu 2019; KrownDesign 2019). All these properties are much sought after in the construction industry worldwide.

Both research and commercial developments in mycelium composites have paved the way for its use in the construction sector in the near future. The hydrophobicity of fungal mycelium material could see the extension of mycelium-based films to coating (Haneef et al. 2017; Jones et al. 2019a) or textile (Janesch et al. 2020) applications. The other applications of importance for mycelium stem from the polymers like chitin, chitosan and β-glucan. Extraction of such polymers for use in 3D printed structures, production of films, production of sheets, reinforcement for polymer nanocomposites, and nanopapers (Nawawi et al. 2019a; Soh et al. 2020), pave way towards new doors in the place of synthetic polymers through these applications, thus in turn enlarge the use of mycelium into the realms of most construction products as traditionally made from synthetic polymers, particularly for semi-structural components and architectural features. In addition, in earthquake prone regions of the world, using lightweight mycelium bricks for housing, where the falling bricks during an earthquake, would no longer pose a threat to human life. This is an attractive proposition indeed, if the issues of strength (compared to conventional building bricks) and water resistance are resolved.

5 Conclusion

This chapter outlines the current virtues and drawbacks of mycelium composites. This biopolymer-based composite is low in embodied energy and processing cost and high in environmentally sustainable and biodegradable material constituents. Applications include thermal and acoustic insulation (given its inherent lossy morphology), flooring and cabinetry. They are also known to exhibit better fire safety and fire reaction qualities than the materials used for traditional construction purposes (e.g. extruded polystyrene insulation and particleboard).

Recent 3D printing of mycelium composites, incorporating chitosan is proposed for the manufacturing process towards deployable semi-structural construction components. In addition, the recent concept of hybridizing live large-scale mycelium structures with conventional construction materials, to harness structural self-healing properties, holds some promise in the at-scale manufacturing space for multi-functional mycelium structures.

However, mycelium composite's lossy morphology results in markedly reduced mechanical strength. In addition, its high moisture uptake and its difficultly in large scale manufacturing at a competitive price point, limits its current industrial deployment.

Hence, future research and advancement of these materials is required to power this 'green' evolution in the construction industry, particularly in strength improvement, coatings to enhance its hydrophobicity, introduction of its multi-functionality, such as self-healing capability and large-scale batch manufacturing. When these challenges are met, the expectation is that mycelium composites will be competitive with currently used construction materials, on a cost-performance basis.

5.1 Sub-Sectional Attribution (to Jones et al. 2020)

Due to the reproduction of Tables 1 and 2 and Figs. 1 and 3 from Jones et al. 2020, parts of Sects. 2.1, 2.2, 3.1, 3.2 and 3.6 are reproduced from Jones et al. 2020 – Under a Creative Commons Attribution 4.0 International License.

References

Abhijith R, Ashok A, Rejeesh CR (2018) Sustainable packaging applications from mycelium to substitute polystyrene: a review. Mater Today: Proc 5(1):2139–2145

Abrams M (2014) Construction materials made from 'Shrooms', http://www.asme.org/engineering-topics/articles/construction-and-building/construction-materials

Alongi J, Carletto RA, Bosco F, Carosio F, Di Blasio A, Cuttica F, Antonucci V, Giordano M, Malucelli G (2014) Caseins and hydrophobins as novel green flame retardants for cotton fabrics. Polym Degrad Stab 99:111–117

Appels FV, Camere S, Montalti M, Karana E, Jansen KM, Dijksterhuis J, Krijgsheld P, Wösten HA (2019) Fabrication factors influencing mechanical, moisture-and water-related properties of mycelium-based composites. Mater Des 161:64–71

Appels FV, van den Brandhof JG, Dijksterhuis J, de Kort GW, Wösten HA (2020) Fungal mycelium classified in different material families based on glycerol treatment. Commun Biol 3(1):1-5.l. https://doi.org/10.1038/s42003-020-1064-4

Appels FVW, Dijksterhuis J, Lukasiewicz CE, Jansen KMB, Wösten HAB, Krijgsheld P (2018) Hydrophobin gene deletion and environmental growth conditions impact mechanical properties of mycelium by affecting the density of the material. Sci Rep 8(1):4703. https://doi.org/10.1038/s41598-018-23171-2

Arifin YH, Yusuf Y (2013) Mycelium fibers as new resource for environmental sustainability. Procedia Eng 53:504–508

Asdrubali F, D'Alessandro F, Schiavoni S (2015) A review of unconventional sustainable building insulation materials. Sustain Mater Technol 4:1–17
Ashby MF, Shercliff H, Cebon D (2018) Materials: engineering, science, processing and design. Butterworth-Heinemann, Oxford, UK
Babrauskas V, Peacock RD (1992) Heat release rate: the single most important variable in fire hazard. Fire Saf J 18(3):255–272
Bartnicki-Garcia S (1968) Cell wall chemistry, morphogenesis, and taxonomy of fungi. Ann Rev Microbiol 22(1):87–108
Bhuvaneshwari S, Hettiarachchi H, Meegoda JN (2019) Crop residue burning in India: policy challenges and potential solutions. Int J Environ Res Public Health 16(5):832
Bultman JD, Chen SL, Schloman WW Jr (1998) Anti-termitic efficacy of the resin and rubber in fractionator overheads from a guayule extraction process. Ind Crop Prod 8(2):133–143
Chand N, Fahim M (2008) Tribology of natural fiber polymer composites. Woodhead Publishing, Cambridge
Chang J, Chan PL, Xie Y, Ma KL, Cheung MK, Kwan HS (2019) Modified recipe to inhibit fruiting body formation for living fungal biomaterial manufacture. PLoS One 14(5):e0209812. https://doi.org/10/ggznw5
Chung S, Suidan MT, Venosa AD (2011) Partially acetylated sugarcane bagasse for wicking oil from contaminated wetlands. Chem Eng Technol 34(12):1989–1996
Costes L, Laoutid F, Brohez S, Dubois P (2017) Bio-based flame retardants: when nature meets fire protection. Mater Sci Eng R Rep 117:1–25
Dai C, Yu C, Xu C, He G (2007) Heat and mass transfer in wood composite panels during hot pressing. Part II. Modeling void formation and mat permeability. Wood Fiber Sci 37(2): 242–257
Defonseka C (2014) Introduction to polymeric composites with rice hulls. Smithers Smithers Rapra Technology
Dell Inc (2016) Green packaging and shipping, http://www.dell.com/learn/us/en/uscorp1/corp-comm/mushroom-packaging2016
DPCleanTech (2019) Understanding rice husk as a biomass fuel, http://www.dpcleantech.com/files/Understanding-rice-husk-as-a-biomass-fuel-EN-V1-2013.9.4.pdf2013
Ecovative Design LLC (2019a) MycoFlex, https://ecovativedesign.com/mycoflex
Ecovative Design LLC (2019b) Mycocomposite—Mycelium—bound Agricultural Byproducts, http://ecovativedesign.com/mycocomposite2019
Elsacker E, Søndergaard A, Van Wylick A, Peeters E, De Laet L (2021) Growing living and multifunctional mycelium composites for large-scale formwork applications using robotic abrasive wire-cutting. Constr Build Mater 283:122732
Elsacker E, Vandelook S, Brancart J, Peeters E, De Laet L (2019) Mechanical, physical and chemical characterisation of mycelium-based composites with different types of lignocellulosic substrates. PLoS One 14(7):0213954
El-Tahlawy K (2008) Chitosan phosphate: a new way for production of eco-friendly flame-retardant cotton textiles. J Text Inst 99(3):185–191
Fumapest Group (2021). https://www.termite.com.au/termites.html
Ghazvinian A, Farrokhsiar P, Vieira F, Pecchia J, Gursoy B (2019) Mycelium-based bio-composites for architecture: assessing the effects of cultivation factors on compressive strength. The eCAADe and SIGraDi Conference, University of Porto, Portugal
Gosden E (2016) Ikea plans mushroom-based packaging as eco-friendly replacement for polystyrene, http://www.telegraph.co.uk/news/earth/business and ecology/recycling/12172439/Ikea-plans-mushroom-based-packaging-as-eco-friendly-re-placement-for-polystyrene.html2016
Griffin DH (1996) Fungal physiology. Wiley, Hoboken, NJ
Haneef M, Ceseracciu L, Canale C, Bayer IS, Heredia-Guerrero JA, Athanassiou A (2017) Advanced materials from fungal mycelium: fabrication and tuning of physical properties. Sci Rep 7(1):1–11. 41292

He J, Cheng CM, Su DG, Zhong MF (2014) Study on the mechanical properties of the latex-mycelium composite. Appl Mech Mater 507:415–420. Trans Tech Publications Ltd
Heveran CM, Williams SL, Qiu J, Artier J, Hubler MH, Cook SM, Cameron JC, Srubar WV III (2020) Biomineralization and successive regeneration of engineered living building materials. Matter 2(2):481–494. https://doi.org/10.1016/j.matt.2019.11.016
Holt GA, Mcintyre G, Flagg D, Bayer E, Wanjura JD, Pelletier MG (2012) Fungal mycelium and cotton plant materials in the manufacture of biodegradable molded packaging material: evaluation study of select blends of cotton byproducts. J Biobaased Mater Bioenergy 6(4):431–439
Hu S, Song L, Pan H, Hu Y (2013) Effect of a novel chitosan-based flame retardant on thermal and flammability properties of polyvinyl alcohol. J Therm Anal Calorim 112(2):859–864
Huynh T, Jones M (2018) Scientists create new building material out of fungus, rice and glass, https://theconversation.com/scientists-create-new-building-material-out- of-fungus-rice-and-glass-98153
Islam MR, Tudryn G, Bucinell R, Schadler L, Picu RC (2017) Morphology and mechanics of fungal mycelium. Sci Rep 7(1):13070
Islam MR, Tudryn G, Bucinell R, Schadler L, Picu RC (2018) Mechanical behavior of mycelium-based particulate composites. J Mater Sci 53(24):16371–16382
Janesch J, Jones M, Bacher M, Kontturi E, Bismarck A, Mautner A (2020) Mushroom-derived chitosan-glucan nanopaper filters for the treatment of water. React Funct Polym 146:104428
Jiang L, Walczyk D, McIntyre G, Bucinell R (2016) A new approach to manufacturing biocomposite sandwich structures: mycelium-based cores, In *International Manufacturing Science and Engineering Conference,* vol. 49897, p. V001T02A025. Am Soc Mech Eng 2016
Jiang L, Walczyk D, McIntyre G, Bucinell R, Li B (2019) Bioresin infused then cured mycelium-based sandwich-structure biocomposites: resin transfer molding (RTM) process, flexural properties, and simulation. J Clean Prod 207:123–135
Jiang L, Walczyk D, McIntyre G, Bucinell R, Tudryn G (2017) Manufacturing of biocomposite sandwich structures using mycelium-bound cores and preforms. J Manuf Process 28(1):50–59
Jones M, Bhat T, Huynh T, Kandare E, Yuen R, Wang CH, John S (2018a) Waste-derived low-cost mycelium composite construction materials with improved fire safety. Fire Mater 42(7): 816–825
Jones M, Bhat T, Kandare E, Thomas A, Joseph P, Dekiwadia C, Yuen R, John S, Ma J, Wang CH (2018b) Thermal degradation and fire properties of fungal mycelium and mycelium-biomass composite materials. Sci Rep 8(1):17583
Jones M, Gandia A, John S, Bismarck A (2021) Leather-like material biofabrication using fungi. Nat Sustainability 4(1):9–16
Jones M, Huynh T, Dekiwadia C, Daver F, John S (2017) Mycelium composites: a review of engineering characteristics and growth kinetics. J Bionanosci 11(4):241–257
Jones M, Mautner A, Luenco S, Bismarck A, John S (2020) Engineered mycelium composite construction materials from fungal biorefineries: a critical review. Mater Des 187:108397
Jones M, Weiland K, Kujundzic M, Theiner J, Kählig H, Kontturi E, John S, Bismarck A, Mautner A (2019a) Waste-derived low-cost mycelium nanopapers with tunable mechanical and surface properties. Biomacromolecules 20(9):3513–3523
Jones MP, Lawrie AC, Huynh TT, Morrison PD, Mautner A, Bismarck A, John S (2019b) Agricultural by-product suitability for the production of chitinous composites and nanofibers utilizing *Trametes versicolor* and *Polyporus brumalis* mycelial growth. Process Biochem 80: 95–102
Kavanagh K (2005) Fungi: biology and applications. Wiley, Hoboken, NJ
KrownDesign (2019) Beautiful products with fungus and biomass, https://www.krown.bio/2019
Li MM, Pan HC, Huang SL, Scholz M (2013) Controlled experimental study on removing diesel oil spillages using agricultural waste products. Chem Eng Technol 36(4):673–680
Logan J, Buckley D (1991) Subterranean termite control in buildings. Elsevier, Amsterdam

López Nava JA, Méndez González J, RuelasChacón X, Nájera Luna JA (2016) Assessment of edible fungi and films bio-based material simulating expanded polystyrene. Mater Manuf Process 31(8):1085–1090
Madurwar MV, Ralegaonkar RV, Mandavgane SA (2013) Application of agro-waste for sustainable construction materials: a review. Constr Build Mater 38(1):872–878
Mogu (2019) Mycelium Technology, https://mogu.bio/technology/2019
MycoTech (2019) Eco Binderless Board, https://www.mycote.ch/biobo2019
MycoWorks (2019) Materials, https://www.mycoworks.com/2019
Nawawi WM, Jones M, Murphy RJ, Lee KY, Kontturi E, Bismarck A (2019b) Nanomaterials derived from fungal sources—is it the new hype? Biomacromolecules 21(1):30–55
Nawawi WM, Lee KY, Kontturi E, Murphy RJ, Bismarck A (2019a) Chitin nanopaper from mushroom extract: natural composite of nanofibers and glucan from a single biobased source. ACS Sustain Chem Eng 7(7):6492–6496
Officina Corpuscoli (2019) Materials, https://www.corpuscoli.com/2019
Papadopoulos AM (2005) State of the art in thermal insulation materials and aims for future developments. Energ Buildings 37(1):77–86
Pelletier MG, Holt GA, Wanjura JD, Bayer E, McIntyre G (2013) An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates. Ind Crop Prod 51:480–485
Pelletier MG, Holt GA, Wanjura JD, Greetham L, McIntyre G, Bayer E, Kaplan-Bie J (2019) Acoustic evaluation of mycological biopolymer, an all-natural closed cell foam alternative. Ind Crop Prod 139:111533
Pheng LS, Hou LS (2019) The economy and the construction industry. In: Construction quality and the economy. Springer, Singapore, pp 21–54
Rinaudo M (2007) Chitin and chitosan—general properties and applications. ChemInform 38(7): 603–632
Roser M, Ritchie H, Ortiz-Ospina E (2019) World population growth. *Our World in Data*. https://ourworldindata.org/world-population-growth#citation
Rothschild L (2018) Myco-architecture off planet: growing surface structures at destination, (2018), https://www.nasa.gov/directorates/spacetech/niac/2018_Phase_I_Phase_II/Myco-architecture_off_planet/
Sartori I, Hestnes AG (2007) Energy use in the life cycle of conventional and low-energy buildings: a review article. Energ Buildings 39(3):249–257
Soh E, Chew ZY, Saeidi N, Javadian A, Hebel D, Le Ferrand H (2020) Development of an extrudable paste to build mycelium-bound composites. Mater Des 195:109058
Sombatsompop N, Chaochanchaikul K (2004) Effect of moisture content on mechanical properties, thermal and structural stability and extrudate texture of poly (vinyl chloride)/wood sawdust composites. Polym Int 53(9):1210–1218
Sun W, Tajvidi M, Hunt CG, McIntyre G, Gardner DJ (2019) Fully bio-based hybrid composites made of wood, fungal mycelium and cellulose nanofibrils. Sci Rep 9(1):3766
Sustainability Victoria (2019) Market summary–recycledglass, https://www.sustainability.vic.gov.au/-/media/SV/Publications/Government/Waste-and-resource-recovery/Recycled-materials-in-pavement/Market-Analysis-Glass-Sept-2014-PDF.pdf?la=en2014
Thakur VK, Siṅgha AS (2013) Biocomposites BB. Smithers Rapra Technology Ltd, Shropshire
Travaglini S, Noble J, Ross PG, Dharan CKH (2013) Mycology matrix composites. In Annual technical conference; 28th, American Society for Composites (pp. 9–11). State College, PA
TUDelft (2019) Mycelium-based Materials for Product Design, https://www.tudelft.nl/en/ide/research/research-labs/emerging-materials-lab/environmentally-sensitive-materials/mycelium-based-materials-for-product-design/2019
Tun MM, Juchelkova D, Win MM, Thu AM, Puchor T (2019) Biomass energy: an overview of biomass sources, energy potential, and management in Southeast Asian countries. Resources 8(2):81

UHPC (UltraHigh Performance Concrete) (2020) Market—Growth, Trends and Forecast (2020–2025)
Utrecht University (2019) Research through design, https://www.uu.nl/en/research/microbiology/mycelium-design2019
Vallas T, Courard L (2017) Using nature in architecture: building a living house with mycelium and trees. Front Archit Res 6(3):318–328
Wei L, Liang S, McDonald AG (2015) Thermophysical properties and biodegradation behavior of green composites made from polyhydroxybutyrate and potato peel waste fermentation residue. Ind Crop Prod 69:91–103
Wessels JGH, Mol PC, Sietsma JH, Vermeulen CA (1990) Wall structure, wall growth, and fungal cell morphogenesis. In: Biochemistry of cell walls and membranes in fungi. Springer, Berlin, Heidelberg, pp 81–95
Xia XC, Chen XW, Zhang Z, Chen X, Zhao WM, Liao B, Hur B (2013) Effects of porosity and pore size on the compressive properties of closed-cell Mg alloy foam. J Magnesium Alloys 1(4): 330–335
Yang Z, Zhang F, Still B, White M, Amstislavski P (2017) Physical and mechanical properties of fungal mycelium-based biofoam. J Mat Civil Eng 29(7):04017030
Zabihzadeh SM (2010) Water uptake and flexural properties of natural filler/HDPE composites. Bioresources 5(1):232–316
Zhu BC, Henderson G, Chen F, Fei H, Laine RA (2001) Evaluation of vetiver oil and seven insect-active essential oils against the Formosan subterranean termite. J Chem Ecol 27(8):1617–1625
Zurich ETH (2019) Mycelium-basedcompositematerials, https://fcl.ethz.ch/research/archipelago-cities/alternative-construction-materials/mycelium.html

Packaging Applications of Fungal Mycelium-Based Biodegradable Composites

Rejeesh C. Rajendran

Abstract In a world that demands the use of sustainable practices for producing more eco-friendly products, the idea of a circular economy prevails through the gradual reduction in consumption of finite resources. Mycelium is that collection of filamentous fibers extending out from the hyphae of any fungus. Mycelium is a biomaterial that is renewable in nature and has the capability to grow quickly on agricultural wastes. Thin fibers grown from the fungus can bind the matrix material to form biocomposite materials that are strong as well as biodegradable. These bio composite materials can be easily molded into various shapes suitable for the manufacturing of shock resistant packaging materials, and can also be used as a construction material or as an insulation material. They use cost-effective raw materials to form the biocomposite and the developed material is a sustainable substitute to synthetic materials like expanded polystyrene (EPS). These attributes make the mycelia-based bio composite material to have every chance of becoming a material of choice in packaging applications. This chapter gives an overview of the current state of the art technologies and the challenges ahead in the development of mycelium-based bio composite materials regenerating from agro-industrial waste. While the main focus is on the packaging applications of fungal mycelium-based biodegradable composites, the chapter also focuses on a variety of applications as a viable substitute for synthetic polymer materials like expanded polystyrene.

Keywords Biocomposites · Biodegradation · Expanded polystyrene · Green material · Insulation foam · Mycelium · Mushroom · Sustainable packaging

R. C. Rajendran (✉)
Department of Mechanical Engineering, Federal Institute of Science and Technology, Angamaly, Kerala, India
e-mail: rejeeshcr@fisat.ac.in

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_11

1 Introduction

One of the few sectors that saw an exponential growth during the COVID-19 pandemic is the e-commerce sector with millions of people opting for a home delivery through online shopping/teleshopping for retail essential goods and even groceries. The pandemic has essentially changed the way people used to shop retail goods. According to recent reports from Salesforce, digital sales have ballooned 71% in the second quarter of 2020 and 55% in the third, which created a surge in the use of packages and packaging materials that are bound to end up in landfill sites, and incineration chambers, or worse in water bodies and the natural environment. The amount of plastic waste generated as a part and parcel of packaging is at a staggering level and is growing at a frightening rate. The excellent versatility and manufacturability of plastic materials have made tremendous improvements to the economic well-being of society; however, a price has to be paid in the form of depleting resources and environmental deterioration (Ashok et al. 2018).

Packaging materials do serve a purpose which is mostly dedicated to protecting things, improving usability and allows safe handling (Ashok et al. 2016). The recyclability of packaging materials like polystyrene or polypropylene is often oversold and it is estimated that only less than 14% of the global annual production of nearly 86 million tons of plastic packaging gets recycled. Instead of getting recycled, a considerable portion of the packaging waste that have been generated is destined for landfill, or to the incineration chamber. It may also end up in the natural environment which unfortunately includes our waterbodies and oceans, where they turn harmful to marine life (Ellen MacArthur Foundation 2017). However, a recent market survey indicates that with increasing awareness about the environment among the customers, they have an affinity towards plastic-free alternatives which drives the companies to adopt alternative materials and strategies to reduce their plastic footprint. In an attempt to minimize the undesirable consequences of packaging waste, research has been in place all over the world that focuses on the development of alternative materials that are sustainable and biodegradable which are mostly derived from renewable agricultural sources (Ashok et al. 2016, Abhijith et al. 2018, Ellen MacArthur Foundation 2017, Ashok et al. 2018).

Expanded Polystyrene or Styrofoam is a lightweight material that is safe for use with food. It is a preferable packaging material for food, electronics and other fragile products all the while being a less eco-friendly petroleum derivative (Rosato and Rosato 2012). While considering factors like emission of greenhouse gases or energy consumption, production of polystyrene leaves a very serious negative impact on the environment. Expanded Polystyrene has become a major constituent in municipal solid waste and marine debris and it is also detrimental to wildlife since they are extensively used for manufacturing disposable products like food plates or food containers. These products made from polystyrene are neither biodegradable nor recycled and are mostly used only once before it turns out to be waste after their useful life (Rosato and Rosato 2012; Arifin and Yusuf 2013).

Fungus is considered as dreadful but they have a major role in the decomposition of biological wastes and thus makes it vital in our ecosystem (Miles and Chang 2004). Manufacturing of biodegradable materials through Bio-design practices is an important leap in this area of research recently. Moist media and living organisms like fungi and algae, are the essential building blocks to grow these designs rather than manufacturing them (Ghazvinian et al. 2019). The fungi-based packaging materials developed from mushroom mycelium have been extensively studied and reported in the past decade. Mycelium based foams developed from agricultural residues or materials like sawdust can be considered as a cost competitive alternative with comparable properties to conventional Styrofoam. Incorporation of mycelium composite materials for packaging applications could reduce the consumption of Styrofoam, and will enhance sustainability through eco-friendly packaging (Vilaplana et al. 2010; Cooper 2013).

Mycelium is a natural glue that binds onto surrounding particles like sawdust, coir pith, hay, or rice husk to create a dense network of hyphae (Miles and Chang 2004). Mycelium biocomposite materials are grown in molds with different shapes as per the final requirement, where they grow quickly into a dense compact material. The material is dehydrated to prevent further growth after it has been grown to the required shape and density. Mycelium-based materials are completely biodegradable and it will decompose within a few weeks without the help of external stimulus. The cost of these materials can be reduced when it is processed on a mass scale in batches and it is easier to biodegrade after its useful life than recycling (Jiang et al. 2013).

This chapter reviews the current state of technology and achievements that have been made on utilizing mycelia for bioremediation of agro wastes. It also reveals the various mycelium packaging materials that have been developed and discusses their characteristics for being projected as a sustainable alternative for conventional packaging materials like Expanded Polystyrene.

2 Demand for Sustainable Materials in Packaging

According to a report, it is estimated that approximately 9.15 billion tons of plastic materials have been manufactured worldwide since the 1950s, of which around 30% are currently in use, only 9% have been reused, and more than 60% are in landfills after their useful life. At this rate of production, it is expected to have around 28.6 billion metric tons of plastic in the world by 2050 (Geyer et al. 2017). Packaging materials made from plastic are non-biodegradable but can be shredded to small fragments. The polymer chain of these fragments gets weakened when they are exposed to sunlight, but the environmental impact caused by these milli fragments is not yet conclusive (Tudryn et al. 2018).

Different materials have different processes suitable for their recycling and the conversion rates also may vary with respect to the plastic (Hopewell et al. 2009). Polyethylene terephthalate (PET) allows repeated recycling with a restoration rate of about 19.5% by weight, while polystyrene plastics like extruded polystyrene or

expanded polystyrene can be recycled only with a recovery rate of just 0.9% by its weight which places them amongst the least recycled plastics. Moreover, freight companies usually charge their services after considering factors like weight and volume of the goods to be transported. Considering that, the transportation cost of the EPS will be relatively very high as it requires a large volume to weight ratio and the light weight structure really makes it a poor choice of material for primary and secondary recycling. For each ton of plastic being turned into waste, the energy consumption required for transportation is approximately 0.49 million BTU all the while emitting 0.04 metric tons of carbon dioxide (Containers and Good 2016; Mojumdar et al. 2021).

Disposing plastic waste through combustion is a common practice which adopts the waste-to-energy (WTE) approach to generate energy. Though the combustion of plastic wastes can release exorbitant amounts of carcinogenic pollutants and heavy metals, other recycling options that involves collection of plastic wastes and their transportation are also harmful to human health and the environment, just like WTE incineration (Rigamonti et al. 2014).

During the 1980s, manufacturers introduced starch-based polymers to polyethylene blends and begin to market them as "ecofriendly" (Iles and Martin 2013). Biodegradable plastic materials from renewable sources are considered as a substitute for synthetic polymers and were extensively studied and used (Ashok et al. 2016). The different types of bioplastic materials that were suitable for packaging application included the

1. Polysaccharides like starch or cellulose extracted from biomass
2. Polymers like Polylactic Acid (PLA) or polyacrylates from chemical synthesis of renewable bio-based monomers
3. Polymers like Polyhydroxyalkanoates (PHA) from microorganisms or genetically modified bacteria (Ashok et al. 2016).

With the growing awareness of environment and sustainability issues caused by packaging materials, there is an increasing demand to find ecofriendly materials derived from renewable sources with comparable properties to substitute traditional packaging materials. Suitability of packaging materials depends on their mechanical and thermal properties which can be improved through steps like using a plasticizer, blending of polymers or reinforcement with nano-materials. More research that focuses on developing environmentally benign and competent alternative materials that are also economical, needs to be investigated in order to fulfill the demand for plastic-based packaging materials.

3 Role of Mycelium in Biocomposite Materials

Mushrooms are unique organisms with body structures and reproductive modes that may look like plants but are in fact fungi. Mushrooms are heterotrophs that must digest food to live, unlike autotrophs like plants that make their own food through photosynthesis. Mycelium (collection of hyphae), fruiting body and spores are the

essential parts of fungi anatomy. Hyphae are long, branched filamentous structures that help in absorbing nutrients from decaying organic matter. While mycelia forms the vegetative part of the fungus and thereby is a growth agent, the fruiting body is a reproductive structure that produces spores. Fungal spores are haploids (carry only one chromosome for each gene) involved in fungal reproduction as they can germinate on damp soil (Mojumdar et al. 2021).

Mycelium gets attached to the medium on which it is grown. The digestive enzymes secreted from hyphae tips have the ability to break down organic waste material to a matter of less complex body plan which allows the hyphae to grow on a substrate. This process of degradation of the substrate materials during which mycelium grows on the substrate by bonding and partially replacing the substrate with a strong biomass of the fungus is called colonization (Appels et al. 2019). Once the mycelium-based bio composite material grows into the required shape and density, further growth of mycelium has to be arrested. Heating and drying are the two ways that can be used to cease the growth of mycelium based bio composite materials. Drying process can temporarily cease the growth of fungi leading to hibernation. It also means that by providing favorable environmental conditions like moisture, the mycelium can grow again. However, heating of mycelium arrests any further growth by permanently destroying them.

A large variety of mycelium based bio composite materials can be developed by varying the fungal species used for inoculation or by changing the type of substrates or additives used. Properties of mycelium bio composite materials can be influenced through optimizing the factors like growing conditions or processing techniques. Several studies have reported the properties of different mycelium biocomposites compared against the conventional plastics like polystyrene (Attias et al. 2017; Haneef et al. 2017; Jones et al. 2017).

Mycelium bio composite materials are made by growing them into the required shapes in customized moulds. Mycelium grows around substrates like sawdust or coir pith by binding them together and the result is a compostable material which is an environmentally benign substitute to expanded polystyrene and other polymers used for cushioning of packaging goods. However, their ability to compete with synthetic materials as a protective packaging material in terms of cost and performance is yet to be established (Ellen MacArthur Foundation 2017).

Fungi grows with a filament like structure to form branches and this architecture helps them to grow quick in soil. Elongation of fungal hyphae is strictly due to the deposition of polysaccharides (Gooday 1995). The forward movement of vesicles with new cell membranes causes the hyphal tips to extend. The excretion of lytic enzymes enables the hyphae to grow through the substrates allowing them to receive nutrients in the form of solutes. The redistribution of internal metabolites throughout the structure is done through translocation. (Olsson 1995). Active hyphae are involved in activities like nutrient uptake, branching and translocation while inactive hyphae are no longer directly involved in those activities. Hyphal tips are the ends of those active hyphae which are extended by using the nutrients inside the fungus. Thus, a hyphal tip extends to become hyphae as it begins to form branches and receive nutrients from adjacent material. Whatever be the environment, fungus needs

the presence of certain elements or their combination for their steady growth. Elements like carbon, hydrogen, nitrogen, sulphur, phosphorus, oxygen and several others including metals are of special interest in this context (Boswell et al. 2003).

4 Potential of Mycelium Biocomposite Materials for Packaging Applications

Labelling of green products and marketing them as such had an appeal only with a smaller customer base until recently, however, an increase in awareness about the positive effects of using sustainable and renewable materials has brought in positive changes in perception to a larger customer base. There has been an increase in purchase power among the eco-conscious consumers who happen to be mostly the younger generation which has made a shift in the sales and marketing landscape and has inspired the manufacturers to focus more on sustainable materials. Consumers are now more inclined towards greener products that advertise sustainability and towards the companies that are keen on corporate social responsibility. The combined effect of these value based thinking has made it look like a viable proposition and has encouraged the manufacturers to incorporate sustainable business practices to their manufacturing chain that consists of stakeholders like suppliers, consumers and employees (Matthews and Rawlings 1999. Mohanty et al. 2005, Kazmierski 2012).

If this increased customer affinity can be channelized to a growth in sales, the companies that switch to sustainable packaging materials from conventional materials like polystyrene could benefit from increased sales revenue. They might also enjoy a young dedicated customer base who is keen on purchasing eco labeled products even after paying a slight premium in price for sustainability. Consumers are now aware of the waste generated as a result of e-commerce and buying consumable goods and they are the more interested to minimize the packaging waste than ever (Abhijith et al. 2018).

Corporates who advertise their commitment to ecological and socially responsible business practices are more likely to get a considerable boost in sales and revenue. Retail sales data of selected goods across several brands in different countries suggests that the products marked with a green labelling on its packaging has experienced an annual increase in average sales by 2%, in comparison with the brands without a green labelling that showed a meager 1% increase in sales. Products having environmental benefits experienced a whooping increase in annual sales by 5%, but obviously with the help of marketing promotions. The increase in sales and revenue of products will help to quickly overtake whatsoever the additional costs are associated with redesigning of their packaging (Abhijith et al. 2018).

Bio design and growing of fungal mycelia in suitable moulds use living organisms to create a viable substitute for polystyrene based foam in conventional packaging. The development and use of mycelium based biocomposite foam

Fig. 1 Mycelium composite material molded for packaging applications

supports a commitment to use renewable materials for better sustainability. Figure 1 shows Mushroom based foam being used for some of the several applications in packaging. At the time when these materials can be developed cost effective, it could change the status quo for good and would reduce the use of non-biodegradable petroleum derived synthetic waste materials that adds to the pollution. Also, the agricultural wastes for growing mycelium biocomposite material could derive additional value from this increased demand. This could very well turn as an added income to the farmers who could utilize these as raw materials instead of incinerating it.

These materials are designed for single use and after its useful life, it will biodegrade either in a compost pit or in landfills instead of remaining there without decomposition forever. Mycelium biocomposite materials are capable of creating an impact in the area of sustainable packaging and will make oil-based plastic materials obsolete and radically change the way the environment has been affected due to non-biodegradable packaging materials. Mycelium based packaging materials have the potential and will make those persistent oil-based plastic materials obsolete and radically change the way industry impacts the environment. Raw materials to be used as substrate to grow mycelium can be any feedstock material (agricultural by-products) that are locally available in abundance, which makes it suitable for manufacturing the biocomposite foam from anywhere in the world. Minimal logistics and transportation is sufficient if the raw materials and finished products are produced locally, using local feed stocks (Abhijith et al. 2018; Jiang et al. 2013).

These shifts in customer behaviour has an impact in global economy and it is being noticed by the corporate giants like Coca Cola and Unilever and they have included sustainability as a core value of their brand. Customers around the world are vocal on local and sustainable products more than ever. The use of mycelium

biocomposite materials in packaging will offer the businesses a new way to tap into the values of potential customers and a reduction in environmental impact.

5 Early Adopters of Mushroom-Based Packaging

Any change in business practices in order to transform the present methods to sustainable packaging might seem like an unnecessary cost, especially for smaller firms with tight margins. However, despite the size of the business, a radical rethinking of the packaging process can be done in order to reduce the harm it does to the environment and focus more on sustainability. While packaging is an important entity in the marketing of any product, a large part of it turns as disposable waste once the item has been unpacked. Mycelia is a natural binder that can grow on agricultural by products like corn, coir pith or oat husks to make sufficiently durable cushioning materials that could substitute expanded polystyrene in practically every application. More and more firms have declared their commitment to reduce dependency on Styrofoam during the past few years and they are actively looking for alternative materials for their products.

Ecovative design is a New York based biotech company and is a pioneer in manufacturing of mushroom based foam. They have gone a long way in making the process efficient, all the while maintaining the key properties of material intact. Corporates like Dell is one of the early adopters that has announced their decision to use the eco-friendly fungal mycelium based foam for shipping their servers in its packing cases. They have extensively tested these material in their labs to ensure that the same amount of safety is provided to their products when compared to traditional expanded polystyrene foam. While they satisfy the necessary requirements, cushioning from mycelium materials are found to be more suited for heavy goods like servers. Mycelium based packaging materials for a standard server can be grown in the mold usually in one to 2 weeks' time. The use of ecofriendly packaging materials comes as a part of the firm's plan for a more sustainable shipping strategy. Not only that mycelium is renewable which makes it a green solution, the organic based mycelium foams can be composted easily after its useful life (Abhijith et al. 2018, Mojumdar et al. 2021).

Ford motor company has decided to make use of mycelium-based foam in automotive parts like bumpers, side doors, and dashboards. The immediate future will see a new generation of cars with more biodegradable components while at present, about 15 kg of synthetic foam is present in each car being manufactured and Ford is likely to replace at least a part of it with eco-conscious alternatives. These materials can be decomposed in a short period after its useful life unlike petroleum derived synthetic foam. The furniture giant Ikea, is also determined to reduce its use of petroleum-based synthetic packaging materials and is keen to substitute polystyrene. Mycelium based cushioning materials is one among the few alternative materials under consideration (Abhijith et al. 2018; Mojumdar et al. 2021).

6 Production of Mycelium Biocomposite Materials

The production of the mycelium biocomposite samples can be divided into two phases. In the first phase, the mycelium/mushroom roots of *Pleurotus ostreatus* species of oyster mushroom are grown and the biocomposite samples are prepared in the second phase.

6.1 Phase 1: Growing of Mycelium

Mycelia is usually grown in a basal medium such as mineral salt, and is supplemented with nutrients like sources of carbon, nitrogen and inorganic compounds (Zhu et al. 2015). Factors like initial pH, carbon source, nitrogen source and presence of inorganic compounds has a heavy influence on the process of microbial mycelia production under submerged fermentation conditions. While factors like fermentation temperature, time, rotation speed and inoculum size affect the fermentation process. A few organic compounds that influences the production of mycelium are $ZnSO_4$, $MgSO_4$, $CuSO_4$, $CaCO_3$, K_2HPO_4, KH_2PO_4, and $Zn(CH_3COO)_2$ (Zhu et al. 2015).

The *Pleurotus ostreatus* species oyster mushroom has been reported as a most favorable choice used for the development of mycelium (Sivaprasad et al. 2021; Hoa and Wang 2015). Oyster mushroom spawns have more life and can endure higher temperatures when compared to other species of mushrooms (Zhu et al. 2015). The growth of mycelium is enhanced by using a medium of potato dextrose agar (PDA), (HI media MH096). The bio composite material has been reported to be prepared in a matrix of growth promoting materials like sawdust or coir pith. Fine particles of the substrate are more suitable for growth of mycelium as the size of particles have a direct impact on binding. Once the required growth of the sample is achieved then it is chemically sterilized and the excess moisture is driven out.

The fruiting body of the mushroom was sliced into tiny parts and was kept at the base of a mixture of steam sterilized Potato dextrose agar (PDA) medium and distilled water. An incubation temperature between 24 °C and 27 °C is important for growth of mycelium and the samples were kept at 25 °C for about 5–7 days. (Islam et al. 2017; Bruscato et al. 2019). Figure 2 shows the various samples of fungal mycelium.

The microscopic images of mycelium under different stages of its growth are shown in Fig. 3. These pictures are captured at an interval of 7 days. The first image is captured on the seventh day just after the mushroom is moved to dextrose agar. The image has been contaminated with the presence of external fungi. Mycelium was again freshly sub cultivated and a second image showing development of mycelia was captured on 14th day. The third image captured on the 21st day shows mycelium growing without contamination (Jose et al. 2021).

.

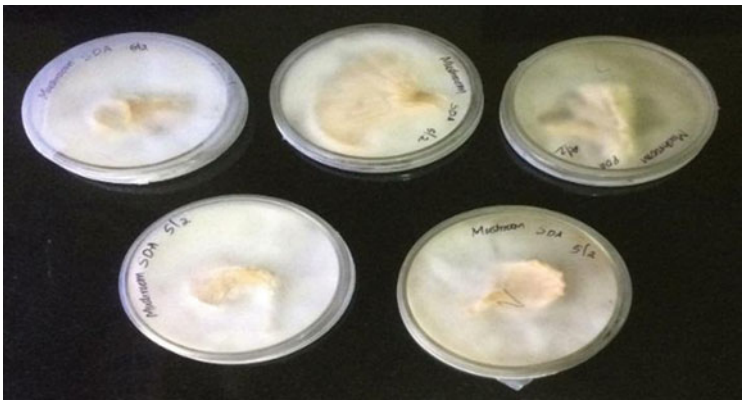


Fig. 2 Mycelium fungus

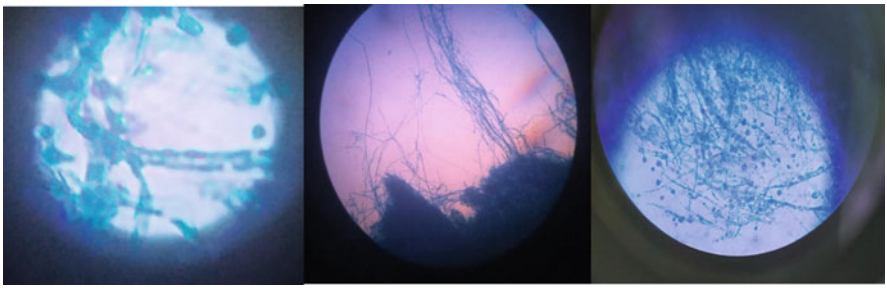


Fig. 3 Microscopic images of growing mycelium

6.2 Phase 2: Preparation of the Biocomposite Materials

Mycelium formed in the first phase is placed in the sterilized substrate at a temperature of 25 °C and relative humidity of 80% and provide a source of nutrition for further growth of mycelium. Sterilization process will kill the microbes present inside the mixture and will cease the growth of mycelia and it takes at least a week for the mycelium to attach entirely to the substrate. It is then transferred to the mold so that the bonds become closer and form a stable structure (Jose et al. 2021). Figure 4 show the biocomposite sample developed from sterilized sawdust and mycelium fungus.

The shelf life of the material is a priority as these bio composite materials can only be a viable substitute to polystyrene if their shelf life is also good as they mainly find their application in the packaging industry, where it might also have to be stored for extended periods. Fungal mycelium-based biocomposite materials are reported to have a lower shelf life and attempts have been made to improve their shelf life by adding coir pith as a substrate material along with saw-dust in the ratio 2:3 (Sivaprasad et al. 2021). The presence of lignin in coir-pith makes it difficult for

Fig. 4 Mycelium-sawdust bio composite foam

the microbes to digest them. Hence the presence of coir-pith can significantly increase the shelf life of mycelium composite materials than when they are prepared alone with sawdust as the substrate. However, any reduction in the amount of saw-dust has led to a reduced growth rate as it is the main nutrient supplier for mycelia in and a decrease in the amount of coir-pith can lead to a decrease in shelf life as the lignin content is reduced (Bruscato et al. 2019; Shashirekha and Rajarathnam 2007; Islam et al. 2018).

Figure 5 shows the process of preparing the mycelium composite material samples. The spawns are evenly distributed in the mold initially before the mold is filled with matrix (saw-dust coir-pith mixture) material. Once the inoculant and substrate are placed in the mold, the mold is fully wrapped but with sufficient holes punctured to maintain enough ventilation and humidity. The molds filled with saw-dust coir-pith mixture and spawns are kept in a laminar airflow cabinet for around 2 weeks and during which, their growth is monitored and water is sprayed every 2 days to ensure the required humidity. On completion of the growth period, the sample is dried in a hot air oven at a temperature of 140 °C for 20 mins (Sivaprasad et al. 2021). Drying of the samples drive out the moisture in it and in the process makes it light in weight. It also inhibits the further growth of mycelium Fig. 6 shows the various samples fabricated with different shapes and size.

Fig. 5 Process of preparing the samples

Fig. 6 Mycelium bio composite samples fabricated in different shapes and size

7 Properties of Mycelium Biocomposite Materials

Promising results were observed when the dense mycelium bio composite samples were subjected to various characterization tests. Tests like thermogravimetric analysis (TGA) and Differential Scanning Calorimetry (DSC) analysis indicated that the thermal stability of mycelium-sawdust bio composite specimens are far more superior when compared to that of expanded polystyrene (Jose et al. 2021). Mycelium bio composites are made of biodegradable raw materials like mushroom roots and sawdust, and this biodegradable nature of mycelium composite materials is a significant influence for its selection as an alternative material to replace expanded polystyrene in packaging applications. Sustainable materials have been an important player in the strategy to reduce environmental pollution. Mycelium bio composite

materials require only minimum energy for their production and thus is a strong competitor to expanded polystyrene in packaging applications.

7.1 Toxicity Test

Incineration is not the best way of waste disposal for either EPS or mycelium bio composite materials. However, the non-biodegradable polystyrene waste is more susceptible to accidental fires in waste landfill sites especially if they are not wholly covered with soil as they are meant to be. Mycelium based bio composite material will quickly biodegrade in outdoor environmental conditions so that the instances involving their burning in a landfill site will be rare (Bruscato et al. 2019; Girometta et al. 2019). When polystyrene is burned, it emits hazardous gases and releases particulate matter to the atmosphere. A toxicity test was performed using a Respirable dust sampler (Envirotech, APM 460 NL) in order to find the extent up to which the amount of particulate matter concentration and hazardous gases has been emitted when polystyrene and mycelium composites were burned. Combustion products contained traces of gases like nitrogen dioxide, carbon monoxide, and sulfur dioxide. The amount of particulate matter released into the atmosphere is shown in Table 1 (Jose et al. 2021).

Results of toxicity tests suggests that burning of expanded polystyrene releases more toxic substances than mycelium bio composite samples. The results are in agreement with the previous results reporting the flame-resistant nature of mycelium-based composites due to high char residue and release of water vapour (Jones et al. 2018).

The burning of mycelium bio composite materials release more carbon monoxide and Sulfur dioxide than when EPS is burned. This is due to the presence of saw dust material as substrate in the bio composite. However, the amount of particulate matter released is very less for the bio composite than that of EPS. Nitrogen dioxide emissions from bio composite material is nearly half that of EPS. Even though mycelium bio composite materials release significant amounts of Sulfur dioxide and carbon monoxide when burned, the instances of mycelium bio composite material catching a fire is very rare as they degrade very quickly leaving little chances of an accidental fire.

Table 1 Combustion products of mycelium sawdust composite material

Sl. No.	Combustion products in $\mu g/m^3$	Expanded Polystyrene	Mycelium bio composite
1	Sulfur dioxide	95.59	167.87
2	Nitrogen dioxide	89.78	43.80
3	Particulate matter	1643.0	153.31
4	Carbon monoxide	49	92

7.2 Scanning Electron Microscopy and Energy Dispersive Spectrum Analysis

The Scanning Electron Microscopy (SEM) image of mycelium composite materials clearly indicate the presence of a network-like microstructure with fine filament distribution in the matrix while polystyrene is reported to have a plate-like structure. It is evident that the mycelium composite also has a well-defined structure when compared to EPS with long polymeric chain structures The open cell structure of mycelium composite material is responsible or the cushioning effect of the foam material which enables it as a suitable alternative for packaging applications (Jose et al. 2021).

The SEM image of mycelium composite (see Fig. 7(a)) at a magnification of 500X shows mycelium filaments binding loosely packed sawdust matrix. The air voids present in the structure is responsible for the lightweight characteristics of the mycelium composite material. Figure 7(b) shows the Energy Dispersive Spectrum (EDS) which reveals the elements present in the sample and they are potassium (K), carbon (C), oxygen (O), magnesium (Mg), Aluminium (Al), and Silicon (Si) (Jose et al. 2021).

The matrix of mycelium bio composite materials has a continuous orientation throughout the material which indicated that their physical properties are consistent throughout the material. (Girometta et al. 2019).

7.3 Density and Moisture Content

The average bulk density for mycelium - sawdust composites were found to be 178.5 kg/m^3 when polystyrene has a density of 1040 kg/m^3. Mycelium composite materials are biologically grown without applying any compressive forces. Samples

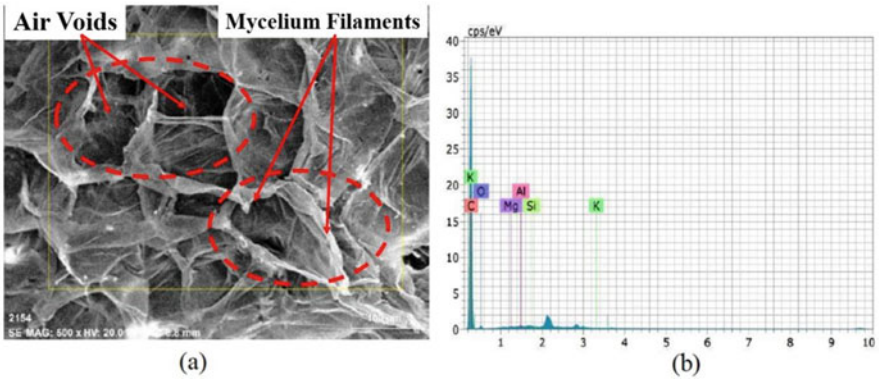


Fig. 7 Mycelium bio composite specimens (**a**) SEM image (**b**) EDS spectrum

with a higher density can be achieved through compressing and densifying of the material. However, this densification can bring out radical changes in other properties of the material. The effect of densification in mycelium based materials is sparsely studied or reported. (Jose et al. 2021).

The moisture content in mycelium composite samples were around 30% while EPS has nearly zero water retention. Moisture retention is a serious determining factor especially when the primary application is in the field of packaging. Applying a thin film of lamination over the surface can greatly increase the moisture resistance of mycelium composites which also increases the shelf life but it comes at the cost of reducing the biodegradability.

7.4 Water Absorption Test

The average density of mycelium-based material after water absorption is about 318.309 kg/m^3. Biocomposite samples remained afloat despite gaining 120–185% mass as shown in Table 2 (Sivaprasad et al. 2021; Attias et al. 2020). The mycelium composite was able to retain its dimensions without any deformation, decomposition or warping even after absorbing water. This indicates that the composite material is a suitable substitute for packaging applications where the product needs to be stored for a limited amount of time.

A biocomposite material that absorbs water is definitely not an ideal feature for a packaging material. However, the mycelium composite material can be considered when the items packed are dry and free from liquids.

7.5 Acoustic Impedance Test

Mycelium composites have superior sound absorbing properties than EPS as evident from Table 3. Mycelium biocomposite material has a higher sound absorption coefficient compared to EPS. Sound waves are either absorbed or reflected and are only partially transmitted through the bio composite (Sivaprasad et al. 2021).

Table 2 Water absorption of mycelium composite material

Sl No.	Time (h)	Weight (kg)	Volume (cm^3)
1	0.5	0.065	157.0796
2	1	0.095	171.5969
3	2	0.115	171.5980
4	4	0.135	181.8390
5	24	0.15	158.319
6	48	0.155	188.832
7	96	0.160	188.832

Table 3 Sound absorption coefficient of mycelium composite

Sl.no	Frequency (Hz)	EPS	Mycelium biocomposite
1	250	0.04	0.17
2	500	0.05	0.19
3	1000	0.10	0.33
4	1600	0.33	0.22

The average sound absorption coefficient for mycelium composite is 0.2275 while that of EPS is 0.13. The average sound absorption coefficient of 0.2 is kept as a standard benchmark for categorizing material as a sound insulator or not. Since the average sound absorption coefficient of composite is above 0.2, mycelium composites have a great potential in the field of sound insulation of walls, doors, and ceilings of concert halls, cinema, auditorium, and broadcasting studio (Girometta et al. 2019; Li and Ren 2011).

7.6 Mechanical Strength of Mycelium Composites

It is observed that the average value of compressive strength and compression modulus of biocomposite material is superior to that of EPS. The flexural modulus of mycelium biocomposite is about five times the flexural modulus of EPS. Since the biocomposite material has a higher compression modulus and compressive strength than EPS, it is safe to assume that the biocomposite material is stronger in compression than EPS. The biocomposite material is capable of taking higher compressive loads when compared to EPS, which will help to reduce the thickness of material to be used for packaging which in turn reduces cost. (Sivaprasad et al. 2021).

7.7 Thermal Conductivity and Limiting Oxygen Index

The thermal conductivity of mycelium biocomposite is a little greater than that of the EPS samples. The mycelium composite has a thermal conductivity of about 0.069950 W/m-k whereas the EPS sample has 0.053984 W/m-k. For the same temperature difference, mycelium biocomposite can transfer more heat compared to EPS, hence expanded polystyrene is marginally a better insulator (Sivaprasad et al. 2021).

The observations from Table 4 indicate that the LOI of EPS is less than 21% and that of the biocomposite is greater than 21%, therefore the biocomposite is self-extinguishing and has fire-retardant properties (Rejeesh and Saju 2017). The LOI of mycelium biocomposite is 23% which is classified as a fire-retardant material whereas EPS has an LOI of 19% which can be classified as a flammable material.

Table 4 Thermal conductivity and LOI

Sample	Material		Thermal conductivity (W/m-K)	LOI (O_2%)
1	Expanded polystyrene (EPS)		0.053984	19
2	Mycelium biocomposite		0.069950	23

8 Application of Mushroom Biocomposites

It was in the 1980s, where Shigeru Yamanka a Japanese scientist reported the gluing power of mycelium to be effective in the paper industry and in manufacture of building materials (Mojumdar et al. 2021). Recently, mycelium is being used in many industries like design, fashion, packaging, architectural design, etc.

The possibility of using mycelium bricks were also exploited to find new applications including the construction sector. Mycelium bricks were used to build a tower as tall as 40 feet and it is the largest structure reported to be made from mushroom materials. Over the past few years, mycelium has been used in building a few architectural projects like Hy-Fi Tower, the Mycotecture alpha, MycoTree (Abhijith et al. 2018; Mojumdar et al. 2021). David Benjamin designed a temporary structure of the Hy-Fi tower in the form of three 13-m tall intersecting cylinders built with 10,000 blocks of mycelium and the tower remained for 3 months in summer (Mojumdar et al. 2021). Phill Ross, the cofounder of MycoWorks designed a small-scale pavilion Mycotecture Alpha. (Karana et al. 2018).

It has also been tried as an insulation material similar to insulation boards, leading to a more energy efficient building It also achieved class A fire rating without using toxic fire retardants. According to Ecovative, their product can be used in many more applications than just packing cases. (Abhijith et al. 2018).

Myco Board is an engineered wood substitute that has the potential to replace wood, plywood or similar engineered wood products (Fig. 8). Engineered wood products that use formaldehyde resins can be substitute by a resin less technology where particles are bonded together with naturally occurring mycelium

Fig. 8 Myco-boards made from mycelia

Myco-boards are expected to substitute popular fabricated wood substitutes like medium density fiber boards and particle boards (Abhijith et al. 2018).

Greensulate is a trade mark fire-retardant board from Ecovative Design that is primarily used for sustainable insulation (Abhijith et al. 2018; Casini 2016). MycoWorks and MOGU Inc. have been successful in producing synthetic leather from mycelium. The product is durable, flexible, sustainable and waterproof. "Future of Plastics" is a project started by OFFICINA CORPUSCOLI that focuses on the development of kitchenware and substitutes to disposable plastics (Mojumdar et al. 2021).

MOGU have been developing mycelium-based wall and ceiling panels (Mojumdar et al. 2021). A Shell Mycelium domed building has been constructed in India for Kochi Muziris Biennale 2016 (Mojumdar et al. 2021).

Various investigations to find newer applications for mycelium composites especially in the field of architecture arein place world wide. The objective is to improve the mechanical, thermal and chemical properties of mycelium-based bio composites and find a way for affordable, and durable, structures which can challenge environmental threats and weather.

9 Conclusion

Fungal mycelium-based bio composites are a centre of attraction not just because it is a sustainable choice instead of the conventional non-biodegradable packaging material, but they are also researched into newer applications like air purification filters or as insulation panels. The manufactured mushroom based packaging material has to maintain a consistent density with a binding material that is a microbe. The real challenge also lies in the scaling up of the production of mycelium composite. In general, factors like reproducibility, or mechanical and thermodynamic properties of mycelium bio composites are still inferior to synthetic materials. These synthetic materials have been in use under extreme conditions for a long time now and are found useful in a variety of applications. However, the investigation on mycelium composites has only recently gained momentum and thus it is safe to conclude that these materials have all the potential to substitute the conventional packaging materials which are mostly petroleum derived synthetic materials. Mycelium bio composite materials as an alternative to expanded polystyrene could largely change the way how packaging industry works currently but it is expected to happen silently than as a radical shift.

References

Abhijith R, Ashok A, Rejeesh CR (2018) Sustainable packaging applications from mycelium to substitute polystyrene: a review. Mater Today Proc 5(1):2139–2145

Appels FV, Camere S, Montalti M, Karana E, Jansen KM, Dijksterhuis J, Krijgsheld P, Wösten HA (2019) Fabrication factors influencing mechanical, moisture-and water-related properties of mycelium-based composites. Mater Des 161:64–71

Arifin YH, Yusuf Y (2013) Mycelium fibers as new resource for environmental sustainability. Procedia Eng 53:504–508

Ashok A, Abhijith R, Rejeesh CR (2018) Material characterization of starch derived bio degradable plastics and its mechanical property estimation. Mater Today Proc 5(1):2163–2170

Ashok A, Rejeesh CR, Renjith R (2016) Biodegradable polymers for sustainable packaging applications: a review. IJBB 2(2):1–11

Attias N, Danai O, Abitbol T, Tarazi E, Ezov N, Pereman I, Grobman YJ (2020) Mycelium biocomposites in industrial design and architecture: comparative review and experimental analysis. J Clean Prod 246:119037

Attias N, Danai O, Ezov N, Tarazi E, Grobman YJ (2017) Developing novel applications of mycelium based biocomposite materials for design and architecture. In: Proceedings of Building with Biobased Materials: Best practice and Performance Specification, 6th–7th September, pp 76–77

Boswell GP, Jacobs H, Davidson FA, Gadd GM, Ritz K (2003) Growth and function of fungal mycelia in heterogeneous environments. Bull Math Biol 65(3):447–477

Bruscato C, Malvessi E, Brandalise RN, Camassola M (2019) High performance of macrofungi in the production of mycelium-based biofoams using sawdust—sustainable technology for waste reduction. J Clean Prod 234:225–232

Casini M (2016) Smart buildings: advanced materials and nanotechnology to improve energy-efficiency and environmental performance. Woodhead Publishing, Sawston

Containers P, Good ND (2016) Documentation for greenhouse gas emission and energy factors used in the waste reduction model (WARM)

Cooper TA (2013) Developments in bioplastic materials for packaging food, beverages and other fast-moving consumer goods. Trends in Packaging of Food Beverages and Other Fast-Moving Consumer Goods (FMCG) 5:108–152

Ellen MacArthur Foundation (2017) The new plastics economy: Rethinking the future of plastics and catalysing action. http://www.ellenmacarthurfoundation.org/publications

Geyer R, Jambeck JR, Law KL (2017) Production, use, and fate of all plastics ever made. Sci Adv 3(7):e1700782

Ghazvinian A, Farrokhsiar P, Vieira F, Pecchia J, Gursoy B (2019) Mycelium-based biocomposites for architecture: assessing the effects of cultivation factors on compressive strength. In: The eCAADe and SIGraDi Conference. University of Porto, Porto

Girometta C, Picco AM, Baiguera RM, Dondi D, Babbini S, Cartabia M, Pellegrini M, Savino E (2019) Physico-mechanical and thermodynamic properties of mycelium-based biocomposites: a review. Sustainability 11(1):281

Gooday GW (1995) The dynamics of hyphal growth. Mycol Res 99(4):385–394

Haneef M, Ceseracciu L, Canale C, Bayer IS, Heredia-Guerrero JA, Athanassiou A (2017) Advanced materials from fungal mycelium: fabrication and tuning of physical properties. Sci Rep 7(1):1–11

Hoa HT, Wang CL (2015) The effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). Mycobiology 43(1):14–23

Hopewell J, Dvorak R, Kosior E (2009) Plastics recycling: challenges and opportunities. Philos Trans R Soc Lond Ser B Biol Sci 364(1526):2115–2126

Iles A, Martin AN (2013) Expanding bioplastics production: sustainable business innovation in the chemical industry. J Clean Prod 45:38–49

Islam MR, Tudryn G, Bucinell R, Schadler L, Picu RC (2017) Morphology and mechanics of fungal mycelium. Sci Rep 7(1):1–12

Islam MR, Tudryn G, Bucinell R, Schadler L, Picu RC (2018) Mechanical behavior of mycelium-based particulate composites. J Mater Sci 53(24):16371–16382

Jiang L, Walczyk D, Mooney L, Putney S (2013) Manufacturing of mycelium-based biocomposites. In: Manufacturing of mycelium-based biocomposites. Proceedings of the International SAMPE Technical Conference, Long Beach, CA, pp 1944–1955

Jones M, Bhat T, Kandare E, Thomas A, Joseph P, Dekiwadia C, Yuen R, John S, Ma J, Wang CH (2018) Thermal degradation and fire properties of fungal mycelium and mycelium-biomass composite materials. Sci Rep 8(1):1–10

Jones M, Huynh T, Dekiwadia C, Daver F, John S (2017) Mycelium composites: a review of engineering characteristics and growth kinetics. J Bionanosci 11(4):241–257

Jose J, Uvais KN, Sreenadh TS, Deepak AV, Rejeesh CR (2021) Investigations into the development of a mycelium biocomposite to substitute polystyrene in packaging applications. Arab J Sci Eng Arab J Sci Eng 46(3):2975–2984

Karana E, Blauwhoff D, Hultink EJ, Camere S (2018) When the material grows: a case study on designing (with) mycelium-based materials. Int J Des 12(2):119–136

Kazmierski C (2012) Growth opportunities in global composites industry 2012–2017. Composites:21–23

Li Y, Ren S (2011) Acoustic and thermal insulating materials. In: Building decorative materials. Woodhead Publishing, pp 359–374. https://doi.org/10.1533/9780857092588.359

Matthews FL, Rawlings RD (1999) Composite materials: engineering and science. Elsevier

Miles PG, Chang ST (2004) Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact. CRC Press

Mohanty AK, Misra M, Drzal LT (2005) Natural fibers, biopolymers, and biocomposites. CRC Press

Mojumdar A, Behera HT, Ray L (2021) Mushroom mycelia-based material: an environmental friendly alternative to synthetic packaging. In: Vaishnav A, Choudhary DK (eds) Microbial polymers. Springer, Singapore, pp 131–141. https://doi.org/10.1007/978-981-16-0045-6_6

Olsson S (1995) Mycelial density profiles of fungi on heterogeneous media and their interpretation in terms of nutrient reallocation patterns. Mycol Res 99(2):143–153

Rejeesh CR, Saju KK (2017) Effect of chemical treatment on fire-retardant properties of medium density coir fiber boards. Wood Fiber Sci 49(3):332–337

Rigamonti L, Grosso M, Møller J, Sanchez VM, Magnani S, Christensen TH (2014) Environmental evaluation of plastic waste management scenarios. Resour Conserv Recycl 85:42–53

Rosato DV, Rosato MG (2012) Injection molding handbook. Springer Science & Business Media

Shashirekha MN, Rajarathnam S (2007) Bioconversion and biotransformation of coir pith for economic production of Pleurotus Florida: chemical and biochemical changes in coir pith during the mushroom growth and fructification. World J Microbiol Biotechnol 23(8):1107–1114

Sivaprasad S, Byju SK, Prajith C, Shaju J, Rejeesh CR (2021) Development of a novel mycelium biocomposite material to substitute for polystyrene in packaging applications. Mater Today Proc 47(15):5038–5044 https://doi.org/10.1016/j.matpr.2021.04.622

Tudryn GJ, Smith LC, Freitag J, Bucinell R, Schadler LS (2018) Processing and morphology impacts on mechanical properties of fungal based biopolymer composites. J Polym Environ 26(4):1473–1483

Vilaplana F, Strömberg E, Karlsson S (2010) Environmental and resource aspects of sustainable biocomposites. Polym Degrad Stab 95(11):2147–2161

Zhu W, Guo C, Luo F, Zhang C, Wang T, Wei Q (2015) Optimization of Calvatia gigantea mycelia production from distillery wastewater. J I Brewing 121(1):78–86

Fungi for Material Futures: The Role of Design

N. Ivanova

Abstract The last decade has seen a rapid growth in design interest, research and development of mycelium-based technologies for various applications across textiles, fashion, product, furniture and architecture domains. Building on an ancient relationship between fungi and humankind—well documented by ethnomycology literature and advanced through both biotechnology and creative practice—a new partnership between design, science and industry leaders has pioneered the market introduction of fungi-derived products. The careful crafting of material, aesthetic and performance properties, paired with an open, collaborative and conscious approach to material innovation, has meant that the early concept designs, protypes, and commercially realised applications, present a holistically considered future of mycelium products, environments and systems. This chapter charts an overview of key moments, considerations and stakeholders in this growing design domain, with a view to providing a resource for the next generation of innovators, who will advance the scope and future applications of fungi in design.

Keywords Mycelium · Materials · Innovation · Biodesign · Biofabrication · Design thinking · Sustainability · Interdisciplinarity

1 Introduction

> We're on a journey to create a more sustainable world. But what if the best way to work for nature was to work with it? And what if the answer was right under our feet. . . (adidas 2021)

Thus begins the launch of *adidas*' latest concept shoe—the *Stan Smith Mylo*^{TM}—a mycelium-based interpretation of their iconic footwear design. The product was launched recently as part of a newly-established fashion consortium between *Stella McCartney*, *adidas*, *lululemon* and *Kering* who, in October 2021, announced an industry-first partnership and investment in consumer biomaterials, to advance the

N. Ivanova (✉)
Helen Hamlyn Centre for Design, Royal College of Art, London, UK
e-mail: ninela.ivanova@rca.ac.uk

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_12

Fig. 1 Mylo™ 'leather' and material swatches. © Bolt Threads

productisation and market realisation of *Mylo*^{TM} (Fig. 1). *Mylo*^{TM} is a fungi-derived alternative to leather. It is developed by engineering the vegetative growth of filamentous fungi (mycelium) into material sheets that could be fashioned into a range of fashion and sportswear products.

Mylo^{TM} is only one of a range of mycelium-based design propositions which began to enter textile technology and design realms since the early nineties, and has since gained rapid momentum due to the parallel advances in three key areas:

Science-led companies, e.g., *Ecovative*, *Modern Meadow* and *Bolt Threads* amongst others, have invested effort, talent and resources towards the development and commercialisation of *victimless*[1] alternatives to animal-based, or ecologically harmful petroleum-based materials and products. This sits well within the growing domains of bioeconomy[2] (Butu et al. 2020; Lee et al. 2020) and circular

[1] 'Victimless Leather' (Catts and Zurr 2004) was a miniature prototype of a stitch-less jacket grown from immortalised cell lines on a biodegradable polymer matrix which explored the future of lab-grown 'leather'.

[2] The bioeconomy aims to *"reduce the dependence on natural resources, transform manufacturing, promote sustainable production of renewable resources from land, fisheries and aquaculture and their conversion into food, feed, fibre, bio-based products and bio-energy, while growing new jobs and industries"*. https://ec.europa.eu/programmes/horizon2020/en/h2020-section/bioeconomy.

economy[3] (Meyer et al. 2020), and is in alignment with the objectives of the UN's Sustainable Development Goals.

The design community developed a keen interest in exploring novel processes of fabrication, namely biofabrication (Lee et al. 2020), and began collaborating with scientists to *'grow'* design products, as opposed to traditional methods of production that rely heavily on extraction of finite natural resources. Biomaterials, bio-inspired design and biofabrication entered design parlance through the works of designers, such as Suzanne Lee (2005, 2011, 2012), Nancy Tilbury (2009), Carole Collet (2012) and Maurizio Montalti (2010a, b, c) amongst others, who went on to educate and inspire many of today's designers working in this field.

A new type of environmentally and socially conscious, value-driven consumer emerged—one, who would not compromise on the quality of products they buy at the expense of planetary health, and would hold companies accountable to sustainable, transparent and ethical processes of resourcing, manufacturing, supply chain, and end-of-use management (Fletcher 2008; Fletcher and Grose 2012; Thackara 2015).

The parallel development in these areas, an overall change in mindset towards open innovation, collaboration and transparency, and the urgency of the environmental crisis, helped create the right context and time to successfully bring to market mycelium-based materials and products.

The following sections provide a design-focused discussion on the value and applications of fungi for materials and consumer products. This is informed by the author's personal interest and design enquiry in the development of novel bio-based materials for applications in textiles, fashion and furniture.

Key questions that are explored throughout this chapter, with a view to opening up thinking and opportunities for further collaborative design-science-industry investigation, include:

What are the benefits of introducing fungi-based[4] *materials and products? How do we ensure integrity of narrative and market realisation?*

Who are the key stakeholders in bringing these products to market? How do we develop an approach that is inclusive of scientific, design, market, corporate and environmental considerations and benefits?

What are the current gaps and opportunity areas in realising a mycelium-based future for consumer products?

What is the value of design thinking, research and practice in advancing engagement with, perception and realisation of consumer biomaterials?

[3] The circular economy aims to *"redefine growth, focusing on positive society-wide benefits. It entails gradually decoupling economic activity from the consumption of finite resources, and designing waste out of the system. Underpinned by a transition to renewable energy sources, the circular model builds economic, natural, and social capital."* https://www.ellenmacarthurfoundation.org/circular-economy/concept.

[4] 'Fungi-based' and 'mycelium-based' are used interchangeably throughout this article, as the fungi currently used in design, which are referenced in this publication, are all filamentous.

2 The Beginnings of Fungi Material Innovation

2.1 The Untapped Material Potential of the Fungi Kingdom

As a natural resource and raw material for design, fungi appear to be relatively untapped (Cooke 1977; Deshmukh and Rai 2005; Kendall 2013; Stamets 2004). The diverse kingdom, comprising the well-known mushrooms, toadstools, bracket fungi, moulds, yeasts and lichens, is estimated to include between 2.2 and 3.8 million species, of which less than 10% have been described (Willis 2018). Their varied morphology, natural properties, and existing biotechnological applications (Hamlyn 1991; Moss 1987; Singh and Aneja 1999; Stamets and Chilton 1983), create scope to consider a multitude of novel uses that bring together mycology, cutting-edge biotechnology, market leaders, and visionary design thinking and craft. As Charaya and Mehrotra (1999) aver, *"In the emerging 'age of biotechnology', the fungi are expected to provide a wider range of useful products and processes for human welfare under the banner of what is called 'fungal biotechnology'"*.

The roles fungi play for life on Earth have been predominantly the subject of scientific research. Their importance as plant, animal and human pathogens is well documented, and their beneficial applications have long been developed by biotechnology sectors, for example, in food and beverages production, medicine, agriculture, and perfumery (Moss 1987; Wainwright 1992). Fungi are key biological factors in sustaining the planet's ecosystem biodiversity and dynamics (Boddy 2013; Boddy and Coleman 2010). Their natural properties to recycle carbon, nitrogen and other essential elements that feed the soil, have formed the basis for applications such as mycofiltration, mycoforestry, mycoremediation and mycopesticides, which Stamets (2004) proposes as a means to *"the mycological rescue of the planet"*.

Charaya and Mehrotra (1999) further emphasise the unique properties of fungi that make them an appropriate and useful natural resource for novel products, e.g., their ability to produce a variety of enzymes, conferring upon them the ability to colonise and degrade various substrates; the potential to synthesise a great variety of metabolites; and the large surface area of the hyphae through which fungi can interchange substances with their environment. In circular design, where a major concern is how we keep products in use for longer, or how we recycle and upcycle materials that are already in use, this indicates a beneficial application for upcycling by-products not only from the design industry itself, e.g., from fashion and architecture, but also from agriculture, food and beverage production.

In a global call to action, to unlock the potential of plants and fungi for sustainable development, Antonelli et al. (2019) assert*: "Humans have been using biodiversity for hundreds of thousands of years, but at no time in our history has it been more crucial to accelerate our exploration of the useful properties of the species that inhabit the world around us."*

For a designer, the access to such an immense and varied resource triggers the imagination of the myriad potential uses for fungi across the design process—from new bio-based raw materials, e.g., fibres and composites, through colour and

finishing agents, e.g., pigments as well as enzymes used to treat denim, to a range of consumer products across fashion, architecture and product design. Furthermore, this enables us to completely reimagine processes of fabrication through the adoption of biodesign processes from the biotechnology and medical industries, as well as the entire product lifecycle—on one hand from the consumer perspective, and on the other, from a systems perspective as a new bioeconomy.

2.2 A Biofabricated Future

Since ancient times developments in fabric production, technology and function have been interwoven with the advances of human society (Trochmé 2002). Historically textile products have been derived from natural plant and animal resources including cotton, linen, wool and silk, which resulted in a well-established, possibly subconscious affinity to materials of natural origin; their physical and aesthetic properties being associated with softness, comfort and luxury: *"[Even] the raw fibre in its natural state is visually evocative of its potential usefulness to man, the small cloud-like formations of the cotton bolls suggestive of a comforting end product."* (Hallett and Johnston 2010).

The multi-disciplinary nature of material and textile production has long triggered the imagination, of both scientists and artists, about how technology and innovation could advance and enrich the composition of our manmade world and everyday objects. Corbman (1985), p. 310) documents the works of English naturalist Robert Hooke who, in 1664, envisaged that it would be possible to make *"an artificial glutinous composition, much resembling, if not full as good, nay better, than that excrement, or whatever other substance it be out of which the silkworm wire-draws his clew"*. Hooke's vision came into realisation through the invention of nitrocellulose by Louis Marie Hilaire Bernigaud de Chardonnet in 1885 as an alternative to silk. This marked the beginning of a line of man-made materials, e.g., viscose rayon (Handley 1999), which aimed to deliver improved quality and performance at a lower economic cost (Corbman 1985; Hallett and Johnston 2010; Trochmé 2002).

Today, the production of man-made, yet bio-derived materials, is being advanced through the use of biotechnology and biofabrication processes traditionally employed in the biomedical industries (Camere and Karana 2018; Lee et al. 2020). Camere and Karana (2018) explain that through the use of low-energy processes that harness the natural properties of living systems, growing design,[5] or biofabrication, offer a process and resulting materials that *"are not only harmless to the environment and biodegradable, but they can even nurture the cultivation of new materials in their end of life."* Furthermore, Camere and Karana assert that, beyond merely

[5] The material design practice which entails growing materials from living organisms to achieve unique material functions, expressions, and sustainable solutions for product design (Montalti 2010b; Camere and Karana 2018).

offering a replacement for environmentally harmful plastics, such materials could extend and advance the functionality of traditional objects through meaningful applications for a long-term and sustainable change.

At the turn of the century, the idea of alternative biobased materials began to attract design attention and experimentation. Advances in biotechnology and synthetic biology were inspiring creatives to apply traditional methods of design thinking and craft, to draw upon a new palette of media such as bacteria, skin and bone tissue, algae and moss. This was the beginning of *'bio'* material innovation (Lee et al. 2020), which set out to explore a range of alternative biobased[6] materials. The new media, often *grown* in the design studio, or cultured in the lab where scientific collaboration was possible, presented a new and exciting process of fabrication—*'growing design'* (Camere and Karana 2018; Montalti 2010c) as a potential method of self-assembly and material construction resulting in *'biofabricated'* materials and products (see Lee et al. 2020, p. 7 for a full list of terms). The concept designs proposed a plethora of new material possibilities, user experiences, production and consumption models.

One of the pioneers in this design domain was Suzanne Lee, who first explored whether one could *'grow'* a material, through experimentation with a kombucha tea fermentation process that dates back to ancient Japanese methods. The idea emerged through conversation with a biologist as part of Lee's research for her book *Fashioning the Future* (Lee 2005), which led to the inception of *BioCouture*. The material—a type of bacterial cellulose—was grown in a vat of the kombucha recipe until a sufficient layer would form on the top, which could be then harvested and either moulded directly into a desirable form, e.g., a tote-bag, or conventionally cut and sewn to create a garment, e.g., a denim jacket. The resulting material resembled the feel and strength of vegetable leather.

To bring to life the potential applications for this novel material in an accessible format, Lee used the bacterial cellulose to recreate iconic fashion articles, e.g., a kimono, a court shoe (Fig. 2), bomber and biker jackets. Sustainable methods of decoration, such as vegetable dyeing and oxidation, were applied to mimic the aesthetic and enhance the cultural familiarity and high-street appeal of these artefacts. The familiarity of the finished prototypes helped stakeholders visualise and understand how *BioCouture* could offer a new line of sustainable, organic, biocompostable and biodegradable products to the market (Lee 2011, 2012).

BioCouture, and similar projects exploring processes of fermentation (Franklin and Cass 2014; Lee 2011), tissue culture (Catts and Zurr 2004; Congdon 2013; Forgacs 2013; Tilbury 2009; Thompson et al. 2006) and synthetic biology (Ginsberg

[6]Biobased materials are *"wholly or partly derived from biomass, such as plants, trees or animals (the biomass can have undergone physical, chemical or biological treatment)"* (https://www.cen.eu/work/areas/chemical/biobased/Pages/default.aspx) and exclude those derived from fossil sources. Traditional examples of biobased materials would include, but are not limited to: natural fibres (e.g., cotton, wool and silk), manmade cellulosics (e.g., viscose), natural polymers (e.g., chitin, keratin and casein), animal leathers and their alternatives, through to polycotton blends (where the biocontent meets the minimum stipulated requirement).

Fig. 2 BioCouture shoe as exhibited at Alive: New design frontiers (Collet 2013) © Author's archive

and King 2009-2011; Collet 2012), raised questions about the meaning of traditional interactions between the human body and clothing, and the moral and ethical considerations around the use of living materials, manipulated or otherwise. They probed what the material makeup of our world post-2050 could be, and highlighted that, in a future where any material could be engineered and programmed at the cellular level, designers had a new role and a great responsibility to bear, as creators of both living and non-living matter.

In light of environmental concerns, sustainability and wellbeing, it was considered that such methods, which built on naturally occurring processes and were advanced by biotechnology, synthetic biology and material science, would require less human labour, fewer natural resources and low-energy production conditions, to be ultimately kinder to the planet and human health.

> Forget harvesting fields of cotton then spinning and weaving cloth. Imagine if we could grow clothing. I grow sheets of bacterial-cellulose in a green tea solution to produce a textile material [. . .] My vision is to produce desirable textiles and clothing with the utmost respect for the natural world. (Lee 2009)

However, in the two decades between 1990 and 2010, few designers the world over were exploring fungi as a potential resource for design fabrication. In fashion in particular, there are only a few well documented examples from that period.

In 1997, Belgian designer Martin Margiela's presented a series of experimental works for his solo exhibition '9/4/1615' at the *Boijmans van Beuningen Museum* of Rotterdam. In collaboration with a microbiologist, Margiela treated one replica garment from each of his previous collections with bacteria and moulds, as a means of critiquing the transient nature of the fashion industry through the natural cycle of creation and decay on Earth.

Fig. 3 Donna Franklin, 'Fibre Reactive' dress, Pycnoporus coccineus, silk organza, 2004. Photographer: Robert Firth. Image courtesy of SymbioticA

Australian designer, Dr. Donna Franklin used the mycelium and fruit bodies of the fungus *Pycnoporus coccineus* (orange bracket fungus) to construct a *'living'* dress (Franklin 2004). The resulting work entitled *'Fibre Reactive'* (Fig. 3) was exhibited at the *Biennale of Electronic Art Perth* (Franklin 2004) and the *Second Skin* exhibition at the *Smithsonian Cooper-Hewitt, National Design Museum* (2006) amongst others.

When the author's investigation of fungi as a potential material for fashion and textiles fabrication began in 2010, the idea that one could design an artefact out of mycelium, which would cohabit our everyday environment, or even replace some of the materials we have used for thousands of years, e.g., cotton, was still novel, foreign, and well situated in the *'What if…?'* sections of speculative exhibitions (Ivanova 2011; Montalti 2010c).

A pertinent example is Maurizio Montalti's conceptual project *'Continuous Bodies: cycles of decomposition triggering a symbiotic partnership between humans and fungi'* (Montalti 2010a) which proposed the use of a mycelium shroud as a

sustainable medium to decompose the human body when buried, and transform the toxins produced during the process of decay into useful compounds for the soil, thereby including the human being as part of nature's eternal cycle. This speaks directly to the work of Stamets (2004) who calls fungi *"the interface organisms between life and death",* in reference to their natural properties to recycle carbon, nitrogen and other essential elements that in turn feed the soil, trees and other plants.

In product design, artist Phil Ross and designers Maurizio Montalti and Eric Klarenbeek were amongst the first to explore the natural properties of mycelium to decay, bind and solidify matter, to grow furniture and architectural elements, e.g., chairs and building blocks. In 2007, the US company *Ecovative Design LLC* was founded by Eben Bayer and Gavin McIntyre after graduating from *Rensselaer Polytechnic Institute (RPI)*, New York (NY), to exploit the properties of mycelium to self-assemble lignin and cellulose. *Ecovative* used mycelium to transform agricultural byproducts into composite materials which could offer sustainable alternatives to packaging and insulation materials. A more detailed description of the extended practice of Ross, Montalti, Klarenbeek and *Ecovative*, as well as others, is provided in Sect. 3 'Fungi applications in contemporary design and bioconsumer products'.

2.3 Ethnomycology

Much less known are some of the miscellaneous ethnomycological[7] uses of fungi outside of food and medicine, which date back at least 8000 years (Boddy and Coleman 2010; Dugan 2011; Harding 2008; Spooner and Roberts 2005).

For example, dried fruit bodies of *Haploporus odorus,* which has anise-like smell, were worn as scented body adornment by indigenous people, to enhance manhood and create a spiritual link with the Gods. Shaggy and common inkcaps (*Coprinus comatus* and *Coprinus atramentarius*) were used to manufacture ink. Timber stained in blue-green by the green elfcup (*Chlorociboria aeruginascens*) was incorporated into small saucer-like objects named Tunbridge ware, after the town of Tunbridge Wells in Kent, UK where they were produced (Harding 2008; Spooner and Roberts 2005). *Fomes fomentarius* and other bracket fungi, e.g., the willow bracket *(Pjellinus ignarius),* the birch bracket *(Piptoporous betulinus),* the maze gill *(Datronia mollis)* and the chicken of the woods *(Laetiporus suplhureus),* were used as tinder to carry fire (Harding 2008). The black shiny rhizomorphs of *Polyporus rhizomorphus* were fashioned into belts in Gabon, and the horse-hair-like hyphae of *Marasmus crinisequi* were used for jewellery strings in Congo and Indonesia (Spooner and Roberts 2005).

[7] Ethnomycology is a field of studies concerned with the various cultural receptions and uses of fungi by humankind throughout history (Dugan 2011, Singh and Aneja 1999).

For the benefit of design, such miscellaneous applications provide an invaluable starting point for thinking and development around novel bioconsumer products. A case in point is the suede-like material Amadou, which is derived from the tinder polypore *Fomes fomentarius*. For hundreds of years craftsmen in Hungary, Bohemia and Romania have extracted the inner, fleshy part of the fruitbody, i.e., the fibrous trama (Cooke 1977; Gandia et al. 2021), and made this into sheets of the leather-like material—varying in size depending on the maturity the fruitbody—which were then used to fashion hats and other wearable artefacts sold at town markets and fares (Boddy and Coleman 2010; Dugan 2011; Gandia et al. 2021; Spooner and Roberts 2005).

The species listed above are some of the fungi that are currently of greatest interest to designers and industry, due to their well-documented beneficial uses and applications. Through a multi-disciplinary exchange in material innovation, what was once taken from nature, as with the *Fomes fomentarius* fruit body to become a hat, could be scientifically engineered and replicated to create mycelium foams, and leather-like materials and composites, that can replace finite resources and environmentally harmful materials and products.

2.4 Fungi for Colour and Paper

An area of growing interest in the creative sector is the use of fungi for dyeing (Fig. 4) and paper. Mushroom dyes which were initially used by indigenous cultures as war paints and early forms of makeup and cosmetics, have a long-standing tradition, which stems from the production of *'orchil'* and *'cudbear'* dyes from lichens (Spooner and Roberts 2005).

Nowadays, a range of fungi species have been tested and described as an alternative to plant-based or chemical dyes, which can be used to dye fibres from plant and animal origin, as well as manmade protein materials. This is thanks to a global movement and an international community of mushroom dyers that began in Mendocino, CA in the 1960s with Miriam Rice (Jan 1918–Aug 2010) who serendipitously discovered that a clump of Sulphur tufts gave a yellow dye. Rice went on to experiment with a range of fungal species and yarns, documenting the process and resulting colours, and passing the knowledge onto others via workshops and symposia (Johansson 2016; Rice and Beebee 1980, 2007).

Using traditional methods of plant-based dyeing which rely on the use of mordants to (1) extract the pigment from the source, and (2) bind the pigment to the fibre, fungi can yield a full spectrum of colour (Fig. 5), and often times a range of different colours from the same fungus. For example, *Dermocybe phoenicea* var. *occidentalis*, could give a range of pastel pinks, bright reds, browns and dark purples by regulating the pH using white vinegar and washing soda (Rice and Beebee 2007).

A known benefit of fungi dyes is the chitin content of the fungal cell wall, which yields dye results that are wash- and colour-fast, compared to some of the outcomes from plant-based dyeing.

Fig. 4 Samples of fungi dyed wool—various species. © Author's archive

Further applications, developed by members of the *International Fungi & Fibre Federation (IFFF)* include the use of fungi to extract pigments for watercolours and myco-stix™ (Johansson 2016; Mushrooms for Color 2017; Rice and Beebee 2007), and to produce hand-crafted paper (Fig. 6) from trimitic polypores such as *Piptoporus betulinus*, *Fomitopsis pinicola*, *Fomes fomentarius, Trametes hirsuta, Trametes ochracea, Cerrena unicolor, Lenzites betulina, Daedalea quercina* and *Ganoderma applanatum* (Johansson 2002; Johansson 2016; Rice and Beebee 2007). Much like the paper made from crustaceans, the paper derived from fungi is chitin-based and of high value. The paper colour would vary depending on the natural colour of the bracket, but could be modified during the making process, e.g., by using waste dye baths from the mushroom dyeing process. An additional benefit of making paper from fungi, is their high lignin content, as also noted by Hamlyn (1991), which means that the production of the paper does not require an additional binding agent, thereby not compromising the wholly natural composition of the paper.

For the author, an engagement with the *International Fungi & Fibre Federation* through their biannual international symposium in Jaca, Spain in 2012, presented a rare opportunity to experience first-hand fungi dyeing, paper-making and watercolour painting processes (Ivanova 2012a). Being part of a global community of artisans who carry a rich knowledge base of mycological science, chemistry, textile technology, spinning, weaving and dyeing, helped identify which fungi

Fig. 5 Silk scarves dye project by the Scottish Fungi Dye Group (Members include: Marilyn Caddell, Patricia Gow, Marilyn Clark, Chris Simpson, Anna S King, Janette McKeown, Rita Barth, Gil Osirio, Joan Gale, Dee Crewdson, Ann Mulley and Jean Mounter). Fungi dyes used: Cortinarius sanguineus (caps) – reds, Phaeolous schweinitzii + iron – soft greens /blue, Innonotus hispidus + iron—khaki greens, Phaeolous schweinitzii—yellow, Cortinarius semisanguineous (stalks)—peach, Innonotus hispidus—gold, Hapilopilus nidulans + ammonia—purples, Paxillus atromentosus, Paxillus atromentosus + iron—silver /dark greys. © The Scottish Fungi Dye Group

species would have the right properties for applications within the fashion and textiles domain (see further Sect. 4.1 'Material experimentation').

2.5 Fungi in Textile Technology: Early Experiments

In 1991, Dr. Paul F Hamlyn and a group of scientists at the British Textile Technology Group (BTTG), were examining the roles of fungi in deteriorating textiles—a particular issue with the uniforms of troops fighting in tropical regions during World War II. Manmade materials were not popular at that time. This meant that uniforms were made of cotton, which easily degraded in hot and very humid conditions. This investigation into the roles of fungi in bio-deterioration of textiles, which in itself had significant implications for the protection of cultural heritage and artifacts (Breuker et al. 2003; Hamlyn and McCarthy 2000), led to a new line of enquiry looking at the potential beneficial applications of fungi in textiles—first published in Textile Technology International (Hamlyn 1991).

The filamentous structure of fungal mycelium indicated the type of materials that could be produced, and the properties of fungi provided a rationale for the range of applications. The novel cell wall chemistry, based on chitin suggested a potential use

Fig. 6 Paper swatches produced from Piptoporus betulinus, Fomitopsis pinicola and Fomes fomentarius. © Johansson 2002 (left) and Mushroom paper created from various species © Johansson 2016 (right)

of fungal materials for wound dressings and medical textiles (Hamlyn and Schmidt 1994). The fine filamentous structure indicated that microfungal nonwovens, or composite mats, could serve a range of applications requiring absorbent, binding, filtration and drug delivery properties. The speed of growth (biomass available in days), low-energy requirements and natural properties, pointed to market opportunities of varying scale and value, i.e., low value products such as absorbent materials, binding agents, filtration products; and high value products, e.g., artificial leather, metal-ion bisorption and would healing.

The fungal species that were tested by Hamlyn and his team included *Aspergillus oryzae*, *Mucor mucedo*, *Rhizomucor miehei* and *Phycomyces blakesleeanus*. Cultures were grown in bioreactors, resulting in a broth of very fine branched filaments which could be fabricated through wet-laying using standard laboratory papermaking equipment, either on their own, or mixed with conventional fibres, e.g., wood pulp or polyester. Alternatively, they could be freeze-dried to produce an absorbent pad of pure mycelium (Fig. 7).

Further research by Hamlyn and Schmidt (1994) explored the potential therapeutic application of fungal filaments in medical textiles for wound dressing materials. Sadly, pursuit of commercial applications was considered futile at the time, due to patents existing in the area of chitin-based medical textiles (Ivanova 2013a). However, in recent years, there has been a renewed interest and research in the use of fungi for high-performance paper-like materials, wound dressing materials, and water purification filters (Gandia et al. 2021).

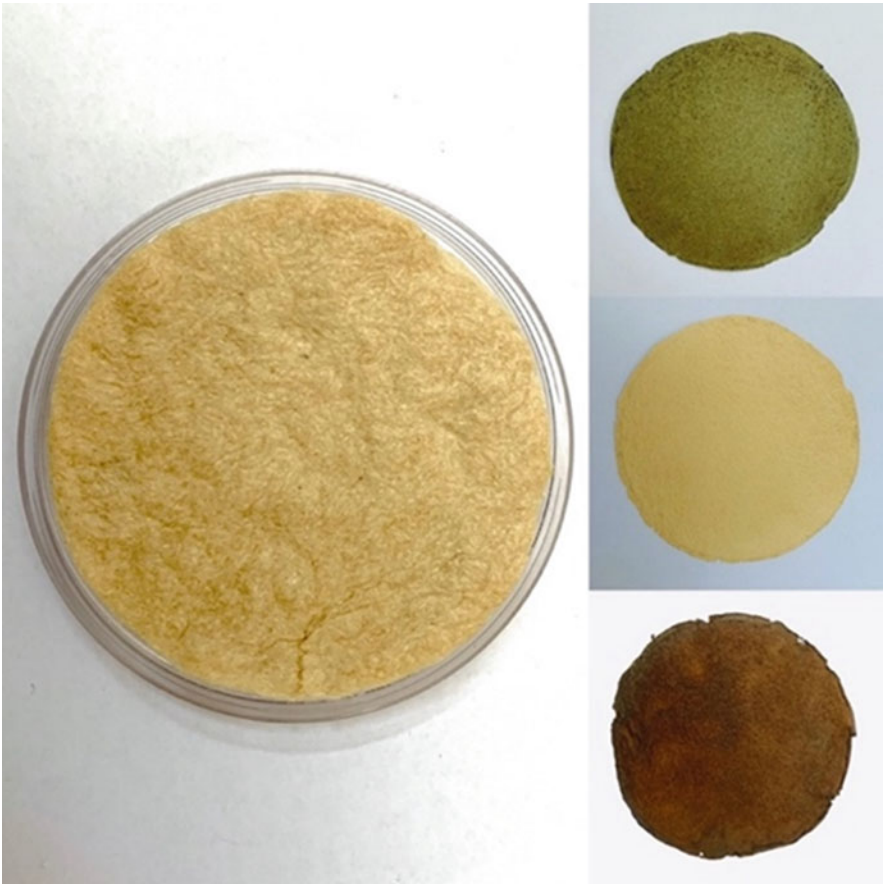


Fig. 7 Absorbent mycelium pad (left) and paper swatches (right) gifted by Hamlyn to the author's collection. © Author's archive

3 Fungi Applications in Contemporary Design and Bioconsumer Products

Over the last decade we have seen a rapid growth in interest and developments in fungi materials across a variety of design applications. Foams and sheets grown from pure mycelium are being made into high-quality paper, textiles and leather-like materials. Biocomposites of mycelium grown on agroindustrial byproducts are finding commercial applications as packaging materials, insulation, building blocks, furniture and interior panels. Due to the beneficial properties of fungi, the scope for environmental impact through a new range of novel sustainable mycelium materials appears enormous: upcycling of byproducts and waste from other industries; a low-energy process associated with heterotrophic fungal growth; versatility of

outputs; and biodegradability of the products at the end of their use. (Appels and Wösten 2020; Camere and Karana 2018; Gandia et al. 2021; Karana et al. 2018; Robertson et al. 2020).

The following section provides an overview of leading fungi applications across domains and at various scales. This list is by no means exhaustive, but rather highlights key practitioners who have paved the way for widespread adoption of mycelium-based materials by designers, industry and consumers alike.

3.1 From Building Blocks to 'Leather'

In the early 90s in San Francisco, artist Phil Ross began a creative practice inspired by the beauty, life cycle, medicinal properties and rich diversity in form, texture and colour of reishi mushrooms (MycoWorks 2021). In 2008, his ongoing experiments in creating living sculptures from fungi, led to the inception of *'mycotecture'*—a term he used to describe the art of designing and building with fungi. The process was used to create strong, yet lightweight structures that could be deployed as building blocks to construct architectural frames, or alternatively moulded into domestic objects, e.g., chairs (Fig. 8), by placing the live mycelium into a desirable shape. The work was exhibited internationally and earmarked fungi as a one-to-watch innovative and sustainable material.

In 2013, Philip Ross partnered with a long-term artistic collaborator—Sophia Wang—to found *MycoWorks*, where, together with a small team of creatives, they began to refine Ross' biotechnique into a patented *Fine Mycelium* technology. This entailed engineering mycelium during the growth phase to create dense, interlocking structures of filamentous growth. The technology was initially used for panels and moulded forms for interior and structural design, similarly to Ross' early works.

Soon after, in 2016, the team began to pursue a new application of this technology for flexible mycelium materials which would have the look and feel of bovine leather. *MycoWorks* assert that, in comparison with other 'mushroom leather' alternatives which are made of compressed mycelium foams,[8] their *Fine Mycelium* technology allows them to engineer the desirable leather strength, performance and aesthetic properties during the mycelium growth phase. This offers a level of control over the finished products, to meet the required quality standards in the fashion and footwear industries.

In parallel, in Green Island (New York), *Ecovative* was founded in 2007 as a mycelium technology company to develop sustainable, biodegradable alternatives to styrofoam packaging and insulation materials (Ecovative 2021). Their early products

[8]Mycelium foams are typically produced via solid-state fermentation on pre-colonised lignocellulosic substrate, e.g., sawdust, whereby a thick foamy mycelium sheet is formed on the surface of shallow moulds, which is then harvested and compressed to decrease its thickness. See further: Gandia et al. (2021).

Fig. 8 'Yamanaka Furniture' by Phil Ross (2022) as exhibited at Alive: New design frontiers (Collet 2013) © Author's archive

were based on a patented mycelium bonding technology, which exploited the natural properties of mycelium to self-assemble lignin and cellulose, in order to transform agricultural by-products, e.g., hemp hurd, into composite materials, which are now marketed under the brand *MycoComposite™*.

Today, *Mushroom® Packaging* is a separate branch of *Ecovative* and is licensed to companies across USA, Europe, the UK and Oceania. The mycelium technology itself has been further developed into a range of materials for applications in the built environment, and for consumer products ranging from high performance footwear, through leather alternatives, to skincare (Ecovative 2021). The latter are based on two new technologies for flexible fungal materials: *MycoFlex™*, which according to Gandia et al. (2021), is the only commercially available pure mycelium foam; and *Forager™*, which uses an aerial mycelium farming technology to create sheets of the material that can be then processed by traditional tanneries into animal leather alternatives.

The most recent company to join the mycelium technology landscape across the Atlantic is *Bolt Threads*. *Bolt Threads* was founded in 2009 by a bioengineer, a biochemist and a biophysicist to develop biobased materials that counter the toxic processes, petroleum-based polymers and non-biodegradable materials of industries such as textiles and fashion. Following on from their first product, *Microsilk™* which was based on an advanced technology to mimic to production of spider silk, in 2018, *Bolt Threads* launched *Mylo™* (Fig. 1, p. 210)—a mycelium-based alternative

to leather (Threads 2021). *Mylo*^{TM} was first prototyped into fashion products by designer Stella McCartney through a recreation of her iconic *Falabella* bag, which was showcased for the first time at the *Fashioned from Nature* exhibit at the *Victoria & Albert Museum* in London (2018–2019).

The collaboration between *Bolt Threads* and Stella McCartney has set the blueprint for the following stages of development and market realisation of Mylo™ through international partnering and multidisciplinary working: with mushroom growers in the Netherlands using vertical farming to minimise ecological footprints; European leather tanneries to meet the aesthetic and performance requirements of traditional leather; and the fashion and sportswear industries who have the capability, resources and talent to bring mycelium-based products to market at scale.

What remains a key driver throughout the entire process of realisation is the sustainability imperative. Whilst the current *Mylo*^{TM} leather alternative is not fully plastic-free[9] and biodegradable, due to the processes involved in the material fabrication and finishing required to meet consumer expectations for softness, strength and suppleness. This level of transparency and attitude towards continuous improvement of the environmental impact of the process and finished product, paired with a realisation that *"a material's potential for impact depends on brand and consumer adoption, and a majority of consumers will not accept big sacrifices in quality compared to leather",* highlights one of the main areas in the domain of mycelium-based consumer products that would require further attention, research and development.

3.2 The Role and Impact of a Single Designer

In parallel, Italian-born designer based in the Netherlands Maurizio Montalti, has led the introduction of fungi-based materials over the last decade in Europe and beyond. His Master's dissertation at Design Academy Eindhoven *'Continuous Bodies: cycles of decomposition triggering a symbiotic partnership between humans and fungi'* (Montalti 2010a) considered the properties of fungi to decompose matter of both natural and synthetic origin. Through a series of material experiments and prototypes, Montalti began to probe a new domestic landscape of *'cultivated'* objects which would replace the environmentally harmful plastics (Montalti 2010b, c).

The early works included various domestic objects which were *'grown'* into the desirable shape and form using moulds, by either utilising local waste, or by biodegrading existing polymeric materials. To describe his process of working with living mycelium, Montalti used the term *'growing design'*, which he compared to a method of *'slow'* 3D printing, whereby the speed of printing would correspond to the time needed for the fungi to grow (Montalti 2014).

[9]Mylo is currently certified as 50–85% bio-based https://boltthreads.com/technology/mylo/.

In Europe, Montalti's work, vision, and openness to collaborate and educate (Corpuscoli 2021) established a more versatile, wholistic and collaborative approach in the pursuit of fungal futures. This had important implications for the uptake and future development of fungi as a novel sustainable material, as well as inspiring and educating the next generation of biodesigners working with mycelium through extensive international lecturing and teaching practice.[10]

3.2.1 A Collaborative and Inclusive Approach Pushing the Boundaries of Material Properties and Applications

In 2010 in Amsterdam, Montalti founded *Officina Corpuscoli* as one of the first design-led consultancies committed to studying and developing mycelium-based technologies. Through collaboration and co-creation with scientists and designers, Montalti not only explored the mechanisms underlying the structural and decorative properties of mycelium, but also identified opportunities for their improvement by assessing natural variations, environmental growth conditions, and genetic qualities of the selected mycelia. Pertinent examples of this include:

- *'The Future of Plastic'* exhibition (2014), which was commissioned by, and exhibited at the *Fondazione PLART* (Napoli). The show comprised a range of everyday objects which provoked the audience to rethink and reimagine the make-up of everyday environments (Fig. 9);
- The collaboration with the Fungal group of the Utrecht University's Microbiology Lab and *Stichting Mediamatic* as part of the NWO-funded project *'Mycelium Design'* (2014–2015), which invited artists and designers to explore the wider material qualities and potential applications of fungi. This provided *Officina Corpuscoli* with multi-perspective feedback about design-led requirements for the properties and performance of mycelium materials for specific products and applications. The outcomes of this engagement were presented as part of the exhibition *'FUNGAL FUTURES / Growing Domestic Bio-Landscapes'* (2016). This inclusive and collaborative approach led to the development of novel material concepts, qualities, and design solutions (Fig. 10);
- A commission by the *Museum of Modern Art (MoMA)* for the exhibition *'Items: Is Fashion Modern?'* (2017–2018) brought about a collaboration with Liz Ciokajlo (OurOwnsKIN) to create a mycelium-based prototype for a *MarsBoot*. This was as a design provocation to explore the evolution of our material culture in the twenty-first century, and specifically the values that drive the creation of new worlds. The fabrication process combined a range of hi-tech and low-tech processes to produce components of both pure and composite mycelium materials, with different physical and technical qualities (Fig. 11).

[10] Including positions as Head and Mentor for the MAD Master (Materialisation in Art and Design) at Sandberg Instituut, Associate Researcher at Design Academy Eindhoven, Artistic Director at dieDAS – Design Akademie Saaleck.

Fig. 9 'The Future of Plastic' exhibition by Maurizio Montalti at Fondazione PLART in Napoli (Montalti 2014) © Corpuscoli / Maurizio Montalti

Fig. 10 Fungal Futures (2016) © Officina Corpuscoli /Maurizio Montalti

3.2.2 Establishing a Company that Diversifies Application

The range of material explorations, prototyping and collaborative effort, led to a realisation of the need to standardise and scale up mycelium technology, in order to

Fig. 11 Caskia/Growing a MarsBoot by Maurizio Montalti (Officina Corpuscoli) and Liz Ciokajlo (OurOwnsKIN). © George Ellsworth

realise the power and responsibility of design to create more sustainable materials and products that positively impact the industry and society at large. In 2015, together with project partners, Montalti founded *Mogu* in Inarzo (Northern Italy), to develop a range of mycelium-based composites and pure mycelium materials which would follow the principles of the circular economy (Mogu 2021). The light, low density, strong, resistant, shock-absorbing, performative applications of fungi-based materials were translated into an ever expanding range of commercial applications such as flooring, acoustic interior panels (Fig. 12), fashion and furniture.

3.3 Mycelium Meets Advanced Technologies

Additive manufacturing, also referred to as 3D printing, has offered an alternative method for biofabrication, which Robertson et al. (2020) assert, could allow for the creation of previously unobtainable design morphologies, as well as extending the capability of 3D printing to provide an economically viable mass production method.

A pioneer in this space is Eric Klarenbeek, of design studio Klarenbeek and Dros (2021), who adapted a 3D-printer to print straw injected with mycelium. This method enables him to create strong, solid and lightweight structures with intricate detail. The work began in 2010 through a series of experiments developing cold paste extrusion-based mycelium printers. Later, several extruders were combined, to enable simultaneous printing with living mycelium and biopolymer scaffolding (FDM printing), to create more robust construction elements and larger structures. These resulted in the creation of a mycelium chair which used PLA scaffolding (Fig. 13).

However, the early prototypes revealed a limitation of the existing biopolymers used in 3D printing, which were not the optimal growth media for mycelium. This

Fig. 12 Mogu—Radical by Nature—Acoustic Mycelium Panels. Photography by A.WORLD PRODUCTIONS © Mogu

resulted in further research and development using algae- and seaweed-based biopolymers, to improve the technology, extrusion methods and suitability for specific strains of fungi, to enhance the speed of growth, and the strength and overall properties of the final designs (Fig. 14).

The improved method also delineated wider applications within architecture. A collaboration with *Biobased Creations* of Dutch design company *New Heroes* and *Dutch Design Foundation,* led to the creation of a fully biobased installation—*'The Growing Pavilion'* (2019), which formed part of *Dutch Design Week 2019*.

The advancement of mycelium technologies, the collaborative and interdisciplinary efforts to refine material properties, the alignment with the sustainability agenda, and the scaling up of production and commercialisation, have shifted the design discourse over the last 10 years—from a *'what if?'* space probing a future where our material world and everyday environments would be made of mushrooms, through the excitement of having a new medium and a new process to *'grow'* design, to a space where consumer engagement and uptake, aesthetic and performance properties, and a wholistic narrative between material properties, application and sustainability, are becoming the key focus of design attention and further development.

The latter question, about how we normalise novel, yet obscure materials from fungi, has been the focal point for the author's design investigation into the use of fungi for bio-based materials and products. The following section provides a personal narrative of the activities and key considerations in developing mycelium-based textiles and domestic objects.

Fig. 13 Mycelium chair made using an adapted 3D printing process by Eric Klarenbeek. © Klarenbeek & Dros

Fig. 14 Mycelium chair made using the improved 3D printing process, exhibited at the Centre Pompidu, Paris (2019). © Klarenbeek & Dros

4 A Design-Led Case Study: The Role of Human Engagement

A starting point for the enquiry was the Russian documentary film *'Плесень' ('Mould')* by Dmitry Vassilev's (2009), which explored the roles fungi play for life on Earth, from fungal diseases caused by *Aspergillus niger*, to beneficial uses such as antibiotics through the discovery of penicillin:

> It first appeared on Earth 200 million years ago. It kills and saves lives. It is often called the "devil's bread" and "God's spit". We don't even think what ancient secrets and hidden powers might be concealed in this cursed and blessed phenomenon. . . mold. (Vassilev 2009)

In 2010, when the applications of fungi in design described above where still in their infancy, the film provoked thinking about the potential role of design in communicating the useful properties and value of fungi, to counter negative associations of fungi with mould, decay, disease and deterioration,[11] which may have been a reason for the initially slow uptake of fungi in the newly emerging field of biofabricated design.

Psychologically, the popular association of fungi with mould brings about a behaviour of disease-avoidance. It is related both to our evolutionary past and the fear of contamination, which Rachman (2004) describes as *"an intense and persistent feeling of having been polluted or infected or endangered as a result of contact, direct or indirect, with a person/place /object that is perceived to be soiled, impure, infectious or harmful. . ."*. This perceived state evokes emotions such as disgust and impurity, which are usually influenced by visual and olfactory stimuli that can be produced by decaying matter (Fig. 15).

The film inspired a year-long design investigation for a Master's project in Fashion at Kingston School of Art (Ivanova 2011). The project explored how, through careful crafting of design and aesthetic properties, we could (1) overcome negative associations of fungi to propose applications within the fashion domain, and (2) educate the public about the importance and value of fungi to humankind. This involved a series of experiments with mould growth on textiles and paper, which were conducted with support from Professor Lory Snyder from the School of Life Sciences, Pharmacy, and Chemistry at Kingston University London.

Through the use of macro photography (Fig. 16), the beauty of microscopic moulds formed the basis for a garment collection that explored the potential value of fungi within sustainable fashion. Different textiles techniques, including digital printing on silk chiffon, flocking and laser cutting, were then used to translate the narrative into garment designs (Fig. 17). The collection also included several pieces with living moulds, encapsulated within sealed PVC components of the dress, to provoke audience engagement with the idea of wearing clothing made from, or still containing, living organisms.

[11] Attitudes, perception and use of fungi can vary dramatically across cultures (Rolfe and Rolfe 1925).

Fig. 15 Aversion response to moulded fruit, expressed by a workshop participant (Kingston School of Art, 2012). © Ezzidin Alwan

In the context of fashion, where there exists a very immediate, intimate and sensorial relationship between the human body and the garment, it appears imperative to consider how we introduce novel bio-based materials from fungi (Ivanova 2013c). This formed the basis for a doctoral design research investigation, which aimed to advance engagement and perception of fungi, alongside any further developments in the chain of market realisation (Ivanova 2015).

The review of science literature and contemporary design practice—some of which is covered in the previous sections of this chapter—provided a starting point for the author's investigation into the use of fungi to fabricate textile-like structures. Preliminary questions that formed the basis for this investigation centred on identifying which fungal species would be best suited to fabricate soft, yet structurally sound materials. The design process was open ended, to allow for material forms and design applications to emerge organically from the natural properties of fungi. An early realisation was the need for partnering and interdisciplinary work, to advance both the fabrication and the uptake of mycelium-based materials.

Answering the above questions entailed a simultaneous pursuit of collaborations with scientists and the development of a design-led methodology to test engagement with fungi in the context of fashion.

4.1 Material Experimentation

Early on in the research process, it became apparent that attempting to 'grow' a material in the design studio, without scientific input and technology, would lead to outcomes with many limitations. Support was therefore sought from several

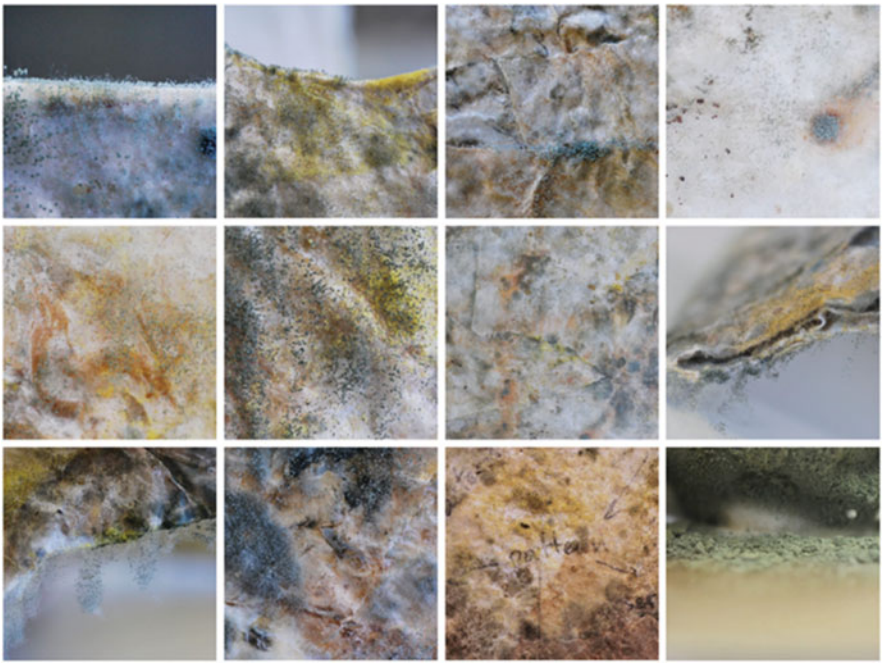


Fig. 16 Mould growth on paper and textiles. © Author's archive

Fig. 17 Garment designs inspired by mould, showcased at Vauxhall Fashion Scout (Freemasons' Hall London, Sept 2011). © Ezzidin Alwan

scientists across the UK who provided invaluable guidance, feedback and training, as well as access to resources and facilities.

Dr. Paul F Hamlyn from the British Textile Technology Group, who had pioneered research on the use of fungi for medical textiles as described earlier in this chapter (Hamlyn 1991; Hamlyn and Schmidt 1994) was interviewed to understand the possibilities of using existing textile technologies in working with fungi, as well as some of the early limitations of fabricating textile forms (Ivanova 2013a).

Prof Lynne Boddy, MBE FRSB FLSW, Professor of Microbial Ecology at Cardiff University, who leads research on the ecology of wood decomposition, helped identify cord-forming fungi as the preferred species for material fabrication (Ivanova 2013b). In nature, these fungi form cords with strong tensile properties in their search for new food sources and interaction with other microorganisms in the soil (Fig. 18).

Dr. Bryn Dentinger, Dr. Begona Aguirre-Hudson and Dr. Heidi Doring—mycologists at the Fungarium at the Royal Botanic Gardens Kew (Ivanova 2012b)—enabled early experiments to be conducted by providing space, resources and in-kind support for the research.

From the above, it appeared possible to imagine that, depending on the morphology of the fungal mycelium and the type of growth media and process, we could fabricate a variety of textile-like structures, e.g., a mycelium cord, a felt-like fungal mat, a membrane, or a lace-like structure. These could be either conventionally fabricated by appropriating existing textile technologies, or grown as bio-films using existing biodesign and biotechnology methods.

This led to series of experiments, conducted at Cardiff University and the Royal Botanic Gardens Kew, aimed at testing the potential of different fungal species to create textile-like structures on their own, and as composite materials with fabrics. Some of the early outcomes (Fig. 19) explored the behaviour and properties of various species in response to traditional textiles, e.g., linen, silk and netting. It was observed that some fungi develop stronger, cord-like mycelium when grown on protein-based textile materials, particularly net-like and open weave fabrics.

Fig. 18 Cord-forming fungi at the laboratory of Professor Lynne Boddy, Cardiff University. Images taken by the author with permission, 2013

Fig. 19 Mycelial textile structures on netting and open weave cotton fabric. © Author's archive 2013

4.2 Advancing Engagement and Perception

In parallel, another experiment conducted with Dr. Simon Park from the University of Surrey explored the potential of cheese-moulds such as *Penicillium roqueforti* and *Penicillium camemberti* to form textile-like biofilms when cultured on milk (Fig. 20). The resulting materials had the appearance of suede and demonstrated both anti-bacterial and water-repelling properties (Fig. 21).

These early experiments began to outline some of the benefits of bio-based materials. For example, 'living garments' could provide a route for re-introducing and re-engaging with specific micro-organisms into our everyday, with a view to re-balancing our environment and relationship with nature, and potentially improving health and wellbeing.[12]

However, if this scenario were to be realised in manufacturing, a pertinent question that would arise would be about how a high-street brand would engage with the complex challenges of selling such a concept to the consumer. Mould, to be converted into a durable material, would face the challenge of overturning its inherent negative associations with decay, disease and deterioration. Shifting this perception would be necessary in order for the material to succeed in a market place that increasingly demands transparency of material sourcing, production, and heightened aesthetic awareness from consumers (Fletcher 2008; Fletcher and Grose 2012).

History provides a pertinent example of the challenging and lengthy process of introducing technical and material inventions to wider fashion and textile markets. At the turn of the twentieth century, an alliance between the three global industries of chemistry, textiles and fashion succeeded in synthesising the first man-made material, viscose rayon, produced from chemically pulped-down wood (Handley 1999). Over the following decades studies of polymerisation in natural fibres, led to the invention of nylon by *DuPont* in the 1930s (DuPont 2014).

[12] Some scientists postulate that the move towards an increasingly hygiene-monitored urban environment, could be a likely cause for some contemporary autoimmune and idiopathic diseases (Dunn 2011; McKenna 1992).

Fig. 20 MycoCouture: cultured form P. roqueforti and P. camemberti (Park and Ivanova 2013). Image courtesy of Dr. Simon Park

Fig. 21 Close up of the MycoCouture surface showing its hydrophobic properties (Park and Ivanova 2013). Image courtesy of Dr. Simon Park

Nylon was introduced to potential markets in 1939, as part of *DuPont*'s *Children of Science* exhibition at New York World's Fair, under the slogan *'better things for better living'* (Handley 1999). The initial promise from science behind this new material was affordable luxury, as an alternative to the relatively more expensive silk. Ultimately, significant time and effort in branding and promoting the new *'beauty fibres'* was required *"in order to convince users and consumers that synthetics were as 'good' and 'luxurious' as silk"* (Handley 1999). Generic man-made textiles, e.g., viscose, acetate, polyamide, polyester and acrylic, were translated into brand names arising from blends of these materials such as *'Dacron', 'Terylene', 'Trelenka', Crimplene', 'Orlon', 'Courtelle', 'Tactel', 'Tencel'*, etc.

However, early synthetic fabrics and their marketing as *'artificial silk'* did not meet consumer expectations, and evoked a negative public perception bound by the *"artificiality, and counterfeiting of the authentic",* as stated in a report commissioned by DuPont in 1927:

> DuPont had realised that selling a new material meant selling an abstract concept, which relied on associations with comfort, performance, economy and luxury. (Handley 1999)

The story of nylon serves as an example of a timeline of the market assimilation process of novel materials, related terminology, and an urgent requirement for a shift in societal perception and attitudes. Negative perception of synthetics and how they were publicly received, was fuelled by the popular perception of science as *'unnatural'* at the time, and was further exacerbated by the poor tactile, visual and behavioural qualities of the early synthetic materials. It took decades for fashion designers, namely the French couture houses, to develop the potential of synthetic materials, and, *"deep within the collective psyche still lingers a suspicion, if not a prejudice, against the words 'plastic' and 'synthetic'."* (Handley 1999).

With hindsight, a more wholistic or a stakeholder approach by the design, science and engineering sectors, with due consideration of all human, material and sensory factors, would have led to a speedier and more fluent integration of synthetic materials on the marketplace.

From the above, the further development of mycelium-based materials by the author, focused on the presentation and education of audience groups via workshops, design probes, seminars and exhibitions (Fig. 22), to best present the advantages and disadvantages of newly developing materials, to make them socially acceptable and desirable (Ivanova 2015). An inclusive approach, which invites stakeholders, e.g., end-users and consumers (Eikhaug and Gheerawo 2010; Sanders and Stappers 2008; Suri 2007), to engage with novel and challenging material concepts and related experience at the early stages of their development, was considered invaluable to inform the design process, and ensure the desirability and market viability of such concepts.

4.3 Design Collaboration: Mycelium + Timber

The potential challenges associated with human engagement and perception with fungi applications for clothing, e.g., questions related to allergies, health and safety, but also concerns about the need to evolve performance and durability—which would require a lengthy process of refinement of material properties and product functionality—led to a consideration of where fungi-based materials may find a more natural, psychologically comfortable, and easy-to-integrate application. This speaks to a bigger vision for designing a material world, environments and objects in a sensitive and sensible way, wherein conscious decision and choice of raw material, processes, design aesthetic, function and presentation becomes a holistic narrative, but also a guidepost for design production and integrity.

Fig. 22 'Mouldy' T-shirts: design probes co-created with research participants, PhD Design Research exhibition, November 2012, Kingston University London. © Author's archive

In 2016, the author met Sebastian and Brogan Cox of furniture design company Sebastian Cox Ltd. (Cox 2017) who were looking to explore the naturally occurring relationship between fungi and wood through the design of domestic objects and furniture. The project was driven by a shared vision of made objects, created in a rational way, with complete alignment and integrity between choice of materials, design process, aesthetic, outcome and application. Through a combined expertise in mycelium, wood and design, the team pursued the development of a furniture collection that would evolve material culture in the context of sustainability and biodiversity, and positively impact the way people choose the objects in their home.

There were several key considerations:

4.3.1 Purpose

The project was initially inspired by an image of two tree branches that were bound together by the black fruitbody of an unidentified polypore, which Sebastian took on one of his trips to the woods in Kent. This suggested a potential application of mycelium as a natural alternative to the glues used in engineered wood products.

> In our workshop we don't use composite wood materials because I've never been quite satisfied with the binding agent holding the wood together [. . .] As a result, I've always had a kind of fantasy interest in ''reinventing' a type of MDF and finding new ways to bind wood

> fibres into either sheets or mounded forms, ideally without glue. (Sebastian Cox in Frearson 2017)

This image became symbolic of the overarching ethos and purpose of the collaboration, which was later named Mycelium + Timber (Cox and Ivanova 2017). The vision for *Mycelium + Timber* focused on the marriage of the naturally occurring properties of fungi with existing carpentry processes and techniques, to create domestic objects that equally celebrate fungi and wood, rather than solely using woodchip waste from the studio mill as a substrate for a composite material.

4.3.2 Species

The team embarked on a series of experiments, set up in a purpose-built lab in the Cox' workshop, which aimed to identify the right match between fungal species and the hardwoods that the studio worked with, to utilise the (waste) resource that was available in the best possible way.

An imperative was to work with locally sourced wood, e.g., birch, ash, sycamore and chestnut, in line with the overarching sustainability narrative of the collection, and in alignment with the ethos of Sebastian Cox Ltd. In addition to the by-products of the workshop's milling and furniture making processes, the team hoped to find a fungus that would grow well on green coppiced hazel (*Corylus avellana*) and goat willow (*Salix caprea*). Goat willow grows in abundance, which presents a challenge to woodland management, but has little economic value and is often considered waste.

Fungal species were selected based on their wood-decaying properties, their ability to grow in culture and their speed of growth, as well as the above guiding principle of being local to the South-East of England. It was considered important that the fungi were safe to use, i.e., with known medicinal, food or other ethnomycological applications, to overcome any future potential challenges with health and safety, or perception.

4.3.3 Process

An imperative was to learn from, and work with the living material, which was realised through an extensive review of literature at the *Fungarium* at the *Royal Botanic Gardens Kew*, and a series of experiments. *Fomes fomentarius* emerged as the most suitable species that worked effectively with coppiced hazel and goat willow (Fig. 23).

The process entailed growing grain culture of *Fomes fomentarius* on sterilised greed wood waste (1.5 h at 60 °C) for 2 weeks, after which time the composite material could be moulded to shape (Fig. 24), and let to grow for a further 2 weeks to take up the desirable form. As previously discussed, this process has no extreme requirements for ambient temperature, humidity or light. Once the growing process

is complete, the finished artefacts are kiln-dried to remove moisture and terminate any further growth.

4.3.4 Properties

Some of the key observations about the behaviour and properties of mycelium that were considered in the design development and pursuit of applications included:

- Changes in colour, texture, and material properties due to variation in growing and environmental conditions;
- Ability of the mycelium to take up both the shape and surface properties of the mould within which it is grown;
- The binding properties of mycelium, which indicated applications for seamless joining of different furniture components;
- The lightness of the composite material which suggested applications for domestic objects that are not weight bearing, unless reinforced with integrated wooden structures;
- The spongelike natural give of the pure mycelium foam, which formed as a top layer in culture vats or moulds, and in turn indicated a suitable application for cushioning and insulation (Fig. 25);
- Sensorial properties including a pleasant, woody or earthy smell, and soft-to-touch velvety texture;
- Hydrophobic and fire-retardant properties.

Fig. 23 Petri dish with *Fomes fomentarius* grown onto wood shavings. © Petr Krejčí

Fig. 24 Preparation of light shade formers filled with 'myceliated' wood for a second growth phase. © Petr Krejčí

4.3.5 Outcomes

The above properties guided the creation of prototypes for two domestic objects: a ceiling pendant in two different sizes, and a stool. These were considered the most appropriate applications that would work well with the natural properties of mycelium, as opposed to trying to enforce a particular shape or design. Further considerations included how to achieve a desired aesthetic without the need for extensive human labour, or the employment of additional hi-tech processes, which would not sit well with the overall sustainability narrative.

The ceiling pendants (Fig. 26) were grown within reusable moulds in the shape of a witch's hat. A hand-turned wooden rose and a woven rim were integrated in the design to translate the natural relationship between fungi and wood into lightweight, incredibly strong and completely compostable pieces of design. A similar process, of growing mycelium around a purpose-made wooden frame were used to create the stool, whereby the stool itself became the mould for the mycelium growth (Fig. 27).

4.3.6 Presentation

The presentation of these early prototypes was a carefully considered process to introduce the collection in a way that would normalise fungi-derived products for applications in the home. *Mycelium + Timber* was first displayed during *London*

Fig. 25 A cross-section of moulded component illustrating different properties of the composite material. © Author's archive

Design Festival 2017 as part of the *Design Frontiers* exhibition at Somerset House. The domestic environment of Somerset House—with its wooden floors, fireplace and large windows (Fig. 28)—was opportune, to allow the audience to engage with, and imagine, what it would feel like to own, and care for, mycelium furniture and domestic objects; a departure from their presentation within a gallery or museum context.

The installation presented the research and creative process—from the raw materials to the finished prototypes—to illustrate the translation of the remarkable and ancient material relationship between wood and fungi into strong, lightweight and entirely compostable forms that can be applied to furniture design through the use of previously overlooked materials. Visitors were able to touch the objects on display, feel their velvety texture, and even smell them if they so wished (Fig. 29).

The transparency of the production process, including objects being 'grown' for the duration of the exhibition, captured the audience imagination, with many questions ensuing about other potential applications and the evolution of the aesthetic properties. Overall, the exhibition was successful in fulfilling its intention to take mycelium-based products out of the lab, or the gallery space, and into a collective appreciation of their value and applications within the home.

Fig. 26 Ceiling pendants made using green wood waste bound by mycelium. © Petr Krejčí

Fig. 27 Two mycelium stools made using green wood waste bound with mycelium to a wooden base and legs. © Petr Krejčí

5 Conclusion and Future Vision

This chapter set out to trace the value and applications of fungi within the domain of design for bioconsumer products. The natural properties of fungi, linked directly to their role within the Earth's ecosystem, have delineated multiple beneficial uses, which biotechnology is converting into commercial applications. In this space,

Fig. 28 Mycelium + Timber display at London Design Festival 2017, Design Frontiers, Somerset House. Image courtesy of London Design Festival. © Ed Reeve

several roles have emerged for design and designers in the entire chain of market realisation.

5.1 Future Casting

Innate to design thinking is the ability to imagine new futures and applications, beyond what is currently scientifically feasible, or viable in relation to the market. Through design methods, e.g., speculative prototypes, design probes, concept drawings and moving image, designers are able to map and visualise the scenarios and systems that would be required to realise mycelium products, as well as raising questions about the wider implications, ethics, and responsible creation of new material futures. A pertinent example here was the *Caskia* MarsBoot by Montalti and Ciokajlo (Sect. 3.2).

5.2 Probing and Prototyping

Unlike other material revolutions, such as the invention of synthetics which was referenced above (Sect. 4.2), the development of mycelium-based materials for

Fig. 29 Audience engagement with Mycelium + Timber artefacts during London Design Festival 2017. Design Frontiers, Somerset House © Author's archive

design applications has been largely championed by artists, designers and design entrepreneurs, e.g., Franklin, Ross, Montalti and Klarenbeek. A curiosity to explore new fabrication processes and media, through design experimentation and

interdisciplinary collaborations, have enabled creatives to prototype early forms and applications for fungi in design.

5.3 Design-Mindedness

Design-mindedness speaks to the ability of designers to think holistically about purpose (need), application, functionality, fabrication, aesthetic and audience engagement and impact, in the development of new market propositions. In the case of fungi, which may bring about negative associations with mould, mildew, decay and rot, it has been imperative to consider how we educate, evolve perception, and normalise mycelium products, in parallel with scientific developments and research to bring such products to market.

5.4 Craft and Narrative

Working with the natural properties of select fungal species, building on traditional craft and making techniques, and introducing advanced technologies, e.g., 3D printing, has enabled designers to advance the material aesthetic, design narrative, and applications for fungi in design. In that, sustainability becomes more than a rationale for the development of mycelium products, but rather an integrity of thought and practice about *how* these products are being brought to market (as evidenced by the Cox and Ivanova case study presented in Sect. 4.3).

5.5 Commercialisation

Over the last decade, design-science-industry partnerships, e.g., *MycoWorks, Ecovative*, *Officina Corpuscoli*, *Mogu* and *Bolt Threads*, have attracted funds, talent, stakeholders and resources, to refine mycelium technologies and scale up materials and products for applications as wide as packaging, insulation, flooring, acoustic panels, apparel and cosmetics amongst others. In this space, the role of design is to lead creatively, and push the boundaries of the visual and material language being developed around these new products.[13] In fashion in particular, where there exists an intimate relationship between the garment and the human body, leaders in the

[13] Beyond the examples of practice referenced in this chapter, designers and design studios who are advancing the applications, material and sensory properties of mycelium-based products include: Valentina Dipietro, Biohm and Mycelium Tectonics (product design and architecture); blast.studio (3D printing); Mycotech Lab, Aniela Hoitink and Kristel Peters (fashion) amongst others.

fashion and sportswear industries have begun to introduce concept artefacts that recreate their iconic designs with fungi-derived materials, e.g., adidas' *Stan Smith Mylo*^{TM} shoe and Stella McCartney's *Falabella* bag, to make mycelium materials more accessible to the wider consumer.

The next stage of development for fungi-based applications in design, is concerned with overcoming current limitations related to scalability and the processing of finished products, to meet the demands of performance and aesthetic as expected of conventional consumer goods. At present, the introduction of additives and finishing agents, e.g., through the use of traditional tanning processes in the production of mushroom 'leather', limits the biodegradability of the final outcomes (Material Innovation Initiative 2020). In the first instance, other natural substances and compounds could be developed to improve the sustainability markers of some mycelium composites; a specific example being the use of fungi as dyes themselves, which is still a commercially underexplored resource. Ultimately, with the advancement of science, desirable properties could be engineered and pre-programmed at the growth phase.

Furthermore, in relation to advancing scalability and market uptake, one approach that has not been fully considered in the context of fungi-derived materials yet, is that of inclusive design. The tools of inclusive design—traditionally employed to include the needs and requirements of people who are excluded from mainstream design consideration based on ability, age and other demographic factors (Coleman 1994, Clarkson et al. 2003, Eikhaug and Gheerawo 2010)—could be deployed in this new context, to advance the sensory properties of mycelium materials, scope new applications, and diversify business models. An inclusive design approach would help realise a mycelium-based future with long-term sustainability in achieving planetary, societal and economic impact.

Acknowledgements This work would not have been possible without the generosity, cooperation and support of the following individuals and organisations: Professor Jane Harris, Dr. Paul Micklethwaite, Professor Lori Snyder and Dr Melanie Flory (PhD supervisory team); Kingston School of Art (funding for doctoral research); the British Mycological Society; Dr. Bryn Dentinger, Dr. Begoña Aguirre-Hudson and Dr. Heidi Doring (Royal Botanic Gardens Kew); Professor Lynne Boddy and Dr. Jennifer Hiscox (Cardiff University); Dr. Simon Park (University of Surrey), Dr. Paul F Hamlyn (British Textile Technology Group); Irene Taylder, Patricia Gow, Marilyn and Frank Caddell, Frances Hudson, Susan Hopkins and Liza Johansson (International Fungi & Fibre Federation) and Ezzidin Alwan (photography). A special thank you to all the designers and photographers who kindly gave permission for the reproduction of images for this publication. And most importantly, to Sebastian and Brogan Cox for our continued collaboration.

References

adidas (2021) Stan Smith Mylo™: made using mushrooms. Available at: https://www.adidas.co.uk/blog/663481. Accessed 4 July 2021

Antonelli A, Smith RJ, Simmonds MSJ (2019) Unlocking the properties of plants and fungi for sustainable development. Nat Plants 5:1100–1102

Appels FVW, Wösten HAB (2020) Mycelium materials. *Encyclopedia of*. Mycology 2:710–718
Boddy L (2013) Fungi - the most important organisms on the planet? at *2008 Earthwatch debate*. Available at: http://www.cardiff.ac.uk/biosi/resources/web%20EW%20Fungi.mov. Accessed 12 Aug 2014
Boddy L, Coleman M (eds) (2010) From another kingdom: the amazing world of fungi. Royal Botanic Gardens, Edinburgh, Edinburgh
Breuker M, McNamara C, Young L, Perry T, Young A, Mitchell R (2003) Fungal growth on synthetic cloth from Apollo spacesuits. Ann Microbiol 53:47–54
Butu A, Rodino S, Miu B, Butu M (2020) Mycelium-based materials for the ecodesign of bioeconomy. Dig J Nanomater Biostruct 15:1129–1140
Camere S, Karana E (2018) Fabricating materials from living organisms: an emerging design practice. J Clean Prod 186:570–584
Catts O, Zurr I (2004) Victimless leather - a prototype of stitch-less jacket grown in a technoscientific "body". Available at: http://www.tca.uwa.edu.au/vl/vl.html. Accessed 4 July 2021
Charaya MU, Mehrotra RS (1999) From Ethnomycology to fungal biotechnology: a historical perspective. In: Singh J, Aneja KR (eds) From Ethnomycology to fungal biotechnology. Springer, Boston, MA
Clarkson J, Coleman R, Keates S, Lebbon C (eds) (2003) Inclusive design: design for the whole population. Springer, London
Coleman R (1994) The case for inclusive design – an overview. In: Proceedings of the 12th Triennial Congress. International Ergonomics Association and the Human Factors Association of Canada
Collet C (2012) BioLace: an exploration of the potential of synthetic biology and living technology for future textiles. Stud Mater Thinking 7:1–12. http://www.materialthinking.org/sites/default/files/papers/SMT_V7_P2_Collet.pdf
Collet C (2013) Alive: New design frontiers. Available at: http://thisisalive.com/. Accessed 10 July 2021
Congdon A (2013) Amy Congdon: Biological Atelier. Available at: http://www.amycongdon.com/. Accessed 30 July 2014
Cooke RC (1977) Fungi, man, and his environment. Longman, New York
Corbman BP (1985) Textiles: fiber to fabric, 6th edn. McGraw-Hill Book Co., Singapore
Corpuscoli O (2021). Available at: https://www.corpuscoli.com. Accessed 10 July 2021.
Cox S (2017) Mycelium + Timber: Exploring biofacture in a new collection of grown furniture. Available at: https://www.sebastiancox.co.uk/news/mycelium-timber-exploring-biofacture-in-a-new-collection-of-grown-furniture. Accessed 11 July 2021
Cox S, Ivanova N (2017) Mycelium + Timber [Exhibition]. London Design Festival 2017: Design Frontiers. 18–26 September 2017
Deshmukh SK, Rai MK (eds) (2005) Biodiversity of fungi: their role in human life. Science Publishers, Enfield
Dugan FM (2011) Conspectus of world ethnomycology: fungi in ceremonies, crafts, diets, medicines and myths. APS Press, St. Paul, Minn
Dunn R (2011) The wild life of our bodies: predators, parasites, and partners that shape who we are today. HarperCollins, New York
DuPont (2014) Innovation Starts: 1939 Nylon. Available at: http://www2.dupont.com/Phoenix_Heritage/en_US/1939_c_detail.html. Accessed 26 April 2014
Ecovative (2021) Ecovative design: we grow materials. Available at: https://ecovativedesign.com. Accessed 10 July 2021
Eikhaug O, Gheerawo R (eds) (2010) Innovating with people: the business of inclusive design. Norwegian Design Council, Oslo
Fashioned from Nature (2018–2019) [Exhibition]. Victoria & Albert Museum, London. 21 April 2018–27 January 2019
Fletcher K (2008) Sustainable fashion and textiles: design journeys. Earthscan, London
Fletcher K, Grose L (2012) Fashion & sustainability: design for change. Laurence King, London

Forgacs A (2013) Leather and meat without killing animals. TED. Available at: https://www.ted.com/talks/andras_forgacs_leather_and_meat_without_killing_animals?language=en. Accessed 4 July 2021
Franklin DM (2004) Fibre reactive a living garment. A synergy of biological technology and art. Masters of arts dissertation, The School of Contemporary Arts, Faculty of Communication and Creative Industries, Edith Cowan University
Franklin D, Cass G (2014) Micro'be' fermented fashion. Available at: http://bioalloy.org/images/projects/microbe/micro_be_technet_paper.pdf. Accessed 20 Jan 2015
Frearson A (2017) Mushroom mycelium used to create suede-like furniture by Sebastian Cox and Ninela Ivanova. Available at: https://www.dezeen.com/2017/09/20/mushroom-mycelium-timber-suede-like-furniture-sebastian-cox-ninela-ivanova-london-design-festival/. Accessed 11 July 2021
FUNGAL FUTURES/Growing Domestic Bio-Landscapes (2016) [Exhibition]. 24 March – 16 May 2016. Available at: https://www.corpuscoli.com/projects/fungal-futures/. Accessed 10 July 2021
Gandia A, van den Brandhof JG, Appels FVW, Jones MP (2021) Flexible fungal materials: shaping the future. Trends Biotechnol 2060:1–11
Ginsberg A, King J (2009-2011) E.chromi. Available at: http://www.echromi.com/. Accessed 20 Jan 2015
Hallett C, Johnston A (2010) Fabric for fashion. Laurence King Publishing Ltd, London
Hamlyn PF (1991) Fabricating fungi. Text Technol Int:254–257
Hamlyn PF, McCarthy BJ (2000) Talking rot. . . and mildew on textiles. Biologist 4(47):194–196
Hamlyn PF, Schmidt RJ (1994) Potential therapeutic application of fungal filaments in wound management. Mycologist 8(4):147–152
Handley S (1999) Nylon: the manmade fashion revolution. Bloomsbury, London
Harding P (2008) Mushroom miscellany. Collins, London
Items: Is Fashion Modern? (2017–2018) [Exhibition]. Museum of Modern Art (MoMA), New York. 1 October 2017–28 January 2018
Ivanova N (2011) Moulded Mind. Available at: http://mouldedmind.blogspot.com/p/moulded-mind.html. Accessed 10 July 2021
Ivanova N (2012a) 12th International Fungi & Fibre Symposium. Unpublished Report
Ivanova N (2012b) The Fungarium. Unpublished Report
Ivanova N (2013a) Interview with Dr Paul F Hamlyn. Unpublished Report
Ivanova N (2013b) Visit to Cardiff University with Prof Lynne Boddy. Unpublished Report
Ivanova N (2013c) The human-mould-fashion relationship: fungi applications for future design scenarios in fashion and textiles fabrication. In: Futurescan 2: collective voices. Sheffield Hallam University., 10–11 January 2013. Association of Fashion and Textiles Courses, pp 34–41
Ivanova N (2015) The T-probe: a fashion-led approach to advance understanding of novel and challenging materials concepts and sensory experiences. PhD Thesis, Kingston University London
Johansson L (2002) Mushroom paper: instruction for your own production using Piptoporus betulinus, Fomitopsis pinicola, Fomes fomentarius. Liza Johansson, Eskilstuna
Johansson L (2016) Pigment & papper av svamp. Vulkan, Sweden
Karana E, Blauwhoff D, Hultink E, Camere S (2018) When the material grows: a case study on designing (with) mycelium-based materials. Int J Des 12(2):119–136
Kendall B (2013) The hidden kingdom of fungi. Radio 4: BBC
Klarenbeek E, Dros M (2021) Klarenbeek & Dros: Designers of the Unusual. Available at: https://www.dotunusual.com. Accessed 11 July 2021
Lee S (2005) Fashioning the future: tomorrow's wardrobe. Thames & Hudson, London
Lee S (2009) Eco Kimono. In: Till C (ed) Warp Factor 09. University of the Arts London, Central Saint Martins

Lee S (2011) Grow your own clothes. TED Available at: https://www.ted.com/talks/suzanne_lee_grow_your_own_clothes?language=en. Accessed 20 Jan 2015
Lee S (2012) Biocouture or how to grow a frock . . . . In: Hemmings J (ed) The textile reader. Berg, London, pp 391–394
Lee S, Congdon A, Parker G, Borst C (2020) Understanding "bio" material innovations: a primer for the fashion industry. Biofabricate and Fashion for Good. https://app.box.com/s/amjq9anszv8hvwdexoxg6wubes4aaxqa
Material Innovation Initiative (2020) Technology assessment: mycelium leather. Available at: https://www.materialinnovation.org/reports. Accessed 11 July 2021
McKenna TK (1992) Food of the gods: the search for the original tree of knowledge: a radical history of plants, drugs and human evolution. Rider, London
Meyer V, Basenko EY, Benz JP et al (2020) Growing a circular economy with fungal biotechnology: a white paper. Fungal Biol Biotechnol 7(5):1–23
Mogu (2021) Radical by Nature. Available at: https://mogu.bio. Accessed 10 July 2021
Montalti M (2010a) Continuous bodies: cycles of decomposition triggering a symbiotic partnership between humans and fungi. MA thesis. Design Academy Eindhoven
Montalti M (2010b) Continuous bodies – Bodies of Change. Available at: https://www.corpuscoli.com/projects/bodies-of-change/. Accessed 4 July 2021
Montalti M (2010c) The Growing Lab. Available at: http://www.corpuscoli.com/projects/the-growing-lab/. Accessed 4 July 2021
Montalti M (2014) The future of plastic. Retrieved from http://www.corpuscoli.com/projects/the-future-of-plastic/
Moss MO (1987) Fungal biotechnology roundup. Mycologist 21(2):1–58
Mushrooms for Color (2017) International Fungi & Fibre Federation (IFFF). Available at: http://www.mushroomsforcolor.com/IFFF.htm. Accessed 10 July 2021
MycoWorks (2021) Grow the Future of Materials. Available at: https://www.mycoworks.com. Accessed 10 July 2021
Park S, Ivanova N (2013) MycoCouture: BioSuede. Available at: http://exploringtheinvisible.com/2013/02/26/mycocouture-biosuede/. Accessed 15 July 2014
Rachman S (2004) Fear of contamination. Behav Res Ther 42(11):1227–1255
Rice MC, Beebee D (1980) Mushrooms for color. Mad River Press, Inc., Eureka, CA
Rice MC, Beebee D (2007) Mushrooms for dyes, paper, pigments and Myco-Stix™, 2nd edn. International Mushroom Dye Institute, Fort Bragg, CA
Robertson O, Høgdal F, McKay L, Lenau T (2020) Fungal future: a review of mycelium biocomposites as an ecological alternative insulation material. In: Proceedings of NordDesign 2020, Lyngby, Denmark, 12th - 14th August 2020
Rolfe RT, Rolfe FW (1925) The romance of the fungus world. Chapman and Hall, London
Ross P (2022) Yamanaka Furniture. In: Alive (2013) [Exhibition]. Espace Fondation EDF, Paris. 26 April – 1 September, 2013
Sanders E, Stappers P (2008) Co-creation and the new landscapes of design. CoDesign 4(1):5–18
Singh J, Aneja KR (eds) (1999) From Ethnomycology to fungal biotechnology. Springer, Boston, MA
Spooner B, Roberts P (2005) Fungi. HarperCollins, London
Stamets P (2004) Mycelium running: how mushrooms can help save the world. Ten Speed, Berkeley, CA
Stamets P, Chilton JS (1983) The mushroom cultivator: a practical guide to growing mushrooms at home. Agarikon Press, Olympia, Washington DC
Suri JF (2007) Involving people in the process. Include, London
Thackara J (2015) How to thrive in the next economy. Thames & Hudson, New York
The Future of Plastic (2014) [Exhibition]. Fondazione PLART, Napoli. 11 July – 27 September, 2014

The Growing Pavilion (2019) [Exhibition]. Dutch Design Week 2019. Available at: https://thegrowingpavilion.com. Accessed 11 July 2021
Thompson I, Stott N, Kerridge T (2006) Biojewellery. Retrieved from http://materialbeliefs.com/biojewellery/images/exhibit/biojewellery-booklet.pdf
Threads B (2021) Bolt technology – meet Mylo™. Available at: https://boltthreads.com/technology/mylo/. Accessed 4 July 2021
Tilbury N (2009) Digital Skins - Body Atmospheres. Available at: http://vimeo.com/9962212. Accessed 6 Aug 2014
Trochmé S (2002) Fabric. Mitchell Beazly, Octopus Publishing Group Ltd, London
Wainwright M (1992) An introduction to fungal biotechnology. John Wiley & Sons Ltd, Chichester
Willis KJ (ed) (2018) State of the World's Fungi. Royal Botanic Gardens, Kew, London
Плесень (Mould) (2009) Directed by D. Vassilev [Video]. Russia: Masterskaya, Channel 1

Part III
Other Macromolecules

Production of Bioresins from Fungal Mycelia

Lai Jiang

Abstract One of the novel ways of producing low-cost and environmentally-friendly bioresins is through the growth of fungal mycelium. In this chapter, the most popular fungal species and cultivation substrates are introduced, followed by factors that affect the synthesis of biopolymers, which include nutritional factors, modes of cultivation, environmental factors, as well as required mineral elements. Finally, a comprehensive literature review is provided which involves most of the research and studies performed regarding the growth of fungal mycelium in making biomaterials to date. Fungal mycelium-based biomaterials are new and promising future engineering materials that are expected to be widely used in the industry in the future.

Keywords Bioresin · Substrates · Mycelium · Basidiomycota · *Pleurotus*

1 Introduction

Mycelium is the root network of fungus, a vegetative part that consists of thread-like hyphae. Filamentous fungi grow through filaments that form an interconnected network known as mycelium. Most fungi use mycelium to absorb nutrients including carbon and nitrogen. Enzymes from fungi will enter the food source through hyphae to biodegrade biological polymers into smaller monomers. These monomers are then absorbed by the mycelium through diffusion and active transport. Mycelium's dense network binds the substrate into a structurally adequate material composite by consuming plant-based waste products (e.g., sawdust). Fungi obtain energy by using organic material as a carbon source and convert it with oxygen just like animals and humans do, with additional demand for nitrogen, sulfur, phosphor, and other micronutrients. All these nutrients can be extracted by fungi from organic waste streams such as plant material. Mycelia go easily undetected by eyes when

L. Jiang (✉)
Department of Mechanical Engineering, Prairie View A&M University, Prairie View, TX, USA
e-mail: lajiang@pvamu.edu

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_13

formed in soil or organic waste. When growing out from the soil and forming fruiting bodies, mycelium can be seen directly, the most conspicuous are mushrooms. Mushrooms only represent a small fraction of the total biomass of the fungus. Mycelia of many mushroom-forming fungi such as *Pleurotus ostreatus* (*PO*; oyster mushroom), *Schizophyllum commune* (*SC*; split gill fungus) and *Trametes multicolor* (*TM*; turkey tail) feed on plant waste material such as straw and wood. The substrate is being biodegraded while being invaded by fungal hyphae, and the non-degraded material was binded by the hyphae together at the same time (Wösten et al. 2018).

Filamentous fungi are indispensable in research and industry and have been used as production organisms in biotechnology. Today, the filamentous fungi mycelium has some new applications. Given the experience of farming edible fungi on plant and animal waste materials as the substrate (Wösten 2018), one attractive extension is the fabrication of structures that contain fungal biomass as the filler binded together by mycelium (Haneef et al. 2017). The filamentous fungi mycelia digest lignocellulosic materials and form entangled networks to improve mechanical strength and other properties (Sun et al. 2019), thus unveiling new ways for implementing fungi as a bioresin in biocomposites productions. Petroleum-based polymers release a large amount of CO_2 along with their entire life cycles. The use of mycelium-based bioresins is regarded as sustainable and contributes to the transformation to a sustainable economy, which is the biggest challenge our society is facing today (Appels et al. 2019). In contrast to the modern fossil-based economy, circular economy approaches allow cycles to be closed by novel recyclable materials that can be generated from wastes and secondary streams (Elsacker et al. 2019). Fungi-related sustainable product development and applications can be found in the packaging and textile industries as isolation material, as well as in the automotive industry with promising properties such as hydrophobicity, low or high density, insulation, or fire retardance.

Mycelium-based biocomposites are composed of plant waste substrates bonded by fungal mycelium. These materials are getting increasingly attractive to the scientific as well as the industrial world. For instance, Jiang et al. (2016, 2017a, b, 2018, 2019) developed a novel manufacturing process that involves myceliated substrates binding textile-based integral tooling that makes fully biodegradable biocomposites and investigated many technical issues involved in the process. Mycelium-based biocomposites offer benefits over other engineering materials as follows (Wösten et al. 2018):

1. Lower manufacturing and energy costs;
2. Complete production cycle following a cradle-to-cradle principle: the waste of other processes (e.g., organic wastes from farming) can be used as the feed for making biocomposites, which can be biodegraded and reused as the soil for plants at the end of their service;
3. Low density therefore can be used for making lightweight products;
4. Fire-proof and good insulation properties;

5. Versatility in producing materials from transparent films to leather and brick-like materials;
6. Possibility for products with complex geometry designs.

The major limitations of mycelium-based biomaterials are:

1. Special growth environment is required (controlled CO_2, and O_2, humidity, temperature, and light, which consumes energy to sustain the environment);
2. Instability and uncertainty of the final material's properties due to the growing process of biological material.

2 Fungal Species and Cultivation Substrates

2.1 Fungal Species

Basidiomycota and Ascomycota are the two divisions considered better for mycelium-based materials in the fungi family because they can form larger and more complex organic structures compared to other fungi (Wösten et al. 2018). Lignocellulosic substrates are colonized by many species due to the various degradative processes in fungi. The most efficient degradation of the lignocellulosic compound has been demonstrated to be the wood-decay fungus, most of which belong to Basidiomycota. Fungi in this phylum have two important properties which make them better suited for producing biocomposites: septa and anastomosis. Septa are special transverse cell walls with an opening. When hypha becomes damaged, this opening can be closed to avoid the draining of cytoplasm through the rapture, which will decrease the colony damage and therefore lead to faster colonization of the substrate. Anastomosis increases the growth speed of mycelium by fusing two different hyphae when they are in touch. Additionally, fast nutrient transport can be achieved through a more homogeneous mycelium network generated with the help of anastomosis (Lelivelt 2015).

The *Pleurotus* genus has about 40 species. This fungus grows on a variety of lignocellulosic substrates and forms nutritious shell-shaped fruiting bodies that are rich in protein, vitamins, and minerals. The *Pleurotus* genus includes several sets of taxa and species distributed worldwide, many of which are of great economic interest. However, their investigation has been repeatedly hampered by problems related to vague initial identification, misuse use of taxonomic names, and conclusions based on fragmentary and incomplete data (Butu et al. 2020).

2.1.1 *Pleurotus* sp.

Pleurotus sp. is the world's third most common species of cultivated mushroom after button mushrooms and shiitake mushrooms at the moment due to its stability of cap and stem, its cooking qualities, and longer shelf life (Golak-Siwulska et al. 2018).

Various components in cultivation substrate, such as basic plant substrates, sawdust, mango, wheat straw, jackfruit, rice rind, straw, coconut, waste cotton, and corn cobs are beneficial for its growth.

Pleurotus sp. mycelia penetrate the substrates by physical pressure and enzymatic secretion to break down biological polymers into a smaller nutrient that is easily absorbed and transported, such as glucose (Haneef et al. 2017). The mycelium and fruiting bodies of *Pleurotus* sp. contain bioactive substances that exhibit anti-diabetic, anti-inflammatory, immunostimulatory, antioxidative, and antibacterial properties. Extracts from *Pleurotus* species can be used for dietary supplements productions, organism's immunity enhancements, and used for cosmetics productions, foods additives as probiotics, or natural preservatives (Girometta et al. 2019).

Great scientific interest is in *Pleurotus* sp. since the important phytochemicals in its structure, but the most important aspect is the enzymes it secretes, which can decompose the substrates, develop interwoven filamentous structures, or decompose plant components that are difficult to be hydrolyzed, like lignin. Girometta et al. (2019) demonstrated that the mycelium of *Pleurotus* sp. can be shaped to produce packaging materials, insulating panels, bricks, and many other design objects like furniture and office products in recent research.

2.1.2 *Pleurotus ostreatus*

Pleurotus pulmonarius and *Pleurotus ostreatu* are the two most economically significant species of oyster mushrooms. Oyster mushroom is a white-rot edible fungus, which is strongly saprophytic and highly adaptable. It is easy to cultivate and can fruit on various agro-industrial wastes. Its production continues to increase rapidly worldwide (Yildirim et al. 2012; Girometta et al. 2019; Liu et al. 2018). The fruiting bodies of oyster mushrooms are highly nutritious and health-promoting. Many species in the *Pleurotus* genus have been used as sources of substances based on their medicinal properties such as high-molecular-weight bioactive compounds (e.g., polysaccharides, peptides, proteins) and low-molecular-weight compounds (Yildirim et al. 2012; Josephine 2015; Girometta et al. 2019).

2.2 Cultivation Substrates

In the structure of bio-composites, mycelium is coupled with other non-fossil materials derived from biological processes, such as plant materials, for the natural growth of the fungal organism on these substrate compounds (Yildirim et al. 2012). Based on substrate degradation and development of reproductive structures, mycelium hyphae show various specializations. The fungal organism produces biomass by using the nutrients degraded from the substrate, resulting in a compact layer of the fungal organism. The chemical, functional, and sensorial characteristics of macromycetes are affected by substrates used in mushroom cultivation (Appels

Fig. 1 Mycelium-based biocomposite (left) and pure mycelium (right) (Karana et al. 2018)

et al. 2019; Bellettini et al. 2019). Mycelium degrades and colonizes the organic substrate, using the products of degradation as feeding elements to extend its hyphae to form a denser network. The substrate must provide the necessary nutrients, such as nitrogen, carbon, vitamins, minerals, and water for the mycelium to grow. Most fungi degrade both cellulose and lignin, although being shifted toward a cellulose or lignin preference. Such a preference is both determined by species and the environment. The choice of substrate is closely linked to the fungus species as each species grows on completely different substrates and a thorough study of the choice of this component is required for the development of this type of composite material (Butu et al. 2020).

The substrate needs to have the appropriate nutrients for the fungus growth, not only to keep them alive but also to improve the mechanical properties the mycelium compound will acquire after growth. Many different substrates have been used for *Pleurotus ostreatus* cultivation, with the most commonly used carbon courses being oats, wheat straw, coffee waste, rye and cotton, and wood shavings in particular birch wood (Golak-Siwulska et al. 2018; Josephine 2015; Elsacker et al. 2020; Pelletier et al. 2017; Attias et al. 2017; Zhang et al. 2017; Agustina et al. 2019; Bruscato et al. 2019).

The most used substrates are common agricultural wastes originating from vegetal for solid biocomposites, except for chicken feathers mentioned in one study (Lelivelt 2015). Forestry waste, including tree wood and bamboo fibers, is another major category being used. Glass fines and plastic waste are mentioned in some studies, however, these may result in problems for recycling and reuse of biocomposites. Silk and wool are also considered in some patents besides vegetal substrates (Cerimi et al. 2019).

Both pure mycelium and a substrate combined with mycelium can be used as bioresins. If mycelium has completely consumed the substrate it grows in, this will create pure mycelium-based bioresins (Fig. 1, right). By varying the environmental growth conditions, and chemical and physical treatments, it is possible to fabricate

materials similar to paper, textile, rubber, and wood out of pure fungal mycelium. The main constraints of fabricating pure mycelium-based materials are the long production process and low yields. On the contrary, it is much faster to produce fungal biocomposites (Fig. 1, left) by combining mycelium with substrates with a high yield. Biocomposites are heated to kill the live fungus in order to stop mycelium from consuming the substrate completely. Depending on the fungus type, plant waste substrates and physical and chemical treatments can be used to produce biocomposite materials similar to cardboard, hardboard, soft board, and brick. The exact factors influencing the mechanical properties of fungal materials (hyphal architecture, cell wall composition, composite constituents, and growth kinetics, etc.) are discussed by Jones et al. (2017b). Haneef et al. (2017) also studied ways to control and tune the physical properties of mycelium materials grown on cellulose and cellulose/potato-dextrose. They concluded that differences in relative concentrations in lipids, polysaccharides, chitin, and proteins of the materials affected their morphology and mechanical properties.

3 Factors Affecting the Synthesis of Biopolymers

3.1 Hypha

A hypha is the long branching filamentous structure of a fungus. They are called mycelium for most fungi and are the main mode of vegetative growth. A hypha consists of one or more cells surrounded by a tubular cell wall. In most fungi, hyphae are divided into cells by internal cross-walls called "septa". The septa are usually perforated with pores large enough to allow ribosomes, mitochondria, and sometimes nuclei to move between cells. The main structural polymers of the fungal cell wall are polysaccharides commonly known as chitin and chitosan. Some fungi contain aseptate hyphae, that is, their hyphae are not separated by a septum (Wikipedia 2020).

3.2 Chitin and Chitosan

Chitin can be easily found in fungi, generated by Ascomycetes, Basidiomycetes, and Zygomycetes. It is an important element of cell walls and membranes of mycelia, stalks, and spores. However, it is not contained by all fungi. It is the major component of primary septa between mother and daughter cells of *Saccharomyces cerevisiae* and also one of the main components of the hyaline outer wall of spores of four arbuscular mycorrhizal glomus species (Sbrana et al. 1995). The chitin of fungi possesses principally the same structure as that of the crustacean chitin. However, fungal chitin is associated with other polysaccharides such as glucans and mannans, which do not occur in the exoskeleton of arthropods (Kaplan 1998). The chitin of the

white-rot fungus *Rigidoporus lignosus* is not detectable during the process of infection due to degradation by enzymes excreted as a defense response by the host cell (Nicole and Benhamou 1991). Another white-rot fungus, *Phellius noxius*, does not contain chitin in its fungal sheath (Nicole et al. 1995). The mycelia, the caps and stalks of fruiting bodies of four edible mushrooms (*Lycophyllum shimeji*, *Lentinus edodes*, *Volvariella volvacea*, and *Caju*) contain chitin as a minor component (Cheung 1996).

Chitosan occurs naturally in Mucorales species such as *Rhizopus*, *Absidia*, and *Mucor*. The presence of chitosan in a basidiomycete has only been reported in *Lentinus edodes* (Shiitake mushroom), while Bacteria (Schizomycetes) and Slime molds (Myxomycetes) are devoid of chitin (Akila 2014).

Because of significant advantages, recently, there has been increased attention on the synthesis of chitin and chitosan from fungal mycelium. Crustacean resources are limited by season and fishery, whereas mushroom mycelium can be obtained through convenient fermentation processes without geographic or seasonal restrictions (White et al. 1979; McClements 2006). Crustacean chitin and chitosan may have different physicochemical properties, whereas fungal chitin and chitosan have relatively constant properties due to controlled fermentation conditions (Rane and Hoover 1993). Fungal chitin and chitosan are more effective in inducing plant immune responses and are potentially more suitable for agricultural purposes (Knorr et al. 1989). Many fungal species including *Absidia coerulea*, *Absidia glauca*, *Absidia blakesleeanus*, *Aspergillus niger*, *Gongronella butleri*, *Lentinus edodes*, *Mucor rouxii*, *Phycomyces blakesleeanus*, *Rhizopus oryzae*, and *Trichoderma reesei* have been investigated for the production of chitin and chitosan (Chatterjee et al. 2005; Arcidiacono and Kaplan 1992; Synowiecki and Al-Khateeb 2003; Hu et al. 1999; Tan et al. 1996). Of all the species studied, the most studied is *Mucor rouxii*, and the amount of chitin and chitosan in the mycelium can reach 35% of the dry mass of the cell wall (Wu et al. 2004). Fungi are usually harvested at later stages of exponential growth to maximize the production of chitin and chitosan (Akila 2014). Although fungi can grow on solid media, cultures to isolate chitin and chitosan are usually performed in potato dextrose broth (PDB), yeast peptone glucose broth (YPG), or salt medium with molasses (MSM) (Chatterjee et al. 2005). The extraction process from fungal sources is similar to that used industrially, except that desalination is not required due to the low mineral content of the fungal mycelium (Akila 2014). This process involves three steps:

(a) Alkaline treatment to remove proteins and alkali-soluble polysaccharides.
(b) Acid reflux to separate chitin and chitosan.
(c) Precipitation of chitosan under alkaline conditions.

Protein removal by alkali treatment is generally performed with 1 M NaOH at 95 °C for 1–6 h and at 121 °C for 0.24–1 h. Separation of chitosan by acid treatment is usually performed with 2–10° ethylenic acid or HCl at 95 °C for 3–14 h. When chitin and chitosan were extracted from *Mucor rouxii*, 2% NaOH was used at 90 °C for 2 h for alkali treatment, and 10% acetic acid was used at 60 °C for 6 h for reflux. Hu et al. (1999) applied to autoclave at 121 °C for both alkaline and acid treatment of *Absidia*

glauca mycelium. However, the temperature and time of the treatment had to be reduced to 25 °C and 1 h to keep away from the polymerization of chitosan while being extracted from zygomycetes. Most of the studies in this field deal with the fermentation process to supply fungal mycelia for chitin and chitosan extraction. Noticeably few researchers have focused on the fungal waste from industrial fermentations or mushroom enterprise. However, thinking about the quantity of waste that accumulates in processing, the citric acid and mushroom industries specifically from *Agaricus bisporus* growing practices can provide plenty of raw materials for fungal chitin and chitosan production. Citric acid is the most extensively used organic acid within the beverage, food, and pharmaceutical industries. Industrial production is based on *Aspergillus niger* submerged fermentation. The contemporary global requirements for citric acid production are around 400,000 tons/year (Kristiansen et al. 1999). Managing this waste means some extra expenses for producers and an alternative answer for mycelial disposal. One of the effective outputs for the spent mycelia is in food supplements, however, this type of feed appears to be not so easy in competing with other low-price feeds (Akila 2014).

3.3 Nutritional Factors and Mode of Cultivation

The production of microbial polymers is usually facilitated by the high carbon or nitrogen content of the medium. Nitrogen sources are growth-limiting nutrients, whose concentrations are adjusted to achieve the desired biomass concentration. As cations can affect the rheological properties of polymer solutions, care must be taken to optimize the concentration of salts used as nutrients in the medium (Papagianni 2016).

Continuous culture is not used for the production of microbial polymers. At higher growth rates desirable for high productivity, and increased ratios of the carbon source is used to produce biomass rather than polymer. In addition, while some microbially produced polymers are unstable in continuous culture, the stability of the strain is not an issue in short-lived batch culture (Anderson and Wynn 2001).

For some microorganisms, the carbon source determines both the quantity and quality of formation and the quantity of the product synthesized. Ordinarily, the carbon source concentration affects the efficiency of carbon source conversion into polymers e.g., the conversion efficiency of glucose to the polymer by *Xanthomonas campestris* by increased concentration of glucose (Rogovin et al. 1961).

A source of nitrogen is required for both cell growth and the synthesis of enzymes that form polymers, but excess nitrogen tends to decrease the conversion of carbohydrate substrates to extracellular polymers (Kang and Cottrell 2012).

3.4 Other Environmental Factors

Temperature is often critical in polymer synthesis. In general, the optimal temperature for cell growth is also the best for product formation. Most of the polymer producers are mesophilic. During fermentation, large volumes of hot air and high levels of agitation are used to avoid inefficient mass transfer caused by high viscosity. Another important factor is pH. The bacterial polymers of possible commercial significance appear to have an optimal pH for synthesis between 6.0 and 7.5 (Cohen and Johnstone 1964). For fungal gum production, the optimum pH lies between 4.0 and 5.5 (Ziegler et al. 2016).

3.5 Mineral Elements

Many microorganisms have strict requirements for specific mineral composition. Among these elements, K, P, Mg, Ca, Mo, Fe, Cu, and Zn are required. However certain elements can inhibit product formation. As a result, the mineral requirements for polymer synthesis vary from species to species (McNeely and Kang 2012).

4 Literature Review on Fungal Species and Substrates Used in Producing Mycelium-Based Bioresins

To date, many research teams have investigated different fungal species and substrates in producing mycelium-based materials. In this section, a comprehensive review is performed for most studies that have been performed so far to help the potential production of fungal bioresins in the future. Table 1 shows a summary of the literature sources this review is based on.

Holt et al. (2012a, b) reported on a process developed by Ecovative Design, LLC for growing fungal species on agricultural biomass to produce environmentally friendly packaging products (EcoCradle™) and insulating panels (Greensulate™). The purpose of this study was to develop and evaluate six mixtures of recycled cotton plant biomass (CPB) materials as substrates for the colonization of selected fungi in the production of molded packaging materials. The mixture consisted of treated CPB, cottonseed husks, starch, and gypsum. The four ingredients had the same mix ratio for all six blends, the only difference being the CPM particle size. The particle sizes of CPB varied from 0.1 to 51 mm. In addition to the six cotton-based blends, two methods, grain and liquid, were used to inoculate the blends with fungal spores resulting in 12 treatments. The blends were inoculated and the test specimens were grown in tools (plastic molds) for 5 days and then dried to remove moisture. The recipe for each blend was identical except for the particle size range of the cotton-based materials. The difference in the inoculums was that one carried the

Table 1 Fungal species and substrates used for producing mycelium-based materials (Butu et al. 2020)

Fungal species	Major components in the substrate	References
Ganoderma sp.	Cotton plant biomass	Holt et al. (2012a, b)
The phylum of Basidiomycetes	Rice straw, hemp pith, kenaf fiber, switch grass, sorghum fiber, cotton bur fiber, flax shive	Pelletier et al. (2013)
Trametes villosa, *Pycnoporus sanguineus*, *Coriolopsis rigida*	Post-consumer waste from bottle caps of PP and EVA, eucalyptus, pine wood flour	Catto et al. (2014)
Coriolus versicolor, *Pleurotus ostreatus*	Wood chips, hemp hurd, loose hemp fiber, and non-woven mats of hemp fiber	Lelivelt et al. (2015)
Ceriporia lacerata	Soybean straw, wheat bran, gypsum	Shao et al. (2016)
Ganoderma sp.	Kenaf/hemp mix and corn stover/ hemp mix	Jiang et al. (2017a, b)
Basidiomycetes based fungi	Cotton burs, switch-grass, rice straw, sorghum stalks, corn stalks, kenaf	Pelletier et al. (2017)
Pleurotus pulmonarius, *Pleurotus ostreatus*, *Pleurotus salmoneo-stramineus*, *Aaegerita agrocibe*	Woodchips of eucalyptus, oak, pine, apple, and vine	Attias et al. (2017)
Daedaleopsis confragosa, *Ganoderma resinaceum*, *Trametes versicolor*	Kenaf, hemp, and corn fibers	Bajwa et al. (2017)
Ganoderma lucidum, *Pleurotus ostreatus*	Cellulose, PDB-cellulose	Haneef et al. (2017)
Trametes versicolor	Rice hulls	Jones et al. (2017a, b)
Basidiomycete fungi	Corn stover	Tudryn et al. (2018)
Irpex lacteus	Birch sawdust, millet grain, wheat bran	Yang et al. (2017)
Trametes versicolor	Rice hulls, glass fines	Jones et al. (2018a, b)
Trametes versicolor	Wheat grain	Jones et al. (2018a, b)
Pleurotus ostreatus, *Pleurotus eryngii*, *Pycnoporus sanguineus*	Coconut powder, wheat bran	Teixeira et al. (2018)
Oxyporus latermarginatus, *Megasporoporia minor*, *Ganoderma resinaceum*	Wheat straw	Xing et al. (2018)
Ganoderma lucidum	Palm sugar fiber, cassava bagasse	Agustina et al. (2019)
Trametes multicolor, *Pleurotus ostreatus*	Beech sawdust, rapeseed straw, cotton fiber	Appels et al. (2019)
Colorius sp., *Trametes* sp., *Ganoderma* sp.	Woodchips (pruning residues) apple, vine crops	Attias et al. (2019)

(continued)

Table 1 (continued)

Fungal species	Major components in the substrate	References
Pycnoporus sanguineus, *Pleurotus albidus*, *Lentinus velutinus*	Sawdust, wheat bran	Bruscato et al. (2019)
Trametes versicolor	Hemp, flax, flax waste, softwood, straw fibers	Elsacker et al. (2019)
Pleurotus ostreatus	Sawdust, wheat bran, straw	Ghazvinian et al. (2019)
Polyporus brumalis, *Trametes versicolor*, *Agaricus bisphorus*	Wheat straw, rice hulls, sugarcane bagasse, blackstrap molasses	Jones et al. (2019)
Ganoderma sp.	Ground corn stover, grain spawn	Pelletier et al. (2019)
Pure white-rot basidiomycete	Mixture of spruce, pine, fir wood particles	Sun et al. (2019)
Fomitopsis pinicola, *Gloeophyllum sepiarium*, *Laetiporus sulphureus*, *Phaeolus schweinitzii*, *Piptoporus betulinus*, *Pleurotus ostreatus*, *Polyporus arcularius*, *Trametes pubescens*, *Trametes suaveolens*, *Trichaptum abietinum*	Sawdust/paper birch, aspen, lodgepole pine, subalpine fir, white spruce	Wimmers et al. (2019)
Ganoderma lucidum	D-Glucose, alkali lignin	Antinori et al. (2020)
Pleurotus ostreatus	Wheat bran, sugarcane, sawdust	Joshi et al. (2020)
Ganoderma lucidum	Cotton stalk	Liu et al. (2020)
Ganoderma lucidum	Bamboo fibers	Ridzqo et al. (2020)
Pleurotus ostreatus	Rubberwood sawdust, corn grain	Shakir et al. (2020)
Pleurotus ostreatus, *Pleurotus citrinopileatus*, *Pleurotus eryngii*, *Ganoderma lucidum*	Husk psyllium, chicken feathers, flour, textile	Silverman et al. (2020)
Trichoderma asperellum, *Agaricus bisphorus*, *Lentinula edodes*, *Pleurotus ostreatus*, *Ganoderma lucidum*, *Kuehneromyces mutabilis*, *Flammulina velutipes*	Oat husk, rapeseed cake	Tacer-Caba et al. (2020)

fungal spores on kernels of grain whereas the other had the fungal spores suspended in the liquid. The liquid inoculum was easier to use in the process and provided a more consistent distribution of fungal spores when applied to the blends. The grain inoculum generally resulted in higher densities due primarily to the added weight of the grain. The densities were higher than desired (32.04 kg/m^3) due in large part to the inclusion of cotton plant particles <2 mm. In future studies, cotton plant material having a diameter < 2 mm will not be used. No single treatment outperformed the other treatments in all categories evaluated. Most of the treatments performed

similarly to each other for the response variables measured. Overall, the use of cotton-based fungal mycelium packaging material is a viable alternative to polystyrene packaging. As refinements in processing and biomass blend development continue, the physical and mechanical properties of the product should improve. The improved physical properties will make agricultural residue-based fungal composites suitable for a variety of applications where fossil fuel-based materials are currently used.

Arifin and Yusuf (2013) studied the production of green composite and unveiled the potential and properties of synthetic foam mycelium using rice husk and wheat grains. Through this research, a composite foam sample was prepared using rice husks and wheat grains as reinforcement materials, and mycelium as the matrix. The percentage effect on the physical properties, microstructure and porosity of rice hulls was studied. Three sample percentages: 50% RH + 50% WS, 70% RH + 30% WS, and 30% RH + 70% WS were tested. This research determined whether the properties of a potential mycelium matrix could replace a major source of polystyrene production. The potential of the mycelium properties, and rice husk and wheat grain mixture characteristics were determined to create a resource and energy-saving approach through 100% natural circulation for insulated packaging.

Pelletier et al. (2013) examined the use of a novel new renewable resource for sound absorption. The new proven material is based on a fungus that grows on semi-hydrophobic substrates of agricultural by-products such as millet, sorghum stalks, rice straw, kenaf, flaxseed and hemp. Because this new composite has limited density control, a variety of substrates have been tested, the main control being component selection. Mycelium was described by Holt et al. (2012a, b), which consisted of the principle processing steps of: (1) fiberized agricultural byproducts, (2) steam processing the fiberized agricultural byproducts to render mold spores inert, (3) inoculating the circulate processed fibers with Basidiomycetesbased fungi, (4) putting the inoculated fibers into 16 × 16 cm molds at a depth enough to generate a final nominal thickness of 2.5 cm, (5) growing fungi on the fiberized byproducts in a controlled environment chamber in a dark, warm, and humid environment for 4–6 days. Compared to standard traditional foam insulation boards, The research results showed mycelium-based boards are a promising bio-based composite alternative. An additional advantage of this new material is that it can be produced at a lower cost in comparison to the traditional petroleum-based foams while being biodegradable at its end of service life.

Catto et al. (2014) evaluated the biodegradability of wood-plastic composites (WPC). The materials used were post-consumer waste from bottle caps of PP and EVA (present in the inner lining of the stoppers supplied by Prisma Montelur Thermoplastics in "flakes" form), and two types of wood flour: eucalyptus and pine of the species *Eucalyptus grandis* and *Pinus elliottii*, respectively. Wood flour was sorted on 32 and 16 mesh Tyler sieve systems with selected particle sizes from 250 to 500 mm. The mixture was processed on a single screw extruder ($L/D = 22$) perforated to a temperature profile of 170–190 °C, a screw speed of 65 rpm and "pellets form". Samples were weighed and placed in duplicate in Erlenmeyer flasks with 30 ml of distilled water and sterilized by autoclaving at

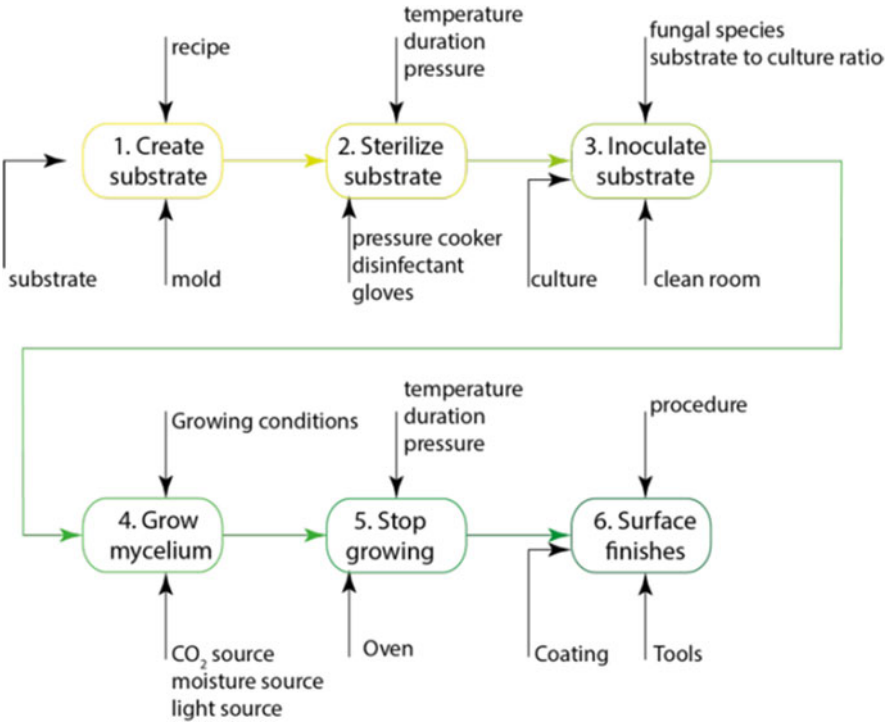


Fig. 2 Standard production process of mycelium-based material (Lelivelt et al. 2015)

127 °C in a vertical autoclave CS-100 Primatec with 100 L capacity and variable working pressure from 1 to 1.5 kgf/cm^2. The fungi selected for the experiments were *Trametes villosa* (TV), *Coriolopsis rigida* (CR), and *Pycnoporus sanguineus* (PS) species, which were previously isolated from Petridi shes from autoclaved and incubator cultured agar malt extract (AEM) in the ambient temperature of 25 °C in the dark. After a week, the fungus was inoculated into each sample (substrate) from the previous culture and then placed in a greenhouse at 25 °C in the dark for 60 days. Monitoring was performed by culturing different types of fungi (basidiomycetes) on different substrates using gravimetric analysis and visual monitoring of composites to evaluate the species of fungi exhibiting increased growth rates and their relationship to different wood types. An assessment of the potential biodegradation of these substances was made Based on the presented results, it was concluded that TV fungus interacts best with wood embedded in the composite in both *Eucalyptus grandis* and *Pinus elliottii*.

Lelivelt et al. (2015) demonstrated a detailed production method for mycelium-based materials and the display of structural properties of these materials. The process of creating mycelium-based materials consists of six steps. It takes four steps to grow mycelium and two more steps to make it a usable material (Montalti 2014). The process is shown in Fig. 2. The first step is to create a habitat for the

Table 2 Growing conditions of Basidiomycota (Lelivelt et al. 2015)

Humidity	90–100% (moist to the touch)
CO_2	High
Light	None
O_2	Necessary for growth
Temperature	<40 °C (heat is generated during growth)

fungus. The substrate can be any cellulosic materials such as straw, wood, and hemp. After substrate selection and mixing, the substrate must be sterilized to prevent contamination by other organisms during growth. This can be done by boiling the substrate in water or by treating it with hydrogen peroxide. After sterilization, the desired fungus spawn was inoculated into the substrate. In order to create a very pure and reliable spawn, it is desirable to use pre-grown spawns raised by specialized companies operating under specific conditions (Montalti 2014). The fungal group for use is preferably Basidiomycota because they can aggregate mycelium into dense masses (Carlile and Watkinson 1995). Inoculation must be done under semi-sterile conditions to prevent contamination and can be performed by mixing the spawn with the substrate. After inoculation, the fungus must be done by mixing the spawn and substrate. After inoculation, the fungus should colonize the substrate (Step 4 in Fig. 2). At this stage, it is important to provide favorable growth conditions. Although these conditions depend on the type of fungus, some conditions are universal to all Basidiomycetes. First, growth must take place in the dark. For Basidiomycetes, the presence of light is a signal that a free surface has been reached. On these surfaces, Basidiomycetes begin to produce fruiting bodies (i.e., mushrooms). When growing fungi for mycelium, it is important to prevent the formation of fruiting bodies. This is because the fruiting body slows down the growth process and creates irregularities in the mycelium. Second, the humidity must be very high. The relative moisture content of the air should be between 90 and 100% (Yadav and Tripathi 1991). However, this is sufficient for the substrate to contain sufficient moisture for the growth phase. The exact moisture content depends on the substrate and species, but a substrate that is 'wet to the touch' is considered sufficient (Wösten 2014). Airflow is also required. Fresh air is essential because mycelium needs oxygen and produces CO_2 as it grows. However, fresh air also increases the likelihood of contamination, so controlling the airflow is a good idea. The low CO_2 content is also an indicator that the basidiomycetes have reached the free surface. This stimulation leads to the formation of fruiting bodies that do not contribute to the growth of mycelium. Therefore, a high CO_2 content must be maintained. The optimum temperature for growth varies depending on the species, but in general, Basidiomycetes do not grow above 40 °C. In most cases, room temperature is sufficient. However, fungi can overheat in an enclosed environment because they generate heat during growth. Under growing conditions optimized for large-scale agricultural mycelium, the growth phase takes 16 days (van der Horst 2014). Typical growth conditions for Basidiomycetes are listed in Table 2. Growth should be stopped after the growth phase, which is essential. Otherwise, the fungus will eventually absorb the entire substrate or begin to form fruiting bodies.

Termination of the mycelium growth phase can be achieved by heating the mycelium. When the growth has stopped, the sample can be removed from the mold. In the final stage, a coating can be added to improve material properties.

Shao et al. (2016) reported the use of an unsterilized substrate consisting mainly of soybean straw used to grow *Ceriporia lacerata* to obtain soybean straw mycelium composite material (MSCM). *C. lacerata* was initially grown on Potato Dextrose Agar (PDA) medium at 25 °C for 7 days and then stored at 4 °C. About 3 cm^2 of the slant culture was inoculated into every 500 ml baffled shake flask containing 150 ml of first-order inoculation culture medium. Then, it was incubated in a rotary shaker incubator shaker at 150 rpm and 25 °C for 3.5 days. 7.5 ml of first-order seeds were inoculated into each 500 ml flask containing 150 ml of culture medium for second-order seeds. They were then incubated for 3 days in a rotary shaker incubator at 150 rpm and 25 °C. A stainless-steel tub (Φ58 × 18 cm) with a lid was used as the culture vessel. Each bath was filled with 1 kg of dry culture substrate and inoculated with 375 ml of second-order seeds without adding a bactericidal or antibacterial/bacteriostatic agent. The moisture content of the culture substrate was about 65%, and it was incubated at 25 °C for 5 days. The culture substrate was inverted and stirred once a day. The inoculated culture substrate was cultured at 25 °C for 5 days to allow the mycelium to completely colonize the culture substrate. After 5 days, the culture medium was crushed into small particles and placed in a mold. For the next 4 days, the molds were incubated at 25 °C to grow mycelium for weaving and binding the individual particles into a solid. After culturing in the mold for 4 days, the culture was taken out of the mold and incubated for 3 days at 25 °C and at least 85% relative humidity. Finally, the culture was dried at 60 °C and MSCM was prepared for the dried culture. The effect of the particle size of soybean straw on MSCM production was evaluated. The thermal conductivity, compressive, and sound absorption properties of MSCM were tested. The results showed that *C. lacerata* could be cultured with unsterilized substrates and used to generate MSCM. This promoted mycelial growth and MSCM formation, and the grain size of soybean straw were larger within a certain range. And the test results showed that MSCM has high compressive strength, good thermal insulation, and good sound absorption properties.

Ziegler et al. (2016) conducted a study to evaluate the potential of innovative biocomposite materials patented by Ecovative Design, LLC. for commercial products and packaging. Biocomposites use fungal mycelium as a matrix to bind plant cellulosic fibers. Test samples were made from various combinations of fiber-fungal strains using cotton waste and hemp cores as well as surface fibrous tissue. The composite is created using a patented process that involves wrapping the shaped fibers between two sets of mats and then incorporating the fungal mycelium seeds into the mold and surface adhesive mats of the composite to initiate fungal growth throughout the material. Biocomposites have a multi-layered structure with two bonding layers on the top and bottom. Surface-bonding mats may consist of natural fiber fabrics such as burlap, which allows the fungus to germinate to form the structure of agricultural fibers. Plant fibers were tightly wrapped between the binding layers. Mycelium was inoculated into this mixture and allowed to germinate into the

binder and core of plant fibers in an environment favorable for fungal growth. Mycelium has evolved into a number of distinct branched filamentous structures called hyphae. Mycelium hyphae usually penetrate the plant fiber matrix and weave the fiber particles together to form a bonding mat. The hyphae grow over time to form a mesh frame densely filled with fibers, effectively tying these fibers together (Bayer 2010). When the mycelium completely enveloped all the plant fibers and achieved optimal growth, the material was exposed to high temperatures to kill the mycelium and make it dormant. At this point, the mycelium provides adequate bonding to the fiber core and completely covers the surface of the bonding mat, leaving a white or grayish-white velvety surface. The mycelium is heat treated (110 °C, 24 h) to inactivate the product before use, making it safe as a packaging material. In addition, the selection of antimicrobial fungal strains can impart these properties to biocomposites. The physico-mechanical properties of biocomposites have been evaluated to gain a complete understanding of the material, which may lead to future applications.

Jiang et al. (2017a, b) tested a non-traditional approach to the production of biocomposite multilayer structures in which all materials are obtained naturally, including fabrics made from natural fibers (jute, hemp, and cellulose) as skin, *Ganoderma* sp. mycelium-bound agricultural waste as the core, and bioresin as the matrix. The core substrate is first combined with the mycelium with pre-grown "regrind" material (kenaf/hemp mixture, then corn straw/hemp mixture (both 50/50 wt%)) and then cut into smaller pieces with a trommel. The growth tray is rinsed with water, wiped dry, and sprayed with an alcohol solution for sterilization. Both layers of the same fiber reinforcement are also sterilized with a 10% hydrogen peroxide solution and then placed appropriately on the bottom of the cultivation tray. The trays were then filled with 120 g of regrind (amount determined to sufficiently fill the mold cavity) and covered with two layers of reinforcement, then covered with mating tray covers to provide light compression and held in place with binder clamps. It is located around the flange of the mold. Each filled and closed growth tray was placed in a semi-permeable polypropylene bag that creates and maintains a high humidity (up to 98% RH) environment for the mycelium fungus to breathe. Incubation procedures lasting 5 days were carried out on growth racks at Ecovatice Design's facility at room temperature (24 °C). If dried without reinforcement skins attached, the 2.5 cm thick wet core should be dried to the specified thicknesses by heat press with slight compression to match the thickness of the reinforcement skin during resin infusion. After the growth phase, the core should be dried at a temperature high enough to remove moisture (∼70–80 wt%) and inactivate (kill) the mycelium. Otherwise, the core will continue to grow and break down the substrate. As a preliminary study for comparison with the thermo-compression process, six cores were completely dried in a convection oven at 82 °C for 12 h and 93 °C for 8 h (standard cycle for Ecovative's packaging ecobatic mycelium products). In addition, 6 other cores were thermo-compressed using Model 2518 Carver press and dried at 250 °C for 20 min to the specified thickness (two samples per reinforcement type). Temperatures >250 °C can lead to unwanted carbonization of the core surface and

20 min was chosen as the upper limit for the cycle time of the Ecovative Design heat press.

Pelletier et al. (2017) investigated the use of novel new renewable resources for sound absorption. The new test substance was based on a fungus grown on semi-hydrophobic substrates of agricultural by-products such as cottonwood, millet, sorghum stalk, corn stalk, rice straw, and kenaf. It forms a light-weight natural bio-composite board. Basidiomycetes were cultures on agricultural by-products pulverized in various proportions using a hammer or grinder and then sieved to exclude particles smaller than 0.853 mm, (#20 mesh sieve) to make subjects. Mycelium was grown from the by-product using the procedure described in (Holt et al. 2012a, b). The main processes include: grinding of agricultural by-products; steaming the fibrous agricultural by-product to inactivate mold spores; Steamed fiber inoculation with Basidiomycetes-based fungi; placing the inoculated fibers in a 16 × 16 cm mold of sufficient depth to obtain a final nominal thickness of 2.5 cm; Fungi are grown on fibrous by-products in a controlled environmental chamber under dark, warm, and humid conditions for 4–6 days to produce low-density fiberboards associated with the fungal mycelium matrix. Converting these low-density boards to new high-density boards requires additional post-processing steps. The compression process starts with a low-density board heat-treated at 100 °C for 10 min at various elevated pressures. No adhesives or binders have been added to the construction of the high-density board. The only binders were chitinous polymers deposited from natural mycelium and the natural growth of fungi. The results of this study indicate that these mycelium-based compression boards are promising alternatives to bio-based composite materials for acoustic baffle panels. This new material offers a completely natural and sustainable alternative to modern composite panels such as Medium Density Fiberboards (MDF) and Oriented Strand Boards (OSB). Attias et al. (2017) reported the cultivation of several fungi species from various local crop wastes to evaluate the best combinations of fungi-plant material for future applications. Four types of fungi: *Pleurotus ostreatus*, *Pleurotus pulmonarius*, *Aaegerita agrocibe*, and *Pleurotus salmoneostramineus* were grown on eucalyptus, oak, pine, apple, and vine chips. Wood chip substrates for this experiment were pruned native trees and crops from the Galilee region of Israel. The average particle size was 5 × 15 mm. The substrate was thoroughly mixed with (50% w/v) water, and then a 14 cm diameter Petri dish (150 g of the wet substrate in each dish) was placed and sterilized in an autoclave (1 h, 121 °C). Each dish was inoculated with a 7 mm diameter mycelium colony grown on agar and then incubated at 25 °C for 4–5 weeks. Samples were tested for selected properties including chemical change (pH, electrical conductivity, water, carbon and nitrogen content) in organic matter, mycelium growth rate, density and quality. The high correlation between the loss of organic matter and growth parameters of fungi helped to evaluate the effectiveness of a set of fungi. The best results are *P. ostreatus* mycelium grown on vine and apple substrates.

Bajwa et al. (2017) evaluated biocomposite boards made from three different cellulosic fibers: kenaf (*Hibiscus cannabinus*) core, hemp (*Cannabis sativa*) core, and corn (*Zea mays*) straw. Cellulose fibers include *Daedaleopsis confragosa*

(*D. conf.*), *Ganoderma resinaceum* (*G. res.*), and *Tramates versicolor* (*T. ver.*). Biocomposite boards were manufactured by Ecovative Design, LLC. in their laboratory located in Green Island, NY. The manufacturing process consisted of pasteurizing and sterilizing the cellulosic material at 115 °C for 28 min, cooling it to 35 °C or less, and then inoculating with fungus strain. The inoculum was transferred to a plastic mold, sealed and incubated at 24 °C for 5 days. Then, the mycelium colonized boards were sterilized in a convection oven at 60 °C for 8 h to inactivate fungi growth and prepare a final product. The details of the manufacturing process are described in a previously published study (Holt et al. 2012a, b). The resulting biocomposite boards were evaluated for termite resistance using four termiticides: guayule resin (*Parthenium argentatum*), vetiver oil (*Vetiveria zizanioides*), cedar oil (*Juniperus virginiana*), and borax, according to ASTM D3345–01 standard. The results showed the effects of different types of termiticides on cellulosic biocomposites associated with fungal mycelium. The results of this study showed that natural vetiver and cedar oils and guayule resin can be used as an effective treatment for mycelium-bonded cellulose composites to increase resistance to termites.

Haneef et al. (2017) obtained films based on the mycelium of two species of edible and medicinal fungi, and *Pleurotus ostreatus Ganoderma lucidum*, grown on different substrates and compared their properties with other bio-based polymers produced by microorganisms such as bacterial cellulose and PHB. Growth of the mycelium material in this study was achieved by feeding two natural polymer substrates, pure amorphous cellulose and a mixture of cellulose and potato dextrose broth (PDB). A polysaccharide-based medium was used as a nutrient. However, those containing PDB was more readily absorbed by the mycelium due to the higher concentration of simple sugars. The mycelium film was heat treated at the end of the growth period to stop fungus growth and obtain a final fibrous film. The physicochemical properties of self-grown films were determined by two types of physiological properties and most importantly by various nutrient substrates. The authors have found that the addition of PDB to the growth medium affects the secondary structure of the *G. lucidum* proteins and increases the protein ratio of *P. ostreatus*. While increasing the relative concentration of lipids in films based on this microorganism since then. Also, the relative presence of chitin was decreased in both species. These deformations had a significant impact on the final mechanical properties of the mycelium-based films. When the nutrient substrate contained PDB, the self-grown fibrous material exhibited lower elastic modulus and increased elongation at break. This substrate makes the mycelium less rigid and more flexible than cellulose-fed materials. In addition, the PDB mycelium had a higher decomposition temperature and stronger hydrophobic properties than the cellulose-fed mycelium. Research has shown that mycelium-derived substances are naturally occurring multicomponent substances that can be modulated by altering their nutrient substrates and represent a novel method for low-cost, high-volume production of functional materials.

Jones et al. (2017a, b) reported that mycelial biocomposite grown from rice husks were evaluated and compared with commercially available extruded polystyrene

Fig. 3 Mycelium based composite material: (**a**) after growth, (**b**) dehydrated (Jones et al. 2017a, b)

(XPS) foam. The fast-growing and commonly available white fungus *Trametes versicolor* (Basidiomycota) was selected for this study based on its lignin degradability and high growth rate. The fungal inoculum was purchased from Aussi Mushroom Supplies (Melbourne, Australia) as the mycelial masses of digested wheat grains sealed in plastic bags with filter patches. Rice husks were selected as the substrate material based on their silica content, low cost and polysaccharide (cellulose and lignin) content and were purchased from CopRice (Melbourne, Australia). Before use, soaked (48 h in Milli-Q® Type 1 ultrapure water) and sterilized (autoclaved for 90 min at 121 °C and 103.4 kPa). It was then combined with a 25 wt% *Trametes versicolor* inoculum using a sterile blender. Low seed weight was chosen to maximize the content of the composite rice hull. The inoculated substrate was then dispensed into sterile plastic molds (duplicates) prior to incubation for 12 days in standard atmospheric conditions (25 °C, 50% RH) to allow the mycelium to colonize the rice husks and produce composites (Fig. 3a). After the incubation period, samples were dried at 50 °C for 48 h to dehydrate and denature the fungal material (Fig. 3b). The fire response properties of these materials were measured under heating conditions with a cone calorimeter, which can produce a correlation for well-developed indoor fires (e.g., heat flux of 50 kW/m^2). This flame retardant set reduces the amount of heat and toxic fumes emitted during combustion, making the mycelium biocomposite safer for use in construction applications where thermal shock resistance is required.

Tudryn et al. (2018) conducted a study that provided insight into the effects of processing methods and composition of biopolymer composites from agricultural waste/fungus on structural and mechanical properties. Composites were obtained by growing mycelium on corn straw. Corn straw particles were sorted using different sieves by size. Three ranges of particle sizes were used: (a) 1.7–6.7 mm, (b) 0.9–1.7 mm, and (c) 0.4–6.7 mm. The average aspect ratio of all three fillers was from 15 to 20 as measured by light microscopy. Corn straw had a low starch content of 1% and a neutral detergent fiber content of 72%, which did not significantly contribute to fungal nutrition or germination. A proprietary type of fungus was inoculated using a vegetative mycelial tissue distributed throughout corn straw with the addition of grains and micronutrients (simple carbohydrates, minerals).

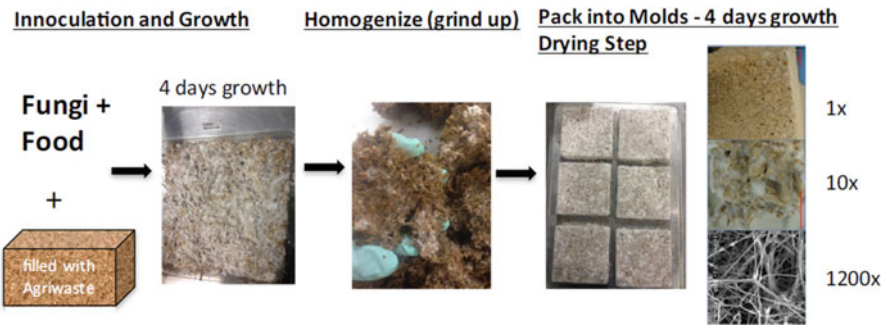


Fig. 4 Flow chart of myceliated corn composites preparation procedure (Tudryn et al. 2018)

Thus, corn straw for the biopolymer matrix is a high-modulus filler. The general procedure for growing the composite material is shown in Fig. 4. The filter patch was filled with 5 l of corn straw in one size range. Calcium and carbohydrates were added to the dry mass of the bag. Water was added to the bag in the amount of 65% of the dry weight of the corn straw. The filter patch bag and its contents were sterilized at 15 psi for 2 h. Corn was inoculated with a millet with mycelial vegetative tissue. The mycelium was grown in the bag for 4 days after which the contents of the bag were removed and manually milled to homogenize the growth to ensure that the mycelium did not colonize just outside the corn straw mass. More carbohydrates were added to promote more mycelial growth during this homogenization step. After fully homogenizing the ratified corn straw, the material was loosely packed into $6'' \times 6'' \times 1''$ tile molds (usually 6–9 tiles are made in one bag). These tiles were left for an additional 4 days. Let it grow, then turn it over and let it grow for an additional 2 days, finally during the tile at 100 °C for several hours to kill any mycelium or biological material in the composite. Since carbohydrates were added as the main material of corn, there was no significant change in the size or aspect ratio of corn straw during growth. The effect of nutrition during inoculation after the homogenization step on flexural modulus and strength was determined. The increase in additional nutrition during inoculation had little effect on the overall strength or elastic modulus of the composites. However, the increase in carbohydrate loading after the homogenization step increased the yield bending stress and bulk flexural modulus. The adjacency of the formed networks was significantly higher in the latter scenario, suggesting that the increase in modulus and strength of the final composite after homogenization was the result of continuous network formation of hyphae, which improved the integrity of the matrix to transmit stress to the filler particles.

Yang et al. (2017) presented an innovative biofoam based on fungal mycelium. Three different mixing protocols using different substrate materials including millet, wood pulp, natural fibers, calcium sulfate, and bran and two packaging conditions were tested to obtain samples for physical, thermal and mechanical properties. In mixing protocol I, the substrate and live fungal culture were mixed, and packaged in molds, and placed in a temperature and humidity controlled incubator. In mixing protocol II, substrate and fungal culture are incubated in filtered polypropylene bags

for a specified period before the mixture is ground and packaged into cylindrical molds to allow them to re-solidify into a structurally more uniform and dense foam. Mixing protocol III is the same as protocol I, but with the addition of natural fibers (50% of the substrate's dry weight) during mixing. Two packaging modes: loose and dense. The former is naturally deposited without compaction and the latter with approximately two times the original volume of substances packed. Birch sawdust from the neighborhood forestry industry and added nutrients have been mixed with a certain quantity of water and pasteurized. Then, the slurry was inoculated with a culture of Basidiomycete saprotrophic fungus, endemic to Alaska, and incubated for some time to achieve full colonization of the nutritive substrate through vegetative mycelium. The inoculated slurry from Protocols I and III was mixed and loaded into cylindrical molds for further incubation. In Protocol II, the inoculated substrate was incubated for a specific period in a filtered polypropylene bag, then crushed and repacked into a cylindrical mold. Incubation took place in a specific range of humidity and temperature. Samples were dried in a dryer before being removed from the mold for testing. The tightly packed sample according to mixing protocol II was found to have the highest dry density, elastic modulus, compressive strength, and similar thermal conductivity, which was equal to or better than the conventional polymer thermal foam, except for the dry density. The results show that this biofoam has great potential as an alternative insulation material for buildings and infrastructure or as a lightweight backfill for geoengineering applications, especially in cold regions.

Jones et al. (2018a, b) conducted a study characterizing the effects of different proportions of agricultural and industrial wastes with high silica content on the combustibility of mycelial composites compared to conventional synthetic building materials. Soak rice husks and wheat grains (primary function to support fungal growth) in Milli-Q® Type 1 ultrapure water for 48 h and sterilize at 121 °C, 103.4 kPa for 40 min before use. The fine glass additives (the main function of modifying material properties) were sterilized as above. Substrates and additives were then combined in various proportions with *Trametes versicolor* wheat grain inoculum using a sterile blender. The inoculated substrate was placed in a sterile plastic mold, closed under standard atmospheric conditions (25 °C, 50% RH) and incubated for 12 days to allow the mycelium to bind to the substrate and additives. After incubation, the sample was dried at 50 °C for 48 h to completely remove the adsorbed moisture and to denature the fungal material (Fig. 5). The results showed that the mycelium composite was safer than the conventional building material considered, resulting in much lower average and maximum heat dissipation rates and a longer time for capping. Also, CO production fluctuated emitted much less smoke and CO_2. Although rice husks produced significant amounts of charcoal and silica ash to improve fire resistance, composites containing fines glass particles had better fire performance due to their significantly higher silica concentrations and lower flammability. Higher concentrations of free fines increase the specific volumetric cost but decrease the cost per weight and specific gravity. The results of this study showed that mycelial composites are very cost-effective alternatives to

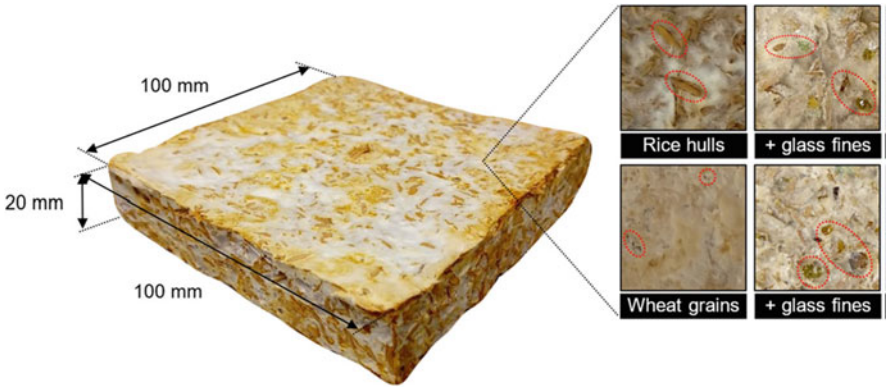


Fig. 5 Typical dehydrated square tile mycelium composites (e.g., rice husk, rice hull + glass flour, wheat, and wheat grains + glass flour) with surface profiles for each composite sample obtained (Jones et al. 2018a, b)

combustible synthetic polymers derived from petroleum and natural gas and woods structures for applications including insulation, furniture, and panels.

Islam et al. (2018) investigated the mechanical behavior of mycelial composites reinforced with biodegradable agricultural waste particles. In the composite, the mycelium acts as a support matrix to bind the reinforcing particles within the fibrous network structure. Composite samples were prepared similarly to the pure mycelium samples as described in (Islam et al. 2017). Here, nutrient mycelial tissues were first inoculated into filter patch bags with corn straw particles and nutrients (calcium and carbohydrates) and water. The mycelium was grown for 4 days at room temperature (~ 25 °C) and then crushed into small pieces to redistribute the growth evenly. The material was then filled into rectangular molds ($60'' \times 60'' \times 1''$) and left for an additional 4 days for growth. In a final step, the sample was heat treated at 100 °C for 4 h to biologically inactivate the material and to deactivate the mycelium. The compression behavior of the mycelium composite was investigated using a comprehensive experimental and computational method. The experimental results show that the composite material mimics the soft elastic response of pure mycelium at small strains and that the compression behavior of hard particles causes large strains. The composite material also exhibited the characteristic stress relaxation and cyclic compression hysteresis previously observed from pure mycelium.

Jones et al. (2018a, b) reported detailed thermal degradation and flame retardant properties of fungal mycelium and biomass mycelium composites. An inoculum of a common fungal species (*T. versicolor*) was purchased from New Generation Mushroom Supplies Pty. Ltd. as a mycelium mass growing from grains of wheat. Wheat grain (E&A Salce Pty. Ltd.) was chosen as the substrate material because of its high nutritional value and consistency with the inoculum composition. The substrate (wheat grain) was immersed in Milli-Q® Type 1 water for 48 h and sterilized (autoclaved at 121 °C, 103.4 kPa for 90 min) before use. Then, a certain amount (25 wt%) of the fungal inoculum was mixed with the substrate using a sterile

Fig. 6 Representative mycelium (*T. versicolor*)—wheat grain composite grown for 18 days and dried at 50 °C for 48 h (Jones et al. 2018a, b)

blender. An inoculum content of less than 25 wt% increased the growth period and the risk of contamination with other competing microbial species. The mixture was then evenly distributed in sterile plastic molds (100 × 100 mm) and incubated at standard atmospheric conditions for 6, 12, and 18 days. After incubation, the samples were dried at 50 °C for 48 h to dehydrate and denature the fungus. A representative composite of wheat mycelium is shown in Fig. 6. Measurements and analyses of key parameters such as decomposition temperature, gases released during pyrolysis, and residual char were performed. According to pyrolysis flow combustion calorimetry (PCFC), the combustion propensity of mycelium is significantly lower compared to polymethyl methacrylate (PMMA) and polylactic acid (PLA), indicating that they have significantly lower ignition and flaming combustion tendencies and are therefore safer to use. The diameter of the mycelium decreases after pyrolysis. The results of the conical calorimeter test showed that the presence of mycelium had a positive effect on the response characteristics of wheat grains to fire. It was found that mycelial growth time did not significantly improve the fire resistance of mycelium-wheat grain composites.

Teixeira et al. (2018) reported a study that selected fungal isolates and evaluated the cultivation period for the mechanical stability of composites made from wheat bran-added coconut powder. The experimental design was fully randomized based on a 5 × 3-factor scheme corresponding to the cultivation of five edible fungal isolates (*Pycnoporus sanguineus*, *Pleurotus ostreatus*, and *Pleurotus eryngii*). Cultures were transplanted in four replicates after complete colonization of the substrate (days 15, 30, and 45). The isolated fungi were grown on a coconut powder-based substrate less than 1 mm in size supplemented with 40% wheat bran and moistened at 60–70% distilled water. The mixture (wet weight = 250 g) was packaged in a 1000 ml cylindrical plastic container. The assembly was autoclaved at 120 °C and 1 atm for 1 h and repeated after 24 h. After cooling, 10 g of the fungal isolates previously obtained on the same substrates (wheat bran and coconut powder) were transferred to a sterile chamber. Fungal isolates were grown at room temperature

until complete colonies were formed. The cultivation length of the isolated fungus can affect overall weight and volume losses. Compressive strength and toughness were dependent on the fungus isolate and cultivation time. The isolates of *P. eryngii*, *Pleurotus ostreatus*, and *Pycnoporus sanguineus* presented potential characteristics for the production of biodegradable composites.

Xing et al. (2018) tried to obtain a mycelium material that can be used as an insulating material. Bio-based materials should have properties similar to conventional alternatives such as expanded polystyrene in terms of physical and mechanical properties but at a high level of biodegradability. Three species of basidiomycete (order Polyforales) were selected because they are known to grow rapidly on agar medium and are powerful colonizers and degraders of lignocellulose (*Oxyporus latermarginatus, Megasporoporia minor, Ganoderma resinaceum*). All were originally isolated from trees (both live and dead) foam in the Nile Delta region of Egypt (El-Gharabawy 2016) and grow rapidly (8.7–13 mm/day incubated at 25 °C on 3% Dark Malt Extract Agar (DMEA)). Fungal cultures were generally cultivated on 3% DMEA and incubated at 28 °C. To increase the inoculum, add 10 g of rye seeds and 10 ml of water to a 25 ml glass vial and autoclave at 115 °C for 15 min to sterilize. After cooling, rye grains were inoculated at 28 °C with three mycelium plugs from cultures on agar plates, with lids loosely closed to allow air exchange. After 14 days, the rye grains were well colonized and used to inoculate straw cultures. The orientation of the straws was random. Straw cultures were grown in polycarbonate plant tissue culture flasks (Magenta GA7; 77 × 77 × 97 mm; Sigma). 20 g straw cut to 3–4 cm in length was dispensed to each vial with 40 ml of water added. The vials were autoclaved at115°C for 15 min and then inoculated with 6–8 colonized rye grains spread around the vial after cooling down. Cultures were incubated at 28 °C for 8 weeks with the vial lids slightly open to ensure air exchange. No resin was used in this process. The straw block was removed from the culture vessel and dried at 70 °C. The test data showed that the mycelium brick had good thermal performances.

Agustina et al. (2019) prepared a composite board of cassava bagasse fiber (CBF) and palm sugar fibers (PSF) bonded with bio-adhesives from *Ganoderma lucidum* mycelium. The original mycelium was transferred to potato dextrose agar (PDA). The PDA plates were inoculated and incubated at room temperature (±30 °C) for 10 days. Four mycelial agars (±5 × 5 mm) from a PDA medium of 10 days old were inoculated into 300 ml flasks containing 100 ml of liquid medium and incubated for 8 days at room temperature (Karana et al. 2018). This material was used as an inoculum for the production process. The production medium was mainly made of a mixture of PSF and CBF supplemented with rice bran, $CaCO_3$, and other ingredients such as gypsum. The production medium was produced in three variations according to the content of PSF and CBF. Medium A = 65:35%; B = 50:50%; and C = 35:65%. Each variant was coded as PRA, PRB, and PRC, respectively. Samples of composite panels were made in two sizes (types):

- Type 1: Approximately 200 g (depending on composition) of the total solid mixture is added with distilled water to final moisture content, then mixed as

homogenously as possible. The mixture was then divided into two portions and placed in a fermentation vessel with approximate dimensions of 12.5 × 12.5 × 8 cm^3. Samples were then sterilized in an autoclave at 121 °C, 15 psi for 60 min, then removed and left overnight before inoculation. Samples of Type 1 were used for Internal Bonding (IB), Density (D), Thickness Swelling (TS), and Water Absorption (WA) tests.

- Type 2: Distilled water was added to about 231 g (depending on composition) of the total solid mixture until the final moisture content was reached, and then mixed as uniformly as possible. The mixture was then placed in a fermentation box measuring approximately 30.5 × 10.5 × 6 cm^3. Samples were then sterilized in an autoclave at 121 °C, 15 psi for 60 min, and removed to left overnight before inoculation. Type 2 specimens were used for moisture content (MC) and dry bending (DB) tests.

The production medium was then inoculated with an amount of liquid inoculum. The samples were then incubated at room temperature for 12 days. After covering with the mycelium, the sample was compressed to a predetermined thickness using a hydraulic press (cool press) and held for 10 min. The compressed samples were then dried in a tray dryer 55–60 °C for about 20 h. The physical and mechanical properties of composite boards conform to Japanese Industry Standards for particle board, so the mycelium of *Ganoderma lucidum* can be used as a biological adhesive in composite board production.

Appels et al. (2019) showed that the density, morphology, tensile, flexural strength, and water absorption properties of mycelium-based composites can be modulated by different types of substrates (sawdust, straw, cotton) and fungal species (*Trametes multicolor* vs. *Pleurotus ostreatus*) and processing techniques (no pressing, cold pressing, or hot pressing). Both types of mycelium were grown for 14 days with beech sawdust and rapeseed straw of 1–3 cm (Gedizo trading BV, Netherlands) by CNC Exotic Mushrooms (Gennep, The Netherlands) and non-woven low-quality cotton fibers by Mogu. By adding bran (CNC Exotic Mushrooms) to rapeseed straw and beech sawdust, the final moisture content was 65–70%, while the final moisture content of low-quality cotton fibers was 55%. In all cases, autoclaved bags (Sac O_2, Belgium) with filter size XL were filled with 3 kg of the substrate, sterilized, and inoculated with *T. multicolor* or *P. ostreatus* (Mycelia, Belgium) spawns. A plastic thermoforming mold (34 × 34 × 4 cm, PETG) was filled with a pre-grown substrate. The substrate was distributed as evenly as possible by hand pressing the material and covered with perforated cellophane foil (0.35 μm, industry-standard PPI). The fungus was further grown at 25 °C for 14 days in the dark. To achieve uniform colonization on both sides, the plate was removed from the mold to increase the growth of the side that had previously been in contact with the mold and held in the opposite direction under the same conditions for an additional 10 days. Heat (150 °C) or cold (20 °C) pressing was performed for 20 min at a 30 kN pressuring force with in a CE-certified mechanical multi-plate press (Vigevano, Italy). The thermo-compressed material was cooled to room temperature, and the unpressed or cold-pressed material was dried at ambient conditions for

24–48 h. The fungal species affected the colonization rate and the thickness of the exposed mycelium called the fungal skin. The level of colonization, skin thickness, and substrate type determine the hardness and water-resistance of the material. Additionally, thermo-compression has been shown to shift properties from foam-like to cork- and wood-like by improving the uniformity, strength, and stiffness of the material. Taken together, these results demonstrate that changes in the properties of mycelium materials can be achieved by modifying the manufacturing process. This study highlighted the possibility of producing various mycelium-based composite materials.

Attias et al. (2019) investigated the effect of substrate composition on mycelial development and derivative characteristics. Three types of fungi were used in this experiment: *Colorius* sp., *Ganoderma* sp., and *Trametes* sp. Apple and vine crop pruning residues were crushed and sieved to a size of approximately 2 mm to obtain a substrate. The woodchips were mixed with 1% wheat flour, 3% wheat straw, and 62% distilled water, then placed in a 700 g bag and autoclaved at 100 °C for 1 h. Then, 3% inoculum was added to the substrate mixture under sterile conditions. The inoculated substrate was divided into two treatments (T1, T2). Sample T1 was transferred to the incubation vessel immediately after inoculation, and in T2 the inoculated substrate was incubated in a filtered bag for 7 days, then mixed again with the addition of 1% wheat flour and transferred to the growth vessel. Experiments included six replicates of each set of fungal substrates in each treatment, for a total of 84 samples. All samples were incubated for 14 days at 23 °C and 95% humidity. After incubation, the samples were dried at 60 °C for 48 h. During incubation, mycelial development on each sample was visually assessed for consistency of mycelium coverage and detection of infection. The mycelial colonization tendency was assessed by visual inspection during incubation. The Relationship between the visual, physical, and chemical properties of mycelium composites has been studied. The results showed clear discrepancies in the material properties of the various configurations tested. By altering these parameters, mycelium-based composites can be modified to accommodate inherently different effects.

Bruscato et al. (2019) developed a bioform to replace expanded polystyrene (EPS) using *Lentinus velutinus*, *Pleurotus albidus*, and *Pycnoporus sanguineus*. The strain was stored on an agar medium (MS) containing sawdust consisting of 2 wt% crushed sawdust (*Pinus* sp.), 2 wt% of crushed wheat bran, 2 wt% agar, and 0.2 wt% $CaCO_3$ dissolved in distilled water. The medium was sterilized in a Phoenix Luferco (Brazil) autoclave at 1 atm for 30 min. After cooling to ambient temperature (18 ± 2 °C), the medium was poured into Petri dishes and inoculated into 1.5 cm discs containing the mycelium of each culture, followed by incubation in an Oxylab® (Brazil) chamber at 28 °C. After a mycelial growth period (~10 days), the dishes were stored at 4 °C. The pre-inoculated medium used for the development of the fungal strain was constructed by adding wheat grains and calcium carbonate (1%) in a glass container and pre-sterilized for 180 min at 1 atm. Once the medium has cooled, the bottle is inoculated by placing about 50% of the agar medium containing the pre-grown mycelium on the surface of the medium in an appropriate glass container. Incubation was continued for 15 days at a temperature of 24 ± 2 °C.

The culture medium for mycelium development and biofoam production was prepared from the main culture medium containing 94 wt% of crushed *Pinus* spp. Sawdust, 5 wt% milled wheat bran, and 1 wt% $CaCO_3$ at 66% humidity. The medium was placed in a 10 × 6 cm (diameter × height) plastic mold and sterilized at 1 atm for 180 min. After cooling, the medium was inoculated by adding a pre-inoculum containing 5 wt% mycelium and incubating at 24 ± 2 °C until complete colonization of the medium. A biofoam was prepared so that the resulting material had the same thickness (25 mm) as that of commercially available polystyrene foam. The biofoam product was then dried at 80 °C for 24 h. Biofoam data classified this material as a sustainable replacement for EPS in some applications while reducing the environmental impact of sawdust and EPS at the same time.

Elsacker et al. (2019) described five different types of lignocellulosic reinforcing fibers (hemp (H), flax (F), flax waste (FW), softwood (S), and straw) and fiber processing (loose (L), chopped (C), dust, pre-compressed (P), and tow (T)) in combination with white-rot fungus, *Trametes versicolor*.

(a) Fiber preparation: The fibers to be cut are first soaked in water for 24 h, then rinsed thoroughly and mixed with fresh water for 10 min. The fibers were sieved with a 5 mm strainer, squeezed by hand, spread out on a tray, and dried at 30 °C for 24 h. The dried chopped fibers were then transferred to an autoclavable microbox measuring 185 × 185 × 78 mm (purchased from Sac O_2, Nevele, Belgium). Loose, tow, and dust fibers were untreated and immediately transferred to the microbox. The fibers were autoclaved at 121 °C for 20 min and the box was left to cool for 24 h.
(b) Mycelium growth: A mixture of 20 wt% fibers, 70 wt% sterile demineralized water and 10 wt% mycelium was placed in PVC molds (Fig. 7a). The mold was filled with several layers and each layer was pressed with a spoon to obtain a compact and dense sample (Fig. 7b), which was then covered with a transparent perforated cellophane foil. Three replicates were made for all groups. Three additional undried samples (HL, FWL, FL) were taken for visual inspection of the cross-sections after being cut in the middle with a knife. Samples were grown in rectangular transparent Petri dishes (120 × 120 × 17 mm) for daily visual monitoring of growth concurrently with the preparation of the composites in the mold.
(c) Incubation: Samples (Fig. 7b) were incubated in a microbox with a depth filtration system providing airflow at 28 °C for 8 days. After that, they were demolded in laminar flow and incubated in a microbox for another 8 days or more without a mold to achieve homogeneous colonization on the side in contact with the PVC mold (Fig. 7c). Samples PHL, PFL, PFWL were pre-compressed after 8 days. They were placed on a base plate fixed to a table and each sample was fitted with a lid as a surface barrier between the sample and screw clamps to distribute the compressive force to the top surface. Each specimen required two screw clamps. One for pressing and the other for holding removable mold parts closed from the side (Fig. 7d). These specimens were pressed to an initial height from 100 mm to 80 mm during the second growth phase for 8 days, then

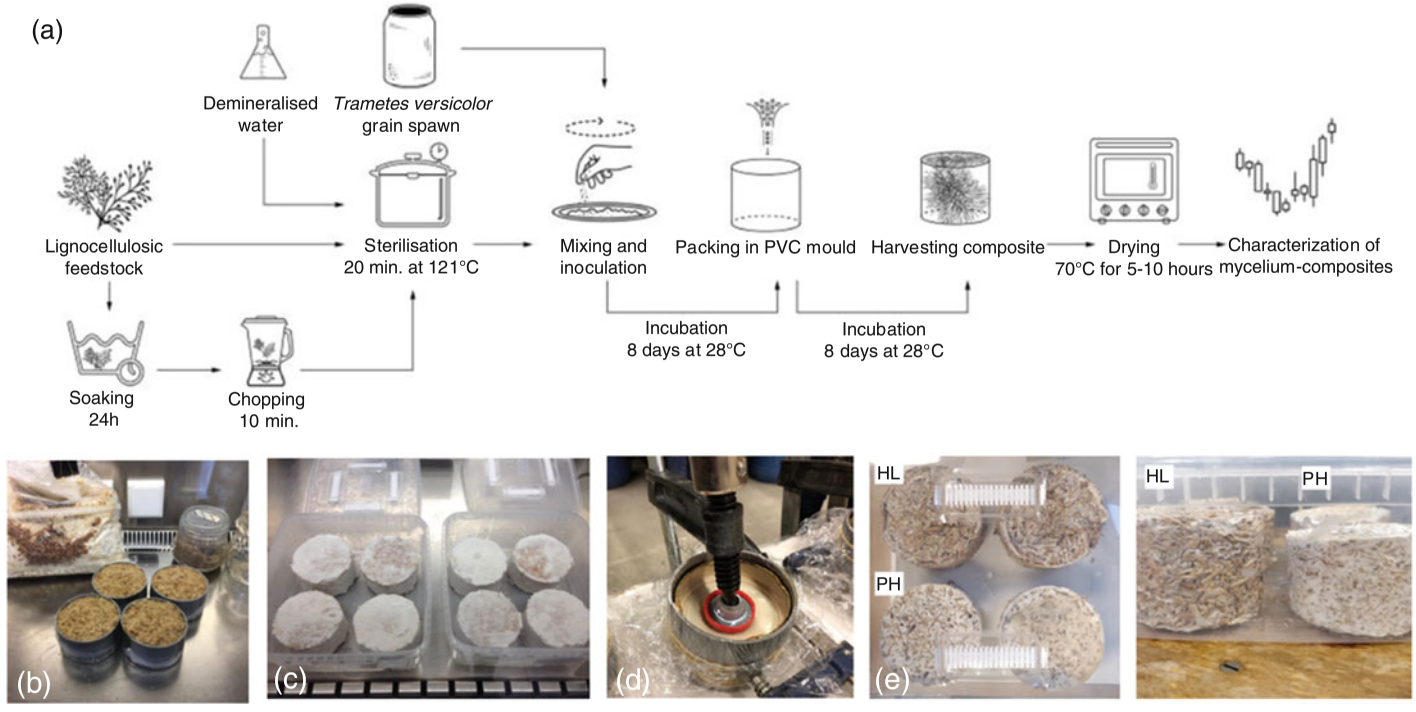


Fig. 7 The process of creating a mycelium-based composite. (**a**) Flowchart showing the method used to make the mycelium-based composite; (**b**) preparation of specimens in molds for compression tests in laminar flow; (**c**) fully grown specimens after 16 days; (**d**) pre-compressed samples PHL, PFL, PFWL; (**e–f**) Formless samples FWL (top) and PFWL (bottom) after 8 days at 28 °C, in a sterile microbox with filters where the second growth phase begins (Elsacker et al. 2019)

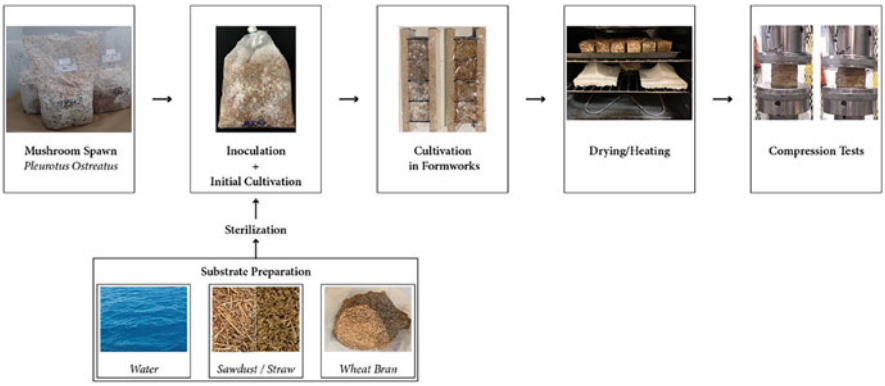


Fig. 8 Experiment workflow by Ghazvinian et al. (2019)

demolded in the laminar flow and cultured in a microbox for an additional 3 days without mold (Fig. 7d–f).

(d) Drying process: All samples were dried in a convection oven at 70 °C for 5–10 h until their weights stabilized.

According to the results of experiments, mycelium composite can meet thermal insulation requirements and can replace fossil-based composites. The methodology used to evaluate the suitability and selection of organic waste streams has proved effective in the production of mycelium-based materials.

Ghazvinian et al. (2019) published a materials study investigating how the types of substrates and additives used to grow mycelium affect the compressive strength of mycelium-based composites used as masonry units in architectural construction. To this end, we tested the compressive strength of samples grown with *Pleurotus ostreatus* (a gray oyster mushroom strain) on three different substrates (sawdust, wheat bran, and straw) with and without additives. The prepared substrates were first sterilized in an autoclave chamber, and then *Pleurotus ostreatus* strain was inoculated. Sterilization grew clean slates and prevented unwanted contamination. The inoculated substrate was placed in a growth chamber where appropriate environmental conditions (humidity and temperature) for growth were created. After the mycelium had been sufficiently incubated for 2 weeks, a sample of the cultured mycelium was transferred to a $5 \times 5 \times 5$ cm^3 plastic mold to initiate the second growth phase. Here, the growth of mycelium made the mixture denser in the form of plastic molds. At the end of the third day, the cube samples were removed from the molds and heated in an oven to kill the mycelium and stop the growing process. The obtained sample was a mycelium-based bio-composite. Finally, mycelium-based biocomposite samples were tested for compressive strength using a 22 kips Instron tester machine, with the complete process shown in Fig. 8.

Jones et al. (2019) conducted a study to determine the suitability of common agricultural by-products as growth media fungi. Growth was measured by quantification of ergosterol, a unique fungal product, in solid and liquid media. Liquid and

pulverized solid agricultural by-products (wheat straw, rice husk, sugarcane offal and molasses) were suspended (1 g/100 ml) in half-strength Carbon-free low Nitrogen Modified Melin-Norkrans (CNMMN) medium in a 250 ml glass bottle and heated at 121 °C for 20 min in an autoclave. Carbon- (glucose) free CNMMN was used to confirm that the mineral nutrition of the fungus was not a limiting factor for growth, and only the use of a carbon source (agricultural byproduct or reference nutrient) was tested fungus. Cultures were cut into inoculum plates (Φ7 mm) using the blunt base of a sterile 1.0 ml pipette tip. A new pipette tip was used for each species. A single inoculum disk was suspended in each glass bottle. Each experiment was performed using four biological replicates in flakes containing one inoculum and incubated at 25 °C in the dark for 7 days on a Paton Scientific OP3422 orbital shaker at 100 rpm. The contents of the glass jars were prepared for growth quantification by vacuum filtration using first-grade chromatography paper and filtration device (EMD Millipore XX104710) and then freeze-drying. Dried frozen samples were weighed on an analytical balance (OHAUS Explorer) and used in whole or in part as aliquots for analysis. The results showed that fungi grow less than commercial wheat grains on agricultural byproducts of rice husks, sugarcane bagasse, and wheat straw. However, liquid molasses, a byproduct of agriculture, has contributed to very high biomass production that surpasses widely used laboratory nutritional malt extracts. The use of these substances provides an inexpensive, renewable, easily isolated, and abundant alternative to the problematic crustacean chitin. When widely implemented, high-value chitinous composites can be quickly converted from low-value agricultural byproducts on a large scale.

Pelletier (2019) tested a natural biopolymer composed of pure fungal mycelium grown at high temperatures of 30–35 °C in an environment with a high CO_2 content of 3–7%. Biopolymer foam originated from an organic substrate inoculated with flat porous fungi of the genus *Ganoderma* belonging to the phylum class of Basidiomycete, order of Polyporaceae and family Ganodermataceae. Mycelium, produced by the fungus was grown in specially designed growth chambers as detailed in (Bayer et al. 2015). The substrate used for inoculation and fungi cultivation consisted of 56.3% chopped corn straw, 27% kernels, 2.4% maltodextrin, 0.8% calcium sulfate, and 13.5% nutrient complex and mineral mixture, details of which are reported in (Bayer and McIntyre 2016). For this experiment, all nutrients were provided by the substrate. Alternatively, however, nutrients can be provided via liquid nutrients in hydroponics-type trophoblast cultures, which will enhance the use of reusable inert substrates (McIntyre et al. 2013). They report that these improvements will provide significant cost savings over current practices and will be investigated further in future studies. They also noted that this improvement could be achieved because the substrate only provides an initial support structure for the first cycle and then a cycle of support using a previously generated mycelium layer. The chamber is designed so that as the CO_2-producing fungus grows and breathes, oxygen is depleted in the upper space of the chamber, creating a CO_2 gradient towards the hollow orifice of the chamber, which is then released into the atmosphere. In this way, the mycelium is stimulated and trained to germinate into the voids and then germinates into the previously grown mycelium layers to find a more

abundant O_2 medium to fill the voids and provide a pure mycelium structure without a growth medium. This new biopolymer has become a suitable alternative to closed-cell foams and synthetic honeycombs for a wide range of applications, from flame-retardant bio-leather, textile-supported foams used in footwear and apparel, to scaffolds for medical-biological organisms and vegetarian meat. Studies have shown that this new class of pure mycelial foam is a sustainable and promising alternative to natural bio-based fibers for acoustic shielding, and an alternative to conventional sound-absorbing, mostly made from petroleum-based adhesives and synthetic fibers.

Sun et al. (2019) conducted two series of experiments: In the first series of experiments, pure white basidiomycete mycelium was grown on softwood (a mixture of spruce, pine, and fir (SPF) particle boards) particles to obtain mycelium-modified wood and then hybridized. Various levels of cellulose nanofibrils (CNF) are used as binders. Another series of experiments was performed on unmodified wood particles mixed with CNF and pure mycelial tissue. To investigate the binding mechanism of the wood mycelium particle system, two different types of wood and mycelium mixtures were used as basic combinations for the production of hybrid composite materials. The first form consists of the direct mixing of wood particles with pure mycelium. The second uses wood mycelium particles obtained by growing mycelium on wood grains. These two main mixtures were combined with CNF in different ratios. The grain size of the wood material used in both groups was 1.40–3.50 mm and went through a vibrating sieve. The target density was 0.6 g cm^{-3}, and the thickness was 9.4 mm, with stops controlled by a thermal press. Composites made from mycelium-modified wood and CNF showed improved physical and mechanical properties compared to composites made from a physical blend of wood, mycelium and CNF. The mycelium modification had a significant effect on reducing the water absorption and expansion of the hybrid composites in thickness, and CNF increased the tensile and elastic moduli by 2.5% under the optimal conditions.

Wimmers et al. (2019) discussed the production feasibility of wood-based insulation panels and whether bio-based composites can become viable alternatives to traditional standard foam insulation panels and more expensive fibreboards (mostly available in the European market) using fungi as the binding agent. Nine fungi species that can biodegrade wood were chosen: *Fomitopsis pinicola*, *Gloeophyllum sepiarium*, *Laetiporus sulphureus*, *Phaeolus schweinitzii*, *Piptoporus betulinus*, *Pleurotus ostreatus*, *Polyporus arcularius*, *Trametes pubescens*, *Trametes suaveolens*, *Trichaptum abietinum*. Another fungal species, *Pleurotus ostreatus* was grown in an oyster mushroom growing kit. Brown rot fungi mainly break down cellulose and hemicellulose, while fungi with white-rot break down all major compounds in wood, including hemicellulose, cellulose, and lignin. Cultures were grown on 2% malt extract agar (20 g malt extract, 1 g yeast extract, 15 g agar, and 1 l water) at 21 °C in an incubator, then transferred to be stored at 4 °C in a refrigerator for further use. Five different wood species were tested as substrates to grow fungal cultures: hardwoods included birch (*Betula papyrifera*) and aspen (*Populus tremuloides*), and softwoods included lodgepole pine (*Pinus contorta*),

subalpine fir (*Abies lasiocarpa*), and white spruce (*Picea glauca*). The wood is native to the Pacific Northwest region in North America and had been oven-dried without any chemical treatments. Experiments were conducted to determine which wood fiber combinations of selected northern tree species, wood-rotting fungus, and growing conditions would be best for making panels. The results showed that under certain optimal growing conditions, *Polyporus arcularius* and *Trametes suaveolens* from birch shavings gave the best combination. In particular, the results of initial physical verification tests, such as thermal conductivity, showed that these panels have comparable performances to traditional insulation materials.

Antinori et al. (2020) showed that *Ganoderma lucidum* was maintained in 100 mm Petri dishes with potato dextrose broth (PDB) as the culture medium, transferring the cultures to fresh medium every 30 days. Mycelium fragments grown for 20 days were inoculated into 100 mm diameter Petri dishes containing 30 ml of either 24 g/l PDB, 24 g/l PDB with 30 g/l D-glucose, or 24 g/l PDB with 2 g/l alkali lignin. *Ganoderma lucidum* was grown in PDB with varying concentrations of D-glucose and alkali lignin until the optimal concentration had been chosen. The tested D-glucose concentrations were always below the aqueous limit of glucose (909 g/l) in water, but significantly exceeded the concentrations used for standard cell culture media with high glucose content (typically around 5 g/l) and ranged from 5 to 50 g/l. Growth was good at all concentrations tested and an intermediate one was selected. Alkali lignin was tested at concentrations between 0.2 and 10.0 g/l as no water solubility limits were reported. The growth of *G. lucidum* growth was significantly slowed down at concentrations >5 g/l, so the intermediate concentration was selected. Water was the solvent used for each medium and all media were autoclaved prior to use. Mycelium was cultured in a climatic chamber (Memmert, HPP 260) at 27 °C and 78% moisture in the dark. Five mycelia were harvested for each condition every 7 days for a total of 28 days and the substrate was washed with a spatula. The mycelia were then dried in an oven at 50 °C for 15 h before further analysis. This study showed that even small changes in the composition of standard fungal growth medium (PDB) can cause significant differences in the morphological, chemical, and hydrodynamic properties of *Ganoderma lucidum* mycelium, and the growth rate of mycelium is also affected by the growth substrate. Mycelium material grown in D-glucose enriched PDB is more porous, thicker, and more prone to water adsorption associated with mycelial material grown on lignin-poor PDBs. The latter, on the other hand, grow very rapidly following a concentric pattern and are denser and less hydrophilic. However, all mycelium is hydrophobic with a contact angle with water of about 120°. Mycelium has interesting properties and can be tuned on the nanoscale, making it suitable for many applications. The methods used in this work are adaptable to a variety of strains and conditions to allow the selection of the optimal mycelium-based materials.

Joshi et al. (2020) studied the kinetics and morphology of *Pleurotus ostreatus* mycelium grown on various agricultural wastes such as wheat bran, sugarcane bagasse, sawdust, and mixtures of these substrates. The culture substrate (potato dextrose agar, PDA) for the primary inoculation was prepared using 2.5% potato dextrose (PD) broth, 1.5% agar, and 1% cellulose, and autoclaved at 121 °C for

20 min. The warm (50–60 °C) lukewarm liquid was poured into sterile Petri dishes until 2/3 of the dish is full. The plates were cooled inside a biosafety cabinet for about 15 min until the medium solidified. A healthy, clean stem of *P. ostreatus* was taken and divided vertically into two parts. Using sterile forceps, a bunch of pure mycelium was harvested and placed in the center of a PDA − cellulose agar plate. The inoculated plates were incubated at 25 °C and 60% humidity for 10–12 days. Agricultural waste was used as a nutrient medium for the substrate along with 1.5% agar for mycelium growth using the same inoculation protocol as the PDA. The agar−substrate solution was prepared by adding 1.5% agar and 1 g of each of these substrate powders to 20 ml of Milli-Q water and mixing. The agar−substrate solution was then autoclaved, poured into Petri dishes and inoculated with fresh *P. ostreatus* mycelium. The inoculated Petri dishes were stored for 10–12 days in an incubator at 25 °C and 60% humidity. The cut and dried bio-substrates were separately placed in a 250 ml beaker. Milli-Q water was added until the substrate was completely wet. These substrates were then autoclaved at 121 °C for 20 min, after which, fully grown mycelium in Petri dishes was inoculated into beakers. These inoculated beakers were stored in the dark at 25 °C for approximately 2 weeks until the mycelium completely colonized the substrate. The mycelium grown with the substrate was then placed in a wooden block mold (8 × 5.5 × 3 cm) and tightly pressed into a mass of 10 kg for 7 days. Final volume reduced by about 50%. Then, the wooden block mold was removed, and the block in which the mycelium was grown was stored in a sterile box for 1 week for surface mycelium growth. After that, the bio-block of the mycelium was baked in an oven at 90 °C for 12 h, and the resulting bioblock showed excellent thermal stability, hydrophobicity, and mechanical strength. The compressive strength of this bio-block is about 6.0–7.5 N/mm^2, which is 5–6 times higher than that of polystyrene packaging materials commonly used. These properties of bio-blocks allow it to replace non-biodegradable materials commonly used for packaging, wall panels, and filtration of toxic waste.

Liu et al. (2020) prepared a kind of novel sustainable mycelium/cotton stem composite in the laboratory by growing *Ganoderma lucidum* on cotton stems, storing them in block mold, and then thermo-compressing them. To improve the mechanical properties, the composites were soaked in water prior to thermo-pressing at different absorption rates (20, 30, 40, and 50%). The results showed that as the water absorption increased, the mechanical properties of the composite material increased first after reaching the maximum absorption of 30 wt% after the cotton stem saturation point. Therefore, the water contributed to a good interface between the mycelium and the cotton stem particles. However, at water uptake >30 wt%, no further improvement occurred due to excessive water in the cell lumen and not the cell wall.

Ridzqo et al. (2020) studied the production of novel bamboo fiber composite boards by a biological binding mechanism using the fungal mycelium *Gigantochloa apus*. There are three types of bamboo stems: long fibers, short fibers, and powder. Water and some extra nutrition were added to the bamboo fibers and sterilized together. These substrates were then inoculated with *Ganoderma lucidum* mycelium seeds. As the mycelium grows, the fibers were bound together. The results showed

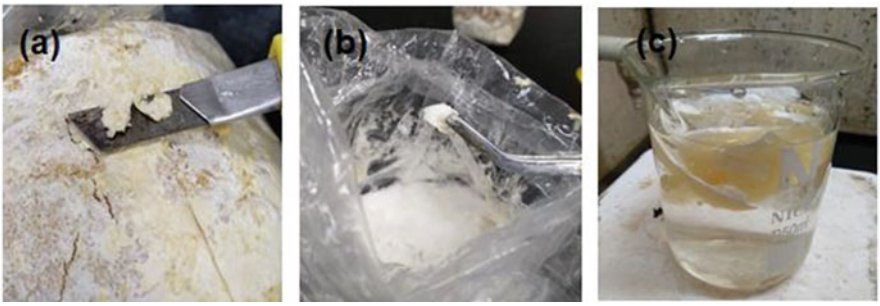


Fig. 9 Mycelial tissue of (**a**) cellulose, (**b**) starch, and (**c**) glucose substrates (Shakir et al. 2020)

that these boards could potentially be used in the interior of buildings, especially in high-rise buildings where there is a high demand for lightweight insulation and partition boards. It is also expected to displace building components made from unsustainable materials and technologies.

Shakir et al. (2020) investigated the mycelium of *Pleurotus ostreatus* as a novel, rewarding and inexpensive biomatrix in biocomposite board production. The mycelial tissues used in this study were inoculated and extracted from different types of substrates such as cellulose, starch, and glucose. For the cellulose and starch substrates, rubberwood sawdust (RWS) and mycelium blocks made from corn kernels were used, respectively. Both substrates were sterilized and inoculated with mycelium. For the glucose substrate, the mycelium was inoculated using sterile 10% glucose agar in a Petri dish. The mycelium grown for each type of substrates was cut and carefully removed after 14 days for analysis. For the glucose substrate, the mycelium was extracted through continuous boiling and filtration using the dry weight method to obtain pure mycelium. In addition, the inoculation time was increased from 14 days to 45 days to prepare a mycelium tissue on the starch substrate. The untreated mycelium tissue obtained from cellulose, starch, and glucose substrates is shown in Fig. 9. The mushroom substrate was obtained from rubberwood sawdust (RWS) and ground. An appropriate amount of RWS was placed in a sterile plastic mold measuring 21 × 15 × 0.6 cm with a target density of 0.80 g/cm^3. RWS were incubated for 45 days in a chamber at room temperature (~25 °C) with 80% humidity. This allowed the mycelium to inoculate and bind the fibers together. The mycelium species *Pleurotus ostreatus* was used in this study. The fully grown biocomposite mycelium board was removed from the mold, and the fabrication process continued using a hot press machine at 130 °C and 5 MPa for 20 and 40 min. The production of biocomposites using mycelium as a binder can be improved by using optimal particle sizes for the inoculation and shaping process.

Silverman et al. (2020) conducted a study on solid waste reduction, resource depletion and material toxicity in the footwear industry. Mycelium composites have been developed using edible mushroom species along with other natural materials. In the 4 × 2 experiment, four types of mushrooms (reishi, oysters, king oysters, and yellow oysters) and two levels of fabric (with or without a natural fabric mat) were

tested. Fabrics, feathers, and growth vessels were sterilized at 80–90 °C in a Stabil-Therm® constant temperature oven (Blue M Electric Co, Blue Island, IL), and laboratory materials were wiped with an alcohol solution prior to contact with the mycelium mixture. Ingredients per 100 g of sawdust block mixture included 1.2 g of wheat flour, 0.3 g of chicken feathers, 2.8 g of psyllium hulls, 50 ml of water, and 1 g of textile for a sample containing a textile mat. These ingredients were added to the sawdust spawn block and thoroughly mixed. This mixture was then added on top of the wet fabric mat layer placed in the mold. A positive, significant linear relationship was found between density and compressive strength, with higher density resulting in improved compressive strength. The compressive strength of mycelium composites, especially those made from oyster mycelium, has paved the way for renewable and biodegradable footwear materials.

Tacer Caba et al. (2020) evaluated the properties of fungal composites obtained from novel fungi, including *Agaricus bisporus* and *Trichoderma asperellum*, grown on oat hulls and rapeseed cakes after oil pressing. The four selected fungal strains were grown in 100 ml of liquid malt extract for 1 week at 21 °C in a 250 ml Erlenmeyer flask, sealed with a cellulose stopper and an aluminum lid, and then sealed with parafilm. Fungal inoculum (1 cm^3) was mixed with nutrient substrates (rapeseed (RS) and oat husks (OH)) and grown in Petri dishes at 21 °C. After 2 weeks, these samples were inspected visually, mixed, and transferred to 4-well plates (1 cm^3/well) (Thermo Scientific, Denmark) to grow for an additional week. Then, the resulting mycelium complex was dried at 40 °C for 48 h to reduce the moisture content to 5.8–8%. The resulting eight mycelium composites were cleaved according to the fungal strain and growth substrate. The results showed that the mycelium composite was hydrophobic and robust, especially when grown on rapeseed cakes. *A. bisporus* grown in rapeseed cake increased its moisture content and then decreased its stiffness. The moisture resistance of this novel mycelium composite contributes to the creation of new environmentally friendly materials.

5 Epilogue

The production of fungal bioresins is still at its very initial stage where most of the production activities took place in research labs. Factors such as fungal species, substrate contents, and environmental controls have significant impacts on the result and qualities of the end product. Pre- and post-processing are very important to the production, too. Many investigations have been performed with various combinations of all these factors, however, mass production of mycelium-based bioresins is still to be studied and properly expended from existing production scales. Overall, mycelium-based bioresins are very promising green materials with lower costs and it is expected to be one of the future materials to be widely used in the industry.

Acknowledgments This work was supported by Award #1909699, the National Science Foundation (NSF), USA.

References

Agustina W, Aditiawati P, Kusumah SS, Dungani R (2019) Physical and mechanical properties of composite boards from the mixture of palm sugar fiber and cassava bagasse using mycelium of Ganoderma lucidum as a biological adhesive. IOP Conf Ser Earth Environ Sci 374:012012

Akila RM (2014) Fermentative production of fungal chitosan, a versatile biopolymer (perspectives and its applications). Adv Appl Sci Res 5(4):157–170

Anderson AJ, Wynn JP (2001) Microbial polyhydroxy alkanoates, polysaccharides and lipids. In: Ratledge C, Kristiansen B (eds) Basic biotechnology. Cambridge University Press, Cambridge, pp 325–345

Antinori ME et al (2020) Fine-tuning of physicochemical properties and growth dynamics of mycelium-based materials. ACS Appl Biol Mater 3:1044–1051

Appels FVW et al (2019) Fabrication factors influencing mechanical, moisture- and water-related properties of mycelium-based composites. Mater Des 161:64–71

Arcidiacono S, Kaplan DL (1992) Molecular weight distribution of chitosan isolated from Mucor rouxii under different culture and processing conditions. Biotechnol Bioeng 39(3):281–286

Arifin YH, Yusuf Y (2013) Mycelium fibers as new resource for environmental sustainability. Proc Eng 53:504–508

Attias N et al (2017) Developing novel applications of mycelium based bio-composite materials for design and architecture. Zagreb, Conference: building with bio-based materials: best practice and performance specification

Attias N et al (2019) Implementing bio-design tools to develop mycelium-based products. Des J 22(1):1647–1657

Bajwa DS et al (2017) Enhancement of termite (Reticulitermes flavipes L.) resistance in mycelium reinforced biofiber-composites. Ind Crop Prod 107:420–426

Bayer E (2010) Are mushrooms the new plastic? [Sound Recording] (Ted.com)

Bayer E, McIntyre G (2016) Method of growing mycological materials. U.S. Patent No. 9394512

Bayer E, McIntyre G, Swersy BL (2015) Panel comprising composite of discrete particles and network of interconnected mycelia cells. U.S., Patent No. 8999687

Bellettini MB et al (2019) Factors affecting mushroom Pleurotus spp. Saudi J Biol Sci 26(4): 633–646

Bruscato C, Malvessi E, Brandalise RN, Camassola M (2019) High performance of macrofungi in the production of mycelium-based biofoams using sawdust—sustainable technology for waste reduction. J Clean Prod 234:225–232

Butu A, Rodino S, Miu B, Butu M (2020) Mycelium-based materials for the ecodesign of bioeconomy. Dig J Nanomater Biostruct 15(4):1129–1140

Carlile MJ, Watkinson CS (1995) The fungi. Academic, London

Catto AL et al (2014) Growth of white rot fungi in composites produced from urban plastic waste and wood. Macromol Symp 344:33–38

Cerimi K et al (2019) Fungi as source for new bio-based materials: a patent review. Fungal Biol Biotechnol 6:17

Chatterjee S, Adhya M, Guha AK, Chatterjee B (2005) Chitosan from Mucor rouxii: production and physico-chemical characterization. Process Biochem 40(1):395–400

Cheung PCK (1996) Dietary fiber content and composition of some cultivated edible mushroom fruiting bodies and mycelia. J Agric Food Chem 44(2):468–471

Cohen GH, Johnstone DB (1964) Extracellular polysaccharides of Azotobacter Vinelandii. J Bacteriol 88(2):329–338

El-Gharabawy HM et al (2016) Analysis of wood decay and ligninolysis in Polyporales from the Nile Delta region of Egypt. Mycosphere 7(4):392–404

Elsacker E et al (2019) Mechanical, physical and chemical characterisation of mycelium-based composites with different types of lignocellulosic substrates. PLoS One 14(7):e0213954. https://doi.org/10.1371/journal.pone.0213954

Elsacker E et al (2020) A comprehensive framework for the production of mycelium-based lignocellulosic composites. Sci Total Environ 725:138431
Ghazvinian A et al (2019) Mycelium-based bio-composites for architecture: assessing the effects of cultivation factors on compressive strength. Architecture in the Age of the 4th Industrial Revolution 2:505–514
Girometta C, Girometta C, Picco A, Baiguera R, Dondi D, Babbini S, Cartabia M et al (2019) Physico-mechanical and thermodynamic properties of mycelium-based biocomposites: a review. Sustainability 11(1):281
Golak-Siwulska I et al (2018) Bioactive compounds and medicinal properties of Oyster mushrooms (Pleurotus sp.). Folia Hortic 30:191–201
Haneef M et al (2017) Advanced materials from fungal mycelium: fabrication and tuning of physical properties. Sci Rep 7:41292
Holt GA et al (2012a) Termite resistance of biocased composition boards made from cotton byproducts and guayule bagasse. Ind Crop Prod 36(1):508–512
Holt GA et al (2012b) Fungal mycelium and cotton plant materials in the manufacture of biodegradable molded packaging material: evaluation study of select blends of cotton byproducts. J Biobaased Mater Bioenergy 6:431–439
Hu K-J, Yeung K-W, Ho KP, Hu K (1999) Rapid extraction of high-quality chitosan from mycelia of Absidia Glauca. J Food Biochem 23(2):187–196
Islam M et al (2017) Morhpology and mechanics of fungal mycelium. Sci Rep 7:13070-1-12
Islam MR et al (2018) Mechanical behavior of mycelium-based particulate composites. J Mater Sci 53:16371–16382
Jiang L, Walczyk D, McIntyre G, Chan WK (2016) Cost modeling and optimization of a manufacturing system for mycelium-based biocomposite parts. J Manuf Syst 41:8–20
Jiang L, Walczyk D, McIntyre G (2017a) A new approach to manufacturing biocomposite sandwich structures: investigation of preform shell behavior. J Manuf Sci Eng 139:021014-1-11
Jiang L et al (2017b) Manufacturing of biocomposite sandwich structures usingmycelium-bound cores and preforms. J Manuf Process 28:50–59
Jiang L, Walczyk D, Li B (2018) Modeling of glue penetration into natural fiber reinforcements by roller infusion. J Manuf Sci Eng 140:041006-1-11
Jiang L et al (2019) Bioresin infused then cured mycelium-based sandwich-structure biocomposites: resin transfer molding (RTM) process, flexural properties, and simulation. J Clean Prod 207:123–135
Jones M et al (2017a) Thermal degradation and fire reaction properties of mycelium composites. Xi'an, 21st International Conference on Composite Materials
Jones M et al (2017b) Mycelium composites: a review of engineering characteristics and growth kinetics. J Bionanosci 11(4):241–257
Jones M et al (2018a) Waste-derived low-cost mycelium composite construction materials with improved fire safety. Fire Mater 42:816–825
Jones M et al (2018b) Thermal degradation and fire properties of fungal mycelium and mycelium—biomass composite materials. Sci Rep 8:17583
Jones MP et al (2019) Agricultural by-product suitability for the production of chitinous composites and nanofibers utilising Trametes versicolor and Polyporus brumalis mycelial growth. Process Biochem 80:95–102
Josephine RM (2015) A review on oyster mushroom (Pleurotus spp). Int J Curr Res 7(1): 11225–11227
Joshi K, Mecher MK, Poluri KM (2020) Fabrication and characterization of bioblocks from agricultural waste using fungal mycelium for renewable and sustainable applications. ASC Appl Bio Mater 3:1884–1892
Kang KS, Cottrell IW (2012) Polysaccharides. In: Perlman D, Peppler HJ (eds) Microbial technology, microbial processes, vol 1. Elsevier Science, Amsterdam, p 420

Kaplan DL (1998) Chitin and chitosan: the chemistry and technology of their use as structure materials. In: Hudson SM, Smith C (eds) Biopolymers from renewable sources. Springer, Berlin, pp 96–118
Karana E, Blauwhoff D, Hultink E-J, Camere S (2018) When the material grows: a case study on designing (with) mycelium-based materials. Int J Des 12(2):119–136
Knorr D, Beaumont MD, Pandya Y (1989) Potential of acid soluble and water soluble chitosan in biotechnology. In: SkjakBrack G, Anthonsen T, Stanford P (eds) Chitin and chitosan. Elsevier Applied Science, London, pp 101–118
Kristiansen B, Linden J, Mattey M (1999) Citric acid biotechnology, 1st edn. Taylor & Frances, London
Lelivelt RJJ (2015) The mechanical possibilities of mycelium materials. Eindhoven University of Technology, Eindhoven
Lelivelt R, Lindner G, Teuffel P, Lamers H (2015) The production process and compressive strength of mycelium-based materials. First International Conference on Bio-based Building Materials, Clermont-Ferrand
Liu S-R, Zhang W-R, Kuang Y-B (2018) Production of stalk spawn of an edible mushroom (Pleurotus ostreatus) in liquid culture as a suitable substitute for stick spawn in mushroom cultivation. Sci Hortic 240:572–577
Liu R et al (2020) Improvement of mechanical properties of mycelium/cotton stalk composites by water immersion. Composite Interfaces 27(10):953–966
McClements DJ (2006) Non-covalent interactions between proteins and polysaccharides. Biotechnol Adv 24(6):621–625
McIntyre G, Bayer E, Palazzolo A (2013) Method of growing mycological biomaterials. US, Patent No. US20130263500A1
McNeely WH, Kang KH (2012) Xanthan and some other biosynthetic gums. In: Whistler RL (ed) Industrial gums—polysaccharides and their derivatives. Elsevier Science, Amsterdam, p 476
Montalti M (2014) Mycelium in design [Interview] (29 April 2014)
Nicole M, Benhamou N (1991) Physiol. Mol Plant Pathol 29:415–431
Nicole M et al (1995) Wood degradation by Phellinus noxius: ultrastructure and cytochemistry. Can J Microbiol 41:253–265
Papagianni M (2016) Microbial Bioprocesses. In: Pandey A, Larroche C, Sanroman MA, Du G (eds) Current developments in biotechnology and bioengineering—bioprocesses, bioreactors and controls. Elsevier Science, Amsterdam, p 65
Pelletier MG et al (2013) An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates. Ind Crop Prod 51:480–485
Pelletier MG et al (2017) An evaluation study of pressure-compressed acoustic absorbers grown on agricultural by-products. Ind Crop Prod 95:342–347
Pelletier MG et al (2019) Acoustic evaluation of mycological biopolymer, an all-natural closed cell. Ind Crops Prod 139:111533
Rane DK, Hoover GD (1993) Production of chitosan by fungi. Food Microbiol 7(1):11–33
Ridzqo IF, Susanto D, Panjaitan TH, Putra N (2020) Sustainable material: development experiment of bamboo composite through biologically binding mechanism. IOP Conf Ser Mater Sci Eng 713:012010
Rogovin SP, Anderson RF, Cadmus MC (1961) Production of polysaccharide with Xanthomonas campestris. J Biochem Microbiol Technol Eng 3(1):51–63
Sbrana C, Avio L, Giovannetti M (1995) The occurrence of calcofluor and lectin binding polysaccharides in the outer wall of AM fungal spores. Mycol Res 99:1249–1252
Shakir MA et al (2020) Preparation and characterization of mycelium as a bio-matrix in fabrication of bio-composite. Journal of Advanced Research in Fluid Mechanics and Thermal Sciences 65(2):253–263

Shao G-B, Yang P, Jiang W-X (2016) Research and preparation of mycelium-soybean straw composite materials. s.l., 2nd Annual International Conference on Advanced Material Engineering (AME 2016)
Silverman J, Cao H, Cobb K (2020) Development of mushroom mycelium composites for footwear products. Cloth Text Res J 38(2):119–133
Sun W et al (2019) Fully bio-based hybrid composites made of wood, fungal mycelium and cellulose Nanofibrils. Sci Rep 9:3766
Synowiecki J, Al-Khateeb NA (2003) Production, properties, and some new applications of chitin and its derivatives. Crit Rev Food Sci Nutr 43(2):145–171
Tacer-Caba Z, Varis JJ, Lankinen P, Mikkonen KS (2020) Comparison of novel fungal mycelia strains and sustainable growth substrates to produce humidity-resistant biocomposites. Mater Des 192:108728
Tan SC, Tan TK, Wong SM, Khor E (1996) The chitosan yield of zygomycetes at their optimum harvesting time. Carbohydr Polym 30:239–242
Teixeira JL et al (2018) Production and mechanical evaluation of biodegradable composites by white rot fungi. Ciênc Agrotecnol 42(6):676–684
Tudryn GJ et al (2018) Processing and morphology impacts on mechanical properties of fungal based biopolymer composites. J Polym Environ 26:1473–1483
van der Horst C (2014) Industrial scale mycelium cultivation [interview] (30 August 2014)
White SA, Farina PR, Fulton I (1979) Production and isolation of chitosan from Mucor rouxii. Appl Environ Microbiol 38(2):323–328
Wikipedia (2020) Hypha [online]. Available at: https://en.wikipedia.org/wiki/Hypha. Accessed 8 June 2021
Wimmers G et al (2019) Fundamental studies for designing insulation panels from wood shavings and filamentous fungi. Bioresources 14(3):5506–5520
Wösten H (2014) Alternate mycelium applications [Interview] (1 May 2014)
Wösten GD (2018) Mushroom cultivation in the circular economy. Appl Microbiol Biotechnol 102(18):7795–7803
Wösten HAB, Krijgsheld P, Montalti M, Läkk H (2018) Growing fungi structures in space. s.l.: Utrecht University, Officina Corpuscoli, ESA ACT
Wu T, Zivanovic S, Draughon FA, Sams CE (2004) Chitin and chitosan—value added products from mushroom waste. J Agric Food Chem 52(26):7905–7910
Xing Y et al (2018) Growing and testing mycelium bricks as building insulation materials. IOP Conf Ser Earth Environ Sci 121:022032
Yadav JS, Tripathi JP (1991) Optimization of cultivation and nutrition conditions and substrate pretreatment for solid-substrate fermentation of wheat straw by Coriolus versicolor. Folia Microbiol 36(3):294–301
Yang Z et al (2017) Physical and mechanical properties of fungal mycelium-based biofoam. J Mater Civ Eng 29(7):04017030
Yildirim NC, Turkoglu S, Yildirim N, Ince OK (2012) Antioxidant properties of wild edible mushroom Pleurotus. Dig J Nanomater Biostruct 7(4):1647–1654
Zhang Z et al (2017) Physical and mechanical properties of fungal mycelium-based biofoam. J Mater Civ Eng 29(7):04017030-1-9
Ziegler AR et al (2016) Evaluation of Physico-mechanical properties of mycelium reinforced green biocomposites made from cellulosic fibers. Appl Eng Agric 32(6):931–938

Laccase Mediated Green Composite Synthesis: A Name Synonymous with Each Other

Komal Agrawal, Shiva Vaishnavi, and Pradeep Verma

Abstract Laccase was identified very early but the potential of the enzyme has cached the eyes of the researchers globally for two decades. The multifarious applications of laccase have enabled its application in various industrial and environmental sectors. The enzyme has been used for the delignification of lignocellulosic biomass, paper, and pulp industries. The manufacture of fibreboard via the chemical treatment releases formaldehyde and pollutes the environment thereby harming flora, fauna, and humans residing in the nearby areas. Thus, the heed for developing non-polluting technologies gained attention amongst the scientific community and laccase was one of the most apt alternatives for the synthesis of the fibreboard via biological treatment methods. As biological treatment methods are used the synthesis process is eco-friendly, non-polluting, and sustainable as well. Thus, the chapter would elaborate the structure of laccase, the general mode of action of laccase, its role in the synthesis of composite and its mechanism of action on plant fiber. Further to gain better insight other reported applications of laccases are also discussed along with its limitations and future prospect.

Keywords Laccase · Fibreboard · Lignocellulosic biomass · Green synthesis · Non-polluting

1 Introduction

Around 2.45 billion years ago, oxygen (O_2) concentration in the biosphere increased which gradually oxidized water-soluble iron (Fe) II to water-insoluble Fe III. Due to Fe III's insolubility, iron was not readily available to the living systems for their metabolic processes. Under this evolutionary pressure, living systems such as aerobic organisms were forced to find naturally available iron-like metals with high redox potentials. As a response, they started utilizing copper (Cu II/Cu I) and

K. Agrawal · S. Vaishnavi · P. Verma (✉)
Bioprocess and Bioenergy Laboratory, Department of Microbiology, Central University of Rajasthan, Kishangarh, Ajmer, Rajasthan, India

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_14

manganese (Mn III/Mn II) which had a similar function as iron (Fe II/Fe III) (Andrews et al. 2003). Copper-containing proteins are mostly are extracellular (Crichton and Pierre 2001). They help with O_2 transport and activation, as well as electron transfer during redox reactions. These proteins are multicopper oxidases (MCOs) and can oxidize huge range of substrates with help of O_2 as an electron acceptor and function as electron transfer proteins (Janusz et al. 2020). One such interesting MCO is laccase. Laccase was first described by Yoshida (1883) that he found in exudates of *Rhus vernicifera*. Laccases along with peroxidases help in the development of plant cell walls. The presence of high levels of laccase-like MCO and its expression in vascular tissues of *Liriodendron tulipifera* indicated the requirement for the uptake of high-efficiency iron pumps in lignified tissues (Hoopes and Dean 2004). Laccases found in Anarcardiaceae resin ducts are thought to aid in defense against herbivores as well as a bacterial and fungal invasion (Mayer and Staples 2002). Most of the high redox potentials laccases are from fungi havng biotechnological and industrial significance (Nunes and Kunamneni 2018). It has been detected in several fungal strains and its production is most efficient in white-rot fungi (Shraddha et al. 2011). Fungal laccases only need oxygen and produce water as a byproduct. Because of their requirements and broad substrate specificity, they are regarded as green catalysts with biotechnological applications, including direct bio-electrocatalysis. Laccase and laccase-mediator system (LMS) have its applications in delignification (Virk et al. 2012), biocomposites (Nasir et al. 2014), biobleaching of pulp (Boruah et al. 2019), removal of aromatic pollutants (Khambhaty et al. 2015), treatment of industrial wastewater (Viswanath et al. 2014), biofuel cells and biosensors (Le Goff et al. 2015; Ribeiro et al. 2014) and degradation of diclofenac (DCF) and chloramphenicol (CAP) by laccase in presence mediators (Nguyen et al. 2014). Laccase has sparked tremendous interest for prospective biotechnological applications due to its catalytic characteristics (Abdel-Hamid et al. 2013). Laccase TEMPO oxidation treatment has been used used on cotton fibers for grafting octadecylamine grafting that enhanced the hydrophobic nature of the fiber (Ding et al. 2016). Bertrand et al. (2002) found the primary catalytic function of laccase in the lignification process. In the following year, laccase was applied in bioremediation processes. Pozdnyakova et al. (2006), demonstrated that laccase was used as a degradation tool to degrade polycyclic aromatic hydrocarbons (PAHs). Thus, with more knowledge and research, laccase was utilized in various industries such as food processing, textile industries, and wine stabilization. Table 1 gives an outline of the research varied out with laccase since 2000 to present 2021.

Thus, the present chapter would discuss the general mechanism of action of laccase, the role of laccase in the synthesis of fibreboard, and the mechanism for the synthesis of biocomposite. Further other reported applications of laccases its limitation and the future prospect have also been elaborated.

Table 1 Timeline and application of laccase

Year	Timeline of laccase	References
2000	Nonphenolic lignin degradation by laccase/1-hydroxybenzotriazole system	Srebotnik and Hammel (2000)
2001	Decolourization of Remazol Brilliant Blue R	Soares et al. (2001)
2002	Crystal structure of laccase	Hakulinen et al. (2002)
2003	Biobleaching of kraft pulp and mediated oxidation of nonphenolic substrate	Arias et al. (2003)
2004	Decolorization of anthraquinone dye	Hou et al. (2004)
2005	Denim washing	Pazarlıoğlu et al. (2005)
2006	Dyes decolorization	Zhang et al. (2006)
2007	Paper pulp delignification	Camarero et al. (2007)
2009	Dyes degradation	Sanghi et al. (2009)
2010	Decolorization of azo dyes	Moya et al. (2010)
2011	Bioremediation of a mixture of pentachlorophenol, 2-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol	Gaitan et al. (2011)
2012	Laccase bio-cathodes	Gutierrez-Sanchez et al. (2012)
2013	Dye removal	Ashrafi et al. (2013)
2014	Laccase and LMS in organic compounds synthesis	Mogharabi and Faramarzi (2014)
2015	Gold nanoparticles synthesis	El-Batal et al. (2015)
2016	Laccase for fruit juice clarification	Lettera et al. (2016)
2017	Immobilized laccase for removal of carbamazepine	Naghdi et al. (2017)
2018	Bisphenol A removal	Barrios-Estrada et al. (2018)
2019	Delignification of agroresidues	Agrawal et al. (2019)
2020	Anthraquinone dye removal	Agrawal and Verma (2020a)
2021	Wastewater decolorization	Amari et al. (2021)

2 A Fascinating MCO-Laccase

Laccases (EC 1.10.3.2) bio-catalyze a electron (e^-) oxidation of substrates and then passes four (e^-) to the catalytic copper (Cu) atoms, that are oxidized without releasing partially reduced O_2 called reactive oxygen species (ROS) (Janusz et al. 2020; Mehra et al. 2018).

2.1 Structure of Laccase

The 3D structure of MCOs is mainly constructed of β-sheets and turns. They contain a 10–20 kDa sized cupredoxin- like domain. MCOs are mainly of 3 types—2-domain, 3-domain, and 6-domain enzymes. Laccase consist of Greek key β barrel topology and it is ~500 amino acid residues structured in three successive domains. The first domain consists of 150 amino acids, second domain from 150 to 300 amino acids, and the third domain from 300 to 500 amino acids. The presence of disulfide bonds in-between the domains I and II and between I and III stabilizes the structure of laccase (Bertrand et al. 2002; Plácido and Capareda 2015). The structure of laccase has been studied using crystallography, isolating plant and animal laccase as crystals had been difficult to obtain due to the unavailability of proper purification protocols. Despite their broad taxonomic distribution and variety of substrates, it has been demonstrated that Cu in laccases exists in four different Cu catalytic forms per protein unit. These four catalytic Cu atoms are type 1 Cu (T1 Cu) and tri-nuclear Cu clusters (T2 Cu, T3α Cu, and T3β Cu) at the T2/T3 site across all multicopper oxidases. These four Cu ions are divided into three types of structures: Type 1 (paramagnetic 'blue' Cu), Type 2 (paramagnetic "normal/non-blue" Cu) and, Type 3 (diamagnetic spin coupled Cu-Cu pair). The majority of the proteins are represented in Table 2.

2.2 General Mode of Action

Catalytic participation of laccase's in coupling reactions is dependent on C–C, C–N, and C–O molecule linkages. Laccase cleaves phenolic components in three ways: Cα–Cβ cleavage, Cα oxidation, and aryl–alkyl cleavage. In laccase-catalyzed oxidation, reaction the initial e^- acceptor is T1 Cu that is situated in the cavity near the enzyme surface. The reduction of T1 Cu is a rate-limiting step and the internal electron then moves from T1 to T2 to T3 Cu. Meanwhile, at T2 and T3 Cu sites, O_2 is reduced to H_2O. Laccase converts phenolic compounds to phenoxyl radicals, which are then polymerized by radical rearrangement or coupling. However, based on the stability of the phenoxyl radicals, redox reversibility with oxidation of a targeted substrate is observed. By acting as mediators, radical-based coupling/redox recycling of phenolic substrates broadens the spectrum of laccase substrates (Patel et al. 2019; Agrawal et al. 2018; Kunamneni et al. 2007).

Table 2 Sources and application of laccase in various industries

Sources	Applications	References
Myceliophthora thermophila	Conditioner for dough	Renzetti et al. (2010)
Pleurotus ostreatus	Polycyclic aromatic hydrocarbons degrdation	Pozdnyakova et al. (2006)
Trametes sp.	Development of bioactive hydrogel dressing	Rocasalbas et al. (2013)
Trametes versicolor	Biosensors	Ardhaoui et al. (2013)
Myrothecium verrucaria	Delignification	Agrawal et al. (2019)
Myrothecium verrucaria	Anthraquinone dye removal	Agrawal and Verma (2020b)
Stropharia sp.	Alizarin Cyanine Green removal	Agrawal and Verma (2019a)
Stropharia sp.	Column bioreactor for the removal of Anthraquinone violet R	Agrawal and Verma (2019b)
Myrothecium verrucaria	Hazardous wastes	Agrawal and Verma (2020c)
Basidiomycete strain PV 002	Decolorization of azo dyes	Verma and Madamwar (2005)
Pleurotus ostreatus and *Phanerochaete chrysosporium*	Dye decolorization	Verma and Madamwar (2002a)
Stropharia sp.	Depolymerization of lignocellulosic biomass	Agrawal and Verma (2020d)
Pleurotus ostreatus	Gold nanoparticles synthesis	El-Batal et al. (2015)
Pleurotus ostreatus	Biosensor	Leite et al. (2003)
Pleurotus ostreatus	Removal of Anthraquinone dye	Hou et al. (2004)

3 Laccase in the Synthesis of Biocomposite

The bio/wood composite is made by the use of two components the i.e., the wood fiber and the adhesive. In the case of synthetic adhesive, formaldehyde and phenol formaldehyde are generally used. However, due to its toxic and harsh effects, the shift has occurred towards the biological synthesis of bio/wood composites (González-García et al. 2011; Moubarik et al. 2010). Also, the Government of Korea stated that the emission level above 4.0 mg/m^2.h for the total volatile organic compound (TVOC) is prohibited (JIS A 1901, small chamber method) (ASTM-D6007-96 1996; Kim et al. 2007). The lignin component of the plants is the second most abundantly available polymer after cellulose. As lignin has structural similarity to the phenol-formaldehyde it has been regarded as a potential substitute for the already available synthetic adhesive (Zhou et al. 2011; Kumar et al. 2009). However, despite the two advantages i.e., high availability and a potential substitute for

synthetic adhesive it has restricted use as most (80–85%) lignin available are either burned or discarded (Vishtal and Kraslawski 2011; Mai et al. 2000; Pizzi 2003). Thus, the use of lignin can be a game-changer for the industry of biocomposites and as it is a renewable resource the fear of its scarcity in the future would not be an issue (Agrawal and Verma 2020d).

4 Mechanism of Action of Laccase on Plant Fiber

The fiber modification has been an integral part of the synthesis of biocomposite and various physical-chemical, methods have been applied and reported in literature e.g., alkaline, microwave, high temperature, and steam treatments (Verma et al. 2005, 2009, 2011; Verma and Mai 2010). However, due to high cost, energy requirement, and less environmental sustainability, the past decades have been diverted towards the biological and enzyme-mediated treatment of fibers. Laccase is ubiquitous and multi-dimensional protein and has been used for the removal of lignin (Agrawal et al. 2019; Agrawal and Verma 2020a). The biological treatment methods are milder, specific, and more sustainable and cause minimal/no damage to the biological structure of the fiber (Kunamneni et al. 2008). Laccase enzyme is large and cannot penetrate the cells of the fiber it only results in surface modification (van de Pas et al. 2011). It acts on phenolic polymers of lignin with the resulting in reduction of O_2 to H_2O (Witayakran and Ragauskas 2009). It is due to these properties that laccase is an intensively studied oxidoreductase having numerous applications and recently in biocomposite synthesis (Agrawal et al. 2019; Agrawal and Verma 2020a, e). Also, laccase-mediated oxidation of lignin, free radicals of phenol and polyphenols are formed. As these free radicals are highly reactive it results in depolymerization, co-polymerization, and grafting (Saastamoinen et al. 2012). Further, the structure of lignin exhibits similarity to phenol-formaldehyde and can thus be a potential adhesive for the synthesis of biocomposite (Zhou et al. 2011; Kumar et al. 2009). Despite the advantage of lignin, the major drawback is its transformation to insoluble lignin and thus requires additional cross-linking e.g., maleic anhydride (Syukri et al. 2021). The laccase mediated treatment also has numerous advantages such as improvement in crystallinity index (Agrawal et al. 2019; Agrawal and Verma 2020a) removal of amorphous phenolic and non-phenolic components with no effect on the microfibril core that ultimately enhances the crystallinity of the cellulose of the fiber along with surface modification to form an effective biocomposite (Nasir et al. 2015) (Fig. 1).

5 Other Applications of Laccases

An overview of the various scientific and industrial applications has been represented in Fig. 2 and has been elaborated in the following section.

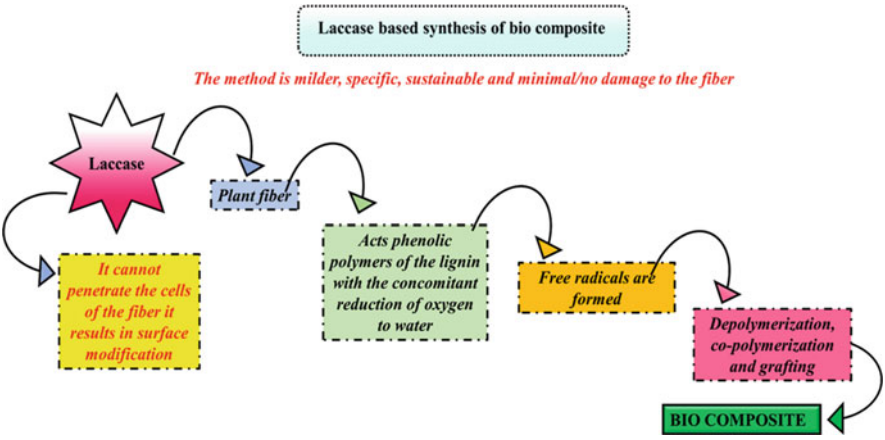


Fig. 1 Schematic representation of the laccase-mediated synthesis of biocomposite

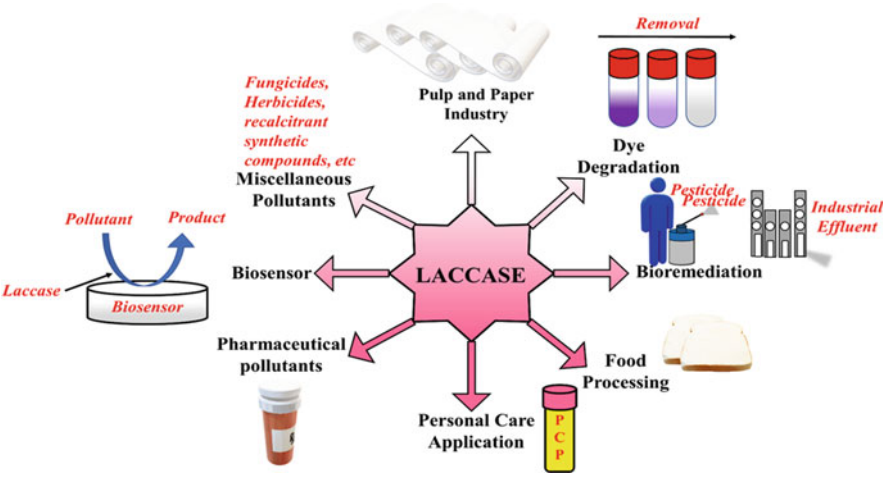


Fig. 2 Applications of laccase in different industrial and scientific sectors

5.1 Paper and Pulp Industry

Wood is made of small wood fibers that are adhered to by lignin. To separate these wood fibers chemical and physical methods of pulping are used. In chemical pulping fibers are separated by dissolving and degrading lignin using chemical agents whereas in physical pulping fibers are physically ripped apart (Bilal et al. 2019a, b; Singh et al. (2015). Pulping is followed by sheeting which results in the production of paper. Chlorine-based chemicals are used for pulp bleaching; as a result, chlorinated aliphatic and aromatic compounds are formed. The compounds are said to be carcinogenic, mutagenic, and toxic. Extensive research has been

undertaken in recent years to develop environmentally sustainable enzymatic bleaching technologies. Pulp bio-bleaching has been demonstrated using laccase-mediated systems, but the lack of proper and cheap mediators has hampered their practicality. Laccases can remove potentially toxic phenols produced during lignin degradation, allowing them to depolymerize lignin and delignify wood pulps. Laccase starts by interacting with small phenolic fragments of lignin, which then react to degrade with the lignin polymer. Moreover, the use of ligninolytic fungi to pretreat wood chips strengthens the pulp while lowering the energy required for mechanical pulping. It is also used to reduce the kappa number of pulp and improve the pulp's papermaking properties. Thus, the use of laccases in bio-bleaching processes in the pulp and paper industry is an environmentally safe approach (Bilal et al. 2019a, b; Virk et al. 2012).

5.2 Dye Degradation

Massive quantities of wastewaters are released by the textile industries which are contaminated by a large spectrum of chemicals for example azo dyes, which are the primary source of environmental pollution (Paździor et al. 2019). For the environment's safety, treatment of industrial wastewater has become very important before its safe release into the environment (Salem et al. 2019). These effluents contain recalcitrant dyes (e.g., azo) that pollute the freshwater with their color and carcinogenic intermediates such as the aromatic amines. These chemical reagents are usually complex, synthetic and are unaffected to decolorization in presence of H_2O, light, and different chemicals. They are also resistant to various existing dye degradation methods e.g., chemical treatments that are ineffective, and results in the production of intermediate compounds that are mutagenic or carcinogenic (Bilal et al. 2019a, b). As a result, the laccase-assisted dye bioremediation has gained interest due to their diverse potential for the degradation of various dyes via sustainable approach (Couto and Toca-Herrera 2006; Verma 2001; Verma and Madamwar 2002a, b). Since traditional treatment methods based on chemical or physical processes are extremely costly and involve massive quantities of resources, various techniques have recently been investigated as alternatives. Laccase due to its ability to catalyze reactions that can degrade a wide variety of pollutants. For textile wastewater treatment, many aerobic and anaerobic bioprocesses have been developed and extensive research has been done fungal laccases for the production of laccase to improve bioprocesses for the degradation of dyes (Verma and Madamwar 2005). The majority of current dye wastewater treatment processes are inefficient and costly. As a result of their ability to degrade dyes fungal laccase mediated remediation of dyes may provide an appealing solution for a sustainable future.

5.3 Bioremediation

Major issues globally today are polluted air, soil, and water that have disastrous consequences. Industrialization and the widespread use of pesticides in agriculture, contamination of the atmosphere is major problem. Industries have been subjected to stringent regulations to handle their waste effluents before their discharge. Numerous remediation strategies have been reported but only a few have been adopted by the industries. Recently, the ability of fungi to transmute diverse chemicals has sparked the interest of the scientific community (Bollag et al. 2003). Also, low cost, high efficiency, and environmental friendliness it has been considered as a feasible alternative to the pre-established chemical-physical methods (Balcázar-López et al. 2016). Further, enzymatic therapy is now being considered as an substitute strategy for the removal of xenobiotics (Balcázar-López et al. 2016). Laccases can remediate polluted soils via immobilization as they can oxidize toxic organic contaminants including chlorophenols, PAHs, etc. (Zhang et al. 2008; Niu et al. 2013). Farnet et al. (2000) investigated the ability of *Marasmius quercophilus* laccase to treatalkylphenols. Saparrat et al. (2010) investigated the detoxification of "alpeorujo," which is a solid by-product from the olive oil extraction industry by *Coriolopsis rigida* laccase. Laccase has been reported for the removal of dichlorodiphenyltrichloroethane (Yuechun et al. 2010) and 2,4-dichlorophenol (Bhattacharya et al. 2009). The degradation of PAHs by *Pleurotus ostreatus* laccase has been reported by Pozdnyakova et al. (2006) and high tannin from wastewater by *Coriolopsis gallica* laccase (Yagüe et al. 2000).

5.4 Food Processing

Laccases have a lot of potential as food additives and manufacturing aids in the food industry (Osma et al. 2010). Laccase-based biocatalysts are energy-efficient and biodegradable, making them ideal for food industries and also to produce low-cost, nutritious foods (Brijwani et al. 2010). Laccases can reduce food processing costs while still being environmentally friendly and to fully realize its ability a detailed understanding of their mode of action is required. Laccase's versatility in action and widespread presence in many fungi species attest to its ease of use in biotechnological processes. Despite the presence of turbidity, after treatment with laccase and active filtration color consistency improved in fruit juices. Also, the phenolic content of juices reduced after laccase treatment along with stability of color (Ribeiro et al. 2010). Dough enhancement additives are added in bread-making process to improve its taste, texture, volume, and freshness. Thus, laccase addition in dough had an oxidizing effect thereby increasing gluten structures strength in baked goods. Also it improved the crumb structure, softness, increased volume, stability, weight, and, reduced stickiness. It also has to be noted that where laccase decreased extensibility in both flour and gluten dough and increased its resistance. The laccase and

proteolytic enzymes when added to oat flour increased loaf specific volume and reduced crumb stiffness, chewiness respectively, and eventually improved its texture. Also, Jurado et al. (2009) stated that the induction of laccase acts as a fermentation inhibitor and increased the output of ethanol from steam-exploded wheat straw and reduced phenolic compounds (Larsson et al. 1999). The polymerization of phenols and polyphenols and the natural co-oxidation reactions have resulted in unwanted fragrance and color changes (Ribeiro et al. 2010). Thus, laccase has been reported and used for the clarification of fruit juices (Narnoliya et al. 2019). Giovanelli and Ravasini (1993) investigated the use of laccase along with filtration for stabilizing apple juice. Phenols were removed more efficiently by laccase treatment over other treatments, such as activated coals (Brijwani et al. 2010). Ribeiro et al. (2010) stated that treatment by laccase significantly decreased the phenolic content of juices while increasing color stability. It has also been found to be more beneficial as compared to traditional treatments e.g., addition of ascorbic acid and sulfites along with the enhancement of its functionality as well as sensory properties. Laccase also contributes in beverage stabilization, role in overall food quality improvement, and use in the baking industry (Manhivi et al. 2018; Di Fusco et al. 2010). Further knowledge of laccase kinetic parameters would be beneficial for functional applications of the enzyme.

5.5 Personal Care Applications

Laccase-generated products contain antimicrobial, detoxifying, or personal care active ingredients and has been used to synthesize anesthetics, anti-inflammatory medicines, etc. (Upadhyay et al. 2016). Couto and Toca-Herrera (2006) stated that the dyeing formula's hydrogen substitution method based on laccase can resolve the inconvenience of chemical dyes by replacing the hydrogen with oxide. In recent years, skin lightening has been also used for cosmetics and dermatological preparations containing staining proteins. Laccase can be used as fragrant agents in personal care items such as toothpaste, mouthwash, detergent, and soap.

5.6 Pharmaceutical Pollutants

Active pharmaceutical ingredients have been detected in wastewater, and no effective method for the removal of are currently in use at large scale. Also these pollutants when released in water severely damages the aquatic environment or drinking water sources (Sui et al. 2010). This perilous condition necessitated the creation of a system for effectively removing pharmaceutical-based pollutants from wastewater. Researchers have confirmed bioremediation and removal of various pharmaceutically active ingredients using laccase (Rana et al. 2017; Xu et al. 2015). Lonappan et al. (2018) confirmed DCF biodegradation by immobilized

laccase and enzyme's binding improved when biochars were pretreated with citric acid. Remarkably, mature pig biochar immobilized laccase demonstrated a notable ability to fully extract DCF (500 μg L^{-1}) in 2 h. Naghdi et al. (2017) investigated the removal of carbamazepine by immobilized laccase. After three cycles of reusability, the immobilized biocatalytic device retained 70% of its original operation and removed 83% of the carbamazepine from the spiked water. In a study by Taheran et al. (2017) used polyacrylonitrile-biochar composite that was home-prepared for laccase immobilization to degrade chlortetracycline from aqueous solution medium. Furthermore, the composite nanofibrous membrane-immobilized laccase demonstrated notable chlortetracycline removal efficacy (Taheran et al. 2017).

5.7 Biosensor

Oxidation of various organic pollutants, present in wastewater, especially phenolic compounds is catalyzed by laccases. It has a significant effect on the production of biosensors for both environmental and clinically relevant metabolites and it does not need any cofactors for e^- transfer reactions. Due to laccase's wide substrate range in biosensor technology, a large range of phenolics and azides can be detected (Rodríguez-Delgado et al. 2015; Sezgintürk et al. 2005). Laccase coupled multi-walled carbon nano tubes-based biosensors are used to calculate the polyphenol index in wines. A bio-sensor based on laccases coupled with multi-walled carbons nano-tubes measures the index of polyphenols in wine. This biosensor gives a clear and fast amperometric response to gallic acid (Di Fusco et al. 2010). The ultrasensitive amperometric detection of nanomolar catecholamine neurotransmitters (dopamine, epinephrine, and norepinephrine) is achieved by co-immobilization-based enzyme electrodes and laccase on glassy carbon electrodes. The enzyme's selectivity to different phenolic compounds has been altered by the hybrid material of Nafion/sol-gel silicate used to immobilize laccase (Abdullah et al. 2007).

5.8 Miscellaneous Pollutants

As the population is increasing, agriculture production is being improved. This has led to heavy industrialization and excessive use of pesticides, which has caused a dreadful environmental condition. This has polluted the soil, water and, air with toxic chemicals which can create havoc on human health and climate. Due to these factors, it has become a major concern for the world. Potentially hazardous substances such as fungicides, herbicides, pesticides pharmaceutical compounds, phenolic compounds, PPCPs, and recalcitrant synthetic compounds can be biodegraded by laccase. Bisphenol-A, which has a carcinogenic effect, can be degraded by glutaraldehyde cross-linked chitosan beads. Laccase can degrade a wide range of substances, including polyvinyl chloride (Sumathi et al. 2016), xenobiotics e.g.,

polynuclear aromatic hydrocarbons (Dias et al. 2003; Cañas et al. 2007), polychlorinated biphenyls (Keum and Li 2004), etc. Laccase catalysis is used to regulate contaminants in the environment where fungal laccases can efficiently degrade and mineralize a variety of environmental contaminants, including BPA (Uchida et al. 2001), chlorophenol (Gaitan et al. 2011), nonylphenol (Tsutsumi et al. 2001), and chlorinated hydroxyl biphenyl (Schultz et al. 2001). It has also been used for the removal of 2,4,6-trinitrotoluene (TNT) (Cheong et al. 2006) and catechol (Tušek et al. 2017).

6 Limitations and Future Aspects

The main limitations of the application aspect using laccase are deactivation factors such as inhibitors, elevated pH, temperature, and non-reusability of free laccase. These drawbacks can be mitigated using new systems such as laccase-mediator or immobilized-laccase catalyzed systems. The lack of capacity to produce large quantities of active enzymes prevents its utility on a large scale. However, these issues can be addressed by recombinant organisms or screening for naturally hypersecretory strains. Thus, strain proficient of producing high titre of a suitable enzyme should be selected followed by optimization of the conditions for laccase production. Recent biotechnological advances, particularly in protein engineering and directed evolution, have enabled essential tools for the efficient development of better enzymes with improved properties with better applicability. Also, the production of new enzymes has been tailored to completely new areas of application where enzymes had not previously been used. Although laccase is still produced in limited quantities, their prospective ability is immense; many of these remain to be revealed. Enzyme immobilization could be used to overcome these limitations while also boosting biodegradation efficiency and enzyme reuse. Since the discovery of laccase, its use has expanded in a variety of sectors and has gained significant interest in the synthesis of biocomposites.

7 Conclusion

Laccase has tremendous potential in the application of biocomposite using plant fibers and the research must now be directed toward less focused aspects of the enzyme to broaden the enzyme's applications. One of the major limitations of using laccase is the high cost of downstream processes such as laccase purification that raises the overall cost of production, preventing it from being commercialized. As a result, research should concentrate on the development of more efficient and cost-effective methods for large-scale production and commercialization of laccase-based applications. It would facilitate the development of a "greener" approach for a

"clean" environment by contributing towards chemical-free treatment in industries, development of a chemical-free biocomposite.

Conflict of Interest Authors have no conflict of interest.

References

Abdel-Hamid AM, Solbiati JO, Cann IK (2013) Insights into lignin degradation and its potential industrial applications. Adv Appl Microbiol 82:1–28

Abdullah J, Ahmad M, Lee YH, Karuppiah N, Sidek H (2007) An optical biosensor based on immobilization of laccase and MBTH in stacked films for the detection of catechol. Sensors 7: 2238–2250

Agrawal K, Verma P (2019a) Biodegradation of synthetic dye Alizarin Cyanine Green by yellow laccase producing strain *Stropharia* sp. ITCC-8422. Biocatal Agric Biotechnol 21:101291

Agrawal K, Verma P (2019b) Column bioreactor of immobilized *Stropharia* sp. ITCC 8422 on natural biomass support of *L. cylindrica* for biodegradation of anthraquinone violet R. Bioresour Technol Rep 8:100345

Agrawal K, Verma P (2020a) Production optimization of yellow laccase from *Stropharia* sp. ITCC 8422 and enzyme-mediated depolymerization and hydrolysis of lignocellulosic biomass for biorefinery application. Biomass Conv Bioref. https://doi.org/10.1007/s13399-020-00869-w

Agrawal K, Verma P (2020b) Myco-valorization approach using entrapped *Myrothecium verrucaria* ITCC-8447 on synthetic and natural support via column bioreactor for the detoxification and degradation of anthraquinone dyes. Int Biodeterior Biodegradation 153:105052

Agrawal K, Verma P (2020c) Potential removal of hazardous wastes using white laccase purified by ATPS–PEG–salt system: an operational study. Environ Technol Innov 17:100556

Agrawal K, Verma P (2020d) Production optimization of yellow laccase from *Stropharia* sp. ITCC 8422 and enzyme-mediated depolymerization and hydrolysis of lignocellulosic biomass for biorefinery application. Biomass Convers Biorefin 9:1–20

Agrawal K, Verma P (2020e) Laccase-mediated synthesis of bio-material using agro-residues. In: Biotechnological applications in human health. Springer, Singapore, p 87

Agrawal K, Chaturvedi V, Verma P (2018) Fungal laccase discovered but yet undiscovered. Bioresour Bioprocess 5:1–12

Agrawal K, Bhardwaj N, Kumar B, Chaturvedi V, Verma P (2019) Process optimization, purification and characterization of alkaline stable white laccase from *Myrothecium verrucaria* ITCC-8447 and its application in delignification of agroresidues. Int J Biol Macromol 125:1042–1055

Amari A, Alzahrani FM, Alsaiari NS, Katubi KM, Rebah FB, Tahoon MA (2021) Magnetic metal organic framework immobilized laccase for wastewater decolorization. Processes 9(5):774

Andrews SC, Robinson AK, Rodríguez-Quiñones F (2003) Bacterial iron homeostasis. FEMS Microbiol Rev 27:215–237

Ardhaoui M, Bhatt S, Zheng M, Dowling D, Jolivalt C, Khonsari FA (2013) Biosensor based on laccase immobilized on plasma polymerized allylamine/carbon electrode. Mater Sci Eng C 33: 3197–3205

Arias ME, Arenas M, Rodríguez J, Soliveri J, Ball AS, Hernández M (2003) Kraft pulp biobleaching and mediated oxidation of a nonphenolic substrate by laccase from *Streptomyces cyaneus* CECT 3335. Appl Environ Microbiol 69:1953–1958

Ashrafi SD, Rezaei S, Forootanfar H, Mahvi AH, Faramarzi MA (2013) The enzymatic decolorization and detoxification of synthetic dyes by the laccase from a soil-isolated ascomycete, *Paraconiothyrium variabile*. Int Biodeterior Biodegrad 85:173–181

ASTM-D6007-96 (1996) Standard test method for determining formaldehyde concentrations in air from wood products using a small scale chamber

Balcázar-López E, Méndez-Lorenzo LH, Batista-García RA, Esquivel-Naranjo U, Ayala M, Kumar VV, Savary O, Cabana H, Herrera-Estrella A, Folch-Mallol JL (2016) Xenobiotic compounds degradation by heterologous expression of a *Trametes sanguineus* laccase in *Trichoderma atroviride*. PLoS One 11:e0147997

Barrios-Estrada C, de Jesús R-AM, Parra AL, Belleville MP, Sanchez-Marcano J, Iqbal HM, Parra-Saldívar R (2018) Potentialities of active membranes with immobilized laccase for Bisphenol A degradation. Int J Biol Macromol 108:837–844

Bertrand T, Jolivalt C, Briozzo P, Caminade E, Joly N, Madzak C, Mougin C (2002) Crystal structure of a four-copper laccase complexed with an arylamine: insights into substrate recognition and correlation with kinetics. Biochemistry 41:7325–7333

Bhattacharya SS, Karmakar S, Banerjee R (2009) Optimization of laccase mediated biodegradation of 2,4-dichlorophenol using genetic algorithm. Water Res 43:3503–3510

Bilal M, Jing Z, Zhao Y, Iqbal HM (2019a) Immobilization of fungal laccase on glutaraldehyde cross-linked chitosan beads and its bio-catalytic potential to degrade bisphenol A. Biocatal Agric Biotechnol 19:101174

Bilal M, Rasheed T, Nabeel F, Iqbal HMN, Zhao Y (2019b) Hazardous contaminants in the environment and their laccase-assisted degradation—a review. J Environ Manag 234:253–264

Bollag JM, Chu HL, Rao MA, Gianfreda L (2003) Enzymatic oxidative transformation of chlorophenol mixtures. J Environ Qual 32:63–69

Boruah P, Sarmah P, Das PK, Goswami T (2019) Exploring the lignolytic potential of a new laccase producing strain Kocuria sp. PBS-1 and its application in bamboo pulp bleaching. Int Biodeterior Biodegradation 143:104726

Brijwani K, Rigdon A, Vadlani PV (2010) Fungal laccases: production, function, and applications in food processing. Enzyme Res 2010:149748

Camarero S, Ibarra D, Martinez AT, Romero J, Gutiérrez A, José C (2007) Paper pulp delignification using laccase and natural mediators. Enzym Microb Technol 40:1264–1271

Cañas AI, Alcalde M, Plou F, Martínez MJ, Martínez ÁT, Camarero S (2007) Transformation of polycyclic aromatic hydrocarbons by laccase is strongly enhanced by phenolic compounds present in soil. Environ Sci Technol 41:2964–2971

Cheong S, Yeo S, Song HG, Choi HT (2006) Determination of laccase gene expression during degradation of 2, 4, 6-trinitrotoluene and its catabolic intermediates in *Trametes versicolor*. Microbiol Res 161:316–320

Couto SR, Toca-Herrera JL (2006) Laccases in the textile industry. Biotechnol Mol Biol Rev 1: 115–120

Crichton RR, Pierre J (2001) Old iron, young copper: from Mars to Venus. Biometals 14:99–112

Di Fusco M, Tortolini C, Deriu D, Mazzei F (2010) Laccase-based biosensor for the determination of polyphenol index in wine. Talanta 81:235–240

Dias AA, Bezerra RM, Lemos PM, Pereira AN (2003) In vivo and laccase-catalysed decolourization of xenobiotic azo dyes by a basidiomycetous fungus: characterization of its ligninolytic system. World J Microbiol Biotechnol 19:969–975

Ding H, Wu Y, Zou B, Lou Q, Zhang W, Zhong J, Lu L, Dai G (2016) Simultaneous removal and degradation characteristics of sulfonamide, tetracycline, and quinolone antibiotics by laccase-mediated oxidation coupled with soil adsorption. J Hazard Mater 307:350–358

El-Batal AI, ElKenawy NM, Yassin AS, Amin MA (2015) Laccase production by *Pleurotus ostreatus* and its application in synthesis of gold nanoparticles. Biotechnol Rep 5:31–39

Farnet AM, Criquet S, Tagger S, Gil G, Le Petit J (2000) Purification, partial characterization, and reactivity with aromatic compounds of two laccases from *Marasmius quercophilus* strain 17. Can J Microbiol 46:189–194

Gaitan IJ, Medina SC, González JC, Rodríguez A, Espejo ÁJ, Osma JF, Sarria V, Alméciga-Díaz CJ, Sánchez OF (2011) Evaluation of toxicity and degradation of a chlorophenol mixture by the laccase produced by *Trametes pubescens*. Bioresour Technol 102:3632–3635

Giovanelli G, Ravasini G (1993) Apple juice stabilization by combined enzyme—membrane filtration process. LWT-Food Sci Technol 26(1):1–7

González-García S, Feijoo G, Heathcote C, Kandelbauer A, Moreira MT (2011) Environmental assessment of green hardboard production coupled with a laccase activated system. J Clean Prod 19:445–453

Gutierrez-Sanchez C, Pita M, Vaz-Dominguez C, Shleev S, De Lacey AL (2012) Gold nanoparticles as electronic bridges for laccase-based biocathodes. J Am Chem Soc 134(41): 17212–17220

Hakulinen N, Kiiskinen LL, Kruus K, Saloheimo M, Paananen A, Koivula A, Rouvinen J (2002) Crystal structure of a laccase from Melanocarpus albomyces with an intact trinuclear copper site. Nat Struct Biol 9:601–605

Hoopes JT, Dean FD (2004) Ferroxidase activity in a laccase-like multicopper oxidase from *Liriodendron tulipifera*. Plant Physiol Biochem 42:27–33

Hou H, Zhou J, Wang J, Du C, Yan B (2004) Enhancement of laccase production by Pleurotus ostreatus and its use for the decolorization of anthraquinone dye. Process Biochem 39:1415–1419

Janusz G, Pawlik A, Świderska-Burek U, Polak J, Sulej J, Jarosz-Wilkołazka A, Paszczyński A (2020) Laccase properties, physiological functions, and evolution. Int J Mol Sci 21:1–25

Jurado M, Prieto A, Martínez-Alcalá Á, Martínez ÁT, Martínez MJ (2009) Laccase detoxification of steam-exploded wheat straw for second generation bioethanol. Bioresour Technol 100 (24):6378–6384

Keum YS, Li QX (2004) Fungal laccase-catalyzed degradation of hydroxy polychlorinated biphenyls. Chemosphere 56:23–30

Khambhaty Y, Ananth S, Sreeram KJ, Rao JR, Nair BU (2015) Dual utility of a novel, copper enhanced laccase from *Trichoderma aureoviridae*. Int J Biol Macromol 81:69–75

Kim S, Kim JA, Kim HJ (2007) Application of field and laboratory emission cell (FLEC) to determine formaldehyde and VOCs emissions from wood-based composites. J Korean Wood Sci Technol 35:24–37

Kumar S, Mohanty AK, Erickson L, Misra M (2009) Lignin and its applications with polymers. J Biobaased Mater Bioenergy 3:1–24

Kunamneni A, Ballesteros A, Plou FJ, Alcalde M (2007) Fungal laccase—a versatile enzyme for biotechnological applications. Communicating current research and educational topics and trends in applied microbiology 1:233–245

Kunamneni A, Camarero S, García-Burgos C, Plo FJ, Ballesteros A, Alcalde M (2008) Engineering and applications of fungal laccases for organic synthesis. Microb Cell Factories 7:1–17

Larsson S, Reimann A, Nilvebrant NO, Jönsson LJ (1999) Comparison of different methods for the detoxification of lignocellulose hydrolyzates of spruce. Appl Biochem Biotechnol 77:91–103

Le Goff A, Holzinger M, Cosnier S (2015) Recent progress in oxygen-reducing laccase biocathodes for enzymatic biofuel cells. Cell Mol Life Sci 72(5):941–952

Leite OD, Fatibello-Filho O, Barbosa ADM (2003) Determination of catecholamines in pharmaceutical formulations using a biosensor modified with a crude extract of fungi laccase (*Pleurotus ostreatus*). J Braz Chem Soc 14:297–303

Lettera V, Pezzella C, Cicatiello P, Piscitelli A, Giacobelli VG, Galano E, Amoresano A, Sannia G (2016) Efficient immobilization of a fungal laccase and its exploitation in fruit juice clarification. Food Chem 196:1272–1278

Lonappan L, Liu Y, Rouissi T, Pourcel F, Brar SK, Verma M, Surampalli RY (2018) Covalent immobilization of laccase on citric acid functionalized micro-biochars derived from different feedstock and removal of diclofenac. Chem Eng J 351:985–994

Mai C, Majcherczyk A, Hüttermann A (2000) Chemo-enzymatic synthesis and characterization of graft copolymers from lignin and acrylic compounds. Enzym Microb Technol 27:167–175

Manhivi VE, Amonsou EO, Kudanga T (2018) Laccase-mediated crosslinking of gluten-free amadumbe flour improves rheological properties. Food Chem 264:157–163

Mayer AM, Staples RC (2002) Laccase: new functions for an old enzyme. Phytochemistry 60:551–565

Mehra R, Muschiol J, Meyer AS, Kepp KP (2018) A structural-chemical explanation of fungal laccase activity. Sci Rep 8:1–16

Mogharabi M, Faramarzi MA (2014) Laccase and laccase-mediated systems in the synthesis of organic compounds. Adv Synth Catal 356(5):897–927

Moubarik A, Allal A, Pizzi A, Charrier F, Charrier B (2010) Characterization of a formaldehyde-free cornstarch-tannin wood adhesive for interior plywood. Eur J Wood Wood Prod 68:427–433

Moya R, Hernández M, García-Martín AB, Ball AS, Arias ME (2010) Contributions to a better comprehension of redox-mediated decoloration and detoxification of azo dyes by a laccase produced by *Streptomyces cyaneus* CECT 3335. Bioresour Technol 101:2224–2229

Naghdi M, Taheran M, Brar SK, Kermanshahi-pour A, Verma M, Surampalli RY (2017) Immobilized laccase on oxygen functionalized nanobiochars through mineral acids treatment for removal of carbamazepine. Sci Total Environ 584:393–401

Narnoliya LK, Agarwal N, Patel SN, Singh SP (2019) Kinetic characterization of laccase from *Bacillus atrophaeus*, and its potential in juice clarification in free and immobilized forms. J Microbiol 57(10):900–909

Nasir M, Gupta A, Beg MDH, Chua GK, Asim M (2014) Laccase application in medium density fibreboard to prepare a bio-composite. RSC Adv 4:11520–11527

Nasir M, Hashim R, Sulaiman O, Nordin NA, Lamaming J, Asim M (2015) Laccase, an emerging tool to fabricate green composites: a review. Bioresources 10:6262–6284

Nguyen LN, Hai FI, Price WE, Leusch FDL, Roddick F, McAdam EJ, Magram SF, Nghiem LD (2014) Continuous biotransformation of bisphenol a and diclofenac by laccase in an enzymatic membrane reactor. Int Biodeterior Biodegradation 95:25–32

Niu J, Dai Y, Guo H, Xu J, Shen Z (2013) Adsorption and transformation of PAHs from water by a laccase-loading spider-type reactor. J Hazard Mater 248:254–260

Nunes CS, Kunamneni A (2018) Laccases-properties and applications. In: Nunes CS, Kumar V (eds) Enzymes in human and animal nutrition: principles and perspectives, Academic Press, London, pp 133–161

Osma JF, Toca-Herrera JL, Rodríguez-Couto S (2010) Uses of laccases in the food industry. Enzyme Res 2010:918761

Patel N, Shahane S, Majumdar R, Mishra U (2019) Mode of action, properties, production, and application of laccase: a review. Recent Pat Biotechnol 13:19–32

Pazarlıoğlu NK, Sariişik M, Telefoncu A (2005) Laccase: production by Trametes versicolor and application to denim washing. Process Biochem 40:1673–1678

Paździor K, Bilińska L, Ledakowicz S (2019) A review of the existing and emerging technologies in the combination of AOPs and biological processes in industrial textile wastewater treatment. Chem Eng J 376:120597

Pizzi A (2003) Natural phenolic adhesives II: lignin. In: Handbook of adhesive technology, 2nd edn. Marcel Dekk, New York

Plácido J, Capareda S (2015) Ligninolytic enzymes: a biotechnological alternative for bioethanol production. Bioresour Bioprocess 2(1):1–2

Pozdnyakova NN, Rodakiewicz-Nowak J, Turkovskaya OV, Haber J (2006) Oxidative degradation of polyaromatic hydrocarbons catalyzed by blue laccase from *Pleurotus ostreatus* D1 in the presence of synthetic mediators. Enzym Microb Technol 39:1242–1249

Rana RS, Singh P, Kandari V, Singh R, Dobhal R, Gupta S (2017) A review on characterization and bioremediation of pharmaceutical industries' wastewater: an Indian perspective. Appl Water Sci 7:1–12

Renzetti S, Courtin CM, Delcour JA, Arendt EK (2010) Oxidative and proteolytic enzyme preparations as promising improvers for oat bread formulations: rheological, biochemical and microstructural background. Food Chem 119(4):1465–1473

Ribeiro DS, Henrique SM, Oliveira LS, Macedo GA, Fleuri LF (2010) Enzymes in juice processing: a review. Int J Food Sci Technol 45:635–641

Ribeiro FWP, Barroso MF, Morais S, Viswanathan S, de Lima-Neto P, Correia AN, Oliveira MBPP, Delerue-Matos C (2014) Simple laccase-based biosensor for formetanate hydrochloride quantification in fruits. Bioelectrochemistry 95:7–14

Rocasalbas G, Francesko A, Touriño S, Fernández-Francos X, Guebitz GM, Tzanov T (2013) Laccase-assisted formation of bioactive chitosan/gelatin hydrogel stabilized with plant polyphenols. Carbohydr Polym 92:989–996

Rodríguez-Delgado MM, Alemán-Nava GS, Rodríguez-Delgado JM, Dieck-Assad G, Martínez-Chapa SO, Barceló D, Parra R (2015) Laccase-based biosensors for detection of phenolic compounds. Trends Anal Chem 74:21–45

Saastamoinen P, Mattinen ML, Hippi U, Nousiainen P, Sipilä J, Lille M, Suurnäkki A, Pere J (2012) Laccase aided modification of nanofibrillated cellulose with dodecyl gallate. Bio Res 7(4): 5749–5770

Salem SS, Mohamed AA, Gl-Gamal MS, Talat M, Fouda A (2019) Biological decolorization and degradation of azo dyes from textile wastewater effluent by *Aspergillus niger*. Egypt J Chem 62: 1799–1813

Sanghi R, Dixit A, Verma P, Puri S (2009) Design of reaction conditions for the enhancement of microbial degradation of dyes in sequential cycles. J Environ Sci 21:1646–1651

Saparrat MCN, Jurado M, Díaz R, Romera IG, Martínez MJ (2010) Transformation of the water soluble fraction from "alpeorujo" by *Coriolopsis rigida*: the role of laccase in the process and its impact on *Azospirillum brasiliense* survival. Chemosphere 78:72–76

Schultz A, Jonas U, Hammer E, Schauer F (2001) Dehalogenation of chlorinated hydroxybiphenyls by fungal laccase. Appl Environ Microbiol 67:4377–4381

Sezgintürk MK, Göktuğ T, Dinçkaya E (2005) A biosensor based on catalase for determination of highly toxic chemical azide in fruit juices. Biosens Bioelectron 21:684–688

Shraddha SR, Sehgal S, Kamthania M, Kumar A (2011) Laccase: microbial sources, production, purification, and potential biotechnological applications. Enzyme Res 2011:217861

Singh G, Kaur K, Puri S, Sharma P (2015) Critical factors affecting laccase-mediated biobleaching of pulp in the paper industry. Appl Microbiol Biotechnol 99:155–164

Soares GM, de Amorim MP, Costa-Ferreira M (2001) Use of laccase together with redox mediators to decolourize Remazol brilliant blue R. J Biotechnol 89:123–129

Srebotnik E, Hammel KE (2000) Degradation of nonphenolic lignin by the laccase/1-hydroxybenzotriazole system. J Biotechnol 81:179–188

Sumathi T, Viswanath B, Sri Lakshmi A, SaiGopal DVR (2016) Production of laccase by *Cochliobolus* sp isolated from plastic dumped soils and their ability to degrade low molecular weight PVC. Biochem Res Int 2016:9519527

Sui Q, Huang J, Deng S, Yu G, Fan Q (2010) Occurrence and removal of pharmaceuticals, caffeine and DEET in wastewater treatment plants of Beijing, China. Water Res 44(2):417–426

Syukri MSM, Rahman RA, Mohamad Z, Illias RM, Mahmood NAN, Jaafar NR (2021) Optimization strategy for laccase immobilization on polyethylene terephthalate grafted with maleic anhydride electrospun nanofiber mat. Int J Biol Macromol 166:876–883

Taheran M, Naghdi M, Brar SK, Knystautas EJ, Verma M, Surampalli RY (2017) Degradation of chlortetracycline using immobilized laccase on Polyacrylonitrile-biochar composite nanofibrous membrane. Sci Total Environ 605:315–321

Tsutsumi Y, Haneda T, Nishida T (2001) Removal of estrogenic activities of bisphenol A and nonylphenol by oxidative enzymes from lignin-degrading basidiomycetes. Chemosphere 42: 271–276

Tušek AJ, Šalić A, Zelić B (2017) Catechol removal from aqueous media using laccase immobilized in different macro-and microreactor systems. Appl Biochem Biotechnol 182: 1575–1590

Uchida H, Fukuda T, Miyamoto H, Kawabata T, Suzuki M, Uwajima T (2001) Polymerization of bisphenol A by purified laccase from *Trametes villosa*. Biochem Biophys Res Commun 287: 355–358

Upadhyay P, Shrivastava R, Agrawal PK (2016) Bioprospecting and biotechnological applications of fungal laccase. 3. Biotech 6:15

van de Pas D, Hickson A, Donaldson L, Lloyd-Jones G, Tamminen T, Fernyhough A, Mattinen ML (2011) Characterization of fractionated lignins polymerized by fungal laccases. Bioresources 6: 1105–1121

Verma P (2001) White rot fungi mediated integrated approach for lignocellulosic waste decomposition and textile dye decolorization. http://hdl.handle.net/10603/77403

Verma P, Madamwar D (2002a) Production of ligninolytic enzymes for dye decolorization by cocultivation of white-rot fungi *Pleurotus ostreatus* and *Phanerochaete chrysosporium* under solid-state fermentation. Appl Biochem Biotechnol 102:109–118

Verma P, Madamwar D (2002) Comparative study on transformation of azo dyes by different white rot fungi. NISCAIR-CSIR, New Delhi, pp 393–396. http://hdl.handle.net/123456789/19893

Verma P, Madamwar D (2005) Decolorization of azo dyes using Basidiomycete strain PV 002. World J Microbiol Biotechnol 21:481–485

Verma P, Mai C (2010) Hydrolysis of cellulose and wood powder treated with DMDHEU by a hydrolase enzyme complex, Fenton's reagent, and in a liquid culture of *Trametes versicolor*. Holzforschung 64(1):69–75. https://doi.org/10.1515/hf.2010.007

Verma P, Mai C, Krause A, Militz H (2005) Studies on the resistance of DMDHEU treated wood against white-rot and brown rot fungi. International Research Group on Wood Protection, Stockholm, Sweden Doc. IRG/WP/05-10566

Verma P, Junga U, Militz H, Mai C (2009) Protection mechanisms of DMDHEU treated wood against white and brown rot fungi. De Gruyter 3:371–378. https://doi.org/10.1515/HF.2009.051

Verma P, Watanabe T, Honda Y, Watanabe T (2011) Microwave-assisted pretreatment of woody biomass with ammonium molybdate activated by H_2O_2. Bioresour Technol 102:3941–3945

Virk AP, Sharma P, Capalash N (2012) Use of laccase in pulp and paper industry. Biotechnol Prog 28:21–32

Vishtal AG, Kraslawski A (2011) Challenges in industrial applications of technical lignins. Bioresources 6:3547–3568

Viswanath B, Rajesh B, Janardhan A, Kumar AP, Narasimha G (2014) Fungal laccases and their applications in bioremediation. Enzyme Res 2014:163242

Witayakran S, Ragauskas AJ (2009) Modification of high-lignin softwood kraft pulp with laccase and amino acids. Enzym Microb Technol 44:176–181

Xu R, Tang R, Zhou Q, Li F, Zhang B (2015) Enhancement of catalytic activity of immobilized laccase for diclofenac biodegradation by carbon nanotubes. Chem Eng J 262:88–95

Yagüe S, Terrón MC, González T, Zapico E, Bocchini P, Galletti GC, González AE (2000) Biotreatment of tannin-rich beer-factory wastewater with white-rot basidiomycete *Coriolopsis gallica* monitored by pyrolysis/gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom 14:905–910

Yoshida H (1883) Chemistry of lacquer (Urushi). J Chem Soc Trans 43:472–486

Yuechun ZHAO, Xiaoyun YI, Minghua LI, Lu LIU, Weijuan MA (2010) Biodegradation kinetics of DDT in soil under different environmental conditions by laccase extract from white rot fungi. Chin J Chem Eng 18:486–492

Zhang M, Wu F, Wei Z, Xiao Y, Gong W (2006) Characterization and decolorization ability of a laccase from *Panus rudis*. Enzym Microb Technol 39:92–97

Zhang J, Liu X, Xu Z, Chen H, Yang Y (2008) Degradation of chlorophenols catalyzed by laccase. Int Biodeterior Biodegradation 61:351–356

Zhou X, Tang L, Zhang W, Lv C, Zheng F, Zhang R, Du G, Tang B, Liu X (2011) Enzymatic hydrolysis lignin derived from corn stover as an intrinstic binder for bio-composites manufacture: effect of fiber moisture content and pressing temperature on boards' properties. Bioresources 6:253–264

Marine Fungi as a Source of Biosurfactants and Bioemulsifiers

Rossana Pitocchi, Alessandra Piscitelli, and Paola Giardina

Abstract The state of knowledge of terrestrial and marine fungi is significantly different, and the causes are diverse. However, there is no doubt that marine fungi have a huge potential in providing new compounds with intriguing characteristics. In this respect, biosurfactants and bioemulsifiers are an emerging class of compounds that, thanks to their unique features, can be applied in a wide range of application fields. Up to now, marine fungi are strongly unattended as effective source of these compounds. Herein some examples of screening of marine fungi able to produce biosurfactants and the characterization of their surface-active compounds are reported.

Keywords Surface tension · Emulsion · Surface active proteins · Screening methods

1 Introduction

The richness of fungal species is generally associated with that of terrestrial fungi. Indeed, marine fungi considerably contribute to the global fungal diversity and recently, marine environment is proving to be an inexhaustible source of new microorganisms producing many useful compounds thanks to their peculiar metabolism. The physical and chemical factors unique to the marine environment, which can mainly influence marine fungi are high salinity and hydrostatic pressure, low temperature, oligotrophic nutrient conditions. Because of these unique physic-chemical properties, marine fungi are endowed with unusual physiological adaptative systems that can be widely exploited in biotechnology.

There is a significant gap between the state of knowledge of terrestrial and marine fungi, and the causes are diverse. One could be the relative difficulty of sampling of marine fungi compared to terrestrial, or problems encountered in growing marine fungi in laboratory conditions, or the lower achievable yield of certain secondary

R. Pitocchi · A. Piscitelli · P. Giardina (✉)
Department of Chemical Sciences, University Federico II, Naples, Italy
e-mail: giardina@unina.it

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_15

metabolites compared with that of bacteria. However, according to Amend et al. (2019), the first challenge of marine mycology is in defining which fungi are truly "marine."

Pang et al. (2016) defined marine fungus as "any fungus that is recovered repeatedly from marine habitats and is able to grow and sporulate on substrates in marine environments or forms symbiotic relationships with other marine organisms or is shown to adapt and evolve at the genetic level and be metabolically active in marine environments".

According to Raghukumar (2008), marine fungi can be classified as obligate and facultative. The first are fungi that grow and sporulate only in sea water and their spores germinate in sea water. On the other hand, facultative fungi have undergone physiological adaptations from other environments to the marine one where they can grow and sporulate.

Marine fungi have been isolated in a huge range of conditions, for example associated with other organisms (plant, algae, sponges) (Kiran et al. 2009), in deep-see sub-surfaces (Rédou et al. 2015), or even associated with dwelling macrofauna and zooplankton (Raghukumar et al. 2010). In addition, fungi can be isolated in oil-contaminated costal area and/or sediments (Bovio et al. 2017; Maamar et al. 2020). Indeed, they can survive even in extreme environmental conditions, in those habitats that appear challenging for life, since they can promptly adapt to changing environments and tolerate several types of pollutants. This peculiar ability is well exhibited in both terrestrial and marine environments, however, literature about bioactive compounds produced by marine fungi is still poor compared with that of terrestrial fungi.

In marine environment the hydrocarbon-degrading capability is widespread. One possible adaptive system to enhance bioavailability and gain access to hydrophobic compounds could be the production of biosurfactants (BS), substances with surface-active properties (Kaczorek et al. 2018). Due to their amphiphilic structure, they can lower interfacial tension and facilitate solubilisation of hydrophobic substances in water. Indeed they can migrate to hydrophobic:hydrophilic interfaces thanks to their amphiphilic nature, aligning their hydrophobic region facing the hydrophobic side and their hydrophilic region facing the polar side of the interface. This alignment balances the interfacial forces and reduces the surface tension (Sunde et al. 2017). The capability to produce BS is most widespread among the hydrocarbon-degrading microbial communities (Perfumo et al. 2010).

Chemical structures of BS produced by different microorganisms are extremely diverse. Hydrophobic and hydrophilic components establish their amphiphilic character, with the hydrophobic part usually composed by fatty acids, or fatty alcohols with variable chain lengths. The hydrophilic components can be small hydroxyl, carboxyl or phosphate groups, or carbohydrate (mono-, oligo-, or polysaccharides) or (poly-)peptide moieties.

According to different studies (Uzoigwe et al. 2015; McClements et al. 2017; Tao et al. 2019), high molecular weight BS, such as hetero/lipopolysaccharides, proteins and lipoproteins are named bioemulsifiers (BE) since they efficiently emulsify immiscible liquids even at low concentrations and are less effective at reducing

surface tension (Kubicki et al. 2019). Nevertheless, it can be reasonably assumed that one kind of activity does not preclude the other. The low molecular weight microbial products generally referred as BS, are composed of sugars, amino acids, fatty acids. These compounds are mostly known to decrease interfacial tension between two or more immiscible liquids, thus possessing a low critical micelle concentration (CMC), however they can also form stable emulsions. BS are mainly anionic and ionic compounds; cationic BS are rare and, in most cases, toxic.

Thanks to their amphiphilic nature, BS can form and stabilize foam, act as detergent and lubricant, solubilize hydrocarbons (Kaczorek et al. 2018). Consequently, they are valuable in a great variety of application fields such as medical, pharmaceuticals, food and beverages, cosmetics, agriculture, detergent, textiles, and petrochemical production. They represent a promising alternative with respect to their synthetic counterparts due to their eco-friendly nature, and temperatures, pH, and salinity resistance. Nevertheless, BS global market is comparatively low, mainly because of poor productivity from microorganisms and expensive downstream processes. To overcome these drawbacks genetic modification and growth condition optimization of microorganisms can be carried out, for example using agricultural wastes as carbon source (Bhardwaj et al. 2013; Fenibo et al. 2019).

BS indeed are considered as "*green molecules*" because of their wide applications in soil bioremediation (Mujumdar et al. 2019). The use of surfactant solution in remediation processes is assessed; different synthetic surfactants had been used for treatment of contaminated soil and/or water. Mechanisms are diverse and depend on the nature of surfactant, and characteristics of soil and/or water. In general, it has been seen that surfactants can increase petroleum, and hydrocarbons solubilisation thus helping its removal from soil and water.

Recently, promising technologies for remediation of petroleum contaminated soil have been developed based on surfactant solution and surfactant foam flushing processes (Karthick et al. 2019). The two main problems of synthetic surfactants are their high toxicity and low biodegradability. Toxicity is the measure of adverse effects caused by surfactants in the soil, whereas biodegradability is the ability of microorganisms present in the environment to completely degrade the surfactant (Aquino et al. 2008). Another serious problem concerning with synthetic surfactants lies in the fact that they can easily accumulate in soil, this accumulation could cause modification in soil characteristics. For example, soil hydrophobicity can change, thus inhibiting the normal fluid retention in the soil and leading to the death of microorganisms (Karthick et al. 2019).

Among marine-BS producing microorganisms, bacteria are widely studied and even exploited in some application fields, for example they had been used in bioremediation processes (Harayama et al. 1999), while the study of marine fungi has been neglected for many years. Among the different genera of bacteria, the best studied and characterized for their BS/BE producing activity are *Acinetobacter spp, Bacillus* and *Pseudomonas* (Satpute et al. 2010; Waghmode et al. 2020). For instance, an extensive and comprehensive collection of BS and BE produced by several *Acinetobacter* strains, is given by Mujumdar et al. (2019).

Only recently the extremely various group of marine fungi has been considered as an excellent source of natural products. Actually, numerous secondary metabolites from marine fungi have been characterized, showing pharmaceutically/medical relevant bioactivities. Among these natural products, BS deserve attention for the unique growing conditions of their producing microorganisms (Gudiña et al. 2016).

BS also display anti-biofilm and anti-adhesive activities, and anticancer activities which are particularly relevant for medical applications. The antiadhesive ability makes them able to decrease adhesion and colonization by pathogens, as well as to remove pre-formed biofilms. Antimicrobial activity of surfactants is well known, but literature mainly concerns about terrestrial microorganisms or synthetic surfactants (Satpute et al. 2010; Coelho et al. 2020). Several studies conducted by Das et al. (2008) show that BS produced by a marine bacterium belonging to Bacillus genera, *Bacillus circulans,* possesses both good surface tension reduction with low CMC and antimicrobial activity. They isolated and identified a BS compound as lipopeptide, trough Thin layer chromatography (TLC) and Fourier-transform infrared spectroscopy (FTIR) experiment. This compound share most of the IR spectrum pattern with a known BS, surfactin. It was found to inhibit growth of some Multi Drug Resistant bacteria (like *Klebsiella pneumoniae* and other two MDR strains of *E. coli*), beside several gram positive and negative bacteria. They also investigate on substrate dependence production, doubling the yield production and antimicrobial activity in the presence of glycerol or starch when compared to glucose (Das et al. 2008).

Mukherjee et al. (2009) moreover, isolated a lipopeptide from another *Bacillus (Bacillus licheniformis* BAS509) able to inhibit most of the tested bacterial (both Gram positive and negative) and fungal strains (*Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*). The antimicrobial action against bacterial strains were checked by agar well diffusion test.

As far as the anticancer activity, many of the drugs involved in chemotherapy are cytotoxic, non-specific and are extracted from natural sources such as plants. The latter represent a big disadvantage because of the high processes' costs and relative low production. The advantages of using microbial surfactant lie for example, in the possibility of genetically engineering microbes, that allows to increase production yield. Furthermore, they exhibit high efficacy, low toxicity, and easy biodegradability, which are relevant features in any anti-cancer agent (Gudiña et al. 2016). As mentioned before, in the search of novel compounds with medical applications, BS from marine fungi are considerably less exploited respect to the bacterial derived ones.

Acute respiratory distress syndrome (ARDS) is a progressive syndrome which result in inefficient oxygen transfer across alveolar membranes into the blood (Matthay et al. 2019). Covid-19 can often cause ARDS, with the resulting lack of oxygen to organs. One of the causes of this alveolar fluid build-up, is surfactant dysfunction which has negative consequences on the emulsification of liquid from this particular region. Future studies will be aimed at using BS as a novel treatment for ARDS, through solubilizing the alveolar substrate (Smith et al. 2020).

2 Methods of Screening and Characterization of Surfactants

2.1 Drop Collapse Test

Drop collapse test is a very rapid, non-expensive and reliable experiment to perform in order to establish if the sample is surface active. In addition, it requires small volumes of sample and no specialized equipment (Bodour and Miller-Maier 1998). The test consists in depositing a sample drop onto a hydrophobic surface and measuring its collapsing area. When in contact with a hydrophobic surface, in fact, BS solution collapses, spreading more extensively than water over it.

Although this test is manly qualitative, Bodour and Miller (1998) proposed an alternative protocol that allows quantification of BS, too. Plotting drop diameter as a function of surfactant concentration, they obtained a calibration curve with a good range of linearity. This curve can be used to determine surfactant concentration of unknown samples.

2.2 Oil Displacement Test

This test is based on the characteristic of surfactants to decrease interfacial tension between different phases, and it is a semi-quantitative method to detect surfactant presence. When oil is added to the surface of a petri dish filled with distilled water and then a drop of surfactant solution is spotted on the surface, oil is displaced, and a clear halo expands (Luepongpattana et al. 2017b). The more the surfactant concentration is, the bigger the oil-displacement area is (Fig. 1). Similarly, to the drop collapse test, this test is very rapid to perform, does not require big volume of sample and does not implies particular equipment. As disadvantage indeed, a big quantity of wastes is produced.

2.3 2,6-Dichlorophenol Indophenol (DCPIP) Colorimetric Assay

The DCPIP based colorimetric assay is not specific to detect the presence of BS, but it is often used as screening method for their production, since it measures the utilization/degradation of hydrocarbons by microorganisms, that is normally related to BS production (Obi et al. 2016).

DCPIP is blue when oxidized and colourless when reduced, being a pH dependant redox indicator. If it is incorporated in a microbial culture during hydrocarbon oxidation, electrons are transferred to DCPIP rather than to O_2, nitrate and sulphate, as usual. Therefore, degradation of hydrocarbons is visualized by monitoring loss of

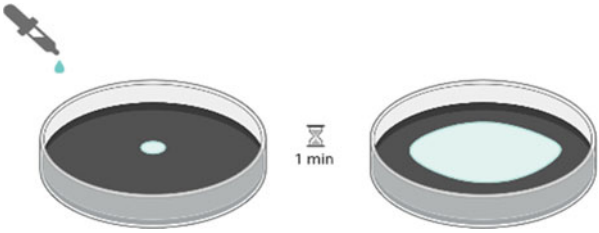


Fig. 1 schematic representation of oil displacement test performed in a petri dish

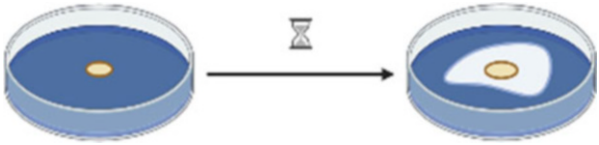


Fig. 2 Schematic representation of the DCPIP discolorization method performed in agar-based culture

colour spectrophotometrically (Hanson et al. 1993; Varjani and Upasani 2013). This test can be performed in agar-based culture, in the presence of hydrocarbons, showing a discoloration halo around fungal hyphae (Fig. 2).

2.4 Emulsification Activity

Ability of surfactants to stabilize emulsions between immiscible phases is commonly reported in terms of Emulsification Index, labelled E_{24}. The experiment is easy to perform and give a rapid and reliable answer. It consists in mixing surfactant solution with a non-polar solvent (kerosene or a 65:35 mix of decane:toluene) and homogenising them to form an emulsion (Fig. 3). Then, the emulsification index is calculated as:

$$E_{24} = \frac{\text{emulsion layer height}}{\text{total volume height}} \times 100$$

To have more detailed information about emulsion nature, thus establishing if it is oil-in-water emulsion or rather water-in-oil, it is possible to analyse emulsions through, for example, confocal microscopy technique. Specific dyes to stain oil and/or surfactant molecules can be used to better visualize emulsions structure (Blesic et al. 2017).

An intriguing feature of this experiment is the possibility to explore emulsification activity varying several parameters besides concentration, such as pH, salinity, temperature, etc. This is important because it allows to understand whether a compound is suitable for a certain application field or not. As an example, for marine bioremediation purpose, a BS must retain its emulsification ability even at high salt concentration or high pressure.

Fig. 3 schematic representation of surfactant-emulsion formation in the presence of an immiscible phase

Table 1 surface tension value of different absolute solvents. (Abe 2019)

Compound	γ (mN/m)
Water	72.75
Glycerin	63.4
Toluene	28.5
Ethanol	22.8
n-hexane	18.4
Olive oil	32.9

2.5 Surface Tension Measurement

Surface tension, indicated as γ, is a force to shrink the surface and has the dimension of force per unit length (mN/m) (Abe 2019). Surface tension measurement is the most accurate technique to detect the presence of surfactant compounds and the most widespread methods are the Du Nouy ring and the Wilhelmy plate. Experimentally these methods are very similar: a platinum ring in the case of Du Nouy method, and a platinum or glass plate for the other, are immersed into the testing liquid to form a liquid–solid–air interface. Then the force necessary to rise the ring and/or the plate is measured by the tensiometer.

This measurement requires a well specialized equipment, big volumes of sample (at least 10 ml) and it is more time-consuming measure. As a pro indeed, it gives no false positive and it allows to calculate Critical Micelle Concentration, that is a very important parameter when characterizing BS. In Table 1 some examples of γ value of absolute solvent are reported.

3 Screening of Marine Fungal Strains Biosurfactant Producers

A limited number of examples about BS producing strain screening, on fungal communities inhabiting in hostile environments, can be found in literature. Indeed, in most of these studies, these BS were only partially characterized, and their

activities analysed using the crude extracts, while only few examples of isolation, identification and characterization of BS compounds from marine fungi are present. Here are briefly reported example of screening on BS producing marine fungal strains, in which the afore described methods are used. In addition, some examples of identification and purification of the BS compounds are reported too.

The *Mycotheca Universitatis Taurinensis* (MUT) represents one of the most important banks of fungal biodiversity in Italy. The purposes of the MUT are the acquisition, identification, preservation and distribution of macro and micromycetes. In particular, it deals with fungal strains isolated from sites of interest, such as landfill, marine chronically contaminated sites etc.

At this regard, in the work of Bovio et al. (2017), 67 taxa were isolated from water samples and sediments, from a contaminated site in the Mediterranean Sea, along the coastal zone of Sicily. For many of the identified species it is the first time of their discovery in seawater and sediments. All isolated fungi were tested primarily for their capabilities to grow on crude Arabian Light oil as sole carbon source, in agar-based culture. Percentage of stimulation was estimated comparing mycelium growth in the presence and absence of crude oil. A percentage stimulation greater than 20% was considered as significant. All the isolated fungi were able to grow on crude oil, although only 24% showed a good percentage of stimulation. Fungi were classified according to their features and by molecular analysis. The most represented genera among the water collected samples were *Penicillium, Aspergillus* and *Trichoderma*. Four strains (*Aspergillus terreus*, *Trichoderma harzianum*, *Penicillium citreonigrum*, Lulworthaiales sp.) were then selected and their capability to degrade oil in liquid cultures was assessed using a modified protocol of the DCPIP colorimetric assay (Varjani and Upasani 2017). Three main parameters were valued: changing from blue to colourless of the culture medium, fungal biomass growth and disappearance of crude oil. Petroleum hydrocarbons degradation by the same fungi was analysed using GC-FID analysis, and two of them, *A. terreus* and *P. citreonigrum*, showed the highest potential for bioremediation.

Another good example of screening on marine fungi BS producing strains, in which simple and fast techniques are involved, is offered by the work of Maamar et al. (2020). Eighty-four filamentous were fungi collected from the port of Oran, Algeria, a chronically contaminated site, and identified through PCR analysis. BS production was proved testing ability of fungi to grow on Arabian Light oil in different concentration and use it as the sole carbon source. Twelve among the fungi selected gave positive results, showing a good growth rate in the presence of 1% and 5% crude oil. No one was able to growth in the presence of 0.1% crude oil. These 12 fungi belong mainly to *Aspergillus* and *Penicillum* genera. Further analysis on the 12 BS producing fungi were performed using different techniques, such as crude oil degradation assay using the decolorization method with DCPIP, oil-drop collapse test and oil-displacement test. Just five among the strains previously selected are the most promising BS producers and all of them belong to *Penicillum* genus.

A very similar investigation on marine fungi as producers of BS was conducted by Luepongpattana et al. (2017b). Sixteen strains were isolated from the coastal

areas of Koh Si Chang (Thailand) and identified. Screening for BS production was performed on the cell-free supernatant of each strain using the oil-displacement test and the Du Nouy method. Among them, the one identified as *Aureobasidium pullulans* (YTP6-14) gave the best results in both the experiments, reaching a surface tension value of 38.4 mN/m. Optimization of growth parameters condition of this strain was then performed.

4 Protein Biosurfactants Produced by Marine Fungi

Proteins are intrinsically partially amphipathic, being composed of amino acids with charged, polar, and nonpolar side chains. However, the amphipilic character of a protein is affected by several factors, such as its fold, conformational stability, flexibility and posttranslational modifications. Surface-active proteins achieve their function through different forms, ranging from peptides to large proteins, from monomers to large assemblies, and from highly ordered structures to disordered regions. Some of them can also undergo major conformational changes.

An array of surface-active proteins expressed during the life cycle of fungi, helps their growth in liquid, semiliquid, and moist substrates, as well as into the air and onto solid surfaces or interfaces.

Hydrophobins (HPB) are probably the most surface-active proteins known and the best studied. They are small, cysteine rich, amphiphilic proteins typical of filamentous fungi. Eight cysteines forming four disulfide bonds are highly conserved, while they show a high sequence diversity, suggesting multiple biological roles. Their amphipathic tertiary structure provides surfactant-like activity and allow their self-assembly into amphipathic layers at hydrophobic-hydrophilic interfaces (Berger and Sallada 2019). This feature can for example, lead to a stabilization of air bubbles and water/oil emulsions. Nonetheless, characterization of HPBs as effective BS and/or BE is scarce.

Class I HPB assemble into layers with a distinctive rodlet pattern. The rodlet characteristics are common to those of amyloid structures (usually associated with amyloid diseases) and their dissolution is only possible by treatment with strong acids. Class II HPB assemblies are less robust and form layers with varied morphology (Ren et al. 2013). The primary structures of Class I HPB are much less conserved (including size variations) than those of class II, which show a high degree of sequence conservation.

Ceratoplatanins (CP) have been included among the small fungal surface-active proteins (Sunde et al. 2017), even if few studies on this kind of activity have been reported so far, and their function is still a matter of debate. They were originally assumed to be HPB-like proteins, while recent studies highlighted structural and biological differences among these two classes of proteins. CP show four conserved cysteines forming two disulfide bridges, and lack of a large hydrophobic patch, differently from HPB. Another issue concerns the ability of CP to change the wettability of surfaces. HPB layers are known to invert the wettability of surfaces,

while CP can increase the polarity of surfaces, as reported by Frishmann et al. (2013). Another important difference concerns the tendency of HPB to form amyloid-like aggregate, that seems to be completely absent for CP.

Among 100 marine fungi from MUT, 23 were selected for their ability to produce foam in shaken cultures and analysed for the identification of new HPB by Cicatiello et al. (2016). Six fungi were then chosen as the best producers of surface-active proteins endowed with different characteristics. In particular, a protein produced by a facultative marine strain of *Penicillium chrysogenum* (SAP-*Pc*) showed remarkable emulsification ability of a water/olive oil mixture. Indeed, according to literature, marine isolates of *P. chrysogenum* were good sources of new interesting bioactive compounds (Imhoff 2016). SAP-*Pc* was identified as a previously unknown surface-active protein, and was able to form amyloid-like fibrils, like class I HPB. Emulsification ability was tested using "Dectol" (Decane:Toluene 65:35 v/v) as a model oil varying conditions as pH and concentrations, and the best result were obtained in aqueous buffer pH 7 ($E_{24} = 70\%$). What is noteworthy is that E_{24} do not vary even increasing protein concentration from 100 μg/ml to 400 μg/ml. Critical micelle concentration was obtained at 28 μg/ml (2 μM) corresponding in a decrease of surface tension of about 55 mN/m. At protein concentrations higher than about 200 μg/ml, a steep decline of the surface tension indicated the occurrence of another aggregation phenomenon, confirmed by Dynamic Light Scattering (DLS) and Atomic Force Microscopy analysis (Cicatiello et al. 2019).

Two Class I HPB from *Penicillium roseopurpur*eum and *Acremonium sclerotigenum* isolated from marine fungi in the screening previously described (Cicatiello et al. 2016) were purified. Different techniques (circular dichroism, dynamic light scattering and Thioflavin T fluorescence assay) were used to analyse their propensity to form fibrils (Cicatiello et al. 2017). It has been demonstrated that the formation of amyloid fibrils and the assembly morphologies depend on their interaction with specific surfaces. Properties of protein layers can be exploited as bio-coating for different materials.

Two of the fungal strains isolated from the oil spill contaminated site in the Mediterranean Sea, *A. terreus* and *T. harzianum* and selected for their ability to grow on crude oil as sole carbon source (Bovio et al. 2017), were exploited to verify the induction of BS production to solubilize and metabolize organic molecules. Among all the secondary metabolites that could possibly be involved in this process, secreted proteins were explored. Only one secreted protein for each fungal strain was purified and identified. Both proteins were ascribed to the family of CP (Pitocchi et al. 2020). For the first time these proteins were characterized as efficient BE and BS. In particular, the protein from *T. harzianum* identified as *ThCP*, showed a very good capability to decrease the surface tension of water to a minimum value of 36 mN/m at a CMC of $8x10^{-7}$ M (10 μg/ml).

4.1 Other Biosurfactants Produced by Marine Fungi

BS of different chemical composition are only rarely isolated and identified from marine fungi.

A marine sponge, *Fasciospongia cavernosa*, was collected from the Bay of Bengal region of the Indian peninsular coast (Kiran et al. 2009). Eight sponge-associated marine fungi were isolated from the sponge and screened for BS production, using different tests, hemolytic assay (Carrillo et al. 1996; Moran et al. 2002), drop collapsing test, oil displacement test. Hemolytic activity in blood agar plate (using both human and sheep blood) is an alternative and less used screening method to select BS producers. Indeed, BS can induce hemolysis at a given concentration and activity is revealed as a distinct clearance zone around colony. Among the eight isolated sponge-associated fungi, only one named MSF3 was selected as good BS producer and further characterized. First, different growth conditions were tested with the aim to improve BS production, varying different parameters. BS production in each condition was evaluated through emulsification activity of the cell free supernatant. Once assessed the best culturable conditions, only a partial chemical characterization was performed through TLC analysis. This led to the classification of the isolated BS as glycolipoprotein.

In the work of Luepongpattana et al. (2017b), only one fungal strain was selected from several strains collected in the Gulf of Thailand, as promising BS producers, *Aureobasidium pullulans* YTP6-14, a dimorphic fungus. Different growth conditions were tested for *A. pullulans* varying carbon sources, among which soybean and palm oil. Nevertheless, the maximum BS production yield (1.26 g/L) was achieved with a mixed carbon source (glycerol and glucose). BS production was first assessed through surface tension and oil displacement area measurement on cultural broth. The minimum surface tension was 32 mN/m (Luepongpattana et al. 2017a). Extraction of the crude BS was then performed using ethyl acetate and a critical micelle concentration of 39 mg/L was evaluated on the crude BS. Purification and characterization of the extracted BS led to identification of the compound as 5-hydroxy-2-decenoic acid delta-lactone, commonly named as massoia lactone (whose structure is reported in Fig. 4). This compound shows a great ability in reducing tension and, what is more, surface tension value does not increase neither varying pH of the solution from 2 to 12, neither increasing NaCl concentration up to 12% (w/v).

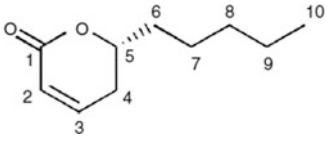


Fig. 4 chemical structure of massoia lactone biosurfactant

5 Future Perspectives

Natural products from some fungal genera, such as *Penicillium* and *Aspergillus*, have been intensively studied, however the great potential of secondary metabolites produced by these fungi has not yet been fully explored. Indeed, the marine microbial world remains largely unexplored even though recent studies have been aimed at exploring the diversity of ecosystems using molecular techniques. The main difficulty concerns the growth of most of marine microorganisms under laboratory conditions (Felczykowska et al. 2012; Jackson et al. 2015). A valid alternative could be culture-independent techniques that can allow the analysis of inaccessible marine microorganisms, avoiding the necessity of culturing them, and the discovery of new natural compounds with important biological activities. One of these molecular techniques is metagenomics that allows culture-independent study of microbial communities through the extraction and analysis of genetic material from any environmental sample, giving access to the total genetic pool of all the microorganisms present in that community and, as consequence, to their biosynthetic capacity (Gudiña et al. 2016).

The growing resource of fungal genome sequences can assist the exploration of new biosynthetic pathways of secondary metabolites and biosynthetic gene clusters. It has been demonstrated that many fungal secondary metabolites show significant differences in bioactivities although closely structurally related. Therefore, it is important to analyse the variety of structures offered by fungi, in order to unravel their biotechnological potential (Imhoff 2016). Interest in BS, as secondary metabolites produced by fungi, has been continuously increasing and the marine environment can be an important source of new and performing compounds.

Recent papers (Sánchez 2020; Spina et al. 2021) have clearly shown that fungi also play a crucial role in the degradation and mineralization of plastics. Indeed, fungi can extend their hyphae through substrates, thus they can explore and grow in places hardly accessible to other microorganisms. Plastics degradation by fungi is favoured not only by their powerful enzymatic system and their ability of adsorption (Karich et al. 2017), but also by the production of natural BS (Sunde et al. 2017), which enable them to use hydrophobic polymers as a source of nutrients.

More recent studies on fungal BS proteins, endowed with very good bioemulsifying activity, can pave the way for their massive production through recombinant expression in highly producing microorganism hosts, thus bringing them closer to their marketability.

References

Abe M (2019) Measurement techniques and practices of colloid and Interface. Springer, Singapore
Amend A et al (2019) Fungi in the marine environment:open questions and unsolved problems. Ecol Evol Sci 10(2):1–15

Aquino SF et al (2008) Methodologies for determining the bioavailability and biodegradability of sludges. Environ Technol 29:855–862. https://doi.org/10.1080/09593330802015508
Berger BW, Sallada ND (2019) Hydrophobins : multifunctional biosurfactants for interface engineering. J Biol Eng 13(10):1–8
Bhardwaj G, Cameotra SS, Chopra HK (2013) Biosurfactants from fungi: a review. J Pet Environ Biotechnol 4(6):1–6. https://doi.org/10.4172/2157-7463.1000160
Blesic M et al (2017) Evaluating the potential of natural surfactants in the petroleum industry: the case of hydrophobins. Pure Appl Chem 2(90):305–314. https://doi.org/10.1515/pac-2017-0703
Bodour AA, Miller-Maier RM (1998) Application of a modified drop-collpase technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. J Microbiol Methods 32:273–280
Bovio E et al (2017) Science of the Total Environment The culturable mycobiota of a Mediterranean marine site after an oil spill : isolation, identi fi cation and potential application in bioremediation. Sci Total Environ 576:310–318. https://doi.org/10.1016/j.scitotenv.2016.10.064
Carrillo PG, Mardaraz C, Giuliett AM (1996) Isolation and selection producing bacteria. World J Microbiol Biotechnol 12(1):62–64
Cicatiello P et al (2016) Marine fungi as source of new hydrophobins. Int J Biol Macromol 92:1229–1233. https://doi.org/10.1016/j.ijbiomac.2016.08.037
Cicatiello P et al (2017) Self-assembly of two Hydrophobins from marine fungi affected by interaction with surfaces. Biotechnol Bioeng 114(10):2173–2186. https://doi.org/10.1002/bit.26344
Cicatiello P et al (2019) Characterization of a surface-active protein extracted from a marine strain of Penicillium chrysogenum. Int J Biol Macromol 20:3242–3255. https://doi.org/10.3390/ijms20133242
Coelho LAS et al (2020) 'Mannosylerythritol lipids : antimicrobial and biomedical properties. Appl Microbiol Biotechnol 104:2297–2318. https://doi.org/10.1007/s00253-020-10354-z
Das P, Mukherjee S, Sen R (2008) Antimicrobial potential of a lipopeptide biosurfactant derived from a marine Bacillus circulans. J Appl Microbiol 104:1675–1684. https://doi.org/10.1111/j.1365-2672.2007.03701.x
Felczykowska A, Bloch SK, Nejman-faleńczyk B (2012) Metagenomic approach in the investigation of new bioactive compounds in the marine environment. ACTA Biochimica Polonia 59(4):501–505
Fenibo EO, Ijoma GN, Selvarajan R (2019) Microbial surfactants:the next generation multifunctional biomolecules for applications in the petroleum industry and its associated environmental remediation. Microorganisms 7:1–29
Frischmann A et al (2013) Self-assembly at air/water interfaces and carbohydrate binding properties of the small secreted protein EPL1 from the fungus Trichoderma atroviride. J Biol Chem 288:4278–4287. https://doi.org/10.1074/jbc.M112.427633
Gudiña EJ, Teixeira JA, Rodrigues LR (2016) Biosurfactants produced by marine microorganisms with therapeutic applications. Mar Drugs 14:38–43. https://doi.org/10.3390/md14020038
Hanson KG, Desai JD, Desai AJ (1993) A rapid and simple screening technique for potential crude oil degrading microorganisms. Biotechnol Tech 7(10):745–748
Harayama S et al (1999) Petroleum biodegradation in marine environments. J Mol Microbiol Biotechnol 1(1):63–70
Imhoff JF (2016) Natural products from marine fungi — still an underrepresented resource. Marine Drugs 14:19–38. https://doi.org/10.3390/md14010019
Jackson SA et al (2015) Metagenomics for the discovery of novel biosurfactants of environmental interest from marine ecosystems. Curr Opin Biotechnol 33:176–182. https://doi.org/10.1016/j.copbio.2015.03.004
Kaczorek E et al (2018) The impact of biosurfactants on microbial cell properties leading to hydrocarbon bioavailability increase. Colloids Interfaces 2:35–57. https://doi.org/10.3390/colloids2030035

Karich A et al (2017) Fungal unspecific Peroxygenases oxidize the majority of organic EPA priority pollutants. Front Microbiol 8(August). https://doi.org/10.3389/fmicb.2017.01463

Karthick A, Roy B, Chattopadhyay P (2019) A review on the application of chemical surfactant and surfactant foam for remediation of petroleum oil contaminated soil. J Environ Manag 243:187–205. https://doi.org/10.1016/j.jenvman.2019.04.092

Kiran GS et al (2009) Optimization and production of a biosurfactant from the sponge-associated marine fungus Aspergillus ustus MSF3. 73. Colloids Surfac B Biointerfac 73:250–256. https://doi.org/10.1016/j.colsurfb.2009.05.025

Kubicki S et al (2019) Marine biosurfactants: biosynthesis, structural diversity and biotechnological applications. Mar Drugs 17:1–30

Luepongpattana S, Thaniyavarn J, Morikawa M (2017a) Production of massoia lactone by Aureobasidium pullulans YTP6-14 isolated from the Gulf of Thailand and its fragrant biosurfactant properties. J Appl Microbiol 132:1448–1497. https://doi.org/10.1111/jam.13598

Luepongpattana S et al (2017b) Screening of biosurfactant production yeast and yeast-like fungi isolated from the coastal areas of Koh Si Chang. In: The 26th Annual Meeting of the Thai Society for Biotechnology and International Conference, pp 469–477

Maamar A et al (2020) Highlighting the crude oil bioremediation potential of marine fungi isolated from the port of Oran (Algeria). Diversity 12:196–215. https://doi.org/10.3390/d12050196

Matthay MA et al (2019) Acute respiratory distress syndrome. Nat Rev Dis Prim 5:18–30. https://doi.org/10.1038/s41572-019-0069-0

McClements DJ, Bai L, Chung C (2017) Recent advances in the utilization of natural emulsifiers to form and stabilize emulsions. Ann Rev Food Sci Technol 8:205–236. https://doi.org/10.1146/annurev-food-030216-030154

Moran AC, Martinez A, Sineriz F (2002) Quantification of surfactin in culture supernatants by hemolytic activity. Biotechnol Lett 24:177–180

Mujumdar S, Joshi P, Karve N (2019) Production, characterization, and applications of bioemulsifiers (BE) and biosurfactants (BS) produced by Acinetobacter spp.: A review. J Basic Microbiol 59:277–287. https://doi.org/10.1002/jobm.201800364

Mukherjee S et al (2009) Antimicrobial biosurfactants from marine Bacillus circulans : extracellular synthesis and purification. Appl Microbiol 48:281–288. https://doi.org/10.1111/j.1472-765X.2008.02485.x

Obi LU, Atagana HI, Adeleke RA (2016) Isolation and characterisation of crude oil sludge degrading bacteria. SpringerPlus 5:1946–1959. https://doi.org/10.1186/s40064-016-3617-z

Pang K et al (2016) " Marine fungi " and " marine-derived fungi " in natural product chemistry research : toward a new consensual definition. Br Mycol Soc 30:163–175. https://doi.org/10.1016/j.fbr.2016.08.001

Perfumo A et al (2010) Production and roles of biosurfactants and bioemulsifiers in accessing hydrophobic substrates. In: Handbook of hydrocarbon and lipid microbiology, pp 1502–1514. https://doi.org/10.1007/978-3-540-77587-4

Pitocchi R et al (2020) Cerato-Platanins from Marine Fungi as Effective Protein Biosurfactants and Bioemulsifiers. Int J Mol Sci 21:2913–2925. https://doi.org/10.3390/ijms21082913

Raghukumar C (2008) Marine fungal biotechnology:an ecological perspective. Fungal Divers 31:19–36

Raghukumar C, Damare SR, Singh P (2010) A review on deep-sea fungi: occurrence, diversity and. Bot Mar 53:479–492. https://doi.org/10.1515/BOT.2010.076

Rédou V et al (2015) Species richness and adaptation of marine fungi from deep-subseafloor sediments. Appl Environ Microbiol 81(10):3571–3583. https://doi.org/10.1128/AEM.04064-14

Ren Q, Kwan AH, Sunde M (2013) Invited review two forms and two faces, multiple states and multiple uses: properties and applications of the self-assembling fungal Hydrophobins. Pept Sci 100(6):601–612. https://doi.org/10.1002/bip.22259

Sánchez C (2020) (2020) 'fungal potential for the degradation of petroleum-based polymers: an overview of macro- and microplastics biodegradation'. Biotechnol Adv 40:107501. https://doi.org/10.1016/j.biotechadv.2019.107501

Satpute SK et al (2010) Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. Biotechnol Adv 28(4):436–450. https://doi.org/10.1016/j.biotechadv.2010.02.006

Smith ML et al (2020) Biosurfactants:a covid-19 perspective. Front Microbiol 11(1341):1–8. https://doi.org/10.3389/fmicb.2020.01341

Spina F et al (2021) Low density polyethylene degradation by fi lamentous fungi *. Environ Pollut 274:116548. https://doi.org/10.1016/j.envpol.2021.116548

Sunde M, Pham CLL, Kwan AH (2017) Molecular characteristics and biological functions of surface-active and surfactant proteins. Annu Rev Biochem 86:585–608. https://doi.org/10.1146/annurev-biochem-061516-044847

Tao W et al (2019) 'Designer bioemulsifiers based on combinations of different polysaccharides with the novel emulsifying esterase AXE from Bacillus subtilis CICC 20034. Microbial Cell Factories BioMed Central 18:1–10. https://doi.org/10.1186/s12934-019-1221-y

Uzoigwe C et al (2015) Bioemulsifiers are not biosurfactants and require different screening approaches. Front Microbiol 6:1–6. https://doi.org/10.3389/fmicb.2015.00245

Varjani SJ, Upasani VN (2013) Comparative studies on bacterial consortia for hydrocarbon degradation. Int J Innov Res Sci Eng Technol 2(10):5377–5383

Varjani SJ, Upasani VN (2017) Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactant. *Bioresour Technol* 232:389–397. https://doi.org/10.1016/j.biortech.2017.02.047

Waghmode S et al (2020) Planococcus species – an imminent resource to explore biosurfactant and bioactive metabolites for industrial applications. Front Bioeng Biotechnol 8(August):1–8. https://doi.org/10.3389/fbioe.2020.00996

Lignin Fungal Depolymerization: From Substrate Characterization to Oligomers Valorization

Shruthi Meenakshisundaram, Estelle Léonard, Claire Ceballos, and Antoine Fayeulle

Abstract Renewable resources obtained via sustainable methodologies provide an alternative to reduce the dependency on fossil resources. Lignin is the second most abundant class of macromolecules on earth. This complex biopolymer is the major source of phenolic compounds since its precursors are three types of phenylpropane units (or monolignols), i.e., conferyl alcohol (G), sinapyl alcohol (S), and *p*-coumaryl alcohol (H). Large amounts of lignin are recovered from the pulping industry using various processes such as Kraft, sulfite, soda anthraquinone, etc., due to its undesirability and high solubility during the cooking process. More recently, lignin is also being recovered during the pretreatment steps (Hydrolysis, Ionic Liquid, Organosolv processes) in biorefinery processes. Based on the extraction procedure and type of biomass used, the molecular structure of lignin will vary and has to be characterized for valorization. Due to the complexity of the structure of lignin, a combination of complementary techniques is used to characterize lignin. Various analytical techniques such as spectroscopy, chemical degradation, thermal degradation, chromatography, and the methods used to visualize the physical structural changes in lignin that help to determine the qualitative and quantitative properties bring different information. Of the techniques elaborated, magnetic resonance and Gel Permeation Chromatography (GPC) are found to be proficient in the elucidation of these complex biopolymers. Aromatic compounds are the basic building blocks in lignin and therefore, their potential in various applications is quite high. However, lignin's recalcitrance makes it difficult to break it down for further applications and requires high temperatures and high pressure for the same. Therefore, fungal depolymerization is a more economical alternative to break down lignin without damaging the cellulose and hemicellulose. Lignin decaying fungi excrete a powerful enzyme cocktail that generates highly reactive mediators which in turn react with the polymer and trigger extracellular depolymerization. The resulting low molecular weight soluble molecules such as vanillin, syringaldehyde, syringic acid, etc. are valuable for further applications and thus the optimization of fungal

S. Meenakshisundaram · E. Léonard · C. Ceballos · A. Fayeulle (✉)
Université de technologie de Compiègne, ESCOM, TIMR (Integrated Transformations of Renewable Matter), Centre de Recherche Royallieu, CS 60 319, Compiègne Cedex, France
e-mail: antoine.fayeulle@utc.fr

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_16

lignin depolymerization and recovery of oligomers before their uptake by fungal cells are ongoing domains of research. Indeed, some of the generated oligomers can be used in the generation of other valuable products such as chemicals and materials through green chemistry. Lignin-derived phenols are also used in bioremediation strategies. Besides, the use of fungal lignin depolymerization has great valorization potential to produce renewable energies such as biomethane, biohydrogen, and bioethanol without generating products that may be inhibitory in the downstream process. Therefore, with the increasing lignin-centric biorefineries, there is strong interest for fungal lignin depolymerization in both valorization and application approaches.

Keywords Analytical techniques · Fungal ligninolytic enzymes · Green chemistry · Lignin extraction · Lignin valorization

1 Introduction

Lignin is a widespread biopolymer entering the composition of all terrestrial plant biomasses with high recalcitrance to biodegradation. Indeed, along with impermeabilization and rigidification of plant tissues, this high molecular weight and hyper-variable polymer protect other polysaccharides of cell walls, i.e., cellulose and hemicelluloses, from microbial enzymatic attacks. In order to obtain such molecules, plants cells excrete different phenyl propanol precursors called monolignols, which are then polymerized in apoplastic domains through a random radical-based polymerization process involving notably laccases (Hoegger et al. 2006) and peroxidases (Tobimatsu and Schuetz 2019). These monolignols are mainly composed of *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, which correspond respectively to H-unit, G-unit, and S-unit in the polymerized lignin structure (Kleinert and Barth 2008).

This potential non-food natural source of biobased aromatics derivatives makes lignin attractive for the development of biorefinery processes (Mahmood et al. 2016). Indeed, large quantities of lignin are produced in nature with estimated amounts ranging from 5 to 36 $\times$ 10^8 tons per year (Gellerstedt and Henriksson 2008). Concerning the significance of industrial residues derived from this total resource, biomass refinery and pulp/paper industries produce yearly 6.2 $\times$ 10^7 and 5 $\times$ 10^7 tons respectively mostly under the form of technical lignin such as Kraft lignin, lignosulfonate, and soda lignin (Zakzeski et al. 2010). However, the recalcitrance of the lignin polymer to degradation complexifies also its valorization in industrial processes, and lignin residues are by now mostly burned for energy supply or wasted (Weng et al. 2021).

Existing lignin depolymerization techniques include thermochemical approaches such as pyrolysis or microwave treatment, and chemical protocols with acid-, base-, metallic, ionic liquid assisted, subcritical/supercritical fluids assisted, or oxidative catalysis. These techniques lead to high lignin depolymerization rates but require severe conditions such as high pressure and temperature leading to high-energy

consumption and the use of environmentally threatening substances. Thus, the third type of approach based on biocatalysts including enzymes or whole microorganisms aims at developing more eco-compatible and chemoselective lignin depolymerization bioprocesses (Chio et al. 2019).

Bacteria attracted interest in particular due to the pre-existence of industrial processes more adapted to bacterial cultivation (Xu et al. 2018). Ligninolytic bacteria described as far belong to *Sphingomonas*, *Pseudomonas*, *Rhodococcus*, *Nocardia,* and *Streptomyces* genera (Bugg et al. 2016) and in particular, *Rhodococcus jostii*, *Pseudomonas putida*, *Acinetobacter* sp. and *Amycolatopsis* sp. were investigated due to their depolymerization abilities of high molecular weight lignin molecules (Salvachúa et al. 2015). However, the lignin depolymerization ability is described as scarcer within the bacterial biodiversity, and the conversion efficiency is much lower than in fungi (Chio et al. 2019).

Thus, the goal of this chapter is to provide up-to-date information required for the development of lignin fungal depolymerization bioprocesses including natural and technical lignin properties, characterization techniques of lignin molecules, mechanisms involved in fungal lignin depolymerization, and main valorization ways for the so produced molecules.

2 Native Lignin Chemical Properties

Lignin is the most abundant polymer based on aromatic moieties in nature (Calvo-Flores and Dobado 2010) and possesses numerous applications. Most of the lignocellulosic biomass is found in the macro- and micro-fibrils of the rigid walls of plant cells, which essentially comprise three biopolymers (Fig. 1): cellulose, hemicellulose, and lignin (Bertella and Luterbacher 2020).

2.1 Native Lignin Chemical Structure

The structure of native lignin is of crucial interest from a long time ago. Indeed, if lignin was considered to be homogeneous with an infinite molecular size, other scientists thought it to be a finite heteropolymer depending on its location within the wood. That is how in 1967, Brown et al. (1967) published in Nature that, when lignin was isolated enzymatically by treatment with the brown rot fungi *Poria monticola* and *Lenzites trabea*, and extracted properly, lignin was found to be a finite polymer with three peaks of characteristic molecular size, and thus differing in size according to its location in wood cell walls.

Chemically speaking, its phenolic high molecular mass biopolymer (600–15,000 kDa) is composed of three monomers, coumaryl-, coniferyl- and sinapyl alcohol (Fig. 2) found with various abundance (Kleinert and Barth 2008) according to the species. As softwoods like pine are composed mainly of guaiacyl

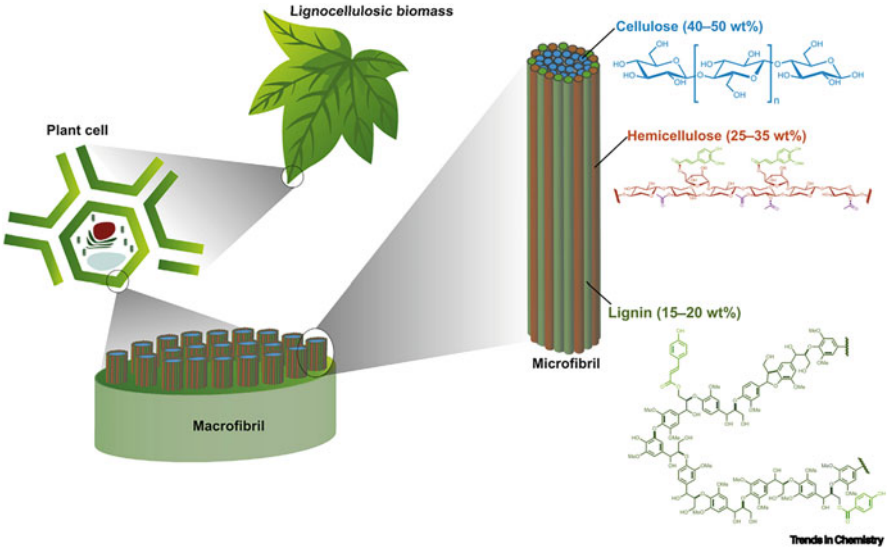


Fig. 1 Overview of the structure of lignocellulosic biomass. (Reuse (print) with permission of Bertella, S., Luterbacher, J.S., 2020. Lignin Functionalization for the Production of Novel Materials. TRECHEM 2, 440–453. https://doi.org/10.1016/j.trechm.2020.03.001. Copyright (2020) Elsevier)

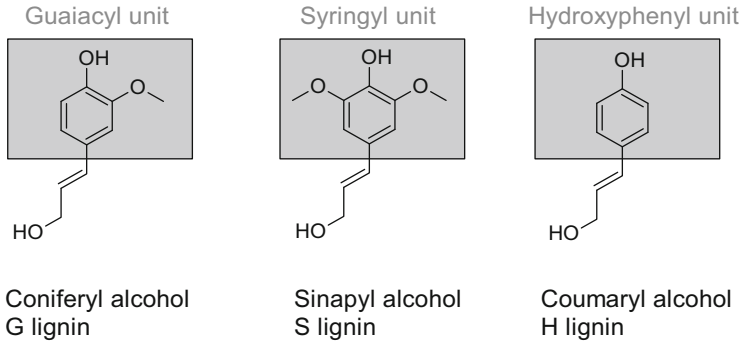


Fig. 2 Structures of three major monomers of lignin and their units

units, hardwoods such as eucalyptus contain guaiacyl but more importantly syringyl units, and for grass lignin, phenolic monomers, together with the syringyl and guaiacyl units, are mostly found (Rinaldi et al. 2016).

These variations in monolignol proportions led to a try for a phylogenetic tree of lignin monomer composition distribution (Vanholme et al. 2010) depending on lineages (Fig. 3).

Indeed, lignin evolved with the need for plants to adapt to terrestrial life. If complete biosynthesis first appeared in moss and was absent from green algae, recent studies detected apparent lignin in *Calliarthron* red alga (Martone et al.

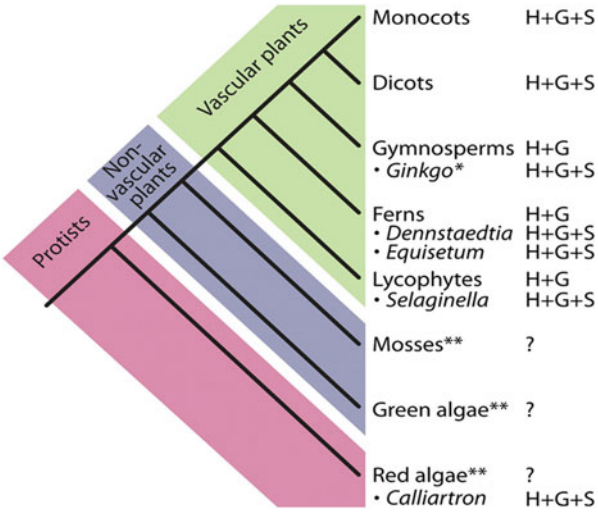


Fig. 3 Phylogenetic tree showing the distribution of lignin monomer composition across major lineages. *, S-units are only found in cell cultures of Ginkgo, not in wood. **, Lignin-like structures are reported in some mosses and green algae, but the presence of real lignin in these nonvascular species remains questionable; red algae have been barely studied. (Reuse (print) with permission of Vanholme, R., Demedts, B., Morreel, K., Ralph, J., Boerjan, W., 2010. Lignin Biosynthesis and Structure. Plant Physiology 153, 895–905. https://doi.org/10.1104/pp.110.155119. Copyright (2010) Oxford Academic)

2009). Logically, lignin in the secondary cell walls of *Calliarthron* may have evolved to resist the breaking waves, similar to lignin in the walls of vascular plants that provides biomechanical support.

So with these complex mixed structures of aromatic units, several expected and unexpected biomolecules (Sederoff et al. 1999) can be extracted, analyzed (Ralph and Landucci 2010), and used as a bioresource for chemistry.

2.2 *Native Lignin Properties*

Lignin possesses antioxidant properties, for example when added to polypropylene (Pouteau et al. 2003). However, if the phenol content and intrinsic reactivity are important, the most important factor to take into account is its solubility.

Another application is related to the enzymatic degradability (Pollegioni et al. 2015) of lignin, and especially the ability of fungal laccase for "lignin-barrier" breakdown of lignocellulose (Leonowicz et al. 2001). Indeed, nanocapsules can be prepared and can be loaded with hydrophilic substances, which can be released for agricultural applications. As seen in Fig. 4, when hollow lignin nanocontainers with varying amounts of cross-linker toluene diisocyanate (TDI) were charged with a fluorescent probe, the presence of laccase from *Xylaria* sp. clearly showed the better

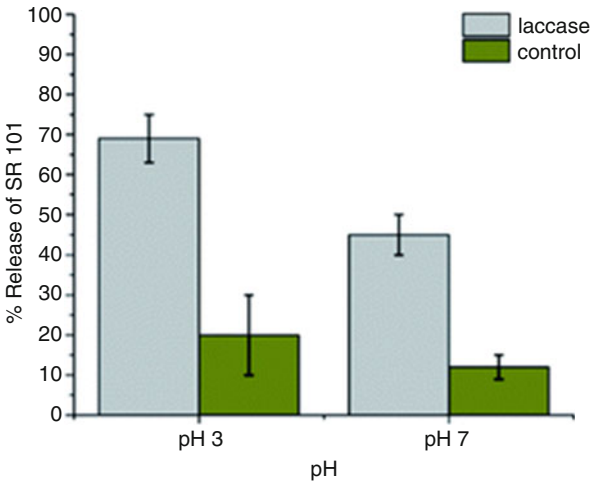


Fig. 4 Release profile of the lignin nanocontainers prepared with 0.2 mM of TDI after degraded by Xylaria sp. (Reuse under Creative Commons Attribution 3.0 Unported Licence. Yiamsawas, D., Baier, G., Thines, E., Landfester, K., Wurm, F.R., 2014. Biodegradable lignin nanocontainers. RSC Adv. 4, 11661–11663. https://doi.org/10.1039/C3RA47971D)

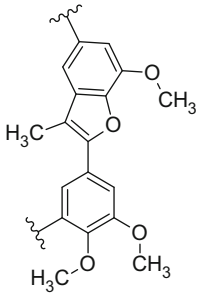


Fig. 5 Phenylcoumarone core

release of the probe in acidic or neutral pH, compared to control (Yiamsawas et al. 2014).

Lignin is also fluorescent (Olmstead and Gray 1997). This could result from the energy transfer from lignin chromophores, excited in 240–320 nm, to an acceptor which seems to be of phenylcoumarone family (Albinsson et al. 1999), as represented in Fig. 5.

3 Impact of Extraction Procedures on Lignin Structure

As presented in part 2, lignin refers to a large group of aromatic biopolymers with an empirical formula of $C_{31}H_{34}O_{11}$. Lignin is the major source of phenolic compounds and its precursors are three types of phenylpropane units (or monolignols) which are connected by C-C and C-O-C (ether) linkages. Lignin has a highly branched chemical structure with different functional groups such as methoxy (CH_3O),

carboxyl (COOH), and carbonyl (C=O) that influence its reactivity. Therefore, lignin is found in different forms and is extracted by different processes. The main target of most of these processes is not lignin but allows isolation and recovery of lignin due to its undesirability. Huge amounts of lignin are produced as a co-product of cooking processes in the paper and pulp industries using industrial practices such as Kraft, sulfite, and soda-anthraquinone. Native lignin is insoluble in water, and the cooking process solubilizes lignin, which helps to separate it from the fiber fraction. Nowadays, lignin is also recovered as part of chemical, physicochemical and enzymatic pretreatment methods used in the biorefinery concepts (Tribot et al. 2019). The structure of the isolated lignin is characterized in terms of aromatic units (GHS), interunit linkages, functional groups (aliphatic alcohols, aromatic alcohols, and carboxylic acids), side chains, and molar mass distribution (Li and Takkellapati 2018).

3.1 Kraft Lignin

Kraft pulping process is still the dominant commercial process as it requires shorter cooking time, provides efficient recovery of pulping chemicals, and is insensitive to the presence of impurities than other chemical pulping processes. From about 130 million tons of Kraft pulp produced worldwide annually, 55–90 million tons of Kraft lignin is produced and only 2% of it is used for value-added products, while the rest are used for energy purposes. Kraft process treats wood chips with a white liquor which is a mixture of sodium sulfide (Na_2S) and sodium hydroxide (NaOH) at high temperatures (150–180 °C) for 2 h. Major portions of lignin get solubilized during the treatment and the spent pulping mixture called the "black liquor" still contains a significant amount of lignin, which is used to produce fuel and Kraft lignin. An acidification process, which lowers the pH of the black liquor is used to precipitate the lignin and is recovered by filtering and washing. The characteristics of the resulting lignin are dependent on the wood source, pulping conditions, and degree of delignification (Baptista et al. 2008; Fernández-Costas et al. 2014; Tribot et al. 2019).

Delignification during the Kraft pulping process occurs in three phases, namely, initial phase (up to 150 °C), bulking phase (150–170 °C), and residual phase (final treatment at 170–180 °C) based on the cooking temperature. During the first two phases, delignification is first-order dependent on the lignin concentration. The degradation reactions occur when the hydroxide and hydrosulfide anions cause the lignin polymer to fragment into smaller units by cleaving linkages between the phenylpropane units. This releases free phenolic hydroxyl groups, which increase the affinity of attachment of hydrophilic groups to the cleaved fragments making them easily dissolvable in alkaline liquor. During the residual phase, delignification is dependent on the hydroxide ion concentration and since the alkalinity is lower than the previous phases, there is a low rate of lignin dissolution. At low alkalinity, condensation reactions occur that increases the molecular size of dissolved lignin

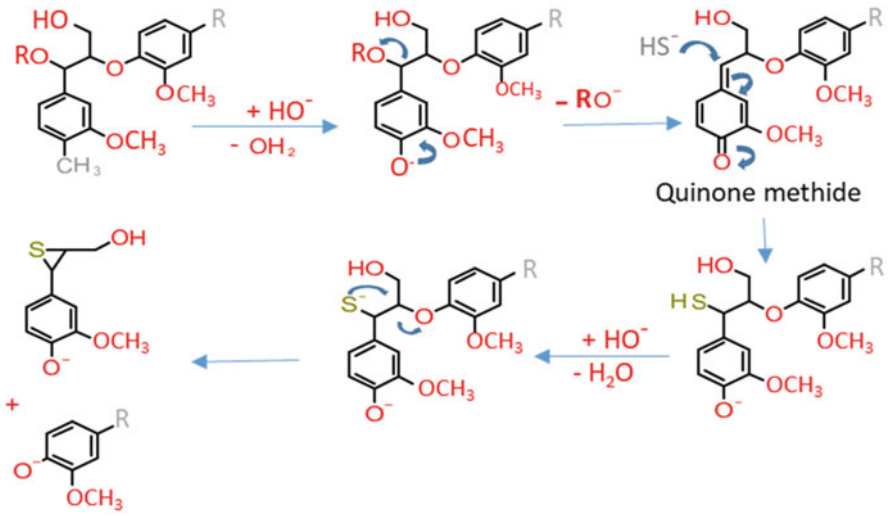


Fig. 6 Phenolic β-*O*-4 cleavage during reactions involved in the Kraft lignin process. (Reuse under Creative Commons Attribution 4.0 Licence. Ekielski, A., Mishra, P.K., 2021. Lignin for Bioeconomy: The Present and Future Role of Technical Lignin. IJMS 22, 63. https://doi.org/10.3390/ijms22010063)

fragments leading to precipitation. The lignin-carbohydrate complexes (LCCs) have both alkali-stable and alkali-sensitive covalent linkages. The alkali-sensitive linkages are broken when the alkalinity is high during the initial phases of the Kraft cooking process. The alkali-stable linkages survive the Kraft cooking process and it has been demonstrated with model compounds that some alkali-stable LCC may be formed during the process. This adds to the difficulty of removing lignin at the residual phase (Axegard and Wiken 1983; Sjostrom 1993; Smook 1992).

The chemical structure of residual lignin changes significantly at the beginning and the end of the cooking process (as shown in Fig. 6). During the initial phase, the cleavage of α-aryl ether and β-aryl ether bonds in the phenolic units resulting in the formation of quinone methide (C_7H_6O, a highly reactive intermediate) is the most prominent degradation mechanism. In the presence of hydrosulfide ions, the quinone methide is restored with the aromaticity of the aromatic ring resulting in the formation of benzyl mercaptide structure. In the absence of hydrosulfide ions, as is the case during soda pulping, the quinone methide is converted into an alkali-stable enol ether structure. Both the reaction pathways are observed in the Kraft process and the degradation process in the initial phase continues until all phenolic units of the α- and β-aryl ether types, both those originally present and those liberated during the initial phase, have reacted. The lignin that is dissolved is of low molecular weight and highly phenolic. During the bulk phase, drastic conditions such as extreme alkaline conditions are required for further lignin degradation, which occurs by the cleavage of β-aryl ether linkages in non-phenolic structures. Though there seems to be very few structural changes of lignin in the bulk phase, this liberates new phenolic

structures. These act as the starting point for the two types of cleavage reactions that occur in the initial phase and continue until the point where dissolution occurs. Simultaneously, condensation reactions occur as and when the quinone methide intermediates are formed to counter-balance the degradation and fragmentation of lignin. Though the process of cross-linking of lignin units have been the subject of debate in many publications, an increase in molecular weight of residual lignin and decrease in the phenolic groups is observed during the residual phase of Kraft pulping (Gellerstedt and Lindfors 1984; Mattsson et al. 2017; Tribot et al. 2019).

3.2 Sulfite Lignin/Lignosulfonate

Aqueous sulfur dioxide (SO_2) and a sulfite base (calcium, sodium, magnesium, ammonium salts) are used in the sulfite cooking process, which occurs over a wide range. The cation base for the pulping process is dependent on the pH. The acid sulfite process (pH 1–2) utilizes calcium while magnesium is used in the neutral sulfite process (pH 5–7). At higher pH (pH 9–13), the alkaline sulfite process utilizes ammonium or sodium cations. In the sulfite cooking process, lignin depolymerization occurs through the incorporation of large amounts of sulfur as sulfonate groups onto lignin. The sulfonate groups are added to the benzylic carbon atom of the lignin phenylpropane (C9) unit (as shown in Fig. 7). In acidic sulfite cooking, calcium salts are used to precipitate the lignosulfonate. Membrane technology by combining ultrafiltration and diafiltration is then used for effective lignin fractionation. Calcium lignosulfonates are used in the tanning, preparation of vanillin as lignosulfonates contain higher sulfur, carbohydrates, and inorganic impurities (Li and Takkellapati 2018; Tribot et al. 2019).

According to a study by Deshpande (2016), the temperature of the cooking process influences the delignification and side reactions. At higher temperatures, the formation of thiosulfate occurs which is an undesired side reaction. Lignin condensation reactions were observed at low total SO_2 content in acid sulfite cooking of spruce and pine. The covalent linkages between lignin and hemicellulose present in native wood were conserved in sulfite pulp samples whereas α-ethers were cleaved in the pulps. The authors also found that even though the LCC are sulfonated, its cleavage is important for sulfonated lignin to be dissolved in the liquor. Therefore, lignin is still retained in pulps (Deshpande 2016).

3.3 Soda Lignin

Pulp industries that use non-wood biomass such as sugarcane and straw produce papers using the soda anthraquinone (AQ) process that results in a by-product called soda lignin. The biomass is treated with NaOH at temperatures below 165 °C and added with 0.1% anthraquinone to prevent the attack of hydrocelluloses in the

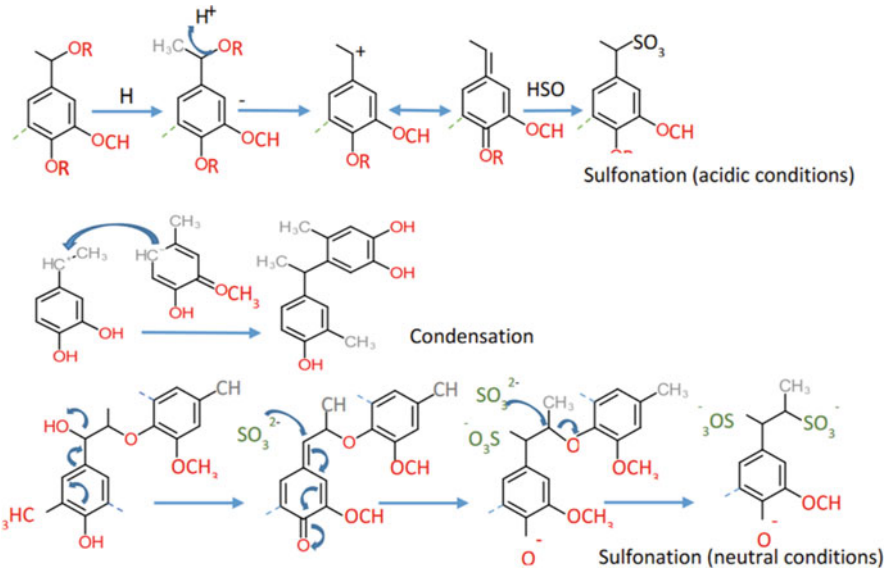


Fig. 7 Reactions involved in the sulfite pulping process under acidic and neutral conditions. (Reuse under Creative Commons Attribution 4.0 Licence. Ekielski, A., Mishra, P.K., 2021. Lignin for Bioeconomy: The Present and Future Role of Technical Lignin. IJMS 22, 63. https://doi.org/10.3390/ijms22010063)

alkaline medium and to catalyze delignification. The black liquor obtained by this process is treated similarly to that of the Kraft process, but the soda lignin obtained as a result is purer than Kraft lignin due to the absence of sulfur in the cooking process (Tribot et al. 2019).

In a study where dissolved lignin was obtained from eucalyptus black liquor, it was observed that the degree of delignification could be controlled by the reaction time at the highest temperature of the process. In the soda-method delignification, condensation reactions were increasingly observed for the first 3 h resulting in high molecular weight lignin. From the third hour to the fourth hour, large amounts of lignin dissolution leading to high lignin removal efficiency were observed. At longer reaction times up to the fifth hour, cleavage of β-*O*-4′ and β-β linkages were the main reactions that led to lignin degradation (as shown in Fig. 8), suggesting that lengthy alkaline treatment cause depolymerization of lignin. The syringyl (S) unit contains two methoxy groups and is easily oxidized in alkaline conditions compared to the guaiacyl (G) unit. The preferential removal of S-unit lignin in soda-AQ pulping results in the decrease of the S/G ratio. Therefore, the S/G ratio of lignin could be used as a parameter to evaluate the biomass recalcitrance, especially in non-wood biomass (Rencoret et al. 2013; Xue et al. 2019).

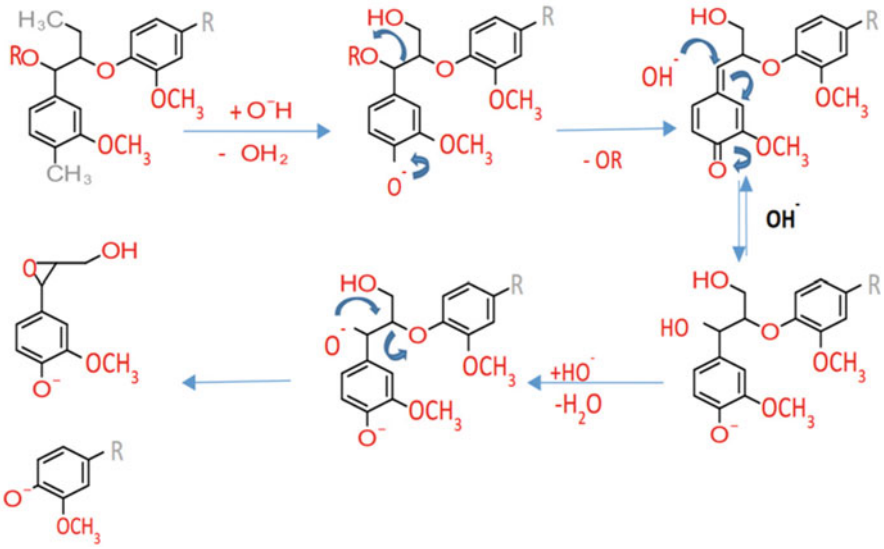


Fig. 8 Reactions involved in the soda anthraquinone (AQ) process (cleavage of the α and β ether linkage during the soda/alkaline process). (Reuse under Creative Commons Attribution 4.0 Licence. Ekielski, A., Mishra, P.K., 2021. Lignin for Bioeconomy: The Present and Future Role of Technical Lignin. IJMS 22, 63. https://doi.org/10.3390/ijms22010063)

3.4 Hydrolysis Lignin

With the increase in investments in cellulosic ethanol, a considerable amount of lignin production is expected in the coming years but its chemical structure is poorly studied. The lignin structural changes during the pretreatment methods employed are important to understand, as the chemical composition of lignin rather than its quantity affects the further enzymatic hydrolysis of cellulose and xylan. Based on the type of pretreatment method employed and the type of biomass, the degree of separation between the lignin and the carbohydrates components varies (Tribot et al. 2019).

Studies on dilute acid pretreatment of corn stover showed that at higher pretreatment temperatures, there is an increase in phenolic groups and carboxylic moieties while there is no change in the number of aliphatic hydroxyl moieties. The cleavage of β-O-4 linkages occurs by protonation, which is followed by dehydration of the benzylic-hydroxyl group, which leads to the formation of benzylic carbocation (as shown in Fig. 9). The cleavage of β-*O*-4 linkages leads to an increase in lower molecular weight lignin fragments initially and at a critical temperature (180 °C), condensation reactions (by the formation of radicals or C-C bonds) overtake the lignin depolymerization reaction by cleavage of aryl ether linkages. This causes the condensed lignin to migrate out and then redeposit as droplets on the surface of the cell walls. This acts as a physical barrier and leads to nonproductive binding of the

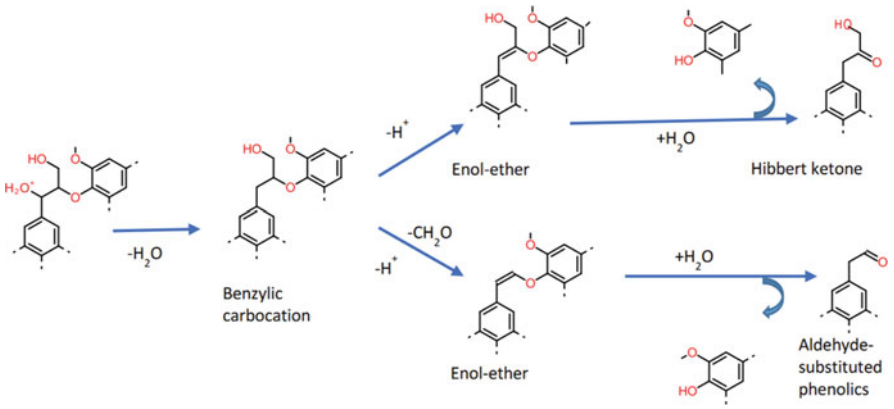


Fig. 9 Reactions involved in acid hydrolysis of lignocellulosic biomass. (Reuse under Creative Commons Attribution 4.0 Licence. Ekielski, A., Mishra, P.K., 2021. Lignin for Bioeconomy: The Present and Future Role of Technical Lignin. IJMS 22, 63. https://doi.org/10.3390/ijms22010063)

enzyme with lignin (Moxley et al. 2012). Similar changes are observed in other thermo-chemical pretreatment methods such as hot water, steam explosion, and ammonia fiber expansion. Therefore, there is only a small extent of lignin removal from various biomass using thermochemical pretreatments. Further catalytic depolymerization methods such as pyrolysis, hydrogenation, or oxidative approaches are needed to complete the fractionation of lignin to aromatic compounds (Chen et al. 2017; Pu et al. 2015).

3.5 Ionic Liquid Lignin

During ionic liquid (IL) pretreatment of wheat straw, cleavage of β-O-4 linkages on S units results in a total increase of phenolic hydroxyl content in lignin with a disproportionate increase in the types of phenolic hydroxyl groups. With the increase in the pretreatment time, the lignin's molecular weight decreases and its molecular weight distribution broadens. Simultaneously, lignin's fragments are recondensed during the ionic liquid pretreatment (Hong et al. 2017). The addition of phenol, ethanol, or mesoporous catalysts is used to prevent repolymerization and char formation. The addition of elements such as Al and Ni to mesoporous catalysts helps to further improve depolymerization by cleavage of ether bonds and stabilizing of highly reactive intermediates (Chen et al. 2017). Lignin recovered by the IL pretreatment exhibits similar properties to that of organosolv lignin but are not as commercially available as organosolv lignin due to the high costs of ILs (Hong et al. 2017).

3.6 Organosolv Lignin

Organosolv extraction process, on the other hand, preferentially cleaves the LCC ether linkages and favors intramolecular condensation reactions. This leads to substantial delignification without significantly altering the chemical structure of lignin. The isolated lignin forms droplets that migrate and are redistributed on the surface of pretreated biomass. This increases the pore volume, initially, which favors the enzymatic adsorption. Later, the lignin droplets inhibit the adsorption of enzymes such as cellulase. The resulting high molecular weight lignin is similar to a complex native lignin structure and can be recovered by precipitating it out with acidic water (Koo et al. 2012). The organosolv lignin has lesser ash than its Kraft counterpart, which helps to have cleaner combustion in the subsequent steps (de la Torre et al. 2013).

Therefore, from all the different methods used to extract lignin, it is observed that delignification is correlated with the formation of functional groups that helps lignin to dissolve in the process liquor (Evstigneyev and Shevchenko 2019).

4 Analytical Methods Adapted to Lignin Characteristics

In the 1960–1970s, prominent structures in softwood and hardwood lignins were proposed by Adler (1977). Since then, many models of each linkage and lignin unit type in literature were built. The difficulties in lignin analysis reside in its complex structure associated with plant cell wall hemicelluloses (Sjostrom 1993) which interfere with the quantification of lignin (Brunow and Lundquist 2010). The acknowledgment concerning the structure of lignin is essential to understand the properties of lignin and the effect of pretreatment. There is no universal method to characterize the lignin but a combination of complementary techniques to provide insights into the structure of the polymer. Some reviews compiled recent innovations in studying lignin (Ghaffar and Fan 2013; Lupoi et al. 2015).

Most of the characterization methods of lignins are summarized in Table 1. The advantages or disadvantages of these techniques are stated. The composition of lignin, its structure and linkages, and destructive analysis techniques are presented. Spectroscopic techniques, thermal and physical methods will be then described. Finally, the last part will be devoted to steric exclusion chromatography, one of the essential techniques for the study of soluble lignins.

4.1 General Composition of Lignin

Lignin as well as its different impurities such as ash, sugar, and moisture are determined by the different standards such as ISO, ASTM, or TAPPI (as shown in

Table 1 Main methods for lignin analysis

Methods	Techniques	Information	Advantages	Disadvantages
Chemical degradation	Thioacidolysis, DFRC, nitrobenzene oxidation	S/G ratio, monolignols quantification	Well-standardized techniques	Tedious Destroys the sample Toxicity
Spectroscopic methods	FT IR	Functional groups	Non-destructive, high throughput	Non-destructive Non-invasive Sensible to water
	Raman	Functional groups	Complimentary to IR Non-destructive	Weak pics, fluorescence masks signal
	UV	Phenol content	Cheap, convenient	The extinction coefficient for lignin quantification difficult to obtain Absorbance in UV of extraneous molecules
	NMR	Aliphatic hydroxyl content Aromatic hydroxyl content Inter-unit bonding patterns S/G ratio	Multi-dimensional NMR Many structural information	Spectral overlap Low throughput Low sensitivity, spectral resolution Expensive Incomplete acetylation of lignin leading not complete NMR
Physical properties	Optical microscopy	Morphology	Fast analysis Non-destructive	Expensive apparatus No structural information
	SEM	Morphology, size, crystalline structure		
	TEM	Crystalline nature		
	X-ray	Ultrastructure, fiber diameter		
	FE-SM	Morphology, surface changes		
	AFM	Morphology		
Molecular structure	SEC	Molecular distribution Weight average molar mass Polydispersity	Large detectable masses	Condensation effects Measurement can vary following the technique
Chromatography	GC/MS, oxidation	S/G ratio, Phenolic content	Selective	Results depend on condensed structures in lignin Destructive Concern volatiles molecules
	Aminolysis	Phenolic content, Aliphatic hydroxyl content		

Table 2 Determination of the general composition of Kraft lignin

	Technique	Notes
Total lignin content Klason lignin	Gravimetry (Tappi T222 om-15)	0–30% wt lignin content for wood and pulp Overestimation of the lignin which condenses with proteins or chitins
Acid soluble lignin	UV-Vis Spectroscopy (Tappi UM-250)	A reliable method for wood and pulp Total lignin corresponds to insoluble and soluble lignin
Van Soest, acid detergent lignin (ADL)	Gravimetry (Hatfield et al. 1994; Van Soest and Wine 1968)	An alternative procedure to Klason lignin and replaces the use of corrosive sulfuric acid by permanganate oxidation to remove protein, hemicelluloses, and other components from the cellulose and lignin
Ash content	Ashing Tappi method T211 om-12	Several temperatures (525–950 °C) used
Carbohydrates	Ion chromatography (SCAN-CM 71) Spectroscopy UV	Measure free or total sugars
Metals	Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	Al, Cu, Fe, Mg, Ca, K...
Sulfur ions	Ion chromatography for sulfur (SCAN-CM 57), for sulfate (ISO-9198)	0–10% wt
Moisture	Gravimetry	

Table 2). The total lignin content is determined according to the NERL/TP-510-42618 protocol (Sluiter et al. 2008) and is considered as the standard method for quantitative lignin analysis. According to this protocol, the sample is hydrolyzed with concentrated H_2SO_4 (72%) then diluted to obtain an acid concentration of 3%. The polymeric carbohydrates are degraded in monosaccharides, leaving behind a lignin-rich residue, named Klason lignin or acid-insoluble lignin (AIL), that is vacuum filtered and measured gravimetrically (Gosselink et al. 2004b; Vishtal and Kraslawski 2011).

4.1.1 Elemental Analysis

The elemental analysis gives access to the various elemental compositions of compounds (including lignin) in terms of carbon, hydrogen, nitrogen, or sulfur. These compositions can sometimes give access to the formula of the tested compounds. The formula is expressed in terms of the element content of the average phenylpropane C9 unit present in the lignin (Norman 1969). The amount of methoxy function is also often incorporated into this formula, for example, $C_9H_{8.83}O_{2.37}(OCH_3)_{0.96}$ for spruce in milled wood lignin (Huang et al. 2019).

The elemental composition of lignins depends on the sources of raw material and separation methods. (Huang et al. 2019). The highest levels of sulfur are observed in the case of lignosulfonates (Svensson 2008).

4.1.2 Linkages

The connectivity of the three randomized phenylpropanoid units H, G, and S (representing coumaryl-, coniferyl- and sinapyl alcohol) is ensured by various linkages, Fig. 10 (Lahive et al. 2020; Li et al. 2015; Mei et al. 2019):

- Alkyl-aryl ether bonds (β-*O*-4 and α-*O*-4),
- Diaryl ether (4-*O*-5), which can be hydrolyzed
- "Condensed" bonds or carbon-carbon bonds (phenylcoumaran (β-5), resinol (β-β), diarylpropane (β-1), and biphenyl (5-5)) more recalcitrant to degradation.

The most common linkage is β(0-4), representing more than 50% of the total linkages in soft and hardwood (Ek et al. 2009; Sjostrom 1993).

4.2 Lignin Degradation Methods

Chemical degradation methods have been for a long time the only methods giving access to the structure of the studied lignin and they were reviewed by Lin and Dence (Dence 1992). Degradation techniques such as nitrobenzene oxidation, acidolysis or thioacidolysis provided a lot of information on the monomeric compositions and the type of bonds involved in the construction of the woody network. In this context, introduced by Freudenberg in 1939, nitrobenzene oxidation is traditional and the oldest method for analytical purposes of lignins (Bose et al. 2009; Chen 1992; Freudenberg et al. 1940), is commonly used to determine their proportion of H, S and G units (Erdtman 1971; Logan and Thomas 1985). Alkaline cupric (II) oxide oxidation (CuO) is another commonly oxidative technique used to analyze the composition of lignins in complex sample matrixes, such as soils and sediments (Bose et al. 2009; Chen 1992). Thioacidolysis is today one of the most used techniques delivering structural information on the aromatic part (H/G/S ratio). Developed by Lapierre (Lapierre et al. 1985), this acid-catalyzed method proceeds in β-*O*-4 cleavage of lignin as lignin is treated with boron trifluoride etherate in ethanethiol and give thioethylated hydroxyphenyl (H), guaiacyl (G), and syringyl (S) (Rolando et al. 1992). The results furnish information on the composition of uncondensed aryl ether structures, the S/G ratios (Lu and Ralph 1997). The main advantage of this method lies in the rapidity and ease of implementation of the protocol. Thioacidolysis has supplanted the techniques of nitrobenzene or alkaline oxidation because compared to alkaline oxidation, thioacidolysis provides more information on core lignin structure without interference from other cell walls phenols. The monomer yielding from alkaline nitrobenzene oxidation, however, is

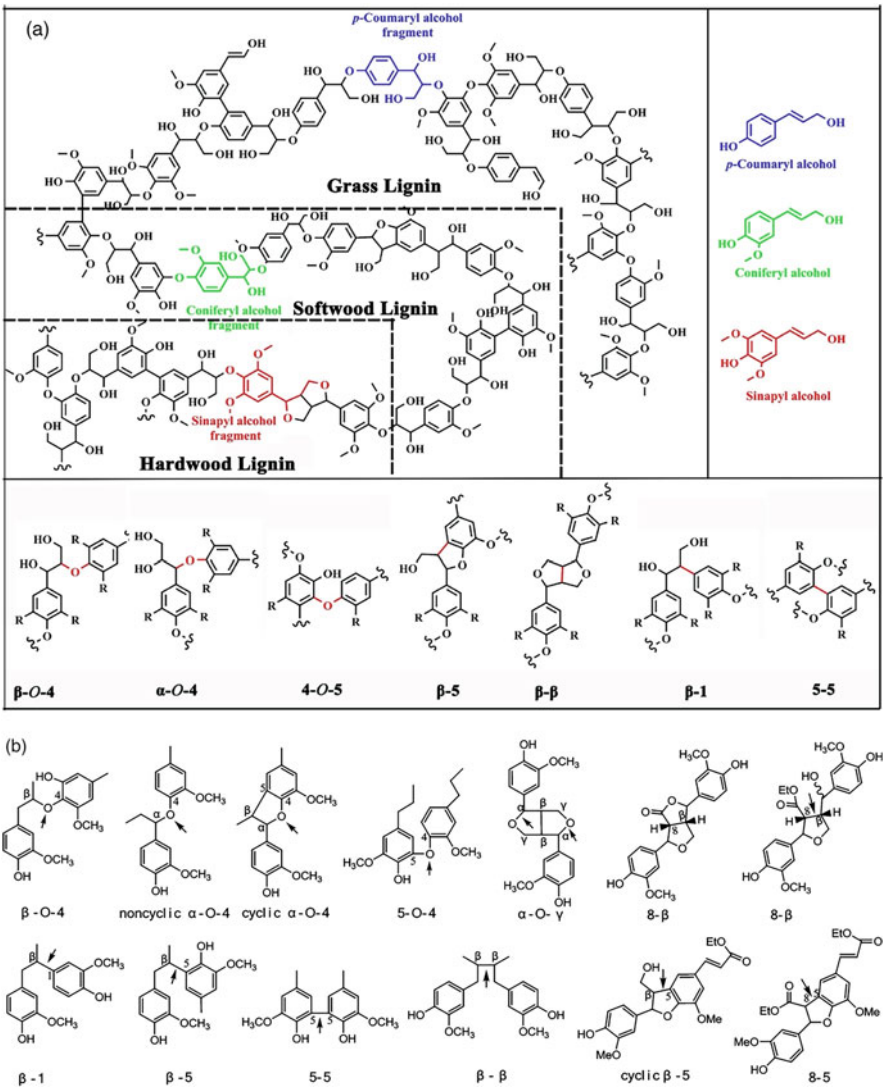


Fig. 10 (**a**) Lignin structure with the main inter-subunit linkages. Reuse (print) with permission Mei, Q., Shen, X., Liu, H., Han, B., 2019. Selectively transform lignin into value-added chemicals. Chinese Chemical Letters 30, 15–24. https://doi.org/10.1016/j.cclet.2018.04.032. Copyright (2019) American Chemical Society. (**b**) Typical linkages between the primary units of lignin. Reuse (print) with permission of Li, C., Zhao, X., Wang, A., Huber, G. W., Zhang, T., 2015. Catalytic Transformation of Lignin for the Production of Chemicals and Fuels. Chem. Rev. 115, 11559–11624. https://doi.org/10.1021/acs.chemrev.5b00155. Copyright (2015) American Chemical Society

higher than from thioacidolysis. But the protocol for DFRC (Derivatization Followed by Reductive Cleavage) is currently preferred to the conventional thioacidolysis because of its simplicity and the use of relatively innocuous reagents (Lu and Ralph 1997). The DFRC degradation consists first in solubilization of lignin by bromination and acetylation with acetyl *bromide* AcBr and then reductive cleavage with zinc dust (Del Río et al. 2012; Matsui and Ohira 2013). This method is performed for selective cleavage of ether linkages in lignins to determine the S/G ratio of biomass. The results obtained from these techniques allowed the statistical reconstruction of lignin models of conifers (Adler 1977; Sakakibara 1980) or hardwoods (Nimz et al. 1981). However, all these techniques have limitations like partial structural information due to the detection of only specific bonds of lignins.

4.3 Spectroscopic Methods

Spectroscopic methods particularly ultraviolet and infrared spectroscopic techniques can be used without treatment of lignins (Gilarranz et al. 2001). Infrared spectroscopy has become a routine technique for the analysis of lignins based on the spectral identity of different types of lignins (Derkacheva and Sukhov 2008; Faix and Beinhoff 1988). NMR is also a very powerful tool for the analysis of native lignins (Ralph et al. 2007).

4.3.1 Fourier Transform Infrared Spectroscopy

FT-IR is a non-destructive spectroscopic technique giving information on functional groups of organic compounds. Infrared is often used in the characterization of lignin due to some advantages: short analysis time, high sensitivity, easy use, direct analysis on solid samples, and convenient data handling (Faix 1991). Lignin contains various functional groups: carbonyl, methoxyl, hydroxyl, and carboxyl groups, aromatics (Fengel and Wegener 2011), and their vibrational bands are summarized in Table 3. The region between 1400 and 1800 cm^{-1} corresponds to carbonyl functions such as the elongation vibrations of non-conjugated and conjugated carbonyl bonds (respectively at 1709–1738 and 1655–1675 cm^{-1}). Their intensity and shift reveal (Faix 1991; Hortling et al. 1997) the efficiency of derivatization reactions and analyze different types of lignin. For example, the study of the infrared spectra from technical lignin by El and Salvadó in 2007 (El and Salvadó 2007) highlighted clear differences between these lignins (Fig. 11) but this method is not quantitative.

Table 3 Assignments of FT-IR absorption bands (cm^{-1})

Absorption bands	Assignment
3400–3600	Free OH stretching
3100–3400	Associated OH
2820–2960	CH stretching of CH, CH_2, CH_3 group
2920	CH stretching of carboxylic OH
2650–2890	CH of CH_3 group in methoxyl
1771	C=O aromatic
1700–1750	C=O stretch in unconjugated ketone, carboxyl group
1722	C=O stretch aliphatic
1650–1680	C=O stretch in conjugated ketone
1500–1600	Aromatic skeletal vibration
1420–1430	Aromatic skeletal vibration
1460	Aromatic methyl group vibrations
1434	Aromatic skeletal vibration
1374	Aliphatic C-H stretch in CH_3
1328	Syringyl ring with C-O stretching
1270–1275	C-O Guaiacyl ring
1242	Aromatic C-O stretching
1215	C-O ether stretching
1165	C-O stretch in ester groups
1135	Aromatic C-H in-plane deformation for syringyl type
1043	Aromatic C-H in-plane deformation for guaiacyl type
750–860	Aromatic C-H out of plane bending

4.3.2 Raman

Raman is vibrational scattering spectroscopy complementing IR. Raman photons are emitted when a sample is illuminated by a laser source (Near IR—Visible and UV) through the phenomenon of inelastic light scattering. Like FTIR, no sample preparation is required and Raman takes short analysis times (similar to FTIR). Several Raman scattering peaks can be attributed to lignin at 1600 cm^{-1} (aromatic ring stretch), at 1620 cm^{-1} (carbonyl content), 1650 cm^{-1}, and at 3070 cm^{-1} (aromatic C-H stretch) (Agarwal 2008; Agarwal et al. 2011; Larsen and Barsberg 2010). The intensities of the peak at 1650 cm^{-1} (C=C) relative to those at 1600 cm^{-1} (phenol ring) may explain the degradation of the lignin (Moosavinejad et al. 2019). Raman spectroscopy allows the measurement of S/G ratios in diverse feedstocks (Lupoi et al. 2015). The major drawback of this technique is that Raman peaks are often of weak intensities and can be completely masked by the fluorescence of lignin. It is however noticeable that the surface-enhanced Raman scattering (SERS) enhances the signal by molecules adsorbed on rough metals surfaces (Agarwal 1999).

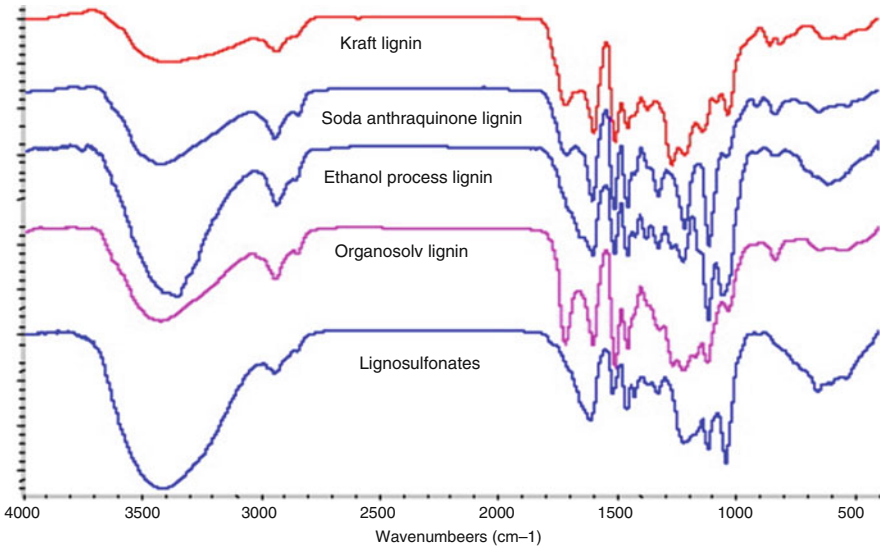


Fig. 11 FTIR spectra of unacetylated lignin samples. (*Reuse (print) with permission of El, M.N., Salvadó, J., 2007. Analytical methods for determining functional groups in various technical lignin. Industrial crops and products. 26, 116–124.* https://doi.org/10.1016/j.indcrop.2007.02.006. *Copyright (2007) Elsevier)*

4.3.3 UV-Spectrometry

Lignin aromatic structure exhibits maximal absorption in the ultraviolet region around 280 nm and at 315 nm if ester groups are present such as in grass lignin. The absorbance difference before and after ionization of phenolic groups in alkaline solution at a wavelength of 300 and 350 nm has been used to measure the content of phenolic hydroxyl groups (Goldmann Valdes et al. 2016). Figure 12 illustrates differences in UV spectroscopy between LignoBoost and alkali-treated lignin samples (Abdelaziz and Hulteberg 2017).

4.3.4 Nuclear Magnetic Resonance

Since the 1970s, NMR analysis of lignins has continuously evolved (Ludwig et al. 1964; Lundquist 1992). NMR spectroscopy is a highly sensitive tool to characterize lignin. The most commonly used NMR probes are ^{1}H, ^{13}C, ^{31}P. However, despite the ease of use, drawbacks remain, as many lignins need acetylation before analysis, because they are insoluble in common deuterated NMR solvents. In this context, Lundquist's research group reported the first ^{1}H NMR to analyze the milled wood lignin (Li and Lundquist 1994; Lundquist et al. 1979a, b, 1980). The ^{1}H NMR of acetylated lignins in $CDCl_3$, as seen in Fig. 13, allows to identify methyl protons in the aliphatic acetyl groups (1.70–2.17 ppm), protons in methoxyl groups

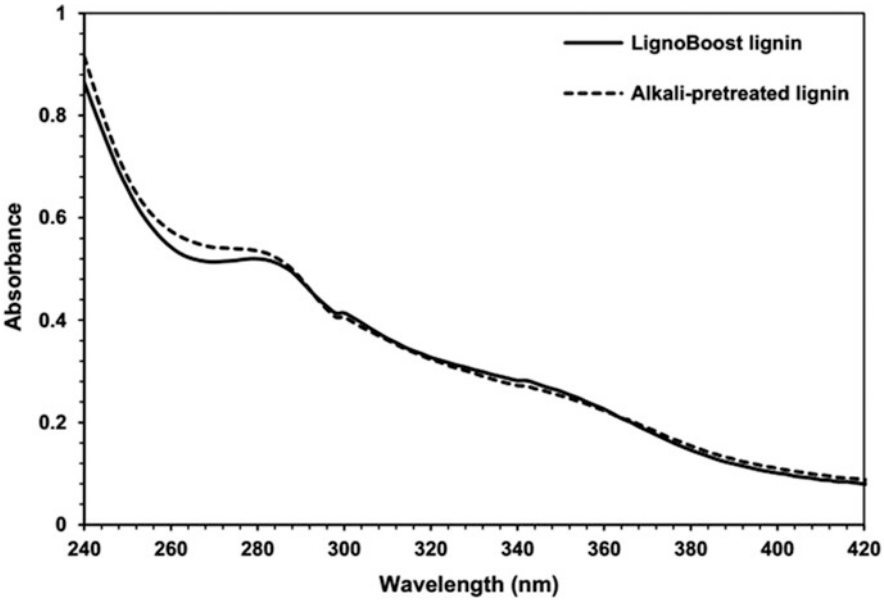


Fig. 12 UV-Visible spectra of lignins obtained from the LignoBoost and alkali pretreatments. (Reuse (print) with permission of Abdelaziz, O.Y., Hulteberg, C.P., 2017. Physicochemical Characterisation of Technical Lignins for Their Potential Valorisation. Waste Biomass Valor 8, 859–869. https://doi.org/10.1007/s12649-016-9643-9. Copyright (2017) Springer)

(3.5–4.0 ppm), aliphatic protons in the linkages of β-*O*-4, β-β, and β-5 (4.0–6.3 ppm), and aromatic protons (6–7.10 ppm) (Chu et al. 2012; Mancera et al. 2011). Anyway, as seen in Fig. 13 (Tejado et al. 2007), lignin analysis by ^{1}H NMR of lignin remains complex due to the significant superposition of several characteristic resonances (Lundquist 1992), making the proton spectra of lignins not really exploitable. The wider spectral window (200 ppm) of carbon NMR allows better discrimination of resonances, thus allowing the identification of the structural motifs of lignins.

The spectroscopic technique of ^{13}C NMR is now widely employed for the study of lignin. ^{13}C NMR spectroscopy of lignins allows the assignment to the various carbons of the lignin skeleton including the presence of aryl ether, condensed and uncondensed aromatic, and aliphatic carbons. The area between 60 and 80 ppm is attributed to the oxygenated aliphatic carbons (carbons carrying the alcohol functions for example), the signals of the aromatic carbons come out between 100 and 160 ppm and finally, the zone between 160 and 180 ppm corresponds to the carbons of carboxylic acids. The attributions are made by comparison with the numerous existing databases (Chen and Robert 1988; Kringstad and Mörck 1983), which were carried out thanks to the analysis of models. For example, the structural features of the lignin fractions from *Eucalyptus pellita* were investigated with ^{13}C NMR spectrometry, Fig. 14 (Yuan et al. 2009). The ^{13}C NMR spectroscopy requires a

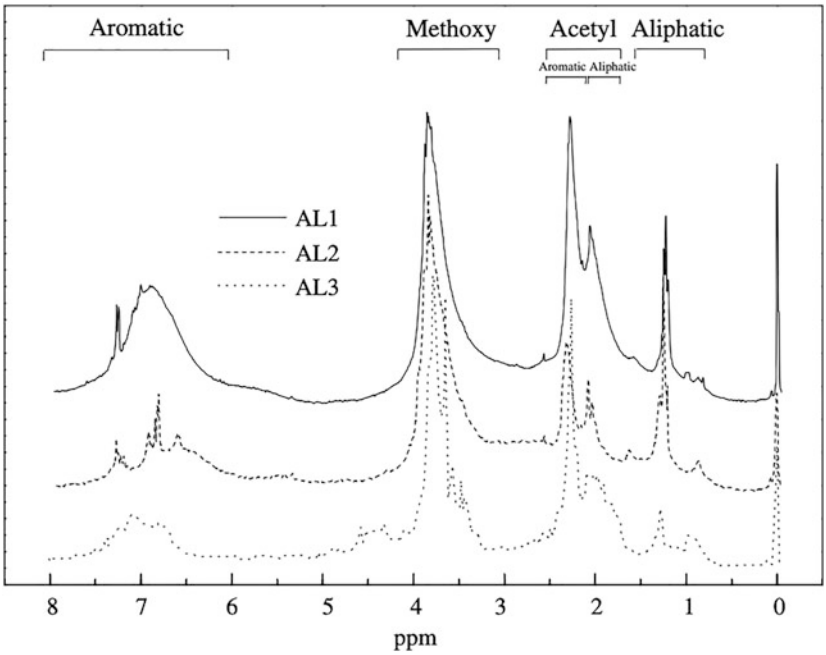


Fig. 13 ^{1}H NMR spectra of acetylated Pine (AL1), Flax (AL2), and Tamarind lignins (AL3) samples. (Reuse (print) with permission of Tejado, A., Peña, C., Labidi, J., Echeverria, J.M., Mondragon, I., 2007. Physico-chemical characterization of lignins from different sources for use in phenol-formaldehyde resin synthesis. Bioresource Technology 98, 1655–1663. https://doi.org/10.1016/j.biortech.2006.05.042 Copyright (2007) Elsevier)

large sample size and a long acquisition time. To overcome the problems of solubility or modifications of lignins, solid-state cross polarization-magic angle spinning ^{13}C NMR is an alternative (Cipriano et al. 2020). In this technique, the sample is spinning very rapidly (up to ~100,000 rpm) at a precise angle, the magic angle ($\theta m \approx 54.7°$), to improve the resolution of the spectrum.

^{31}P NMR can be used as a complementary analysis to ^{13}C NMR. Phosphorylation of the hydroxyls carrying labile protons of lignin with 2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane (TMDP) in anhydrous pyridine/$CDCl_3$ (1.6/1 v/v) solution give phosphite products (Argyropoulos 1994; Pu et al. 2011). For example, a typical ^{31}P NMR spectrum of an organosolv lignin isolated from bamboo is shown in Fig. 15. Total aliphatic hydroxyl, phenolic hydroxyl, and carboxyl groups, as well as the G/S/H ratio of lignin samples, are established by quantitative ^{31}P NMR using published procedures (Cateto et al. 2008; Faix et al. 1994). Anyway, ^{31}P NMR analysis requires intensive sample preparation.

Interpretation ^{1}D NMR (one-dimensional NMR) is based on 3000 structures of lignins and derivatives (from monomers to tetramers) database (Ralph et al. 2004) but is limited to simple patterns or specific band assignments. Thus, researchers turn

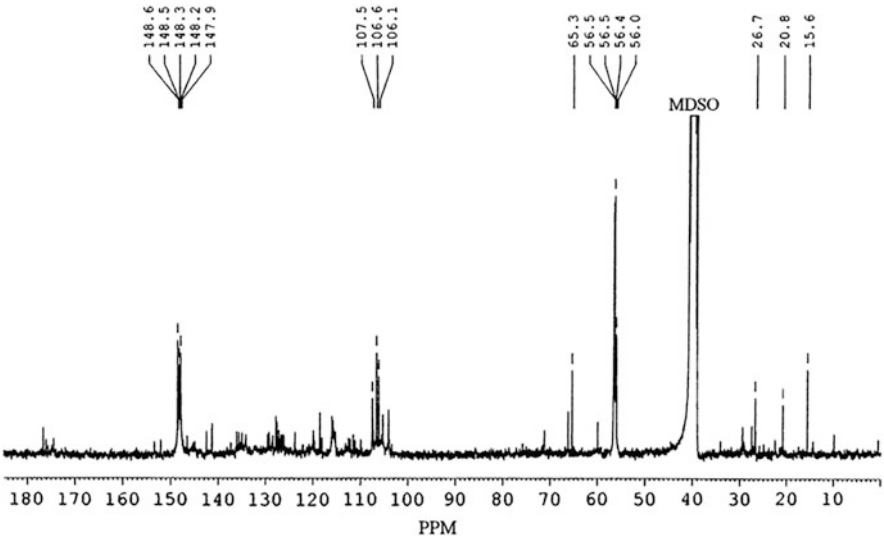


Fig. 14 ^{13}C NMR spectrum of the degraded lignin fraction. (Reuse (print) with permission of Yuan, T.-Q., He, J., Xu, F., Sun, R.-C., 2009. Fractionation and physico-chemical analysis of degraded lignins from the black liquor of Eucalyptus pellita KP-AQ pulping. Polymer Degradation and Stability 94, 1142–1150. https://doi.org/10.1016/j.polymdegradstab.2009.03.019. Copyright (2019) Elsevier)

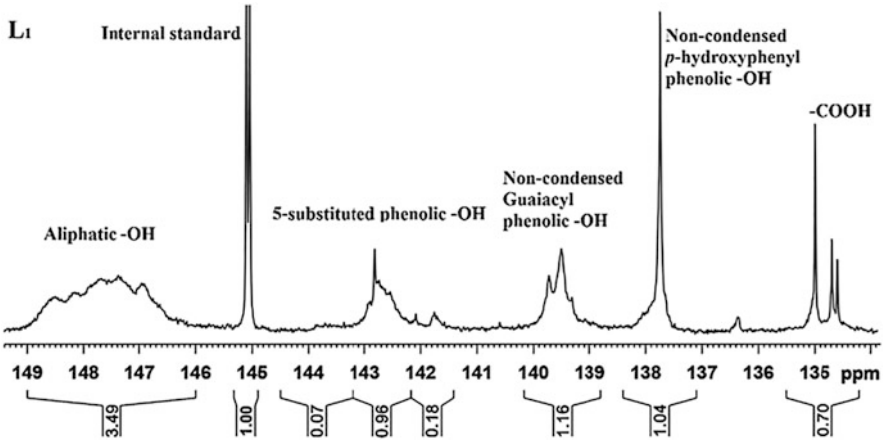


Fig. 15 ^{31}P NMR spectrum of organosolv lignin isolated from bamboo. (Reuse (print) with permission of Liao, J., Latif, N., Trache, D., Brosse, N. and Hussin, M., 2020. Current advancement on the isolation, characterization and application of lignin. *International Journal of Biological Macromolecules*, 162, pp. 985–1024. https://doi.org/10.1016/j.ijbiomac.2020.06.168 Copyright (2020) Elsevier)

to two-dimensional NMR techniques to explore more structural elucidation and the presence of some particular structures, in low abundances such as spirodienone.

The two-dimensional measurements (heteronuclear single quantum coherence, HSQC; heteronuclear multiple quantum coherence, HMQC) combine both ^{13}C and ^{1}H NMR. As illustrated, the identification of different substructures present in lignins polymer is achieved through HSQC as displayed in Fig. 16 (Ibarra et al. 2007; Liitiä et al. 2003; Ralph et al. 2004; Rencoret et al. 2008). Database enrichment allows confirmation of correlation assignment on HSQC and HMBC spectra of native lignins (Ibarra et al. 2007; Liitiä et al. 2003; Ralph et al. 2004) and also determines the nature of different bonds and structures present in lignins.

4.4 Thermal Analysis

Thermal analyses reveal the structure-properties relationships of polymers as a function of temperature. Thermogravimetric analysis (TGA) and differential enthalpy analysis (DSC) are widely used to study the thermal behavior of lignins (Poletto 2017). TGA allows monitoring the lignin weight loss when a sample is heated, cooled, or maintained at a fixed temperature. For most lignins, three stages of mass loss are observed when the analysis is performed under inert atmosphere (N_2): drying (20–150 °C), pyrolysis (200–500 °C), and carbonization (500–900 °C). DSC is a thermoanalytical method where the temperature of a sample and reference material is increased at a constant rate and allows to determine the glass transition temperature (Tg) and the enthalpy of lignins due to changes in the lignin polymer (Fig. 17). For example, lignin Tg determined by DSC is usually comprised between 110 and 160 °C for milled wood lignin and 124–174 °C for Kraft lignins (Glasser 1999) but is influenced by several factors such as the aromatic structure, hydrogen bonding type interactions, the molar mass, the type of inter-unit bonds and the degree of cross-linking (Köhnke et al. 2019). Comparison of the glass transition temperatures (Tg) of different modified lignins are reported (Ding et al. 2015).

4.5 Pyr/Gas-Chromatography MS

Analytical pyrolysis is an efficient technique for the analysis of the chemical composition of wood and in particular for the characterization of lignin of various origins. Pyrolysis/gas chromatography-mass spectrometry (Pyr/GC-MS) is very interesting to evaluate the ratios S/G in lignin (Lopes et al. 2011). Pyrolysis consists of heating a sample to a high temperature (>500 °C) in an inert atmosphere to cleave the constituent polymers into small fragments. These more volatile products formed flushed by helium can be separated by chromatography generally coated with an apolar silicone phase. Peaks of the pyrolysis products on GC give a specific

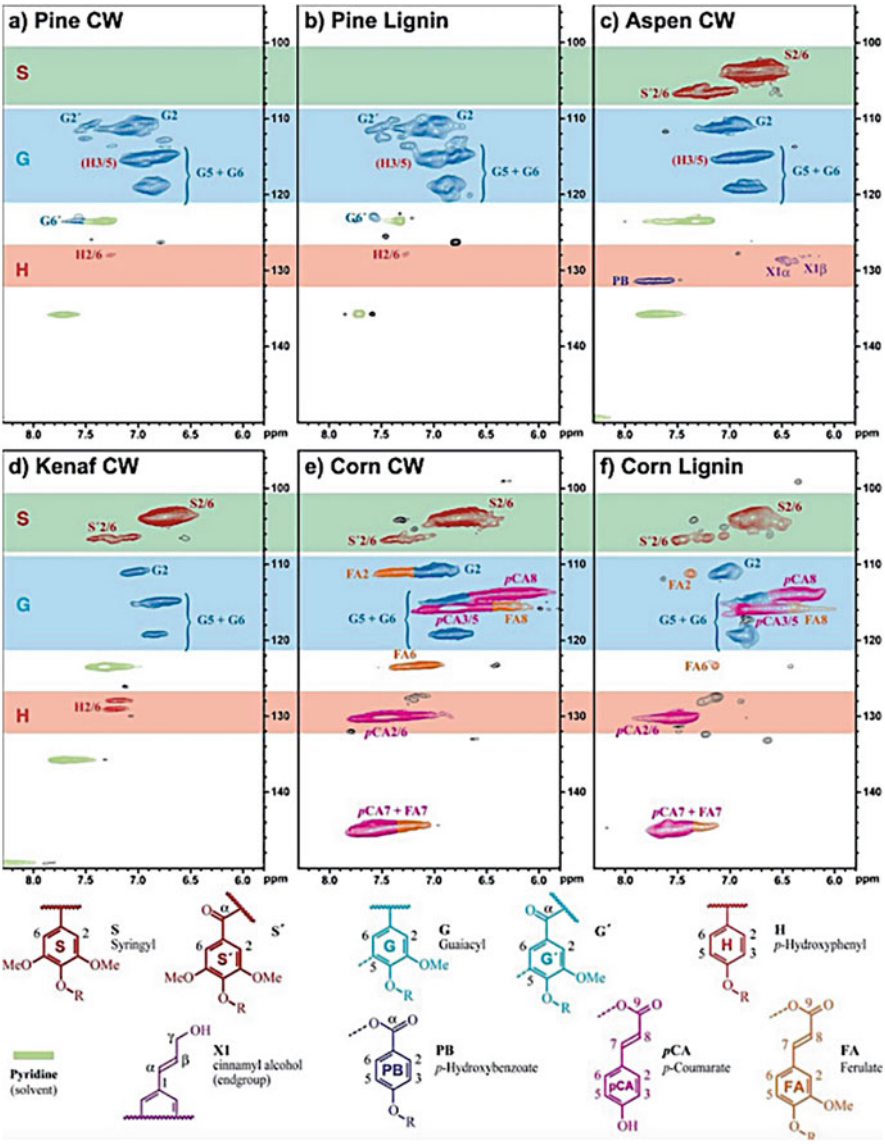


Fig. 16 Aromatic regions of 2D-HSQC NMR spectrum from cell wall gels and soluble lignins from various samples: (**a**) pine, (**b**) pine isolated lignin, (**c**) aspen, (**d**) kenaf bast fiber, (**e**) corn stems, and (**f**) corn isolated lignin. (Reuse (print) with permission of Liao, J., Latif, N., Trache, D., Brosse, N. and Hussin, M., 2020. Current advancement on the isolation, characterization and application of lignin. International Journal of Biological Macromolecules, 162, pp. 985–1024. https://doi.org/10.1016/j.ijbiomac.2020.06.168 Copyright (2020) Elsevier)

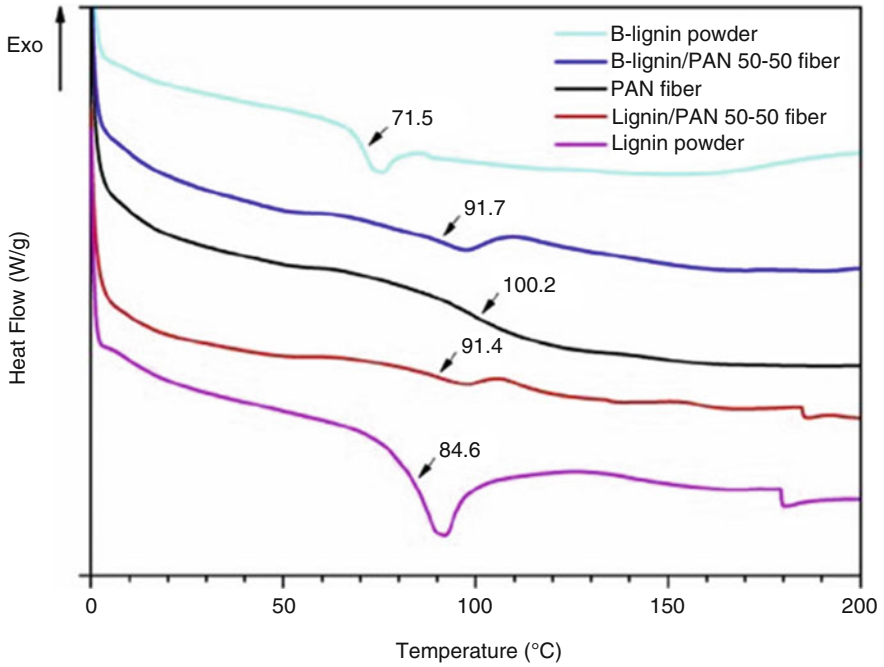


Fig. 17 Glass transition temperature (Tg) of lignin, B-lignin (Butyrate organosolv lignin), and precursor fibers based on PAN (polyacrylonitrile), lignin/PAN 50–50, and B-lignin/PAN 50–50 measured by DSC. (Reuse (print) with permission of Ding, R., Wu, H., Thunga, M., Bowler, N., Kessler, M., 2015. Processing and Characterization of Low-Cost Electrospun Carbon Fibers from Organosolv Lignin/Polyacrylonitrile Blends. Carbon 100. https://doi.org/10.1016/j.carbon.2015.12.078. Copyright (2015) American Chemical Society)

pyrogram (Fig. 18) whose peaks can be identified according to their mass spectra obtained by electronic impact (70 eV) (Galletti and Bocchini 1995).

Studies confirmed the ability of the Py-GC/MS technique to provide useful data for the characterization of lignin (Lima et al. 2008; Lucejko et al. 2020). Whereas being a sensitive analysis used for the examination of lignin, Py/GC-MS presents major drawbacks: greater expense instrumentation, long analysis times (typically between 40 min and 2 h per sample), and complex interpretation of data.

4.6 Physical Qualitative Properties

Optical microscopy is an easy method to observe morphological aspects, the particle size of biomass, and the lignin distribution, for example within straw. Scanning electron microscopy (SEM) is used to observe its morphology and size (Mandlekar 2019), and small-angle or ultra-small-angle X-ray scattering can be used to study the supramolecular structure of lignin in both solid-state and in solution. Fromm et al.

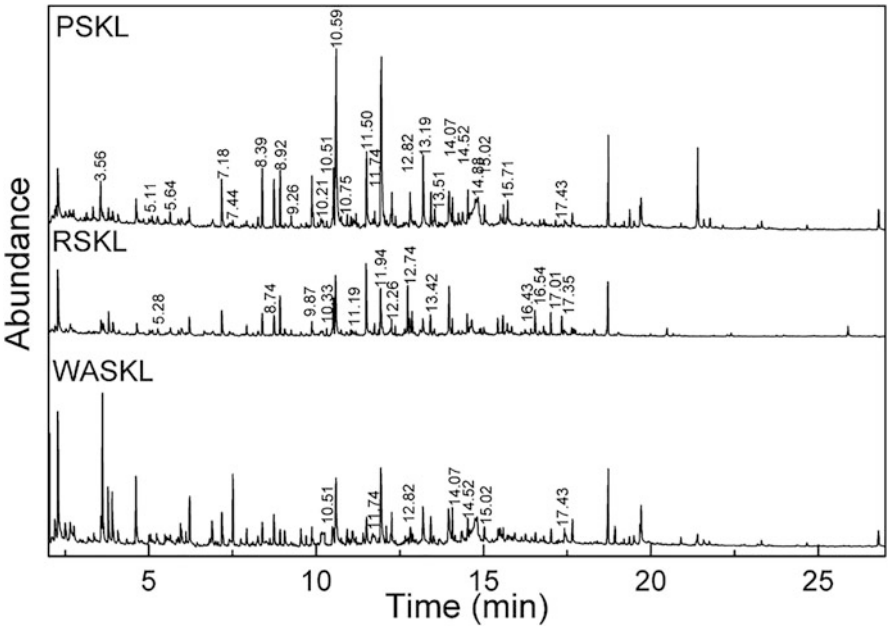


Fig. 18 Typical ion chromatograms from pyrolysis of the G, GS, and HGS type lignin from variant biomass resources peanut shell walnut shell (PSKL), rape straw (RS), wheat straw (WASKL). (Reuse (print) with permission of Chen, L., Wang, X., Yang, H., Lu, Q., Li, D., Yang, Q., Chen, H., 2015. Study on pyrolysis behaviors of non-woody lignins with TG-FTIR and Py-GC/MS. Journal of Analytical and Applied Pyrolysis 113, 499–507. https://doi.org/10.1016/j.jaap.2015.03.018. Copyright (2015) Elsevier)

reported also the distribution of lignin in wood cell walls by field emission scanning electron microscopy (FE-SEM) (Fig. 19) (Fromm et al. 2003), which is a technique used for qualitative analysis of biomass. Transmission electron microscopy is also useful to analyze lignin nanoparticles allowing a clear contrast between the core and the shell. Atomic Force Microscope (AFM) is another method that enables evaluate surface structures of different lignin at nanometer scales.

4.7 Molecular Structure

Molar mass distribution of lignins is important to understand the fundamental properties of lignins and to know their exact molecular weight (Gellerstedt 1992). The determination of molecular weight distribution is usually performed by steric exclusion chromatography (SEC) or also called gel permeation chromatography (GPC) when an organic solvent is used as a mobile phase. Dissolved molecules are eluted through a specific column filled with a nanoporous gel (stationary phase) and separated by size. Recent work by Baumberger et al. focused on standardizing

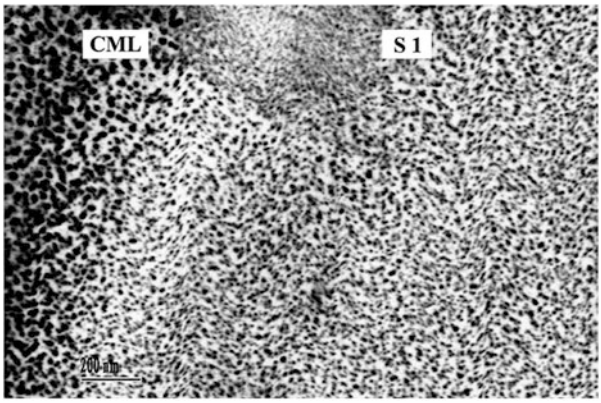


Fig. 19 FE-SEM micrograph. The transverse section shows lamellae of bright cellulose microfibrils and dark lignin aggregates. *CML* compound middle lamella, *S1* secondary wall 1. (Reuse (print) with permission of Fromm, J., Rockel, B., Lautner, S., Windeisen, E., Wanner, G., 2003. Lignin distribution in wood cell walls determined by TEM and backscattered SEM techniques. Journal of Structural Biology 143, 77–84. https://doi.org/10.1016/S1047-8477(03)00119-9. Copyright (2003) Elsevier)

the SEC method for lignin analysis (Baumberger et al. 2007). The molar mass of acetylated Kraft lignins obtained from various black liquors varies mainly in the range of 200–15,000 g/mol. To improve their solubility, acetylation of lignins is sometimes done (Asikkala et al. 2012). In most cases, polystyrene gel with THF is used to calibrate molar mass distribution for different lignins (Baumberger et al. 2007). GPC spectra of soda lignin (SL) extracted with organic solvents are displayed for example in Fig. 20. Results exhibited that soda lignin (SL) was sequentially fractionated by organic solvents (ethyl acetate: F1, methanol: F2, acetone: F3, dioxane/water: F4) (Kim et al. 2017).

4.8 Lignin Functional Groups-Based Approaches

Lignin has various functional groups such as phenolic hydroxyl, aliphatic hydroxyl, benzyl alcohol, noncyclic benzyl ether, carbonyl groups, carboxylic acids, and methoxyl groups (Fig. 21). The abundance of these functional groups directly affects the reactivity of the lignin in various chemical reactions. The use of selective reagents is often needed to analyze lignin functional groups and Table 4 summarizes their determination methods (El and Salvadó 2007; Gosselink et al. 2004a).

To summarize, the synergistic combination of many methods each bringing partial but complimentary information is compulsory to understand the complex and heterogeneous lignin. Due to its extreme heterogeneity, the nature of lignin carbohydrate linkages and occurrence is not completely resolved. Many studies and techniques were led until now as SEM, SEC, FT-IR, TGA/DSC, NMR, and DFRC.

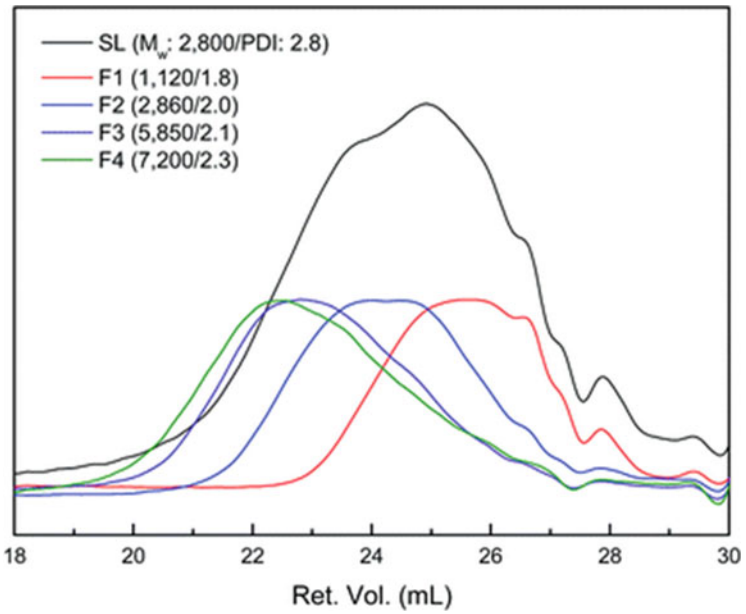


Fig. 20 GPC curves of lignin fractions (the peak maxima were normalized to same value except for SL) (Kim et al. 2017). (Reuse (print) with permission of Kim, J.-Y., Park, S.Y., Lee, J.H., Choi, I.-G., Choi, J.W., 2017. Sequential solvent fractionation of lignin for the selective production of monoaromatics by Ru catalyzed ethanolysis. RSC Adv. 7, 53117–53125. https://doi.org/10.1039/C7RA11541E. Copyright (2017) Royal Society of Chemistry)

But, the problem inherent in the structural investigation of lignins is still important. Nevertheless, the development of new methodologies has improved our understanding of the complex structure of these polymers. In particular, the magnetic resonance techniques and GPC have proved to be efficient analytical tools for the structural elucidation of these complex biopolymers.

5 Fungal Enzymes and Mechanisms Involved During Lignin Depolymerization

Due to the recalcitrance of lignin to biodegradation, the ability to depolymerize it and to use the obtained oligomers as sources of carbon and energy is a true evolutionary advantage and represents a key activity for the carbon cycle in the ecosystems. Fungi are reported as the most efficient microorganisms for lignin degradation (Chio et al. 2019; Weng et al. 2021) and developed the capacity to produce ligninolytic enzymes in relation to their ecological role as wood degraders, soil litter-decomposers, or pathogens (Hofrichter 2002; Choi et al. 2014). The use of these natural fungal activities to depolymerize lignin appears attractive for the development of new

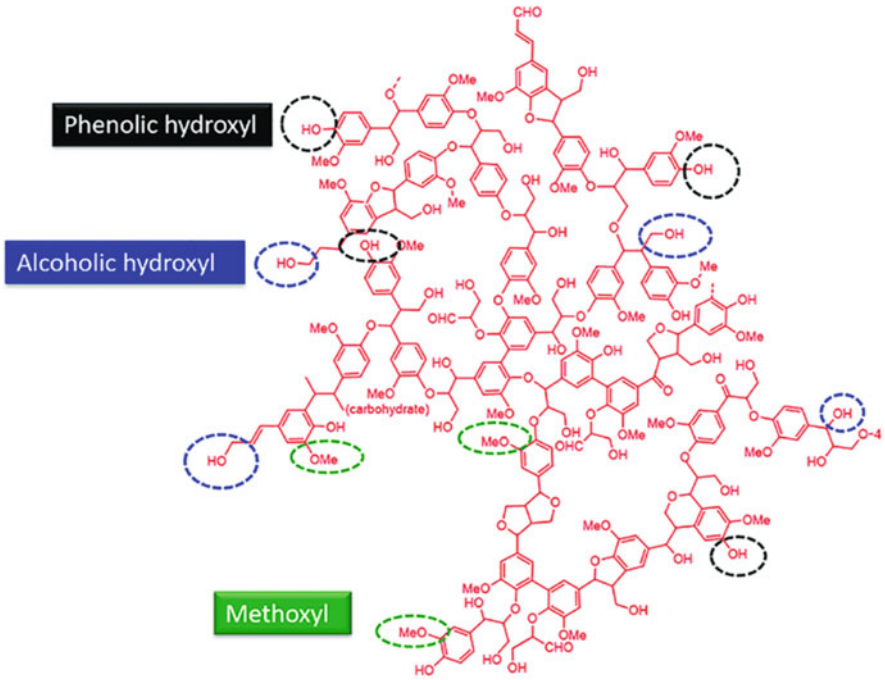


Fig. 21 Main functional groups of lignin (Poursorkhabi et al. 2020). (Reuse (print) with permission of Poursorkhabi, V., Abdelwahab, M., Misra, M., Khalil, H., Gharabaghi, B., Mohanty, A., 2020. Processing, Carbonization, and Characterization of Lignin Based Electrospun Carbon Fibers: A Review. Frontiers in Energy Research 8, 208. https://doi.org/10.3389/fenrg.2020.00208. Copyright (2017) National Renewable Energy Laboratory (DOE))

bioprocesses with a reduced environmental impact compared to the physicochemical approaches. Thus, the functioning of the cellular and enzymatic mechanisms involved in lignin biodegradation was widely investigated in the past and is still the object of active research nowadays.

5.1 Lignin Depolymerizing Enzymes from Fungi

Lignin decaying fungi excrete enzymes able to depolymerize the high molecular weight and hyper-variable molecule of lignin. Indeed, these enzymes do not directly interact with lignin but generate highly reactive oxidizing mediators able to chemically react with this polymer and trigger degradation ranging from its partial depolymerization to mineralization in CO_2 and H_2O (Li and Zheng 2020). These enzymes belong either to phenol oxidases with laccases or to heme peroxidases with lignin-, manganese-, versatile and dye decolorizing peroxidases (Table 5). Along with these main enzyme types, the role of polyphenol oxidases was also described

Table 4 Main analytical methods to characterize the functional groups of lignin

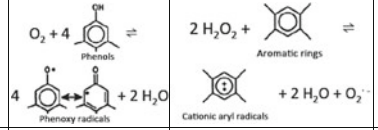
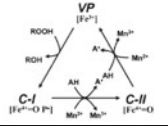
Functional groups	Technique
Phenolic and Aliphatic Hydroxyl groups	Aminolysis
	UV visible spectrometry
	Potentiometric titration
	Acetylation/quantitative ^{13}C NMR
	Periodate oxidation
	^{1}H NMR/^{31}P NMR methods
Benzyl Ether and Benzyl Alcohol groups	Acid hydrolysis in methanol
	NMR
Carbonyl groups	Hydroxylamine hydrochloride method
	$NaBH_4$
Carboxylic acid groups	Conductimetry with titration
	– Titration in the aqueous phase
	– Titration in non-aqueous phase by tetra-n-butylammonium hydroxide (TnBAH)
Methoxyl content	Gaz chromatography by indirect measurement

for lignin degradation by some fungi such as *Lentinula edodes* and *Pleurotus ostreatus* (Dong et al. 2013). Other enzymes such as aromatic peroxygenases have also been suggested to be involved in the conversion of lignin oligomers (Hofrichter et al. 2010). The oxidative reactions involved in lignin decomposition and catalyzed by this mixture of oxidoreductases include the cleavage of C-C bonds and ether linkages as well as the removal of side chains and aromatic rings (Zabel and Morrell 2020).

Laccases are copper-containing enzymes that use molecular oxygen as a final electron acceptor for the cleavage of aromatic rings (Munk et al. 2015) or for the oxidation of phenolic substrates to phenoxy-radicals, which can then react with the lignin molecule (Gianfreda et al. 1999) and promote Cα oxidation, alkyl-aryl cleavage, and Cα-Cβ cleavage (Kawai et al. 1988). Consequently, laccases are reported to trigger various lignin modifications such as polymerization, depolymerization, Cα oxidation, and demethylation. Laccases can also oxidize non-phenolic compounds with the help of mediators such as 2,2′-azino-*bis*(3--ethylbenzothiazoline-6-sulfonic acid) (ABTS), 3-hydroxyanthranilate (HAA), or 1-hydroxybenztriazole (HBT) which stabilize the reaction intermediates (Hilgers et al. 2018). The oxidized non-phenolic compounds coupled with these mediators can promote the aromatic ring cleavage, Cα-Cβ cleavage, Cα oxidation, and β ether cleavage (Kawai et al. 2004). Thus, with these kinds of mediators, laccases can degrade up to 90% of the lignin structure (Camarero et al. 2004).

Lignin peroxidases (LiP) are glycoproteins (38–43 kDa) using hydrogen peroxide generated by extracellular oxidases such as glyoxal or aryl-alcohol oxidases to perform one-electron oxidation of different phenolic and non-phenolic structures related to lignin (Weng et al. 2021), resulting in the formation of aryl cation radicals. These reactive cationic radicals are in turn capable of chemically attacking the lignin

Table 5 Main characteristics of major fungal ligninases families (phylogenetic trees were generated using the data from the fungal peroxidase database fPoxDB through the common tree tool of the taxonomy database of the NCBI website, catalytic reaction of VP reused (print) from Pérez-Boada, M., Ruiz-Dueñas, F.J., Pogni, R., Basosi, R., Choinowski, T., Martínez, M.J., Piontek, K., Martínez, A.T., 2005. Versatile Peroxidase Oxidation of High Redox Potential Aromatic Compounds: Site-directed Mutagenesis, Spectroscopic and Crystallographic Investigation of Three Long-range Electron Transfer Pathways. Journal of Molecular Biology 354, 385–402. https://doi.org/10.1016/j.jmb.2005.09.047. Copyright (2005) Elsevier)

Enzyme identification	Laccase (EC 1.10.3.2)	Lignin-peroxidase (EC 1.11.1.14)	Manganese-peroxidase (EC 1.11.1.13)	Versatile peroxidase (EC 1.11.1.16)	Dye decolorizing peroxidase (EC 1.11.1.19)	
Alternative names	Urishiol oxidase	Diarylpropane oxygenase, diarylpropane peroxidase, ligninase I, LiP	Mn-dependent peroxidase, MnP	Hybrid peroxidase, polyvalent peroxidase, VP	DyP-type peroxidase, DyP	
Catalyzed reaction			$2Mn^{2+} + 2H^+ + H_2O_2 \rightleftharpoons 2Mn^{3+} + 2H_2O$		Reactive Blue 5 + $2H_2O_2$ ⇌ phthalate + 2,2′-disulfonyl azobenzene + 3-((4-amino-6-chloro-1,3,5-triazin-2-yl)amino)benzenesulfonate + $2H_2O$	
Producing fungal species (reported in fPoxDB)	Almost all wood decaying **Ascomycota** and **Basidiomycota** (Hoegger et al. 2006)	Fungi +-**Ascomycota** | ++Pezizomycetes | | \-*Pyronema omphalodes* | \+Sordariomycetes | \-*Fusarium oxysporum* \+**Basidiomycota** \-Polyporales ++Meruliaceae | \-*Phlebia brevispora* +-Phanerochaetaceae | +-Phanerodontia | | \-*Phanerodontia chrysosporium* | +-Phlebiopsis | | \-*Phlebiopsis gigantea* | \-Bjerkandera | \-*Bjerkandera adusta* \+Polyporaceae \-*Trametes versicolor*	Fungi ++**Ascomycota** | \-*Pyronema omphalodes* \-**Basidiomycota** \-Agaricomycetes ++Trechisporales | \-*Sistotremastrum suecicum* ++Hymenochaetales | \-*Fomitiporia mediterranea* ++Corticiales | \-*Punctularia strigosozonata* ++Jaapiales | \-*Jaapia argillacea* ++Amylocorticiales | \-*Plicaturopsis crispa* ++Geastrales | \-*Sphaerobolus stellatus* +-Russulales | ++Stereaceae | | \-*Stereum hirsutum* | \-Bondarzewiaceae | \-Heterobasidion | +-*Heterobasidion irregulare* | \-*Heterobasidion annosum* +-Polyporales | ++Meruliaceae | | \-*Phlebia brevispora* | +-Phanerochaetaceae | | +-Phanerodontia | | | \-*Phanerodontia chrysosporium* | | +-Phlebiopsis | | | \-*Phlebiopsis gigantea* | | \-Bjerkandera | | \-*Bjerkandera adusta* | \-Polyporaceae | +-Ganoderma | | \-*Ganoderma sp.* | +-Dichomitus	Fungi +-**Ascomycota** | ++Pezizomycetes | | \-*Pyronema omphalodes* | \+Sordariomycetes | \-*Fusarium oxysporum* \-**Basidiomycota** \-Agaricomycetes +-Polyporales | ++Phanerochaetaceae | | \-*Bjerkandera adusta* | \-Polyporaceae | +-Dichomitus | | \-*Dichomitus squalens* | +-Lentinus | | \-*Lentinus tigrinus* | \-Trametes | \-*Trametes versicolor* \+Agaricales \-*Pleurotus ostreatus*	**Ascomycota** +-Lecanoromycetes | ++Teloschistales | | \-*Gyalolechia flavorubescens* | \+Lecanorales | \-Cladonia | +-*Cladonia macilenta* | \-*Cladonia metacorallifera* ++Dothideomycetes | \-*Cenococcum geophilum* +-Leotiomycetes | \-Helotiales | ++Gelatinodiscaceae | | \-*Ascocoryne sarcoides* | \-Sclerotiniaceae | +-Botrytis | | \-*Botrytis cinerea* | \-Sclerotinia | \-*Sclerotinia sclerotiorum* ++Sordariomycetes | \-Sordariaceae | +-Sordaria | | \-*Sordaria macrospora* | \-Neurospora | +-*Neurospora tetrasperma* | +-*Neurospora discreta* | \-*Neurospora crassa* \-Eurotiomycetes ++Verrucariales | \-*Endocarpon pusillum* \-Eurotiales \-Aspergillaceae +-Penicillium | \-*Penicillium chrysogenum* \-Aspergillus +-*Aspergillus fumigatus* +-*Aspergillus carbonarius* +-*Aspergillus fischeri*	**Basidiomycota** +-Pucciniomycetes | \-Pucciniales | ++Melampsoraceae | | \-*Melampsora larici-populina* | \+Pucciniaceae | \-*Puccinia graminis* \-Agaricomycetes ++Hymenochaetales | \-*Fomitiporia mediterranea* ++Corticiales | \-*Punctularia strigosozonata* ++Jaapiales | \-*Jaapia argillacea* +-Cantharellales | ++Botryobasidiaceae | | \-*Botryobasidium botryosum* | \+Tulasnellaceae | \-*Tulasnella calospora* ++Atheliales | \-*Piloderma croceum* ++Geastrales | \-*Sphaerobolus stellatus* ++Sebacinales | \-Serendipita | +-*Serendipita vermifera* | \-*Serendipita indica* +-Russulales | ++Stereaceae | | \-*Stereum hirsutum* | \-Bondarzewiaceae | \-Heterobasidion | +-*Heterobasidion irregulare* | \-*Heterobasidion annosum* +-Boletales | ++Paxillaceae | | \-*Hydnomerulius pinastri* | ++Sclerodermataceae

			| |\-*Dichomitus squalens* | +-Polyporus | | +-*Polyporus brumalis* | | \-*Polyporus arcularius* | +-Lentinus | | \-*Lentinus tigrinus* | \-Trametes | \-*Trametes versicolor* +-Agaricales | ++Cortinariaceae | | \-*Hebeloma cylindrosporum* | +-Omphalotaceae | | +-Gymnopus | | | \-*Gymnopus luxurians* | | \-Omphalotus | | \-*Omphalotus olearius* | +-Strophariaceae | | +-Galerina | | | \-*Galerina marginata* | | \-Hypholoma | | \-*Hypholoma sublateritium* | +-Tricholomataceae | | \-Laccaria | | +-*Laccaria amethystina* | | \-*Laccaria bicolor* | ++Agaricaceae | | \-*Agaricus bisporus* | \+Pleurotaceae | \-*Pleurotus ostreatus* \-Auriculariales ++Auriculariaceae | \-*Auricularia subglabra* \+Exidiaceae \-*Exidia glandulosa*		+-*Aspergillus terreus* +-*Aspergillus oryzae* +-*Aspergillus flavus* \-*Aspergillus clavatus*	| | \-*Scleroderma citrinum* | ++Pisolithaceae | | \-*Pisolithus tinctorius* | \-Suillaceae | \-Suillus | +-*Suillus brevipes* | \-*Suillus luteus* +-Polyporales | ++Meruliaceae | | \-*Phlebia brevispora* | ++Dacryobolaceae | | \-*Postia placenta* | +-Phanerochaetaceae | | +-Phlebiopsis | | | \-*Phlebiopsis gigantea* | | \-Bjerkandera | | \-*Bjerkandera adusta* | \-Polyporaceae | +-Ganoderma | | \-*Ganoderma sp.* | +-Dichomitus | | \-*Dichomitus squalens* | +-Polyporus | | +-*Polyporus brumalis* | | \-*Polyporus arcularius* | +-Lentinus | | \-*Lentinus tigrinus* | \-Trametes | \-*Trametes versicolor* +-Agaricales | ++Marasmiaceae | | \-*Moniliophthora perniciosa* | ++Cortinariaceae | | \-*Hebeloma cylindrosporum* | +-Omphalotaceae | | +-Gymnopus | | | \-*Gymnopus luxurians* | | \-Omphalotus | | \-*Omphalotus olearius* | +-Strophariaceae | | +-Galerina | | | \-*Galerina marginata* | | \-Hypholoma | | \-*Hypholoma sublateritium* | +-Amanitaceae | | \-Amanita | | +-*Amanita thiersii* | | \-*Amanita muscaria* | +-Tricholomataceae | | \-Laccaria | | +-*Laccaria amethystina* | | \-*Laccaria bicolor* | \+Pleurotaceae | \-*Pleurotus ostreatus* \-Auriculariales ++Auriculariaceae | \-*Auricularia subglabra* \+Exidiaceae \-*Exidia glandulosa*

polymer (Pelmont 1995). The catalytic cycle uses in particular veratryl alcohol (VA) as an electron donor and cofactor (Wong 2009). LiPs are considered as the most efficient peroxidase type because it has a high redox potential enabling it to oxidize a higher variety of substrates (Sigoillot et al. 2012). Thus, LiPs are mostly described as the major enzyme responsible for lignin depolymerization and degradation (Weng et al. 2021). However, some studies hypothesized this kind of enzyme to have more of a role of detoxification of low molecular weight phenolic compounds released during lignin depolymerization or of functionalization of the lignin polymer to facilitate its depolymerization by other enzymes (Sarkanen et al. 1991).

Manganese peroxidases (MnP) are glycosylated heme enzymes very similar to LiP, but they use hydrogen peroxide to oxidize Mn^{2+} in the presence of chelators to Mn^{3+}, which in turn oxidizes a variety of phenolic substrates. In particular, Mn^{3+} catalyzes alkyl-aryl cleavage and α-carbon oxidation in lignin (Tuor et al. 1992) and forms other radicals involved notably in the degradation of non-phenolic parts of lignin through the oxidation of organic sulfur compounds and unsaturated fatty acids (Wariishi et al. 1989; Kapich et al. 1999). By oxidizing phenolic compounds of lignin, Mn^{3+} also generates phenoxy-radicals, which are involved themselves in lignin depolymerization (Hofrichter 2002). MnPs are classically described to only oxidize phenolic compounds, but they were shown to oxidize also non-phenolic lignin model compounds in the presence of additional Mn^{2+} (Chio et al. 2019). Concerning their consequences on lignin structure, MnP and laccases from *Ceriporiopsis subvermispora* have been reported to mainly cleave β-*O*-aryl ether linkages in the lignin of *Pinus taeda* (Guerra et al. 2004). MnP was also shown to trigger loss of methoxyl groups in ^{14}C-labeled lignin enhancing its reactivity by increasing the phenolic content (Ander et al. 1992). In cell-free systems, MnP was shown to generate the formation of water-soluble fragments and even to mineralize 5% of ^{14}C-labeled lignin (Kapich et al. 1999). MnP is the most common ligninolytic peroxidases of Basidiomycetes (Hofrichter 2002).

Versatile peroxidases (VP) are also designated under the term hybrid peroxidases in the sense that these bifunctional enzymes present similar catalytic activities to both lignin and manganese-peroxidases. Indeed, the structure of VP provides multiple binding sites for different substrates enabling the oxidation of Mn^{2+} as well as both phenolic and non-phenolic aromatic compounds without a mediator. However, VP can directly degrade high reduction potential substrates without VA or oxidize independently Mn^{2+} in contrast to LiP and MnP respectively (Camarero et al. 1999). Other substrates described for VP are a-keto-g-thiomethylbutyric acid (KTBA), veratryl alcohol, dimethoxybenzenes, different types of dyes, substituted phenols, and hydroquinones (Caramelo et al. 1999; Heinfling et al. 1998).

Dye decolorizing peroxidases (DyP) share similar catalytic properties with other previous peroxidases in the sense that they use hydrogen peroxide and mediators for substrate oxidation (Sugano et al. 2007; Liers et al. 2014). However, a DyP from *Pleurotus sapidus* was shown to be able to oxidize the substrate with the oxygen from the air and without the presence of hydrogen peroxide (Avram et al. 2018). DyP are phylogenetically distinct from other lignin-modifying peroxidases (Sugano et al. 2007) and have different sequences and structures with an α + β ferredoxin-like fold

(Singh and Eltis 2015). More in detail, DyPs catalyze the oxidation of a variety of substrates such as phenolic and nonphenolic aromatic compounds including high-redox anthraquinone dyes, lignin model compounds, and polymeric lignocellulose. However mostly DyPs demonstrate high activity against phenolic compounds but do not always accept non-phenolic compounds or more complicated lignin molecules. This catalysis occurs in the active site or on the surface of the enzyme according to the size of the substrate and on the existence of radical transfer pathways available in the enzyme (Catucci et al. 2020). According to their structures, DyPs are classified into four classes namely A, B, C, and D (Fawal et al. 2013). Unlike other lignin-degrading peroxidases, DyP are produced by both bacteria (types A, B, C) and fungi (type D) (Abdel-Hamid et al. 2013).

Some studies showed synergy between different extracellular oxidoreductases in the lignin depolymerization process for instance between MnP and laccase (Galliano et al. 1991) or between MnP and LiP (Thompson et al. 1998). Thus, now that the catalytic mechanisms are better understood for each lignin-modifying enzyme, a promising research trail is at the level of the cooperation between them and with sugar-oxidizing enzymes to better understand the lignin degradation in the lignocellulosic biomass context (Hofrichter 2002).

5.2 *Ligninases Phylogenetic Repartition Within the Fungal Kingdom*

5.2.1 The Different Types of Wood Rots

Wood decaying fungi are generally divided into three main non-monophyletic groups according to the degradation mechanism involved: white-rot, brown-rot, and soft-rot fungi. The name of the different groups refers to the aspect of wood after the action of the different fungi, i.e., the majority degradation of lignin let the lighter-colored cellulose, the preferential degradation of cellulose let the brown oxidized lignin, and the action of some molds soften the wood surface (Deacon 2013).

White-rot fungi are the most numerous wood rots and the most efficient in lignin degradation since this group is the only one able to carry out the complete mineralization to CO_2 (Andlar et al. 2018). This capacity relies on the fact that this group produces most of the different reported extracellular ligninases. The evolution of white rots peroxidase was even proposed to be driven by the evolution of wood lignin since the appearance of more efficient peroxidases with solvent-exposed catalytic tryptophan correlates with the diversification of angiosperms with hardwoods including dimethoxylated syringyl lignin units (Ayuso-Fernández et al. 2019). White-rot fungi include the terms white rot, corrosive rot, simultaneous rot, pocket rot, and uniform rot and are often defined as all species in the Basidiomycotina that have the capacity to degrade lignin (Blanchette 1991). However other references also include a few species of Ascomycetes within this category

(Sigoillot et al. 2012; Deacon 2013). White-rot fungi can either selectively delignify wood, simultaneously degrade lignin and wood polysaccharides throughout the decayed wood or cause a combination of selective delignification and simultaneous decay within the same substrate (Blanchette 1991). Indeed, *Ceriporiopsis subvermispora*, *Phellinus pini*, *Ganoderma australe*, and *Phlebia tremellosa* specifically degrade lignin and hemicellulose but not cellulose, whereas *Phanerochaete chrysosporium, Trametes versicolor*, *Heterobasidion annosum,* and *Irpex lacteusare* can simultaneously degrade cellulose, hemicellulose, and lignin (Weng et al. 2021).

Brown-rot (formerly red-rot) fungi represent 7% of wood-rotting Basidiomycetes and can rapidly hydrolyze the component of cellulose and hemicellulose while just partially oxidize lignin through hydroxyl radicals resulting from Fenton oxidation chemistry (Bugg et al. 2011a). Indeed, these fungi produce extracellular hydroquinones that can reduce Fe^{3+} of Fe–oxalate complex to Fe^{2+} able to generate hydroxyl radicals by reacting with hydrogen peroxide (Jensen Kenneth et al. 2001). Brown rots were shown to be able to non-selectively break the intermonomer side-chain linkages of lignin (Yelle et al. 2008) and decrease the arylglycerol-β-aryl ether linkage (Yelle et al. 2011) through this type of Fenton reactions primarily in conifer softwoods (Weng et al. 2021). Besides, brown rots have been found to generate extensive oxidative demethylation, significant side-chain oxidation, lignin depolymerization, potentially repolymerization, limited aromatic ring cleavages, hydroxylation of aromatic rings, Cβ-ether cleavage, partial side chains hydroxylation, formation of new aryl-*O*-aryl ether and aryl-aryl or side-chain linkages. However, in spite of all these alterations, brown-rotted lignin remains polymeric (Arantes and Goodell 2014). Despite the different mechanisms involved in lignin degradation, DNA analyses have shown that brown rots are not separated from white rots in the Basidiomycota phylogeny (Hibbett and Donoghue 2001). Indeed, the phylogenetic analysis of ligninolytic peroxidases showed that the mix of concerted and birth-and-death evolution processes led to the alternation of white and brown rots in the same lineages (Zhou et al. 2014). The reductive evolution of brown rots from white-rot ancestors is hypothesized to have enabled to develop a less energy-demanding wood attack strategy based on non-enzymatic processes compared to the production of complex biomass deconstructing enzymes (Arantes and Goodell 2014).

Despite the name of the group, soft-rot fungi designate the characteristic penetration and growth of hyphae within the secondary cell walls of wood whether or not softening of the surface (Levy 1966). Soft rots are mainly Ascomycetes decaying preferentially angiosperm hardwoods (Kuhad et al. 1997) and degrading lignin by attacking the syringyl units (Zabel and Morrell 2020). Soft rots have been described to degrade vanillic acids and phenols, but little is known about the enzymes involved in lignin degradation and it is hypothesized that this group modifies rather than mineralize lignin (Weng et al. 2021).

5.2.2 Ligninases Producers

Laccases belong to the blue copper proteins and more precisely to the family of multicopper oxidases (MCOs) with ferroxidases, ascorbate oxidase, and ceruloplasmin. A phylogenic comparison and classification of sequences within this family showed that laccases *sensu stricto* are produced by Basidiomycetes and Ascomycetes at least partially according to their lifestyle, whereas laccases by larger sense originate from bacteria, fungi, plants, and insects (Hoegger et al. 2006). In this study, the laccases *sensu stricto* cluster clearly according to the taxonomical association with two groups associated either to Homobasidiomycetes or to filamentous Ascomycetes. Within Homobasidiomycetes, laccases genes appeared to group according to their function notably in wood decay.

Lignin peroxidases have their genes quite limited to white-rot fungi according to the fungal peroxidase database (fPoxDB; Choi et al. 2014). Indeed only 1 gene has been assigned to each Ascomycète *Pyronema omphalodes* and *Fusarium oxysporum* over the total 43 genes reported in fPoxDB (2021). So almost all genes documented in this bank are associated with the Polyporales order of Basidiomycetes (Table 5).

Manganese peroxidases have drawn attention as alternative ligninolytic peroxidases to LiP due to their wider distribution among Basidiomycetes (Choi et al. 2014), although still limited to the Agaricomycetes class (Table 5). These Basidiomycetes belong to two ecophysiological groups, namely wood-decaying fungi causing white rot and certain soil litter-decomposing fungi. Indeed, both typical wood colonizers belonging to phylogenetically older families including Meruliaceae, Coriolaceae, or Polyporaceae, and soil-litter colonizers such as Strophariaceae and Tricholomataceae produce MnP (Hofrichter 2002). Whereas the fPoxDB lists 33 species presenting MnP genes, Hofrichter (2002) reports 56 species as producing MnP in culture according to enzyme purification or enzyme activities measurements. Thus, the number of annotated genes is likely to increase in the future.

Versatile peroxidases genes are mainly described in white-rot fungal genera *Pleurotus* and *Bjerkandera* in the literature (Hofrichter et al. 2010). However, fPoxDB reports genes in some other Basidiomycetes white rots, namely *Dichomitus squalens*, *Lentinus tigrinus*, *Trametes versicolor*, and even two Ascomycetes (Table 5). In contrast, *Pleurotus eryngii* is not cited in fPoxDB, whereas VP was well described in this species (Camarero et al. 1999).

Dye decolorizing peroxidases are not only restricted to the fungal kingdom but are also found widely in various bacteria (Chio et al. 2019). Within fungi, the fPoxDB data show that the genes of this type of peroxidases are also widely distributed within both Ascomycetes and Basidiomycetes phyla (Table 5). Besides, DyP structures were also characterized in the two Basidiomycetes *Termitomyces albuminosus* and *Marasmius scorodonius* (Hofrichter et al. 2010) and there is a report on DyP-like proteins in the *Auricularia auricula-judae* (Liers et al. 2010).

5.3 *Products of Fungal Lignin Depolymerization*

The analysis of residual lignin at various stages of decay by white-rot fungi indicates a steady loss of methoxyl groups and increases in oxygen and hydroxyl content. Main white-rot lignin degradation products reported by Kirk et al. (1975) include low molecular weight soluble molecules such as vanillin, syringaldehyde, coniferyl aldehyde, vanillic acid, syringic acid, and a wide range of aliphatic or aromatic acids and phenols. More recent researches enabled us to distinguish three main steps in lignin biodegradation namely depolymerization, biological funneling, and ring cleavage (Li and Zheng 2020). After lignin depolymerization, a heterogeneous mixture of low-molecular-weight aromatic compounds is generated with certain toxicities against microorganisms. Within this mixture, three categories of monolignol can be distinguished and correspond to the major monomers enabling lignin synthesis, as previously described, G-lignin, H-lignin, and S-lignin (Weng et al. 2021). The oligomers generated by lignin depolymerization are then taken up by fungal cells, converted to other intermediates through funneling pathways, and further degraded notably through ring cleavage and citric acid cycle (Li and Zheng 2020). Thus, the inhibition of oligomers cellular uptake has been proposed as a promising approach to increase oligomers production yields through fungal lignin depolymerization (Leriche-Grandchamp et al. 2020). The biological funneling step may involve several chemical transformations catalyzed by diverse enzymes such as acryl CoA synthetase, acryl-CoA hydratase, dehydrogenase, decarboxylases, and *O*-demethylase system according to the structure of lignin monomers and environmental conditions (Abdelaziz et al. 2016). The H-lignin derivative *p*-coumaric acid is described to be converted ultimately into *p*-hydroxybenzaldehyde, 4-hydroxybenzoate, and protocatechuic acid during biological funneling processes through CoA-dependent β-oxidation, CoA-dependent non-β-oxidation or CoA-independent pathways. The G-lignin derivative ferulic acid is converted through different metabolic pathways (non-oxidative decarboxylation, CoA-dependent non-β-oxidation, CoA-dependent β-oxidation, side-chain reduction) into vanillin, vanillic acid, and protocatechuic acid. The S-lignin derivative syringic acid is described to be converted into 3-*O*-methyl gallate and gallic acid (Li and Zheng 2020).

6 Applications of Fungal Lignin Depolymerization

Thanks to the strong ability of extracellular enzymes produced by fungi for delignification and lignin depolymerization, these enzymes have been used in various applications such as the production of platform chemicals, bioremediation, and biofuel production.

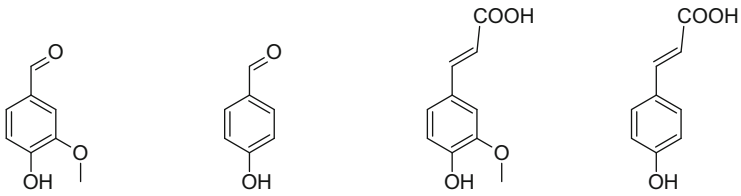


Fig. 22 Phenolic compounds from lignin microbial degradation

6.1 Fungal Lignin Depolymerization Products in Green Chemistry

Various molecules of phenolic class can be obtained from the fungal or bacterial degradation of lignin (Bugg et al. 2011b; Sainsbury et al. 2013) and can give access to small molecules of interest such as vanillin, *p*-hydroxybenzaldehyde, ferulic acid, or even *p*-coumaric acid (Fig. 22).

These compounds contain several similarities in terms of function, as they are all phenols, with an *o*-methoxy group (Vanillin, Ferulic acid) or not (*p*-Hydroxybenzaldehyde, *p*-Coumaric acid). They also possess functionalizable moieties, such as aldehyde (Vanillin, *p*-Hydroxybenzaldehyde) or an acrylic acid (Ferulic acid, *p*-Coumaric acid). All these parameters are giving strong possibilities for using them as given or to be able to functionalize them, especially under green conditions.

Indeed, the concept of green chemistry was developed by Paul Anastas and John Warner in 1998 (Anastas and Warner 1998), and is based on 12 principles including (examples given):

- **Prevention**, as it is better to prevent waste than to treat or clean up waste after it has been created (Jimenez-Gonzalez et al. 2011)
- **Atom economy**, as a synthetic method, should be designed to maximize the incorporation of all materials used in the process into the final product (Lim and Dolzhenko 2020)
- **Less hazardous chemical syntheses**, as methods should be designed to use or produce substances with little or no toxicity for Human health or the environment (Anastas and Warner 2005)
- **Designing safer chemicals**, as they should preserve the efficacy of function while reducing their toxicity (Ariëns 1980)
- **Safer solvents and auxiliaries**, as these agents should be made unnecessary when possible and innocuous when used (Inamuddin et al. 2021)
- **Design for energy efficiency**, as environmental and economic impact, should be minimized, and methods should be conducted at ambient pressure and temperature.

- **The use of renewable feedstocks**, as raw material, should be renewable whenever practicable (Tursi and Olivito 2021)
- **Reduce derivatives**, as additional steps such as protection/deprotection generate waste
- **Catalysis**, as catalytic reagents are superior to stoichiometric ones, when selective (Sheldon et al. 2007)
- **Design for degradation**, like chemicals should not persist in the environment at the end of their function
- **Real-time analysis for pollution prevention**, as in-process and control analyses, should be developed before hazardous substances formation
- **Inherently safer chemistry for accident prevention** as releases, explosions, and fires should be avoided by choosing the right substances or formed substances during the process (Gao et al. 2021)

So, by trying to approach the maximum of the ideal 12 green chemistry concepts, some examples using fungal depolymerization products of lignin are given. First, some examples will concern the vanillin transformation, followed by ferulic compounds and coumaric moieties, and then dyes recovery from hydroxybenzaldehyde or vanillin will be discussed.

6.1.1 Vanillin

Vanillin being a valuable depolymerization product, its use as starting material in green chemistry or using a green process is of great interest. Some examples of these syntheses are given in Fig. 23.

In the domain of materials, resins made of methacrylated vanillin-glycerol dimethacrylate can give access, without any reaction solvent to a low viscosity polymer, using a two-step, one-pot synthesis. Indeed, each step was conducted without solvent, even for the reaction of vanillin to the methacrylated product of step one (Stanzione et al. 2012).

But small molecules can also be of great interest from vanillin, such as benzothiazoles where the "on-water" process has been used for its synthesis, particularly in 81% yield from vanillin (Chakraborti et al. 2007). In the same way, condensation with semicarbazide gives access to thiazolidine-thione in aqueous ethanol (88–91% yield from vanillin), where thiazole can be a central tool for drug-like candidates (Ramesh and Lalitha 2016). Vanillin-based ethers are of great interest in perfumery, and their recovery with 100% selectivity is important. Then, a triphasic reaction (Fig. 24) involving a phase-transfer catalyst tetrabutylammonium bromide (TBAB) gave excellent 100% conversion of vanillin, but also a great 100% selectivity into the reached ether (Yadav and Lande 2005).

When synthesis is performed in water, some surfactants can be useful to catalyze the reaction. That is the case for the one-pot formation of 1,8-dioxo-decahydroacridines in aqueous media, where the best catalyst was *p*-dodecylbenezenesulfonic acid (DBSA) compared to the couple SDS (Sodium

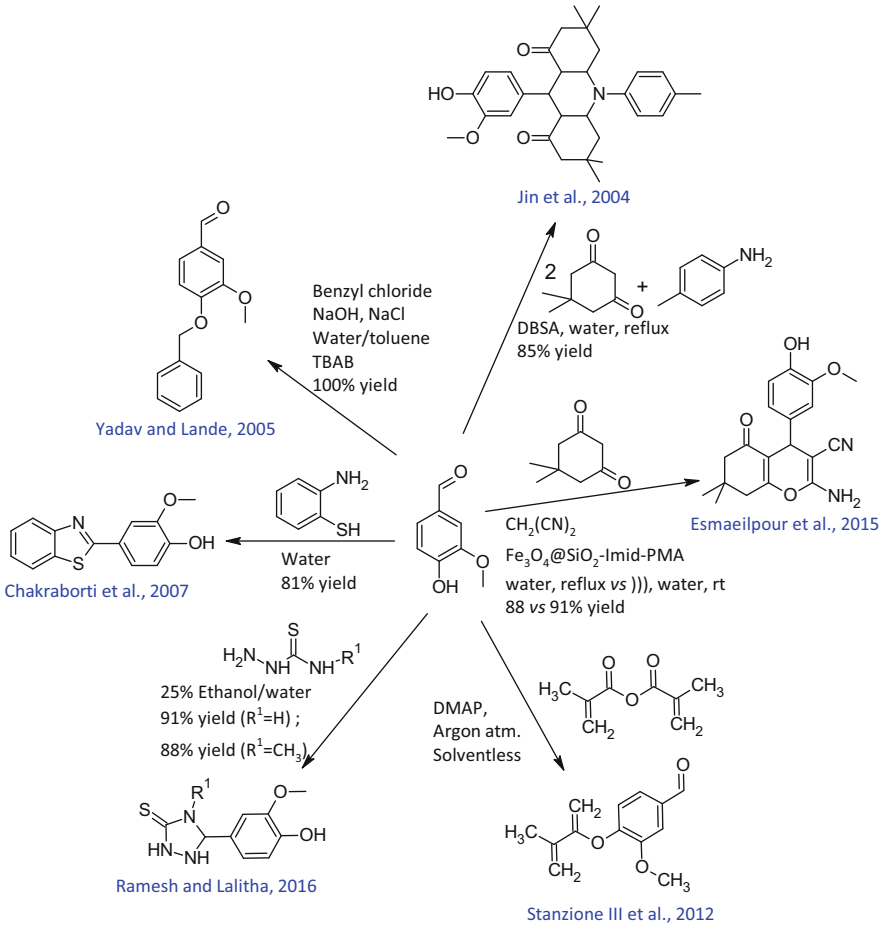


Fig. 23 Some examples of green syntheses from Vanillin

Dodecyl Sulfate) + TsOH (Tosylic acid), only TsOH or fatty acid for example (Jin et al. 2004). Another way of using vanillin for chromene derivatives is to use magnetic nanocatalyst under ultrasonic irradiation or underwater reflux conditions. That is what was done in 2015 by a green one-pot three-component synthesis (Esmaeilpour et al. 2015).

Moreover, some original examples can be given (Fig. 25) using lemon juice or laccase as reactants. In the first case, lemon juice extract from *Citrus limonium* contains moisture (85%), citric acid (5–7%), carbohydrates (11.1%), vitamin C (0.4%), protein (1%), fat (0.9%), minerals (0.3%), fibers (1.7%), and some free and combined organic acids. It is obviously the natural acidic lemon juice extract content that will act as a natural acidic catalyst (Deshmukh et al. 2012). In the second example, the vanillin dimerization will occur using laccase from *Trametes versicolor*

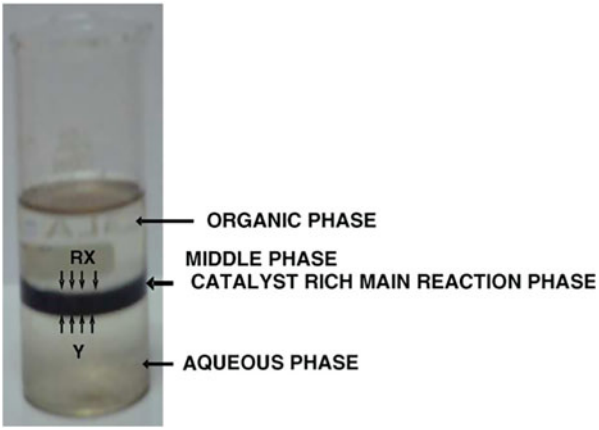


Fig. 24 Photograph of the liquid-liquid–liquid phase-transfer catalysis. (Reuse (print) with permission of Yadav, G.D., Lande, S.V., 2005. Novelties of reaction in the middle liquid phase in tri-liquid phase transfer catalysis: Kinetics of selective O-alkylation of vanillin with benzyl chloride. Applied Catalysis A: General 287, 267–275. https://doi.org/10.1016/j.apcata.2005.04.006. Copyright (2005) Elsevier)

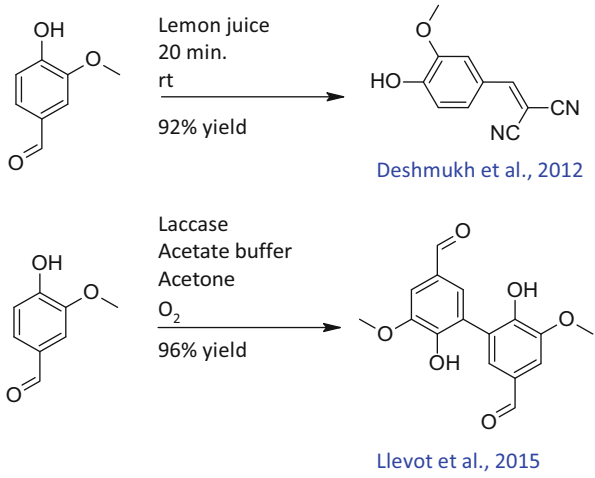


Fig. 25 Original examples of vanillin green modification

as the catalyst. This leads to a dimerization into the *ortho* position compared to the hydroxyl group (Llevot et al. 2015).

6.1.2 Ferulic and Coumaric Acids

Ferulic and coumaric acids are of great interest for green functionalization, and some examples are given below (Fig. 26). For instance, ferulic acid can be functionalized

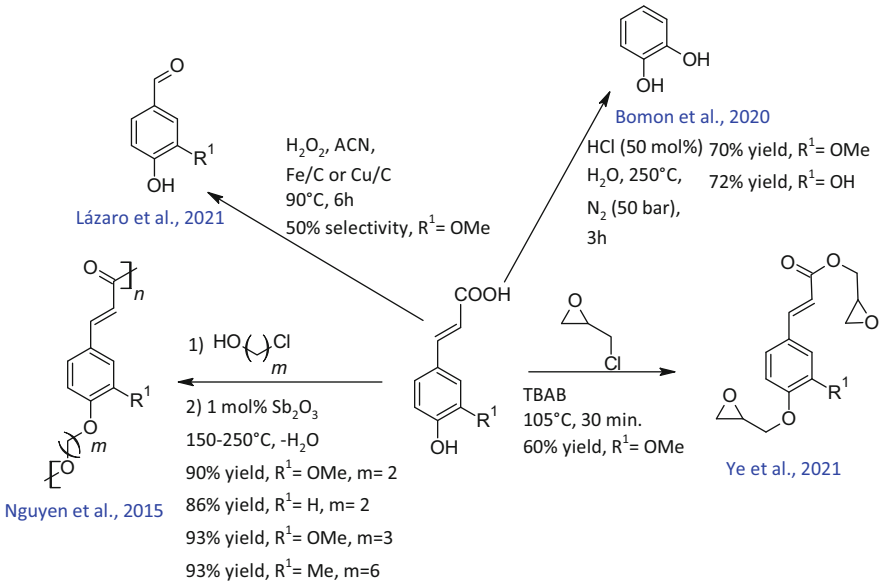


Fig. 26 Some examples of green syntheses from ferulic (R^1 = OMe) and coumaric (R^1 = H) acids

with epoxy groups and polymerized. Such bio-based epoxies can lead to a green alternative to fossil-sourced bisphenol A (Ye et al. 2021). Other polymers are also of great interest, such as polyethylene ferulate (PEF), or polyethylene coumarate (PEC), which have the thermal range of fossil polystyrene (Nguyen et al. 2015).

Degradation of ferulic acid can also lead to derivatives such as bio-catechol (Bomon et al. 2020). In this example, both O- and C-dealkylation occur, and the authors proposed a mechanism for this dealkylation (Fig. 27). Indeed, treatment of the ferulic acid or caffeic acid derivatives with hydrochloric acid in water leads to the formation of benzylic alcohol **A** through the addition of water to the alkene. Protonation of the phenolic compound leads to the intermediate **B** then allows C-C bond cleavage via a retro vinylogical aldol reaction, delivering guaiacol **C**. The O-demethylation reaction of **C** is initiated by protonation of the arene into **D** followed by the addition of water to give hemiacetal **E**, which upon elimination of methanol and aromaticity restoration give the final catechol.

Ferulic acid can also be transformed into valuable vanillin (Fig. 26), with green catalysts obtained by Mimosa tannins transformed and functionalized into Cu/Fe-OMC or Cu/Fe-DMC (Ordered/Disordered Mesoporous Carbons) by mechanochemistry (Lázaro et al. 2021).

The global work presentation of these authors (Fig. 28) and the proposed mechanism of ferulic transformation (Fig. 29) are given below.

Indeed, first, ferulic acid is decarboxylated while heated to get vinylguiaiacol, whose vinylic part is oxidized into an epoxide moiety. The epoxide opening into diol generated vanillin.

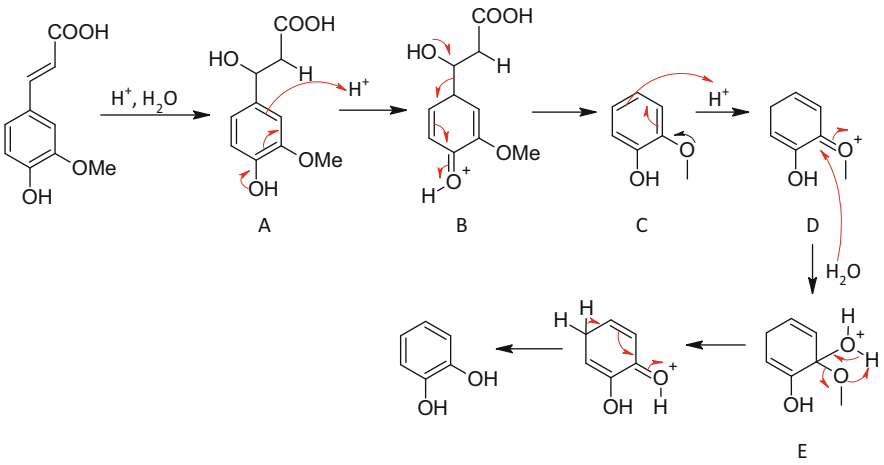


Fig. 27 Proposed mechanism for O- and C-dealkylation (Bomon et al. 2020)

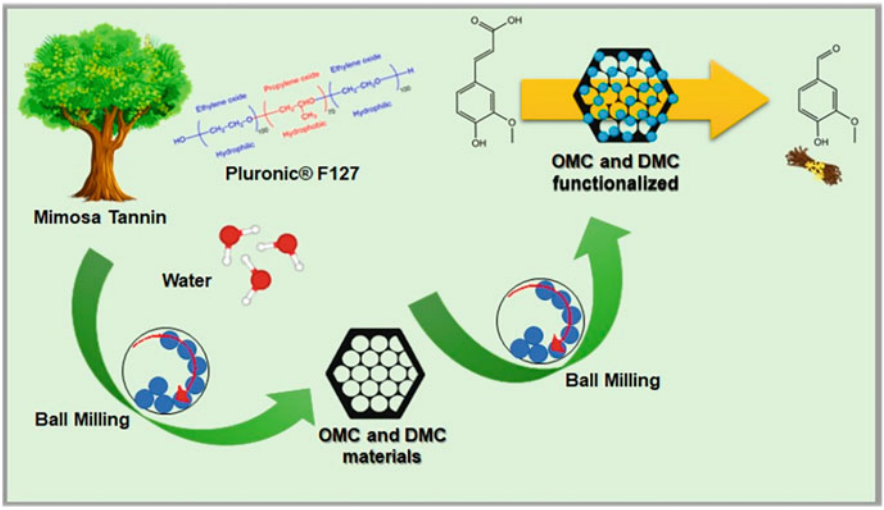


Fig. 28 Global approach for green ferulic acid transformation into vanillin. (Reuse (print) with permission of Lázaro, N., Castro-Gutiérrez, J., Ramírez-Vidal, P., Celzard, A., Fierro, V., AlGarni, T.S., Pineda, A., Luque, R., 2021. Mechanochemical Functionalization of Mesoporous Carbons for the Catalytic Transformation of trans-Ferulic Acid into Vanillin. ACS Sustainable Chem. Eng. 9, 4704–4710. https://doi.org/10.1021/acssuschemeng.0c09028. Copyright (2021) American Chemical Society)

As previously described for vanillin, laccase can be used to graft or modify ferulic acid or ferulate. This is the case for bio-composites elaboration when ferulic acid molecules were grafted on cellulose using laccase from *Trametes versicolor*. The

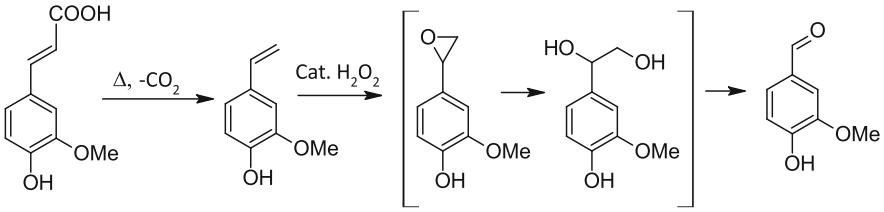


Fig. 29 The proposed mechanism by Lázaro et al. (2021) for the formation of vanillin from ferulic acid

resulting bio-composites possessed an elongation at maximal stress of 23% improvement, corresponding to a material mechanical resistance. This result was also confirmed by bio-composite elaboration with natural fibers (Morin et al. 2019). Oxidation of ferulic acid or ethyl ferulate with *Myceliophthora thermophila* laccase led to an eco-friendly procedure to synthesize new active molecules possessing antioxidant properties (Aljawish et al. 2014).

Finally, green sunscreen can be obtained when an ester from ferulic acid is transesterified with soybean oil. Indeed, this transesterification from ethyl ferulate molecule with soybean oil thanks to *Candida antarctica* lipase B reactivity can lead to feruloylated monoacyl- and diacylglycerols with small quantities of ferulic acid, feruloyl glycerol, and fatty acid ethyl esters. These two major compounds are known as SoyScreen™ (Laszlo et al. 2003).

6.1.3 Organic dyes

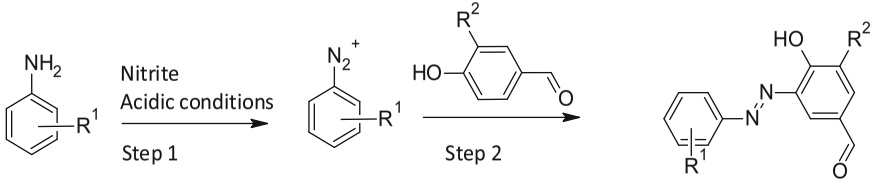
Organic dyes are mainly composed of azobenzene molecules, that are smart compounds able to isomerize while illuminated and many applications can be found for these molecules such as dyes (Rizk et al. 2015; Tanizaki et al. 1966; Teimouri et al. 2013), antibacterials (Banaszak-Leonard et al. 2021; Velema et al. 2015), for retina recovery (Laprell et al. 2017) in the electrical storage domain (Zhu et al. 2019) or even for smart micellar catalysis (Drillaud et al. 2012).

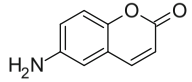
The simplest way for obtaining unsymmetrical azobenzenes from hydroxybenzaldehyde or vanillin is using diazonium salt of the corresponding aniline on the phenolic compound, as shown in the general figure of Table 6 below.

First, during step 1, sodium nitrite and acid (usually HCl—entries 1, 4, and 5) affords the unstable nitrous acid that reacts with aniline to afford the diazonium salt (Franche et al. 2020; Léonard and Fayeulle 2021). It is noticeable that this step works well with bulk anilines (entry 1) as well as smaller ones, but with electronic effects on the final yield. For example (entries 2, 3, and 4) an electron-withdrawing group such as the 4-nitro group gives a better final yield than an electron-donating group, mainly because of the availability of the electrophilic diazonium salt in step 2.

During step 2, vanillin or hydroxybenzaldehyde are deprotonated with a base ($NaHCO_3$, KOH, NaOH, K_2CO_3) to give the corresponding sodium or potassium

Table 6 General scheme of diazotation and examples of diazo compounds obtained from vanillin or hydroxybenzaldehyde



Entry	R^1-NH_2	Step 1	R^2	Step 2	Yield	Reference
1		$NaNO_2$ HCl Water	OMe	$NaHCO_3$ Water	90%	Guha et al. (2011)
2	R^1 = 4-NO_2	Butyl nitrite PTSA Water	H	K_2CO_3 Ethanol	88%	Khaligh et al. (2019)
	R^1 = 2-NO_2				84%	
	R^1 = H				80%	
	R^1 = 4-Me				77%	
	R^1 = 4-Cl				82%	
	R^1 = 2-Cl				80%	
	R^1 = 4-Br				81%	
	R^1 = 4-C(O)-Me				83%	
	R^1 = 4-OMe				79%	
3	R^1 = 4-NO_2	*Tert*-butyl nitrite Acetic acid *Bis*(trifluoromethanesulfonyl)imide	H	K_2CO_3 Ethanol	88%	Khaligh (2017)
	R^1 = 2-NO_2				82%	
	R^1 = H				81%	
	R^1 = Me				78%	
	R^1 = 4-Cl				80%	
	R^1 = 2-Cl				78%	
	R^1 = 4-Br				80%	

	R^1 = 4-C(O)-Me				81%	
	R^1 = 4-OMe				77%	
4	R^1 = 4-NO_2	$NaNO_2$ HCl Water	H	KOH Water	85%	Kumar et al. (2013)
5	R^1 = H	$NaNO_2$ HCl Water	H	Na_2CO_3 Water	38%	Amaravathi et al. (2013)
	R^1 = 4-Me				33%	
	R^1 = 4-OMe				35%	
	R^1 = 4-NO_2				56%	

PTSA *p*-toluenesulfonic acid

phenolate, which will act, after an electronic delocalization, as a nucleophile on the unstable diazonium salt, and will thus form the desired azobenzene. It is noticeable that in all the syntheses presented here, the solvents have a null or minor impact on the environment (water or ethanol).

To conclude, various molecules of phenolic class are obtained from the fungal or bacterial degradation of lignin and the small molecules having some useful functional groups can be used as extracted, or can be further functionalized. In this context, and having in mind the 12 principles of green chemistry, the diverse modifications of vanillin, hydroxybenzaldehyde, ferulic or coumaric acids were presented giving access to useful synthons, polymers, or dyes.

6.2 Bioremediation

Even after the lignin extraction processes used in the paper and pulp industry, the wastewater still contains high amounts of complex lignin-derived chlorinated phenolics and sulfonated pollutants. The ligninolytic fungal enzymes such as laccase and peroxidases are capable of catalyzing the modification of phenolic and non-phenolic lignin-derived substances. Due to the non-specificity of lignin-degrading enzymes, they are capable of degrading organic and chloro-organic compounds found in the effluents. The presence of phenoloxidases in the white-rot fungi is directly related to lignin degradation ability. During the initial phase, the fungi produce the ligninolytic enzyme system, followed by lignin degradation during the secondary idiophasic metabolism. White-rot fungi such as *Phanerochaete chrysosporium* are the most commonly used in the degradation of paper and pulp industry waste. As fungi, unlike bacteria, do not require water phases for active dispersal, solid wastes can be used to bioremediate by layering the sludge or soil with fungi. While for liquid effluents, fungi are applied either as agar blocks, spores, mycelium, enzymes directly or immobilized. Batch or continuous flow bioreactors are also used because of their advantages such as augmented bioremediation due to a controlled environment. Batch treatment of lignin-containing Kraft effluent using *Coriolus versicolor* was found to be successful in remediating using both adsorption and oxidation mechanisms (Harms et al. 2011; Kulshreshtha et al. 2013; Singh et al. 2021; Singh 2017).

However, laccases are only capable of degradation of low redox potential phenolic compounds and not dyes, which are recalcitrant aromatics. Thus, a lignin-mediator concept is studied for dye decolorization of effluents from the textile, pulp and paper industry. The use of naturally occurring laccase mediators, which are compounds involved in the natural degradation of lignin by white-rot fungi presents some environmental and economic benefits. These low molecular weight compounds are oxidized by laccase to stable radicals that then act as redox mediators to oxidize other compounds, which are not direct substrates of the enzyme. In addition to mediating the oxidative reactions catalyzed by laccases, the mediators are capable of diffusing to distant sites that are difficult to reach by the mycelium or

the enzymes themselves. Lignin-derived phenols such as syringaldehyde and acetosyringone are observed to be efficient mediators for laccase degradation of different types of dyes and other recalcitrant compounds in a shorter time (Camarero et al. 2005).

6.3 *Potential Applications of Ligninases in Melanin Degradation*

Ligninases produced by white-rot fungi have an important potential in the degradation of melanin. Melanin is a biological dark pigment found in fungi, bacteria, helminths, and mammals. Polymerization of phenolic and/or indolic compounds that form complexes with proteins and carbohydrates results in negatively charged, hydrophobic pigments called melanin. Fungi synthesize two types of melanin, namely, dihydroxy naphthalene (DHN) melanin and L-3,4-dihydroxyphenylalanine (L-dopa) melanin. DHN melanin is produced by a polyketide pathway that uses a series of redox reactions and the final step of DHN polymerization to DHN melanin is catalyzed by the laccase enzyme. L-dopa melanin biosynthesis uses a variety of diverse enzymes, including polyphenol oxidases (i.e. tyrosinase, laccase, catechol oxidase) (Belozerskaya et al. 2017). Therefore, laccase is an important enzyme in the synthesis of melanin. Apart from the remarkable properties of melanin to facilitate the organism to tolerate extreme environments and having various functions, their role in microbial pathogenesis is important to be considered. Melanin provides pathogenicity by reducing the pathogen's susceptibility to host antimicrobial mechanisms by influencing the host immune response to infection (Nosanchuk et al. 2015). So, there are two ways to degrade melanin to reduce the pathogenicity of the microbes. One is to silence the lac gene of the pathogen which inhibits the production of melanin and thereby acts as a disease control (Lǔ et al. 2017). Other, is to introduce ligninase producing white-rot fungi or the isolated enzymes. LiP and MnP because of their high oxidation potential are found to bleach melanin. Melanin bleaching is equivalent to melanin degradation as it is understood that if the pigment is not black, its protective properties are changed and it is no longer melanin. Therefore, isolated enzymes such as LiP have also found applications in skin lightening products but difficulties in purification and volumetric production are the limitations in commercial applications (Butler and Day 1998; Sadaqat et al. 2020).

6.4 Renewable Energies

6.4.1 Biomethanation

Coal rejects that are a major cause of environmental concerns can be bio-solubilized and further converted to value-added products such as methane and volatile fatty acids. *Neurospora discreta* was observed to produce the highest laccase activity compared to *Trichoderma viride* and *P. chrysosporium* during fungal solubilization of coal rejects. The profile of degradation products as a result of bio-solubilization varies with time and fungal species. Bio-solubilization occurs by the breakdown of hydrophobic biomass matrix to hydrophilic products with the increase in polarity. The heterogeneity of the products also decreases with time. The polar nature makes it easy for methanogens and bacteria to utilize the simpler compounds and significantly improve the production of VFAs and methane in subsequent steps (Ahmed and Sharma 2021).

During pretreatment of lignocellulosic biomass such as with dilute acid or steam explosion, hydrolysates rich in xylose are generated and are a great source for bioenergy production. Nevertheless, toxic byproducts such as hydroxymethylfurfural (HMF) and lignin depolymerization products such as vanillin and syringaldehyde are also generated in the process. These byproducts have been shown to inhibit microorganisms used in ethanol production and thereby the ethanol production itself. However, Barakat et al. (2012) concluded that the anaerobic digestion of xylose in the presence of lignin polymers or inhibitory compounds on its own did not inhibit methane production. The methane potential of the natural lignin polymers was observed to be positively correlated with their S/G ratio and negatively correlated with their molecular weights. Therefore, though pretreatment of lignocellulosic biomass is important to maximize bioenergy production, the presence of inhibitory products makes it less adverse to produce biomethane than ethanol (Barakat et al. 2012). Fungal pretreatment of agricultural waste, horticultural waste, and industrial fiber pulp has been reported to provide an environment-friendly approach to decrease the lignin content and improve the biogas yield. However, the residence time for fungal treatment is quite long. By combining these chemical and fungal pretreatment strategies, the severity of the disadvantages of the individual methods can be reduced. Combined fungal-chemical pretreatment approaches have been reported to improve methane yield by decreasing the production of the inhibitory products due to less intensity of chemicals used and shorter residence time needed for fungal pretreatment (Meenakshisundaram et al. 2021).

6.4.2 Biohydrogen Production

In the last decade, fungal treatment of high-lignin-containing biomass has been studied to improve biohydrogen production. They are of particular interest because of their capacity to detoxify several lignocellulosic inhibitors simultaneously.

Pleurotus pulmonarium was used for 60% delignification of sugarcane top without production of inhibitors that helped to increase the yield of biohydrogen. Further, the spent medium of dark fermentation was used for methane production that resulted in higher gaseous energy recovery (Kumari and Das 2016). It has been reported that by using fungal pretreatment (*Gymnopus contrarius*) for rice straw, no further addition of nutrients was needed to be supplemented for hydrogen-producing bacteria as the fungi provided the prerequisite nutrients. Fungal pretreatment showed high lignin removal with only a small loss of hemicellulose and cellulose in rice straw. Therefore, fungal depolymerization of biomass has great potential to produce high hydrogen yields under mild conditions (Sheng et al. 2018).

6.4.3 Bioethanol Production

Several white-rot fungi have been identified to ferment oligosaccharide materials to ethanol without acid or enzymatic hydrolysis. *Phlebia* sp. MG-60 has been reported to be capable of converting lignocellulosic biomass directly to ethanol with high yields. *Phlebia* sp. MG-60 not only possesses delignification ability but also anaerobic saccharification and ethanol fermentability. This is because of the presence of genes related to glucose uptake, metabolism, production of pyruvate, and ethanol synthesis that are upregulated under fermentation conditions. Other white-rot fungi such as *Peniophora cinerea* and several *Trametes* strains are reported to directly and efficiently ferment hexoses to ethanol without a hydrolysis step (Okamoto et al. 2014; Wang et al. 2016). Fungal (*Aspergillus nidulans* FLZ10) and yeast consortia (*Saccharomyces cerevisiae*) are also used for simultaneous detoxification, delignification, and fermentation of steam-exploded corn stover to ethanol. Therefore, fungi depolymerization of biomass is seen to be very advantageous for bioethanol production with low energy requirements and providing a detoxification step of the inhibitors generated by non-biological pretreatments either in a separated or in situ process (Moreno et al. 2015).

7 Conclusion and Future Perspectives

Lignin is a highly complex, variable and high molecular weight biopolymer enclosing monomers with potential high added value applications. This structural complexity makes the characterization of lignin difficult and requires the combination of advanced techniques as shown in this chapter. The industrial processes producing technical lignins, which are among the main valorization targets, can reinforce the natural variability of lignins resulting from the random radical polymerization of monolignols in plant cell walls. Thus, the development of more adapted and effective analytical techniques appears mandatory for both the characterization of initial substrates, and the monitoring of bioconversion and biodegradation during the process. Besides physicochemical depolymerization approaches,

which are effective but with a low ecological compatibility, biological techniques try to overcome these challenges with prospect to produce bio-based synthons for green chemistry, help in bioremediation processes or improve the development of renewable energies. The fungal kingdom was widely investigated for the diversity and the particularity of its enzymatic machineries, which are considered as the most effective for lignin biodegradation as far. As a result, fungi attract attention for the development of lignin valorization processes through fungal depolymerization. However, most of the studies were carried out at bench scale as far and many challenges persist for process scale-up with yields and conversion efficiencies, which meet the industrial expectations. Envisaged ways to answer to these current limitations are: (1) the use of bioprocess engineering strategies promoting microbial diversity with complementary enzymatic activities such as aerobic granular sludge or sequencing batch biofilm bioreactor, (2) the enhancement of enzymatic catalysis through coupling with electrochemical cell, pH-responsive membranes bioreactor, graphene electron conduction, (3) improve the bioavailability and biodegradability of lignin through either biological approaches using fungal secretomes or thermochemical strategies. Fundamental studies about lignin-derived aromatics metabolism are also still needed in both bacteria and fungi to enable the development of bioprocesses involving mixed cultures and to improve microbial biodegradation efficiency as well as resistance to generated inhibitors through metabolic and genetic engineering strategies.

References

Abdelaziz OY, Hulteberg CP (2017) Physicochemical characterisation of technical lignins for their potential valorisation. Waste Biomass Valor 8:859–869

Abdelaziz OY, Brink DP, Prothmann J, Ravi K, Sun M, García-Hidalgo J, Sandahl M, Hulteberg CP, Turner C, Lidén G, Gorwa-Grauslund MF (2016) Biological valorization of low molecular weight lignin. Biotechnol Adv 34:1318–1346

Abdel-Hamid AM, Solbiati JO, Cann IKO (2013) Insights into lignin degradation and its potential industrial applications. Adv Appl Microbiol 82:1–28

Adler E (1977) Lignin chemistry—past, present and future. Wood Sci Technol 11:169–218

Agarwal U (1999) An overview of Raman spectroscopy as applied to lignocellulosic materials. In: Advances in lignocellulosics characterization. TAPPI Press, Norcross, pp 201–225

Agarwal UP (2008) Raman spectroscopic characterization of wood and pulp fibers. Characterization of lignocellulosic materials. Blackwell, Oxford, pp 17–35

Agarwal UP, McSweeny JD, Ralph SA (2011) FT–Raman investigation of milled-wood lignins: softwood, hardwood, and chemically modified black spruce lignins. J Wood Chem Technol 31: 324–344

Ahmed A, Sharma A (2021) Fungal solubilisation and subsequent microbial methanation of coal processing wastes. Appl Biochem Biotechnol 193:3970–3982. https://doi.org/10.21203/rs.3.rs-612910/v1

Albinsson B, Li S, Lundquist K, Stomberg R (1999) The origin of lignin fluorescence. J Mol Struct 508:19–27

Aljawish A, Chevalot I, Jasniewski J, Paris C, Scher J, Muniglia L (2014) Laccase-catalysed oxidation of ferulic acid and ethyl ferulate in aqueous medium: a green procedure for the synthesis of new compounds. Food Chem 145:1046–1054

Amaravathi M, Kanakaraju S, Chandramouli GVP (2013) Synthesis and characterization of azobenzene–porphyrins. J Heterocycl Chem 50:268–271

Anastas PT, Warner JC (1998) Green chemistry: theory and practice. Oxford University Press, Oxford

Anastas ND, Warner JC (2005) The incorporation of hazard reduction as a chemical design criterion in green chemistry. Chem Health Saf 12:9–13

Ander P, Hatakka A, Lundell T, Petterson B, Stalmasek M, Volc J (1992) Demethoxylation of lignin by peroxidases from *Phlebia radiata* and *Phanerochaete chrysosporium*. In: Kennedy JF, Phillips GO, Williams PA (eds) Lignocellulosics: science, technology, development and use. Ellis Horwood Limited, Chichester, pp 109–119

Andlar M, Rezić T, Marđetko N, Kracher D, Ludwig R, Šantek B (2018) Lignocellulose degradation: an overview of fungi and fungal enzymes involved in lignocellulose degradation. Eng Life Sci 18:768–778

Arantes V, Goodell B (2014) Current understanding of brown-rot fungal biodegradation mechanisms: a review. In: Deterioration and protection of sustainable biomaterials. ACS Publications, Washington, DC, pp 3–21

Argyropoulos D (1994) Quantitative phosphorus 31 NMR analysis of lignins, a new tool for the lignin chemist. J Wood Chem Technol 14:45–63

Ariëns EJ (1980) Design of safer chemicals. In: Ariëns EJ (ed) Medicinal chemistry, drug design. Elsevier, Amsterdam, pp 1–46

Asikkala J, Tamminen T, Argyropoulos DS (2012) Accurate and reproducible determination of lignin molar mass by acetobromination. J Agric Food Chem 60:8968–8973

Avram A, Sengupta A, Pfromm PH, Zorn H, Lorenz P, Schwarz T, Nguyen KQ, Czermak P (2018) Novel DyP from the basidiomycete *Pleurotus sapidus*: substrate screening and kinetics. Biocatalysis 4:1–13

Axegard P, Wiken J (1983) Delignification studies—factors affecting the amount of residual lignin. Sven Papperstidn 86:178–184

Ayuso-Fernández I, Rencoret J, Gutiérrez A, Ruiz-Dueñas FJ, Martínez AT (2019) Peroxidase evolution in white-rot fungi follows wood lignin evolution in plants. Proc Natl Acad Sci U S A 116:17900

Banaszak-Leonard E, Fayeulle A, Franche A, Sagadevan S, Billamboz M (2021) Antimicrobial azo molecules: a review. J Iran Chem Soc 18:2829–2851. https://doi.org/10.1007/s13738-021-02238-4

Baptista C, Robert D, Duarte AP (2008) Relationship between lignin structure and delignification degree in Pinus pinaster Kraft pulps. Bioresour Technol 99:2349–2356

Barakat A, Monlau F, Steyer JP, Carrere H (2012) Effect of lignin-derived and furan compounds found in lignocellulosic hydrolysates on biomethane production. Bioresour Technol 104:90–99

Baumberger S, Abaecherli A, Fasching M, Gellerstedt G, Gosselink R, Hortling B, Li J, Saake B, de Jong E (2007) Molar mass determination of lignins by size-exclusion chromatography: towards standardisation of the method. 61(4):459–468. https://doi.org/10.1515/HF.2007.074

Belozerskaya TA, Gessler NN, Averyanov AA (2017) Melanin pigments of fungi. In: Mérillon J-M, Ramawat KG (eds) Fungal metabolites. Springer International Publishing, Cham, pp 263–291. https://doi.org/10.1007/978-3-319-25001-4_29

Bertella S, Luterbacher JS (2020) Lignin functionalization for the production of novel materials. Trends Chem 2:440–453

Blanchette RA (1991) Delignification by wood-decay fungi. Annu Rev Phytopathol 29:381–403

Bomon J, Broeck EVD, Bal M, Liao Y, Sergeyev S, Speybroeck VV, Sels BF, Maes BUW (2020) Brønsted acid catalyzed tandem defunctionalization of biorenewable fzerulic acid and derivates into bio-catechol. Angew Chem 132:3087–3092

Bose SK, Francis RC, Govender M, Bush T, Spark A (2009) Lignin content versus syringyl to guaiacyl ratio amongst poplars. Bioresour Technol 100:1628–1633

Brown W, Falkehag SI, Cowling EB (1967) Molecular size distribution of lignin in wood. Nature 214:410–411

Brunow G, Lundquist K (2010) Functional groups and bonding patterns in lignin (including the lignin-carbohydrate complexes). In: Heitner C, Dimmel D, Schmidt J (eds) Lignin and lignans. CRC Press, Boca Raton, pp 267–299

Bugg TD, Ahmad M, Hardiman EM, Singh R (2011a) The emerging role for bacteria in lignin degradation and bio-product formation. Curr Opin Biotechnol 22:394–400

Bugg TDH, Ahmad M, Hardiman EM, Rahmanpour R (2011b) Pathways for degradation of lignin in bacteria and fungi. Nat Prod Rep 28:1883–1896

Bugg TDH, Rahmanpour R, Rashid GMM (2016) Bacterial enzymes for lignin oxidation and conversion to renewable chemicals. In: Fang Z, Smith J, Richard L (eds) Production of biofuels and chemicals from lignin. Springer Singapore, Singapore, pp 131–146

Butler MJ, Day AW (1998) Destruction of fungal melanins by ligninases of *Phanerochaete chrysosporium* and other white rot fungi. Int J Plant Sci 159:989–995. https://doi.org/10.1086/314093

Calvo-Flores FG, Dobado JA (2010) Lignin as renewable raw material. ChemSusChem 3:1227–1235

Camarero S, Sarkar S, Ruiz-Dueñas FJ, Martínez MJ, Martínez ÁT (1999) Description of a versatile peroxidase involved in the natural degradation of lignin that has both manganese peroxidase and lignin peroxidase substrate interaction sites. J Biol Chem 274:10324–10330

Camarero S, García O, Vidal T, Colom J, del Río JC, Gutiérrez A, Gras JM, Monje R, Martínez MJ, Martínez ÁT (2004) Efficient bleaching of non-wood high-quality paper pulp using laccase-mediator system. Enzym Microb Technol 35(2–3):113–120. ISSN 0141-0229. https://doi.org/10.1016/j.enzmictec.2003.10.019

Camarero S, Ibarra D, Martínez MJ, Martínez ÁT (2005) Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. Appl Environ Microbiol 71:1775–1784

Caramelo L, Martinez M, Martinez A (1999) A search for ligninolytic peroxidases in the fungus pleurotus eryngii involving alpha-keto-gamma-thiomethylbutyric acid and lignin model dimers. Appl Environ Microbiol 65:916–922

Cateto CA, Barreiro MF, Rodrigues AE, Brochier-Salon MC, Thielemans W, Belgacem MN (2008) Lignins as macromonomers for polyurethane synthesis: a comparative study on hydroxyl group determination. J Appl Polym Sci 109:3008–3017

Catucci G, Valetti F, Sadeghi SJ, Gilardi G (2020) Biochemical features of dye-decolorizing peroxidases: current impact on lignin degradation. Biotechnol Appl Biochem 67:751–759

Chakraborti AK, Rudrawar S, Jadhav KB, Kaur G, Chankeshwara SV (2007) "On water" organic synthesis: a highly efficient and clean synthesis of 2-aryl/heteroaryl/styryl benzothiazoles and 2-alkyl/aryl alkyl benzothiazolines. Green Chem 9:1335–1340

Chen CL (1992) Nitrobenzene and cupric oxide oxidations. In: Lin SY, Dence CW (eds) Methods in lignin chemistry, Springer series in wood science. Springer, Berlin, pp 301–321

Chen CL, Robert D (1988) Characterization of lignin by 1H and 13C NMR spectroscopy. In: Methods in enzymology. Elsevier, Amsterdam, pp 137–174

Chen L, Wang X, Yang H, Lu Q, Li D, Yang Q, Chen H (2015) Study on pyrolysis behaviors of non-woody lignins with TG-FTIR and Py-GC/MS. J Anal Appl Pyrolysis 113:499–507

Chen P, Zhang Q, Shu R, Xu Y, Ma L, Wang T (2017) Catalytic depolymerization of the hydrolyzed lignin over mesoporous catalysts. Bioresour Technol 226:125–131

Chio C, Sain M, Qin W (2019) Lignin utilization: a review of lignin depolymerization from various aspects. Renew Sust Energ Rev 107:232–249

Choi J, Détry N, Kim KT, Asiegbu FO, Valkonen JP, Lee YH (2014) fPoxDB: fungal peroxidase database for comparative genomics. BMC Microbiol 14:117
Chu S, Subrahmanyam A, Huber G (2012) The pyrolysis chemistry of a β-O-4 type oligomeric lignin model compound. Green Chem 15:125–136
Cipriano D, Chinelatto L, Nascimento S, Rezende C, Menezes S, Freitas J (2020) Potential and limitations of 13C CP/MAS NMR spectroscopy to determine the lignin content of lignocellulosic feedstock. Biomass Bioenergy 142:105792
de la Torre MJ, Moral A, Hernández MD, Cabeza E, Tijero A (2013) Organosolv lignin for biofuel. Ind Crop Prod 45:58–63
Deacon JW (2013) Fungal biology. Wiley, New York
Del Río JC, Rencoret J, Prinsen P, Martínez ÁT, Ralph J, Gutiérrez A (2012) Structural characterization of wheat straw lignin as revealed by analytical pyrolysis, 2D-NMR, and reductive cleavage methods. J Agric Food Chem 60:5922–5935
Dence CW (1992) The determination of lignin. In: Lin SY, Dence CW (eds) Methods in lignin chemistry, Springer series in wood science. Springer, Berlin, pp 33–61
Derkacheva O, Sukhov D (2008) Investigation of lignins by FTIR spectroscopy. Macromol Symp 265:61–68
Deshmukh MB, Patil SS, Jadhav SD, Pawar PB (2012) Green approach for Knoevenagel condensation of aromatic aldehydes with active methylene group. Synth Commun 42:1177–1183
Deshpande R (2016) The initial phase of sodium sulfite pulping of softwood: a comparison of different pulping options (Doctoral dissertation, Karlstads universitet)
Ding R, Wu H, Thunga M, Bowler N, Kessler M (2015) Processing and characterization of low-cost electrospun carbon fibers from organosolv lignin/polyacrylonitrile blends. Carbon 100:126–136
Dong XQ, Yang JS, Zhu N, Wang ET, Yuan HL (2013) Sugarcane bagasse degradation and characterization of three white-rot fungi. Bioresour Technol 131:443–451
Drillaud N, Banaszak-Léonard E, Pezron I, Len C (2012) Synthesis and evaluation of a photochromic surfactant for organic reactions in aqueous media. J Org Chem 77:9553–9561
Ek M, Gellerstedt G, Henriksson G (eds) (2009) Wood chemistry and wood biotechnology. Walter de Gruyter, Boston
Ekielski A, Mishra PK (2021) Lignin for bioeconomy: the present and future role of technical lignin. Int J Mol Sci 22:63. https://doi.org/10.3390/ijms22010063
El MN, Salvadó J (2007) Analytical methods for determining functional groups in various technical lignin. Ind Crop Prod 26:116–124
Erdtman H (1971) In: Sarkanen KV, Ludwig CH (eds) Lignins: occurrence, formation, structure and reactions. Wiley, New York, p 916
Esmaeilpour M, Javidi J, Dehghani F, Dodeji FN (2015) A green one-pot three-component synthesis of tetrahydrobenzo[b]pyran and 3,4-dihydropyrano[c]chromene derivatives using a Fe3O4@SiO2–imid–PMAn magnetic nanocatalyst under ultrasonic irradiation or reflux conditions. RSC Adv 5:26625–26633
Evstigneyev EI, Shevchenko SM (2019) Structure, chemical reactivity and solubility of lignin: a fresh look. Wood Sci Technol 53:7–47
Faix O (1991) Classification of lignins from different botanical origins by FT-IR spectroscopy. Holzforschung 45:21–28
Faix O, Beinhoff O (1988) Ftir spectra of milled wood lignins and lignin polymer models (DHP's) with enhanced resolution obtained by deconvolution. J Wood Chem Technol 8:505–522
Faix O, Argyropoulos DS, Robert D, Neirinck V (1994) Determination of hydroxyl groups in lignins evaluation of 1H-, 13C-, 31P-NMR, FTIR and wet chemical methods. Holzforschung 48:387–394
Fawal N, Li Q, Savelli B, Brette M, Passaia G, Fabre M, Mathé C, Dunand C (2013) PeroxiBase: a database for large-scale evolutionary analysis of peroxidases. Nucleic Acids Res 41:D441–D444
Fengel D, Wegener G (2011) Wood: chemistry, ultrastructure, reactions. Walter de Gruyter, New York. https://doi.org/10.1515/9783110839654

Fernández-Costas C, Gouveia S, Sanromán MA, Moldes D (2014) Structural characterization of Kraft lignins from different spent cooking liquors by 1D and 2D nuclear magnetic resonance spectroscopy. Biomass Bioenergy 63:156–166

Franche A, Fayeulle A, Lins L, Billamboz M, Pezron I, Deleu M, Léonard E (2020) Amphiphilic azobenzenes: antibacterial activities and biophysical investigation of their interaction with bacterial membrane lipids. Bioorg Chem 94:103399. https://doi.org/10.1016/j.bioorg.2019.103399

Freudenberg K, Lautsch W, Engler K (1940) Die Bildung von Vanillin aus Fichtenlignin. Berichte der deutschen chemischen Gesellschaft (A and B Series) 73:167–171

Fromm J, Rockel B, Lautner S, Windeisen E, Wanner G (2003) Lignin distribution in wood cell walls determined by TEM and backscattered SEM techniques. J Struct Biol 143:77–84

Galletti GC, Bocchini P (1995) Pyrolysis/gas chromatography/mass spectrometry of lignocellulose. Rapid Commun Mass Spectrom 9:815–826

Galliano H, Gas G, Seris JL, Boudet AM (1991) Lignin degradation by rigidoporus lignosus involves synergistic action of two oxidizing enzymes: Mn peroxidase and laccase. Enzym Microb Technol 13:478–482

Gao X, Abdul Raman AA, Hizaddin HF, Bello MM, Buthiyappan A (2021) Review on the inherently safer design for chemical processes: past, present and future. J Clean Prod 305:127154. https://doi.org/10.1016/j.jclepro.2021.127154

Gellerstedt G (1992) Gel permeation chromatography. In: Lin SY, Dence CW (eds) Methods in lignin chemistry, Springer series in wood science. Springer, Berlin, pp 487–497

Gellerstedt G, Henriksson G (2008) Lignins: major sources, structure and properties. In: Monomers, polymers and composites from renewable resources. Elsevier, Amsterdam, pp 201–224

Gellerstedt G, Lindfors EL (1984) Structural changes in lignin during Kraft pulping. Holzforschung 38:151–158

Ghaffar SH, Fan M (2013) Structural analysis for lignin characteristics in biomass straw. Biomass Bioenergy 57:264–279

Gianfreda L, Xu F, Bollag JM (1999) Laccases: a useful group of oxidoreductive enzymes. Bioremediat J 3:1–26

Gilarranz MA, Rodríguez F, Oliet M, García J, Alonso V (2001) Phenolic OH group estimation by FTIR and UV spectroscopy. Application to organosolv lignins. J Wood Chem Technol 21:387–395

Glasser WG (1999) Classification of lignin according to chemical and molecular structure. In: Lignin: historical, biological, and materials perspectives, ACS symposium series. American Chemical Society, Washington, DC, pp 216–238

Goldmann Valdes W, Ahola J, Mankinen O, Kantola A, Komulainen S, Telkki VV, Tanskanen J (2016) Determination of phenolic hydroxyl groups in technical lignins by ionization difference ultraviolet spectrophotometry (Δε-IDUS method). Period Polytech Chem Eng 61. https://doi.org/10.3311/PPch.9269

Gosselink R, Abaecherli A, Semke H, Malherbe R, Käuper P, Nadif A, Dam J (2004a) Analytical protocols for sulfur-free lignin characterization. Ind Crop Prod 19:271–281

Gosselink RJA, Snijder MHB, Kranenbarg A, Keijsers ERP, de Jong E, Stigsson LL (2004b) Characterisation and application of NovaFiber lignin. Industrial crops and products. In 6th international lignin institute conference, vol 20, pp 191–203

Guerra A, Mendonça R, Ferraz A, Lu F, Ralph J (2004) Structural characterization of lignin during Pinus taeda wood treatment with Ceriporiopsis subvermispora. Appl Environ Microbiol 70:4073–4078

Guha S, Lohar S, Hauli I, Mukhopadhyay SK, Das D (2011) Vanillin-coumarin hybrid molecule as an efficient fluorescent probe for trace level determination of Hg(II) and its application in cell imaging. Talanta 85:1658–1664

Harms H, Schlosser D, Wick LY (2011) Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nat Rev Microbiol 9:177–192

Hatfield RD, Jung HJG, Ralph J, Buxton DR, Weimer PJ (1994) A comparison of the insoluble residues produced by the Klason lignin and acid detergent lignin procedures. J Sci Food Agric 65:51–58

Heinfling A, Ruiz-Dueñas FJ, Martínez MJ, Bergbauer M, Szewzyk U, Martínez AT (1998) A study on reducing substrates of manganese-oxidizing peroxidases from Pleurotus eryngii and Bjerkandera adusta. FEBS Lett 428:141–146

Hibbett DS, Donoghue MJ (2001) Analysis of character correlations among wood decay mechanisms, mating systems, and substrate ranges in homobasidiomycetes. Syst Biol 50:215–242

Hilgers R, Vincken JP, Gruppen H, Kabel MA (2018) Laccase/mediator systems: their reactivity toward phenolic lignin structures. ACS Sustain Chem Eng 6:2037–2046

Hoegger PJ, Kilaru S, James TY, Thacker JR, Kües U (2006) Phylogenetic comparison and classification of laccase and related multicopper oxidase protein sequences. FEBS J 273: 2308–2326

Hofrichter M (2002) Review: lignin conversion by manganese peroxidase (MnP). Enzym Microb Technol 30:454–466

Hofrichter M, Ullrich R, Pecyna MJ, Liers C, Lundell T (2010) New and classic families of secreted fungal heme peroxidases. Appl Microbiol Biotechnol 87:871–897

Hong S, Lian H, Pan M, Chen L (2017) Structural changes of lignin after ionic liquid pretreatment. Bioresources 12:3017–3029

Hortling B, Tamminen T, Kenttä E (1997) Determination of carboxyl and non-conjugated carbonyl groups in dissolved and residual lignins by IR spectroscopy. Holzforschung 51:405–410

Huang J, Fu S, Gan L (2019) Structure and characteristics of lignin. In: Lignin chemistry and applications. Elsevier, Amsterdam, pp 25–50

Ibarra D, Chávez MI, Rencoret J, Del Río JC, Gutiérrez A, Romero J, Camarero S, Martínez MJ, Jiménez-Barbero J, Martínez AT (2007) Lignin modification during eucalyptus globulus Kraft pulping followed by totally chlorine-free bleaching: a two-dimensional nuclear magnetic resonance, Fourier transform infrared, and pyrolysis−gas chromatography/mass spectrometry study. J Agric Food Chem 55:3477–3490

Inamuddin, Boddula R, Ahamed MI, Asiri AM (2021) Green sustainable process for chemical and environmental engineering and science. Elsevier, Amsterdam. https://doi.org/10.1016/C2019-0-03692-8

Jensen Kenneth A, Houtman Carl J, Ryan Zachary C, Hammel Kenneth E (2001) Pathways for extracellular Fenton chemistry in the brown rot Basidiomycete *Gloeophyllum trabeum*. Appl Environ Microbiol 67:2705–2711

Jimenez-Gonzalez C, Ponder CS, Broxterman QB, Manley JB (2011) Using the right green yardstick: why process mass intensity is used in the pharmaceutical industry to drive more sustainable processes. Org Process Res Dev 15:912–917

Jin TS, Zhang JS, Guo TT, Wang AQ, Li TS (2004) One-pot clean synthesis of 1,8-dioxodecahydroacridines catalyzed by p-dodecylbenezenesulfonic acid in aqueous media. Synthesis 2004:2001–2005

Kapich AN, Jensen KA, Hammel KE (1999) Peroxyl radicals are potential agents of lignin biodegradation. FEBS Lett 461:115–119

Kawai S, Umezawa T, Shimada M, Higuchi T (1988) Aromatic ring cleavage of 4,6-di(tert-butyl) guaiacol, a phenolic lignin model compound, by laccase of *Coriolus versicolor*. FEBS Lett 236: 309–311

Kawai S, Iwatsuki M, Nakagawa M, Inagaki M, Hamabe A, Ohashi H (2004) An alternative β-ether cleavage pathway for a non-phenolic β-O-4 lignin model dimer catalyzed by a laccase-mediator system. Enzym Microb Technol 35:154–160

Khaligh NG (2017) Telescopic synthesis of azo compounds via stable arenediazonium bis (trifluoromethane)sulfonimide salts by using tert-butyl nitrite. Dyes Pigments 139:556–560

Khaligh NG, Hamid SBA, Johari S (2019) Telescopic synthesis of azo compounds via stable arenediazonium tosylates by using n-butyl nitrite as diazotization reagent. Polycycl Aromat Compd 39:346–352

Kim JY, Park SY, Lee JH, Choi IG, Choi JW (2017) Sequential solvent fractionation of lignin for selective production of monoaromatics by Ru catalyzed ethanolysis. RSC Adv 7:53117–53125
Kirk TK, Chang H, Lorenz LF (1975) Topochemistry of the fungal degradation of lignin in birch wood as related to the distribution of guaiacyl and syringyl lignins. Wood Sci Technol 9:81–86
Kleinert M, Barth T (2008) Phenols from lignin. Chem Eng Technol 31:736–745
Köhnke J, Gierlinger N, Prats Mateu B, Unterweger C, Solt P, Mahler K, Schwaiger E, Liebner F, Gindl-Altmutter W (2019) Comparison of four technical lignins as resource for electrically conductive carbon particle. Bioresources 14:1091–1109
Koo BW, Min BC, Gwak KS, Lee SM, Choi JW, Yeo H, Choi IG (2012) Structural changes in lignin during organosolv pretreatment of Liriodendron tulipifera and the effect on enzymatic hydrolysis. Biomass Bioenergy 42:24–32
Kringstad K, Mörck R (1983) 13C-NMR spectra of Kraft lignins. Holzforschung 37:237–244
Kuhad RC, Singh A, Eriksson KE (1997) Microorganisms and enzymes involved in the degradation of plant fiber cell walls. Adv Biochem Eng Biotechnol 57:45–125
Kulshreshtha S, Mathur N, Bhatnagar P (2013) Mycoremediation of paper, pulp and cardboard industrial wastes and pollutants. In: Goltapeh EM, Danesh YR, Varma A (eds) Fungi as bioremediators, soil biology. Springer, Berlin, pp 77–116
Kumar A, Pandey R, Gupta RK, Pandey DS (2013) Fluorescent azophenol-quinazoline dyad as multichannel reversible pH indicator in aqueous media: an innovative concept on diazo based dyads. Tetrahedron Lett 54:6164–6167
Kumari S, Das D (2016) Biologically pretreated sugarcane top as a potential raw material for the enhancement of gaseous energy recovery by two stage biohythane process. Bioresour Technol 218:1090–1097
Lahive CW, Kamer PCJ, Lancefield CS, Deuss PJ (2020) An introduction to model compounds of lignin linking motifs; synthesis and selection considerations for reactivity studies. ChemSusChem 13:4238–4265
Lapierre C, Monties B, Rolando C, de Chirale L (1985) Thioacidolysis of lignin: comparison with acidolysis. J Wood Chem Technol 5:277–292
Laprell L, Tochitsky I, Kaur K, Manookin MB, Stein M, Barber DM, Schön C, Michalakis S, Biel M, Kramer RH, Sumser MP, Trauner D, Van Gelder RN (2017) Photopharmacological control of bipolar cells restores visual function in blind mice. J Clin Invest 127:2598–2611
Larsen KL, Barsberg S (2010) Theoretical and Raman spectroscopic studies of phenolic lignin model monomers. J Phys Chem B 114:8009–8021
Laszlo J, Compton D, Eller F, Taylor S, Isbell T (2003) Packed-bed bioreactor synthesis of feruloylated monoacyl- and diacylglycerols: clean production of a "green" sunscreen. Green Chem 5:382–386
Lázaro N, Castro-Gutiérrez J, Ramírez-Vidal P, Celzard A, Fierro V, AlGarni TS, Pineda A, Luque R (2021) Mechanochemical functionalization of mesoporous carbons for the catalytic transformation of trans-ferulic acid into vanillin. ACS Sustain Chem Eng 9:4704–4710
Léonard E, Fayeulle A (2021) Azo-dyes-grafted oligosaccharides—from synthesis to applications. Molecules 26:3063. https://doi.org/10.3390/molecules26113063
Leonowicz A, Cho N, Luterek J, Wilkolazka A, Wojtas-Wasilewska M, Matuszewska A, Hofrichter M, Wesenberg D, Rogalski J (2001) Fungal laccase: properties and activity on lignin. J Basic Microbiol 41:185–227
Leriche-Grandchamp M, Flourat A, Shen H, Picard F, Giordana H, Allais F, Fayeulle A (2020) Inhibition of phenolics uptake by ligninolytic fungal cells and its potential as a tool for the production of lignin-derived aromatic building blocks. J Fungi 6:363. https://doi.org/10.3390/jof6040362
Levy J (1966) The soft rot fungi: their mode of action and significance in the degradation of wood. In: Preston RD (ed) Advances in botanical research. Academic, New York, pp 323–357
Liao J, Latif N, Trache D, Brosse N, Hussin M (2020) Current advancement on the isolation, characterization and application of lignin. Int J Biol Macromol 162:985–1024. https://doi.org/10.1016/j.ijbiomac.2020.06.168

Li S, Lundquist K (1994) A new method for the analysis of phenolic groups in lignins by 1H NMR spectroscopy. Nord Pulp Paper Res J 9:191–195
Li T, Takkellapati S (2018) The current and emerging sources of technical lignins and their applications: sources of technical lignins. Biofuels Bioprod Biorefin 12:756–787
Li X, Zheng Y (2020) Biotransformation of lignin: mechanisms, applications and future work. Biotechnol Prog 36:e2922. https://doi.org/10.1002/btpr.2922
Li C, Zhao X, Wang A, Huber GW, Zhang T (2015) Catalytic transformation of lignin for the production of chemicals and fuels. Chem Rev 115:11559–11624
Liers C, Bobeth C, Pecyna M, Ullrich R, Hofrichter M (2010) DyP-like peroxidases of the jelly fungus Auricularia auricula-judae oxidize nonphenolic lignin model compounds and high-redox potential dyes. Appl Microbiol Biotechnol 85:1869–1879
Liers C, Aranda E, Strittmatter E, Piontek K, Plattner DA, Zorn H, Ullrich R, Hofrichter M (2014) Phenol oxidation by DyP-type peroxidases in comparison to fungal and plant peroxidases. J Mol Catal B Enzym 103:41–46
Liitiä TM, Maunu SL, Hortling B, Toikka M, Kilpeläinen I (2003) Analysis of technical lignins by two- and three-dimensional NMR spectroscopy. J Agric Food Chem 51:2136–2143
Lim FPL, Dolzhenko AV (2020) Atom economy in green organic synthesis. In: Inamuddin, Boddula R, Asiri AM (eds) Green sustainable process for chemical and environmental engineering and science. Elsevier, Amsterdam, pp 1–12
Lima CF, Barbosa LCA, Marcelo CR, Silvério FO, Colodette JL (2008) Comparison between analytical pyrolysis and nitrobenzene oxidation for determination of syringyl/guaiacyl ratio in *Eucalyptus* spp. lignin. Bioresources 3:701–712
Llevot A, Grau E, Carlotti S, Grelier S, Cramail H (2015) Renewable (semi)aromatic polyesters from symmetrical vanillin-based dimers. Polym Chem 6:6058–6066
Logan KR, Thomas B (1985) Distribution of lignin derivatives in plants. New Phytol 99:571–585
Lopes F, Silvério F, Baffa D, Loureiro M, Barbosa M (2011) Determination of sugarcane bagasse lignin S/G/H ratio by pyrolysis GC/MS. J Wood Chem Technol 31:309–323
Lu F, Ralph J (1997) Derivatization followed by reductive cleavage (DFRC method), a new method for lignin analysis: protocol for analysis of DFRC monomers. J Agric Food Chem 45:2590–2592
Lǔ Z, Kang X, Xiang Z, He N (2017) Laccase gene *Sh-lac* is involved in the growth and melanin biosynthesis of *Sclerotrula shiraiana*. Phytopathology 107:353–361. https://doi.org/10.1094/PHYTO-04-16-0180-R
Lucejko J, Tamburini D, Modugno F, Ribechini E, Colombini M (2020) Analytical pyrolysis and mass spectrometry to characterise lignin in archaeological wood. Appl Sci 11:240. https://doi.org/10.3390/app11010240
Ludwig CH, Nist BJ, McCarthy JL (1964) Lignin. XII. The high resolution nuclear magnetic resonance spectroscopy of protons in compounds related to lignin. J Am Chem Soc 86:1186–1196
Lundquist K (1992) Proton (1H) NMR spectroscopy. In: Lin SY, Dence CW (eds) Methods in lignin chemistry, Springer series in wood science. Springer, Berlin, pp 242–249
Lundquist K, Mannervik B, Nordfors K, Nishida T, Enzell CR, Reid WW, Yanaihara N, Yanaihara C (1979a) NMR studies of lignins. 3. 1H NMR spectroscopic data for lignin model compounds. Acta Chem Scand 33b:418–420
Lundquist K, Sjöholm R, Teien G, Pakkanen T, Servin R, Sternerup H, Wistrand LG, Nørskov L, Schroll G (1979b) NMR studies of lignins. 2. Interpretation of the 1H NMR spectrum of acetylated birch lignin. Acta Chem Scand 33b:27–30
Lundquist K, Aasen AJ, Daasvatn K, Forsgren B, Gustafsson JÅ, Högberg B, Becher J (1980) NMR studies of lignins. 4. Investigation of spruce lignin by 1H NMR spectroscopy. Acta Chem Scand 34b:21–26
Lupoi JS, Singh S, Parthasarathi R, Simmons BA, Henry RJ (2015) Recent innovations in analytical methods for the qualitative and quantitative assessment of lignin. Renew Sust Energ Rev 49:871–906

Mahmood N, Yuan Z, Schmidt J, Xu C (2016) Depolymerization of lignins and their applications for the preparation of polyols and rigid polyurethane foams: a review. Renew Sust Energ Rev 60:317–329

Mancera C, Ferrando F, Salvadó J, El Mansouri NE (2011) Kraft lignin behavior during reaction in an alkaline medium. Biomass Bioenergy 35:2072–2079

Mandlekar N (2019) Integration of wood waste to develop multifunctional fully biobased textile structure. Thesis, University of Lille. https://doi.org/10.13140/RG.2.2.21810.35529

Martone PT, Estevez JM, Lu F, Ruel K, Denny MW, Somerville C, Ralph J (2009) Discovery of lignin in seaweed reveals convergent evolution of cell-wall architecture. Curr Biol 19:169–175

Matsui N, Ohira T (2013) Analysis of broad leaf lignin of Japanese angiospermous trees by DFRC (derivatization followed by reductive cleavage) method. Plant Physiol Biochem 72:112–115

Mattsson C, Hasani M, Dang B, Mayzel M, Theliander H (2017) About structural changes of lignin during Kraft cooking and the kinetics of delignification. Holzforschung 71:545–553

Meenakshisundaram S, Fayeulle A, Leonard E, Ceballos C, Pauss A (2021) Fiber degradation and carbohydrate production by combined biological and chemical/physicochemical pretreatment methods of lignocellulosic biomass—a review. Bioresour Technol 331:125053. https://doi.org/10.1016/j.biortech.2021.125053

Mei Q, Shen X, Liu H, Han B (2019) Selectively transform lignin into value-added chemicals. Chin Chem Lett 30:15–24

Moosavinejad SM, Madhoushi M, Vakili M, Rasouli D, Moosavinejad SM, Madhoushi M, Vakili M, Rasouli D (2019) Evaluation of degradation in chemical compounds of wood in historical buildings using FT-IR and FT-Raman vibrational spectroscopy. Maderas Cienc tecnol 21:381–392

Moreno AD, Ibarra D, Alvira P, Tomás-Pejó E, Ballesteros M (2015) A review of biological delignification and detoxification methods for lignocellulosic bioethanol production. Crit Rev Biotechnol 35:342–354

Morin S, Bockstal L, Jacquet N, Richel A (2019) One-step enzymatic grafting of ferulic acid with cellulose to enhance matrices–fibres compatibility in bio-composites. J Mater Sci 54:13314–13321

Moxley G, Gaspar AR, Higgins D, Xu H (2012) Structural changes of corn Stover lignin during acid pretreatment. J Ind Microbiol Biotechnol 39:1289–1299

Munk L, Sitarz AK, Kalyani DC, Mikkelsen JD, Meyer AS (2015) Can laccases catalyze bond cleavage in lignin? Biotechnol Adv 33:13–24

Nguyen HTH, Reis MH, Qi P, Miller SA (2015) Polyethylene ferulate (PEF) and congeners: polystyrene mimics derived from biorenewable aromatics. Green Chem 17:4512–4517

Nimz HH, Robert D, Faix O, Nemr M (1981) Carbon-13 NMR spectra of lignins, 8. Structural differences between lignins of hardwoods, softwoods, grasses and compression wood. Holzforschung 35:16–26

Norman AG (1969) Constitution and biosynthesis of lignin. K. Freudenberg and A. C. Neish. Springer-Verlag, New York, 1968. x + 132 pp., illus. $7. Molecular Biology, Biochemistry and Biophysics, vol. 2. Science 165, 784

Nosanchuk JD, Stark RE, Casadevall A (2015) Fungal melanin: what do we know about structure? Front Microbiol 6:1463. https://doi.org/10.3389/fmicb.2015.01463

Okamoto K, Uchii A, Kanawaku R, Yanase H (2014) Bioconversion of xylose, hexoses and biomass to ethanol by a new isolate of the white rot basidiomycete *Trametes versicolor*. Springerplus 3:121. https://doi.org/10.1186/2193-1801-3-121

Olmstead JA, Gray DG (1997) Fluorescence spectroscopy of cellulose, lignin and mechanical pulps: a review. Pulp Pap Sci 23:J571–J581

Pelmont J (1995) L'attaque de la lignine. In: Presses Universitaires de Grenoble (ed) Enzymes : Catalyseurs du monde vivant. EDP Sciences, Les Ulis, pp 897–903

Perez-Boada M, Ruiz-Duenas FJ, Pogni R, Basosi R, Choinowski T, Martínez MJ, Piontek K, Martínez AT (2005) Versatile peroxidase oxidation of high redox potential aromatic

compounds: site-directed mutagenesis, spectroscopic and crystallographic investigation of three long-range electron transfer pathways. J Mol Biol 354(2):385–402
Poletto M (2017) Assessment of the thermal behavior of lignins from softwood and hardwood species. Maderas-Cienc Tecnol 19:63–74
Pollegioni L, Tonin F, Rosini E (2015) Lignin-degrading enzymes. FEBS J 282:1190–1213
Poursorkhabi V, Abdelwahab M, Misra M, Khalil H, Gharabaghi B, Mohanty A (2020) Processing, carbonization, and characterization of lignin based electrospun carbon fibers: a review. Front Energy Res 8:208. https://doi.org/10.3389/fenrg.2020.00208
Pouteau C, Dole P, Cathala B, Averous L, Boquillon N (2003) Antioxidant properties of lignin in polypropylene. Polym Degrad Stab 81:9–18
Pu Y, Cao S, Ragauskas AJ (2011) Application of quantitative 31P NMR in biomass lignin and biofuel precursors characterization. Energy Environ Sci 4:3154. https://doi.org/10.1039/c1ee01201k
Pu Y, Hu F, Huang F, Ragauskas AJ (2015) Lignin structural alterations in thermochemical pretreatments with limited delignification. Bioenergy Res 8:992–1003
Ralph J, Landucci LL (2010) NMR of lignins. CRC Press, Boca Raton, p 137
Ralph S, Landucci L, Ralph J (2004) NMR database of lignin and cell wall model compounds
Ralph J, Brunow G, Boerjan W (2007) Lignins. In: ELS. ACS, Washington, DC. https://doi.org/10.1002/9780470015902.a0020104
Ramesh R, Lalitha A (2016) Facile and green chemistry access to 5-aryl-1,2,4-triazolidine-3-thiones in aqueous medium. ChemistrySelect 1:2085–2089
Rencoret J, Marques G, Gutiérrez A, Ibarra D, Li J, Gellerstedt G, Santos J, Jiménez-Barbero J, Martínez N, del Río J (2008) Structural characterization of milled wood lignin from different eucalypt species. Holzforschung 62:514–526
Rencoret J, Marques G, Gutiérrez A, Jiménez-Barbero J, Martínez ÁT, del Río JC (2013) Structural modifications of residual lignins from sisal and flax pulps during soda-AQ pulping and TCF/ECF bleaching. Ind Eng Chem Res 52:4695–4703
Rinaldi R, Jastrzebski R, Clough MT, Ralph J, Kennema M, Bruijnincx PCA, Weckhuysen BM (2016) Paving the way for lignin valorisation: recent advances in bioengineering, biorefining and catalysis. Angew Chem Int Ed 55:8164–8215
Rizk HF, Ibrahim SA, El-Borai MA (2015) Synthesis, fastness properties, color assessment and antimicrobial activity of some azo reactive dyes having pyrazole moiety. Dyes Pigments 112:86–92
Rolando C, Monties B, Lapierre C (1992) Thioacidolysis. In: Lin SY, Dence CW (eds) Methods in lignin chemistry, Springer series in wood science. Springer, Berlin, pp 334–349. https://doi.org/10.1007/978-3-642-74065-7_23
Sadaqat B, Khatoon N, Malik AY, Jamal A, Farooq U, Ali MI, He H, Liu FJ, Guo H, Urynowicz M, Wang Q, Huang Z (2020) Enzymatic decolorization of melanin by lignin peroxidase from *Phanerochaete chrysosporium*. Sci Rep 10:20240. https://doi.org/10.1038/s41598-020-76376-9
Sainsbury PD, Hardiman EM, Ahmad M, Otani H, Seghezzi N, Eltis LD, Bugg TDH (2013) Breaking down lignin to high-value chemicals: the conversion of lignocellulose to vanillin in a gene deletion mutant of Rhodococcus jostii RHA1. ACS Chem Biol 8:2151–2156
Sakakibara A (1980) A structural model of softwood lignin. Wood SciTechnol 14:89–100
Salvachúa D, Karp EM, Nimlos CT, Vardon DR, Beckham GT (2015) Towards lignin consolidated bioprocessing: simultaneous lignin depolymerization and product generation by bacteria. Green Chem 17:4951–4967
Sarkanen S, Razal RA, Piccariello T, Yamamoto E, Lewis NG (1991) Lignin peroxidase: toward a clarification of its role in vivo. J Biol Chem 266:3636–3643
Sederoff RR, MacKay JJ, Ralph J, Hatfield RD (1999) Unexpected variation in lignin. Curr Opin Plant Biol 2:145–152
Sheldon RA, Arends IWCE, Hanefeld U (2007) Green chemistry and catalysis. Wiley, New York. https://doi.org/10.1002/9783527611003

Sheng T, Zhao L, Gao L, Liu W, Wu G, Wu J, Wang A (2018) Enhanced biohydrogen production from nutrient-free anaerobic fermentation medium with edible fungal pretreated rice straw. RSC Adv 8:22924–22930

Sigoillot JC, Berrin JG, Bey M, Lesage-Meessen L, Levasseur A, Lomascolo A, Record E, Uzan-Boukhris E (2012) Fungal strategies for lignin degradation. In: Jouanin L, Lapierre C (eds) Advances in botanical research. Academic, New York, pp 263–308

Singh L (2017) Biodegradation of synthetic dyes: a mycoremediation approach for degradation/decolourization of textile dyes and effluents. J Appl Biotechnol Bioeng 3:430–435. https://doi.org/10.15406/jabb.2017.03.00081

Singh R, Eltis LD (2015) The multihued palette of dye-decolorizing peroxidases. Arch Biochem Biophys 574:56–65

Singh AK, Bilal M, Iqbal HMN, Meyer AS, Raj A (2021) Bioremediation of lignin derivatives and phenolics in wastewater with lignin modifying enzymes: status, opportunities and challenges. Sci Total Environ 777:145988. https://doi.org/10.1016/j.scitotenv.2021.145988

Sjostrom E (1993) Wood chemistry: fundamentals and applications. Gulf Professional Publishing, Houston

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D (2008) Determination of structural carbohydrates and lignin in biomass. In: Laboratory analytical procedure (LAP). National Renewable Energy Laboratory, Golden

Smook GA (1992) Handbook for pulp & paper technologists, 2nd edn. Angus Wilde Publications, Vancouver

Stanzione JF III, Sadler JM, Scala JJL, Reno KH, Wool RP (2012) Vanillin-based resin for use in composite applications. Green Chem 14:2346–2352

Sugano Y, Muramatsu R, Ichiyanagi A, Sato T, Shoda M (2007) DyP, a unique dye-decolorizing peroxidase, represents a novel heme peroxidase family: ASP171 replaces the distal histidine of classical peroxidases. J Biol Chem 282:36652–36658

Svensson S (2008) Minimizing the sulphur content in Kraft lignin

Tanizaki Y, Kobayashi T, Hoshi T (1966) The absorption spectra of dyes. XI. A note on the color change in some azobenzene dyes with variation in the acidity. Bull Chem Soc Jap 39:558–562

Teimouri A, Chermahini AN, Ghorbani MH (2013) The green synthesis of new azo dyes derived from salicylic acid derivatives catalyzed via baker's yeast and solid acid catalysis. Chemija 24:59–66

Tejado A, Peña C, Labidi J, Echeverria JM, Mondragon I (2007) Physico-chemical characterization of lignins from different sources for use in phenol-formaldehyde resin synthesis. Bioresour Technol 98:1655–1663

Thompson DN, Hames BR, Reddy CA, Grethlein HE (1998) In vitro degradation of natural insoluble lignin in aqueous media by the extracellular peroxidases of Phanerochaete chrysosporium. Biotechnol Bioeng 57:704–717

Tobimatsu Y, Schuetz M (2019) Lignin polymerization: how do plants manage the chemistry so well? Curr Opin Biotechnol 56:75–81

Tribot A, Amer G, Abdou Alio M, de Baynast H, Delattre C, Pons A, Mathias JD, Callois JM, Vial C, Michaud P, Dussap CG (2019) Wood-lignin: supply, extraction processes and use as bio-based material. Eur Polym J 112:228–240

Tuor U, Wariishi H, Schoemaker HE, Gold MH (1992) Oxidation of phenolic arylglycerol beta-aryl ether lignin model compounds by manganese peroxidase from *Phanerochaete chrysosporium*: oxidative cleavage of an alpha-carbonyl model compound. Biochemistry 31:4986–4995

Tursi A, Olivito F (2021) Biomass conversion: general information, chemistry, and processes. In: Rahimpour MR, Kamali R, Amin Makarem M, Manshadi MKD (eds) Advances in bioenergy and microfluidic applications. Elsevier, Amsterdam, pp 3–39

Van Soest PJ, Wine RH (1968) Determination of lignin and cellulose in acid-detergent fiber with permanganate. J Assoc Off Anal Chem 51:780–785

Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W (2010) Lignin biosynthesis and structure. Plant Physiol 153:895–905

Velema WA, Hansen MJ, Lerch MM, Driessen AJM, Szymanski W, Feringa BL (2015) Ciprofloxacin–photoswitch conjugates: a facile strategy for photopharmacology. Bioconjug Chem 26:2592–2597

Vishtal AG, Kraslawski A (2011) Challenges in industrial applications of technical lignins. Bioresources 6:3547–3568

Wang J, Suzuki T, Dohra H, Takigami S, Kako H, Soga A, Kamei I, Mori T, Kawagishi H, Hirai H (2016) Analysis of ethanol fermentation mechanism of ethanol producing white-rot fungus Phlebia sp. MG-60 by RNA-seq. BMC Genomics 17:616. https://doi.org/10.1186/s12864-016-2977-7

Wariishi H, Valli K, Gold MH (1989) Oxidative cleavage of a phenolic diarylpropane lignin model dimer by manganese peroxidase from *Phanerochaete chrysosporium*. Biochemistry 28:6017–6023

Weng C, Peng X, Han Y (2021) Depolymerization and conversion of lignin to value-added bioproducts by microbial and enzymatic catalysis. Biotechnol Biofuels 14:84. https://doi.org/10.1186/s13068-021-01934-w

Wong DWS (2009) Structure and action mechanism of ligninolytic enzymes. Appl Biochem Biotechnol 157:174–209

Xu R, Zhang K, Liu P, Han H, Zhao S, Kakade A, Khan A, Du D, Li X (2018) Lignin depolymerization and utilization by bacteria. Bioresour Technol 269:557–566

Xue Y, Li Y, Liu Z, Hou Y (2019) Structural changes of lignin in soda delignification process and associations with pollution load. Bioresources 14(4):7869–7885

Yadav GD, Lande SV (2005) Novelties of reaction in the middle liquid phase in tri-liquid phase transfer catalysis: kinetics of selective O-alkylation of vanillin with benzyl chloride. Appl Catal A Gen 287:267–275

Ye J, Ma S, Wang B, Chen Q, Huang K, Xu X, Li Q, Wang S, Lu N, Zhu J (2021) High-performance bio-based epoxies from ferulic acid and furfuryl alcohol: synthesis and properties. Green Chem 23:1772–1781

Yelle DJ, Ralph J, Lu F, Hammel KE (2008) Evidence for cleavage of lignin by a brown rot basidiomycete. Environ Microbiol 10:1844–1849

Yelle DJ, Wei D, Ralph J, Hammel KE (2011) Multidimensional NMR analysis reveals truncated lignin structures in wood decayed by the brown rot basidiomycete Postia placenta. Environ Microbiol 13:1091–1100

Yiamsawas D, Baier G, Thines E, Landfester K, Wurm FR (2014) Biodegradable lignin nanocontainers. RSC Adv 4:11661–11663

Yuan TQ, He J, Xu F, Sun RC (2009) Fractionation and physico-chemical analysis of degraded lignins from the black liquor of *Eucalyptus pellita* KP-AQ pulping. Polym Degrad Stab 94:1142–1150

Zabel RA, Morrell JJ (2020) Chemical changes in wood caused by decay fungi. In: Zabel RA, Morrell JJ (eds) Wood microbiology, 2nd edn. Academic, San Diego, pp 215–244

Zakzeski J, Bruijnincx PCA, Jongerius AL, Weckhuysen BM (2010) The catalytic valorization of lignin for the production of renewable chemicals. Chem Rev 110:3552–3599

Zhou LW, Wei YL, Dai YC (2014) Phylogenetic analysis of ligninolytic peroxidases: preliminary insights into the alternation of white-rot and brown-rot fungi in their lineage. Mycology 5:29–42

Zhu Y, Ma F, Ma C, Han H, Sun R, Peng H, Xie M (2019) Enhanced dielectric and electrical energy storage capability of polymers with combined azobenzene and triphenylamine side groups by ring-opening metathesis polymerization. Polymer 184:121886. https://doi.org/10.1016/j.polymer.2019.121886

From Blue Pigment to Green Technology: Properties and Applications of Fungi-Derived Pigment Xylindein

G. Giesbers, M. J. Brodeur, R. C. Van Court, S. C. Robinson, and O. Ostroverkhova

Abstract We overview properties of fungi-derived pigments of interest for artistic and technological applications, with a focus on xylindein, a blue-green spalting pigment produced by *Chlorociboria* species. Optical and electronic properties of xylindein are reviewed, and underlying mechanisms behind its attractive properties (which include enhanced photostability and electronic characteristics) and how they are enabled by the hydroxyl groups in the molecular structure of xylindein are discussed. Potential applications of xylindein as a sustainable material for (opto)-electronic and electrochemical devices which include solar cells, electrochemical transistors, and batteries, are described. The features of xylindein and accompanying challenges depending on the application are outlined, and the outlook is presented.

Keywords Fungi-derived pigments · Organic semiconductors · Organic electronics · Bioelectronics

1 Introduction

1.1 Spalting Fungi and Fungi-Derived Pigments

The art of spalting involves using wood stained by a variety of soft-rotting fungi for decorative and artistic work, such as marquetry, intarsia, and woodturning. Spalted wood is used across the globe, but is mostly documented in Western Europe from around the mid-1400s to the late 1600s. It was particularly used in Italy and Germany for elaborate marquetry works (Fig. 1). Spalting is generally broken down into three categories—white rot (a lightening of the wood area due to decay), zone lines (lines of melanin secreted into the wood to act as a physical

G. Giesbers · M. J. Brodeur · O. Ostroverkhova (✉)
Department of Physics, Oregon State University, Corvallis, OR, USA
e-mail: Oksana.Ostroverkhova@oregonstate.edu

R. C. Van Court · S. C. Robinson
Department of Wood Science and Engineering, Oregon State University, Corvallis, OR, USA

S. K. Deshmukh et al. (eds.), *Fungal Biopolymers and Biocomposites*,
https://doi.org/10.1007/978-981-19-1000-5_17

Fig. 1 Bureau, South Germany, 1560–1570, from the Bilbao Fine Arts Museum. The blue-green stain seen is from xylindein, the pigment produced by soft rotting fungi in the *Chlorociboria* genus

barrier between species), and pigmentation (broadly produced, colorfast pigment that deeply penetrates the wood). There are several commonly used spalting fungi in each category, such as *Trametes versicolor* and *Polyporus brumalis* for white rots, *Xylaria polymorpha* for zone lines, and *Chlorociboria* species and *Scytalidium cuboideum* for bright colors (blue-green and red, respectively). Of particular interest is xylindein, the blue-green pigment produced by *Chlorociboria* species (elf's cup), which has a long history of historic use in Western European artwork, and is favored by today's artisans for the long-lasting, UV stable color (Fig. 2).

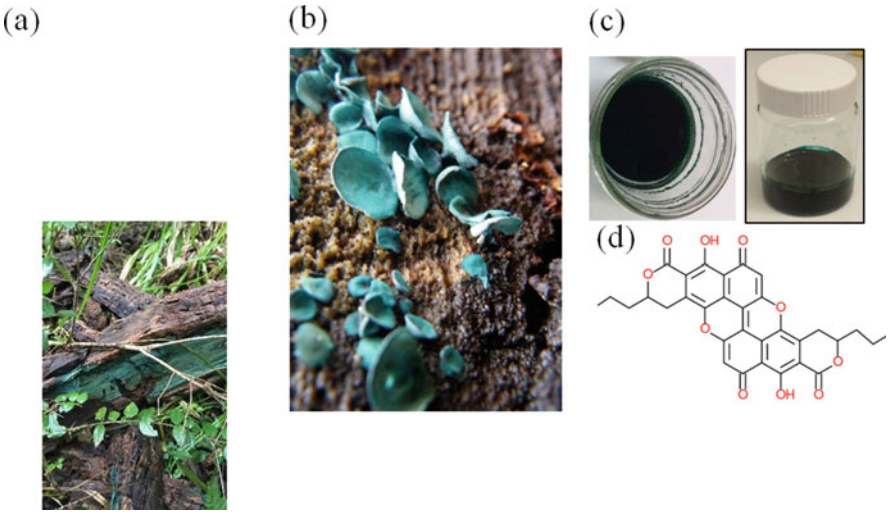


Fig. 2 (**a**) Blue-green stain of *Chlorociboria* on a downed log in Chile, South America. Photo by Dr. Patricia Vega Gutierrez. (**b**) The fruiting cup of *Chlorociboria* spp. on a downed hardwood log in Oregon, USA. Photo by Dr. Seri C. Robinson. (**c**) Xylindein extraction from liquid cultures and pigment in solution. (**d**) Molecular structure of xylindein

In addition to arts and crafts, xylindein has found use in a number of colorant contexts. It has been used as a textile dye (Weber et al. 2014; Hinsch et al. 2015, Fig. 3), decking coating (Vega Gutierrez et al. 2021, Fig. 4), and paint (Robinson et al. 2017, 2018, Fig. 5). Outside of usage as a colorant, over the past ~5 years xylindein has also been investigated as a sustainable (opto)electronic material (Giesbers et al. 2018, 2019a, b, 2021) as discussed extensively in this chapter.

Although the present chapter focuses on xylindein, particularly the mechanisms behind its attractive properties (photostability, enhanced electronic characteristics, and device applications), it is not the only fungi-derived pigment of interest for artistic and technological applications. The orange-red pigment dramada—produced by the spalting fungus *Scytalidium cuboideum*—is a naturally occurring naphthoquinonic crystal (Vega Gutierrez et al. 2018). This pigment has not been as widely studied as xylindein but has been successfully turned into an ink for textile inkjet printing (He et al. 2021, Fig. 6), a decking protectant (Vega Gutierrez et al. 2021, Fig. 4), a paint and oil colorant (Robinson et al. 2017; Robinson et al. 2018, Fig. 5), and has been investigated for hand dyeing of fabrics (Hinsch et al. 2015, Fig. 3). In addition, *Scytalidium ganodermophthorum* produces a range of colors including yellow, green, purple, and red (Vega Gutierrez et al. 2020) which seem to be due to the presence of multiple pigments (Van Court et al. 2020b). The identity of these pigments is so far unknown. Yellow extracts from *S. ganodermophthorum* have shown to produce porous films with morphology similar to those of xylindein (Vega Gutierrez and Robinson 2017) and interesting optical properties (Wiesner 2020). These fungi-derived pigments may have potential for electronic and/or

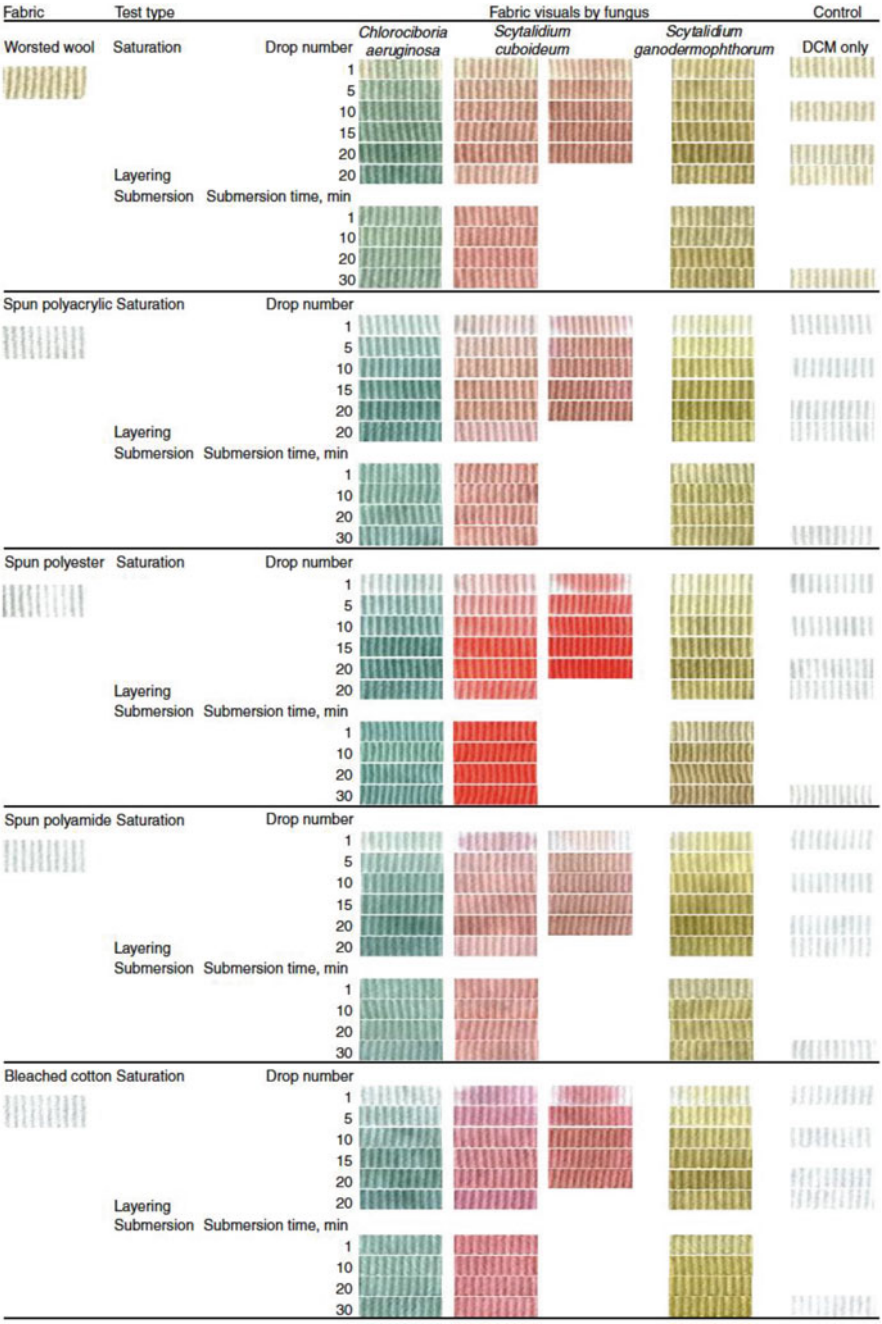


Fig. 3 Extracted pigment from *Chlorociboria* species (blue-green xylindein), *Scytalidium cuboideum* (red dramada), and *Scytalidium ganodermophthorum* (yellow unknown pigment) applied to five different fabric types, showing saturation changes by fabric. Polyester, notoriously difficult to dye, held the most color. From Weber et al. (2014)

Fig. 4 Douglas-fir (*Pseudotsuga menziesii*) and white pine (*Pinus strobus*) wood treated with linseed oil, aniline dyes, or the pigment xylindein (*Chlorociboria* species) / dramada (*Scytalidium cuboideum*) after an accelerated weathering test. The linseed oil mixed with the fungal pigments significantly decreased the cracking and greying of the wood. From Vega Gutierrez et al. (2021)

Fig. 5 Xylindein (blue-green), dramada (red), and the unknown yellow pigment from *Scytalidium ganodermophthorum* can be carried in natural oils, thereby eliminating the need for a solvent carrier. Image shows the color range available. B = xylindein, BR = xylindein + dramada, BY = xylindein + yellow pigment, R = dramada, RY = dramada + yellow pigment, Y = yellow pigment. L = raw linseed oil, D = Danish oil, W = walnut oil

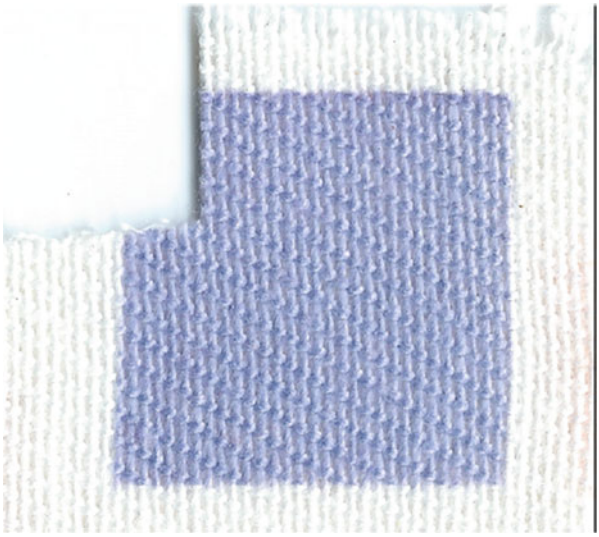


Fig. 6 Dramada (red) pigment from *Scytalidium cuboideum* printed on cotton using an inkjet printer with CTAB-based ink. The pigment was successfully made into an inkjet-compatible ink, which allowed for precise color printing without bleeding edges. From He et al. (2021)

electrochemical applications discussed here in the context of xylindein and require further studies.

1.2 Motivation for Exploring Xylindein as an Organic Semiconductor

There are several technological challenges that humanity must face in the wake of climate change and the need for more sustainable development. Ever since the discovery of conductivity in polyacetylene by Heeger et al. in the 1970s (Shirakawa et al. 1977), organic semiconducting polymers and small-molecule organic materials have been well positioned as a viable replacement for inorganic materials in a variety of (opto)electronic applications (Ostroverkhova 2016). Today, many applications of organic semiconductors have been commercialized, which include organic light-emitting diode (OLED)-based displays and lighting and organic solar cells (Ostroverkhova 2018). The benefits frequently touted are their structural flexibility, ease of processing, and low-cost, to name a few. Organic semiconductor materials that are also sustainable and non-toxic are of special interest.

Many natural products-derived pigments that have been used in art, wood staining, textile dyes, etc. over the centuries (Irimia-Vladu et al. 2011) have a conjugated molecular structure (i.e. containing alternating single and double bonds). However, their utility as organic semiconductors (for which the conventional requirement for the molecular structure is the presence of conjugation) has been limited until recently (Głowacki et al. 2013a, b; Głowacki et al. 2014), although there has been some early work on excited states and charge carrier dynamics in

carotenes (Ehrenfreund et al. 1992) and chlorophyll (Kamat et al. 1986). Even less work has been done on utilizing *fungi-derived* pigments in devices, with only a few reports on using, for example, *Cortinarius* and *Monascus* fungi as sources for sensitizers in dye-sensitized solar cells (Zalas et al. 2015; Ito et al. 2010).Unexpectedly strong performance of naturally derived indigo and other hydrogen (H)-bonded dyes and pigments as organic electronic materials in the past decade has been referred to as a "paradigm shift in molecular electronics design" (Irimia-Vladu 2014). These dyes exhibit high stability with respect to heat degradation and chemical oxidation (which prompts their utility as textile dyes). They also exhibit long-range order and have remarkable photophysical characteristics due to both intra- and intermolecular hydrogen (H-) bonding. Work of Sariciftci and co-workers (Głowacki et al. 2012, 2013a, b, 2014, 2015; Irimia-Vladu et al. 2011; Sytnyk et al. 2014) has documented that H-bonded building blocks, such as indigoids (derived from indigo dye obtained from *Indigofera tinctoria* and *Isatis tinctoria* plants and traditionally used for coloring blue jeans), may contribute good optoelectronic performance, *in spite of lack of intramolecular conjugation,* and stability. These materials owe their performance to intermolecular order that promotes charge delocalization resulting in enhanced charge transport properties that are the key to the electronic device performance. For example, electron and hole mobility of 0.3 $cm^2/(Vs)$ was observed in organic field effect transistors (OFETs) of Tyrian purple, a 6,6′-dibromoindigo pigment originally derived from sea snails and shellfish, and hole mobility of 1.5 $cm^2/(Vs)$ was obtained in epindolidione, a structural isomer of indigo used as a yellow colored toner for printing (Głowacki et al. 2013a, b). Furthermore, power conversion efficiencies of up to 8.2% were obtained from organic solar cells containing indigoids (Deng et al. 2014). Strong (opto)electronic performance of these dyes is coupled with high stability in air (Głowacki et al. 2013a, b); for example, Tyrian purple diode performance showed no signs of degradation even after a month of continuing operation in air, likewise, epindolidione OFET was stable for at least 140 days of operation in air (Gospodinova and Tomšík 2015).

Another high-performance naturally derived building block is H-bonded diketopyrrolopyrrole (DPP) pigments. These are known for their utility in outdoor and automotive paints (e.g. in a famous "Ferrari Red"). However, when used in organic electronic devices, they exhibited ambipolar transport in OFETs with mobilities of up to 0.06 $cm^2/(Vs)$ (Głowacki et al. 2014). Furthermore, DPP-based co-polymers exhibited charge carrier mobilities of >12 $cm^2/(Vs)$, at least four orders of magnitude higher than those in traditional conjugated polymers (e.g. poly (p-phenylene vinylene), or PPV, derivatives) and approaching those in molecular crystals (Ostroverkhova 2016). Finally, some derivatives such as quinacridone (widely used as a magenta colored toner for ink-jet printers) exhibited ambipolar charge carrier mobilities of ~0.1 $cm^2/(Vs)$ (Głowacki et al. 2013a, b) and extraordinarily high photocurrents characterized by an external quantum efficiency (EQE) of 10% in single-component Schottky diodes (i.e. ITO/quinacridone/Al), three orders of magnitude higher than that in similar pentacene (benchmark organic semiconductor) devices (Głowacki et al. 2012).

The work described above served as a motivation to explore H-bonded naturally-occurring spalting fungi-derived pigments as sustainable materials for electronic applications. Xylindein, which exhibits enhanced photostability and high electron mobility (Giesbers et al. 2019a, b), both attractive properties for electronic device applications, is a representative material of this class of promising sustainable organic electronic materials and is the focus of the present chapter. The structure of xylindein was first reported in the 1960s (Blackburn et al. 1962; Edwards and Kale 1965) but its absolute configuration remained unknown for decades, leading to a systematic re-examination in 2000 to obtain the xylindein tautomeric structure (Saikawa et al. 2000). Attempts at xylindein synthesis have so far proven incomplete (Donner et al. 2012), and the work presented in this chapter has been carried out with xylindein extracted from liquid cultures.

The chapter is organized as follows. Section 2 discusses (opto)electronic properties of xylindein and underlying mechanisms. Section 3 introduces challenges that need addressing for viability of xylindein-based electronic devices. Section 4 highlight preliminary work on utilizing xylindein in electrochemical and energy storage devices, and Sect. 5 concludes and presents an outlook.

2 Properties of Xylindein as an (Opto)Electronic Material

2.1 Optical Properties

The important property that motivated exploration of xylindein as an (opto)-electronic material is a rare combination of its enhanced photostability (as compared to conventional organic semiconductor molecules) with promising electronic characteristics (Harrison et al. 2017; Giesbers et al. 2018, 2019a, b, 2021;

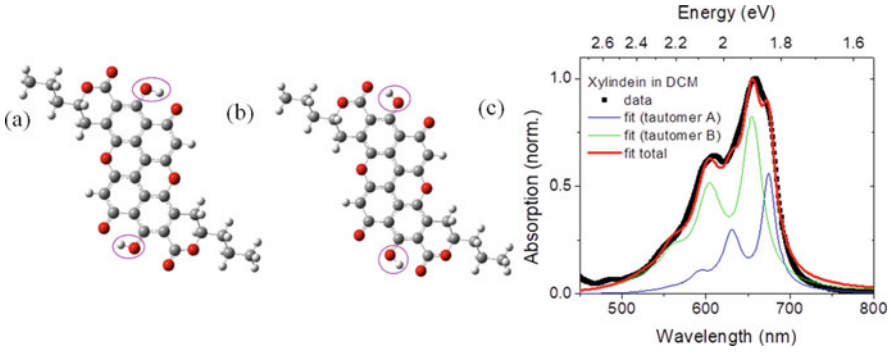


Fig. 7 Molecular structures for xylindein tautomers A (**a**) and B (**b**) with optical properties shown in (**c**). The difference in the orientations of the OH groups between the two tautomers is emphasized by encircling. (**c**) Absorption spectrum of xylindein in dichloromethane (DCM) fit using two vibronic progressions. The sum of the contributions of the two tautomers to the spectra is also included (total fit). From Giesbers et al. (2019a)

Krueger et al. 2021a, b), as summarized in this section. Therefore, for molecular design of next-generation organic electronic materials, it is important not only to characterize the optical and electronic properties of xylindein but also to understand how the molecular structure of xylindein enables these properties.

Optical absorption spectrum of xylindein solution in dichloromethane (DCM) is shown in Fig. 7. The spectrum structure offers an insight into the molecular structure of xylindein and how the molecule interacts with light. In particular, the spectrum exhibits a rather complicated structure that can be described by a sum of two vibronic progressions (Fig. 7). A vibronic progression (a combination of optical transitions offset by a vibrational energy, seen in Fig. 7 as a set of three peaks of varying intensity) is due to the coupling of excitation (electron-hole pair on the photoexcited molecule) to a vibrational mode of the molecule, in this case a C-C stretching mode. The need for two such progressions for properly describing the optical absorption spectrum of xylindein in solution indicates the presence of two dominant tautomers (A and B in Fig. 7) with slightly offset energies of optical transitions (as confirmed by the density functional theory) that contribute to the spectra at a ratio of 30:70 (Giesbers et al. 2019a). Interestingly, these two tautomers exhibit considerable differences in the excited state dynamics, as only one of them undergoes efficient excited-state intramolecular proton transfer (Krueger et al. 2021a). Note that tautomerization of xylindein is related to the OH groups in the xylindein molecular structure; for example, xylindein derivative dimethylxylindein in which the OH groups are replaced by OCH_3 groups does not have tautomers and as a result has a simpler absorption spectrum which fits well with one vibronic progression (Giesbers et al. 2021). The photoluminescence quantum yield of xylindein is low (<0.1%), due to efficient non-radiative decay pathways as discussed in Sect. 3.2.

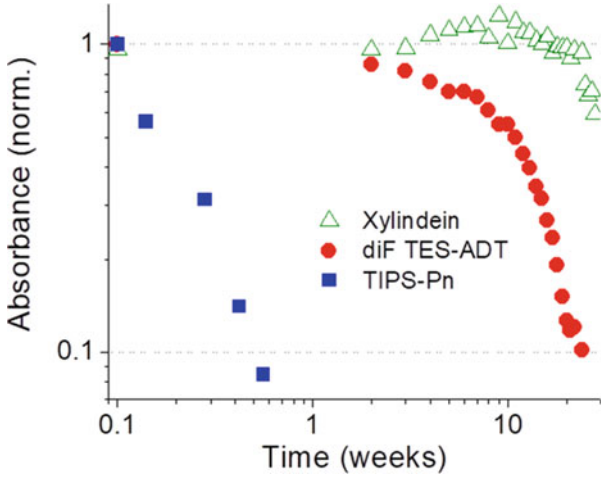


Fig. 8 Integrated optical absorption S_0-S_1 spectra, normalized at time t = 0, of dilute solutions of xylindein and of benchmark organic semiconductors TIPS-Pn and diF TES-ADT continuously illuminated by white light in air, illustrating superior photostability of xylindein in air. From Giesbers et al. (2019a)

2.2 *Photostability*

Figure 8 illustrates that xylindein in solution has a considerably higher photostability as compared to solutions of benchmark organic semiconductors functionalized pentacene (TIPS-Pn) and anthradithiophene (diF TES-ADT) (Ostroverkhova 2016). In particular, under continuous white-light illumination in air, the TIPS-Pn molecules in solution decomposed within 3 days. The fluorinated ADT derivative, diF TES-ADT, which is photostable enough to enable its use as a fluorophore in single-molecule fluorescence spectroscopy (Shepherd et al. 2015), showed a gradual degradation over the period of first several weeks followed by an accelerated degradation starting at about 5 weeks. Under the same illumination conditions, no degradation in optical absorption of xylindein was observed over the period of about 25 weeks, after which partial degradation occurred (Fig. 8) (Giesbers et al. 2019a). In addition, xylindein in solution exhibited enhanced photostability in comparison with other H-bonded pigments, which are known for their enhanced photostability (Yamazaki et al. 2011), including indigo and alizarin (Giesbers et al. 2021). On the molecular level, the photostability of xylindein is enabled by OH groups in its molecular structure and the fast nonradiative excited state processes these groups facilitate such as excited-state intramolecular proton transfer (Sect. 3.2). In particular, a deprotonated xylindein and xylindein derivative where OH groups are replaced by OCH_3 groups (dimethylxylindein), degrade considerably faster than xylindein (Sect. 3.2).

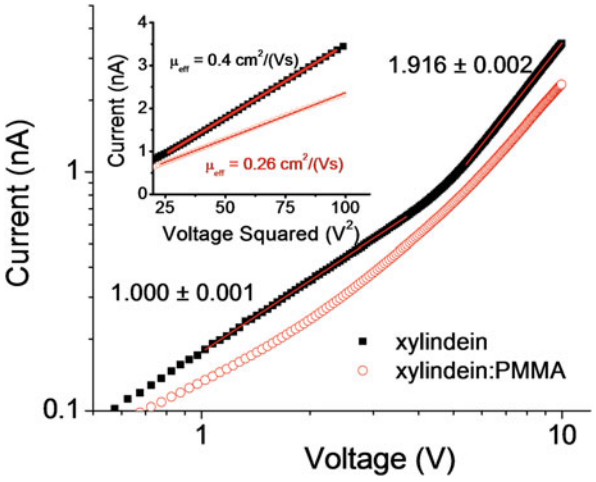


Fig. 9 Current-voltage (I-V) characteristics for pristine xylindein and xylindein:PMMA films on coplanar Al electrodes with a 200 μm gap, exhibiting transition from the linear (Ohmic) to the quadratic (SCLC) regime. Inset shows the current (I) replotted as a function of voltage-squared (V^2) and linear fits slopes of which were used to calculate the effective electron mobilities assuming the thin-film approximation of the SCLCs in the planar electrode geometry. From Giesbers et al. (2019a)

2.3 Electronic Properties

Figure 9 shows current-voltage (I-V) characteristics for a pristine xylindein film on coplanar aluminum (Al) electrodes exhibiting ohmic response followed by a characteristic transition from the linear ($I \sim V$) to the space-charge-limited current (SCLC) ($I \sim V^2$) regime (Day et al. 2009). From SCLC currents (inset of Fig. 9), the values for the effective electron mobility, which is one of the defining characteristics of an electronic material, in the 0.1–0.4 $cm^2/(Vs)$ range were obtained, depending on the device, in pristine xylindein films. These values are considered to be lower bound estimates to the "true", intrinsic value for the electron mobility, as the trap-free SCLC regime (characterized by a sharp, step-like increase in the current at higher voltages, followed by the $I \sim V^2$ behavior but at considerably higher levels of currents) was not reached in these measurements and thus, the intrinsic mobility is higher. Also note that the performance of xylindein-based devices depends on the fungi growth and purification protocols (Van Court et al. 2020a; Giesbers et al. 2019b), and higher mobilities could be possible with improved protocols. Achieving effective mobility values above 0.1 $cm^2/(Vs)$ is rather remarkable given that xylindein films are amorphous, as these values approach the value of ~1 $cm^2/(Vs)$ in amorphous silicon while most amorphous organic semiconductor films have charge carrier mobilities below 10^{-3} $cm^2/(Vs)$. This illustrates benefits of an interplay of intermolecular hydrogen bonding and π-π stacking, made possible by the molecular structure of xylindein but not present in conventional organic semiconductors with molecular structures that lack OH groups and rely only on π-π stacking to promote charge transport. This observation is further supported by four orders of magnitude higher charge carrier mobilities obtained in xylindein films as compared to dimethylxylindein (Giesbers et al. 2021).

As xylindein tends to form porous and inhomogeneous films (Sect. 3.3, Giesbers et al. 2018), in order to improve film processability, blends of xylindein with a polymer polymethylmethacrylate (PMMA) were explored. The PMMA exhibits negligible electric currents in the absence of xylindein, thus providing a non-conductive scaffold for the xylindein molecules (Giesbers et al. 2018, 2019a). The xyindein:PMMA blend yielded considerably smoother films as compared to xylindein films, with optical and electronic properties similar to those of pristine xylindein film (Fig. 9), exhibiting, for example, SCLC effective mobilities of 0.26 $cm^2/(Vs)$ in the xylindein:PMMA film (as compared to 0.4 $cm^2/(Vs)$ in pristine xylindein), inset of Fig. 9. Thus, blending with polymers represents one of the promising routes for improving processability, reproducibility, and performance of xyindein-based films for electronic devices. Blending of xylindein with nature-derived materials such as nanocrystalline cellulose has also been explored (Giesbers et al. 2019a). Although electronic characteristics from these blends were inferior to those from pristine xylindein and xylindein:PMMA blends, this was attributed to non-uniform distribution of xylindein which prevented formation of efficient conductive pathways. This issue could potentially be resolved by improvements in film

processing methods, towards creating a fully nature-derived sustainable organic electronic material.

3 Challenges

3.1 Impurities in Fungi-Derived Pigments and their Effect on the (Opto)Electronic Properties

As spalting is likely a stress response by fungi, pigments are not the only compounds secreted. A host of other compounds (and mycotoxins) are produced both intra and extracellularly by fungi under stress, the makeup of which is not only species specific, but also strain and conditions specific. This mix of secondary metabolites makes purification of xylindein difficult. The preferred solvent for pigment extraction, dichloromethane (DCM),solubilizes other compounds along with xylindein, as seen in high-performance liquid chromatography (HPLC) analysis—most of which are not readily visible to the naked eye and are well hidden in the blue-green extract. While the peaks of such compounds separate out on HPLC, physically removing them has proven difficult (Van Court et al. 2020a; Giesbers et al. 2019b) as illustrated below. As xylindein is produced by many species in the *Chlorociboria* genus, output amount and types of secondary metabolites differ, both across species and within strains (Van Court et al. 2020a). Strain was found to be more important than species in terms of selecting high xylindein output and relative purity (Van Court et al. 2020a). (In contrast, purification of the dramada crystal from *Scytalidium cuboideum* is relatively simple—allowing the DCM solution to evaporate slowly so that the crystals can lengthen. These crystals can then be harvested from the substrate (usually glass) without any competing secondary metabolites (Vega Gutierrez et al. 2018). There also do not appear to be clear strain effects on the amount of dramada produced by *S. cuboideum* (Weber et al. 2016).)

Growth conditions affect metabolite production of all fungi, but are particularly challenging when obtaining pigments from spalting fungi. High nutrient growth conditions lead to mycelial generation but not necessarily to pigment production (Van Court et al. 2020a; Stange et al. 2019), hence conditions must be maintained that stress the fungi without killing them. For cooler-climate fungi, such as *Chlorociboria* species (Richter and Glaeser 2015), this can mean growth at warmer temperatures. For pH sensitive fungi, like *Scytalidium* species, this can mean additions to the growth media to stimulate the desired color output.

Figure 10 illustrates the effect of impurities, most likely secondary metabolites mentioned above, present in xylindein extracted from liquid cultures on the optical properties and electronic characteristics. A simple protocol (“ethanol wash”) was developed to remove some, but not all, impurities, which was observed using mass spectrometry and has manifested into optical and electronic properties (Fig. 10). In particular, Fig.10a, b show a comparison between the absorption and

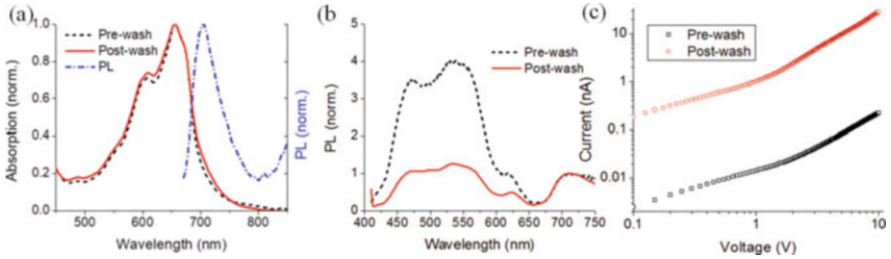


Fig. 10 (**a**) Absorption and (**b**) Emission spectra before and after ethanol wash. In (**b**), PL emission (normalized at its values at 712 nm) was collected with a 400 nm excitation, exhibiting large difference in contribution of contaminants responsible for emission below ~600 nm. (**c**) Current-voltage (I-V) characteristics for the "pre-wash" and "post-wash" xylindein film on coplanar Al electrodes with a 50 μm gap, showing transition from the linear to the quadratic (SCLC) regime. Effective charge carrier mobilities extracted from these SCLC characteristics are 1.6×10^{-3} $cm^2/(Vs)$ for "pre-wash" and 0.19 $cm^2/(Vs)$ for "post-wash" xylindein. From Giesbers et al. (2019b)

photoluminescence (PL) spectra obtained from "pre-wash" (i.e. as extracted) and "post-wash" (i.e. purified using "ethanol wash") xylindein solutions. While the absorption in the 500–700 nm region corresponding to that from the lowest electronic (S_0-S_1) transition of xylindein is preserved (as shown in Fig. 10a), a dramatic reduction in the UV-absorbing species (referred to as contaminants) was observed as a result of the ethanol wash (Giesbers et al. 2019b; Van Court et al. 2020a). The PL of xylindein occurs in the >680 nm wavelength region (Fig. 10a, b) and is weak, characterized by a quantum yield of <0.1%. A considerably stronger PL emission was observed from contaminants in the "pre-wash" sample, occurring in the broad (400–600 nm) spectral region upon 400 nm excitation (Fig. 10b). As seen from Fig. 10b, the ethanol wash substantially reduced the PL emission from the contaminants - by nearly 75% (as compared to the PL emission in the "pre-wash" sample), but it did not eliminate it completely.

Figure 10c shows a comparison between electronic properties of films deposited from "pre-wash" and "post-wash" xylindein solutions. An increase in the electric current (and associated charge carrier mobility) of more than two orders of magnitude was observed in samples made with a "post-wash" xylindein as compared to "pre-wash" xylindein. This large difference in the electronic performance demonstrates importance of development of effective purification protocols for achieving as high purity as possible to boost electronic properties.

Additionally, more than an order of magnitude difference in charge carrier mobility was observed in xylindein films made using xylindein produced by different *Chlorociboria aeruginascens* strains (Van Court et al. 2020a), due to the varying type and concentration of impurities. This further highlights the multitude of variables that could be optimized to achieve the best device performance.

3.2 *Photophysics: High Photostability Versus Photosensitivity for Optoelectronics*

The molecular core of xylindein has a structure of a peri-xanthenoxanthene (PXX). However, the photophysical and electronic properties of xylindein are considerably different from those of unsubstituted PXX and its derivatives studied in the literature, which indicates importance of side groups in xylindein, not present in commonly studied PXX derivatives. For example, xylindein is non-fluorescent (PL quantum yield (QY) of<0.1%), and it favors electron (n-type) transport, in comparison with PL QYs of 0.5–0.97 and hole transport in PXX (Al-Aqar et al. 2017; Kobayashi et al. 2009). Therefore, in order to better understand properties of xylindein and relate them to the specifics of its molecular structure, a molecule with a structure closer to that of xylindein as compared to PXX was necessary. An example of such molecule is a methylated derivative of xylindein (dimethylxylindein), which has most of the features of xylindein's molecular structure except for the OH groups that are substituted with OCH_3 (OMe) groups. A side-by-side comparison of optical and (opto)electronic properties of these two compounds was performed, revealing dramatic differences in their photophysics and (photo)conductivity. In particular, it was demonstrated that the OH groups in xylindein enable processes that are critical both for its remarkable photostability and enhanced electronic properties (Giesbers et al. 2021).

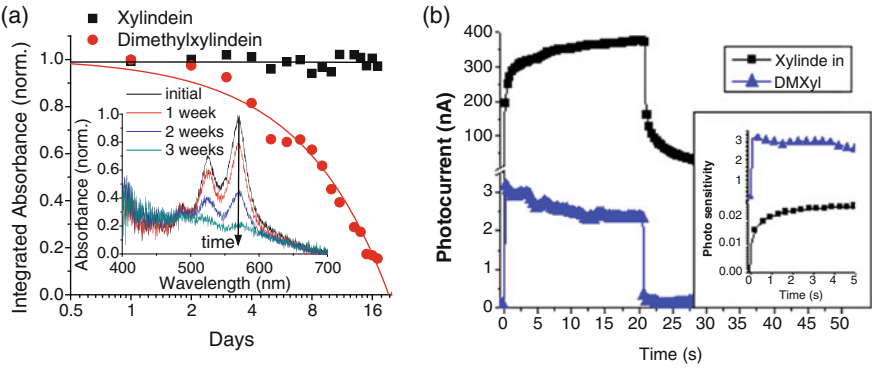


Fig. 11 (**a**) Integrated absorption spectra of xylindein and dimethylxylindein solutions in dichloromethane versus time during exposure to white light in air. Lines provide the guide to the eye. Inset shows time evolution of dimethylxylindein absorption spectra as the compound degrades upon exposure to light. (**b**) Photocurrent from pristine xylindein and dimethylxylindein (DMXyl) films on interdigitated electrodes with a 25 μm gap at the applied voltage of 100 V under 532 nm continuous wave laser illumination turned on at time t = 0 and off after 20 s. Inset shows the photosensitivity of the same films, defined as a ratio of the photocurrent to dark current. Note that although the photocurrent is higher in xylindein (main figure), due to xylindein's superior charge carrier mobility, the photosensitivity is higher in dimethylxylindein (inset), due to dimethylxylindein's more efficient charge photogeneration. From Giesbers et al. (2021)

Figure 11a shows integrated absorption over the S_0-S_1 transition for xylindein (600–700 nm) and dimethylxylindein (520–590 nm) in solution under continuous white light illumination in air. Importantly, dimethylxylindein degraded by more than 80% after only two weeks of exposure, while xylindein showed no evidence of photodegradation under identical illumination conditions during this period of time. Significant photodegradation was also observed in deprotonated xylindein, further demonstrating the importance of the hydroxyl (OH) groups to the photostability of the molecule. Transient absorption spectroscopy (which probes excited-state dynamics on the ultrafast time scales) provides further insight intothe stark difference between the stability of xylindein and dimethylxylindein molecules (Fig. 11a) which revealed fast excited-state decay which depopulates the photoexcited state on picosecond time scales in xylindein as compared to long-lived excited state that persists in dimethylxylindein after many tens of nanoseconds after photoexcitation (Krueger et al. 2021a; Giesbers et al. 2021; Krueger et al. 2021b).

In particular, Fig. 12 shows the potential energy surfaces in xylindein, highlighting efficient ultrafast pathways for energy relaxation both in solution and thin film. In solution (Fig. 12a), fast conformational motions of xylindein lead to a conical intersection (CI) and excited state deactivation on the picosecond time scales, which prevents chemical reactions (such as photo-oxidation) responsible for photodegradation that require a longer-lived excited state.

Figure 11b shows photoconductive properties, important for devices that rely on the ability of materials to generate charge carriers under illumination (e.g. photovoltaic cells or phototransistors), of xylindein and dimethylxylindein films under a 532 nm continuous-wave light excitation. Although the photocurrent is about two orders of magnitude higher in xylindein films than in dimethylxylindein, this is a combined effect of a four orders of magnitude *higher* charge carrier mobility, but two orders of magnitude *lower* charge photogeneration efficiency in xylindein, both of which contribute to the photocurrent resulting in a net higher photocurrent in xylindein. While the higher charge carrier mobility in xylindein is due to a combination of hydrogen bonding promoting intermolecular charge transfer and more favorable film morphology as compared dimethylxylindein, the question arises of why the charge photogeneration efficiency in xylindein is so much lower than that of the dimethylxylindein. The lower photogeneration efficiency in xylindein is illustrated by a comparison of the photosensitivity of xylindein and dimethylxylindein, which is defined as the ratio of photocurrent to dark current. As seen from the inset of Fig. 11b, the photosensitivity was considerably higher in dimethylxylindein. This is due to the differences in the excited state dynamics of these two compounds which are responsible for higher photostability of xylindein (Fig. 11a) but manifest into a lower charge photogeneration efficiency (Fig. 11b) as compared to dimethylxylindein. In particular, as shown in Fig. 12b, c, the photoexcitation of xylindein films is followed by the sub-picosecond excimer formation. This, in turn, is followed by an efficient non-radiative decay which dramatically reduces exciton diffusion lengths which are often necessary for high charge photogeneration efficiency. Therefore, there appears to be a trade-off between photostability and charge photogeneration efficiency in

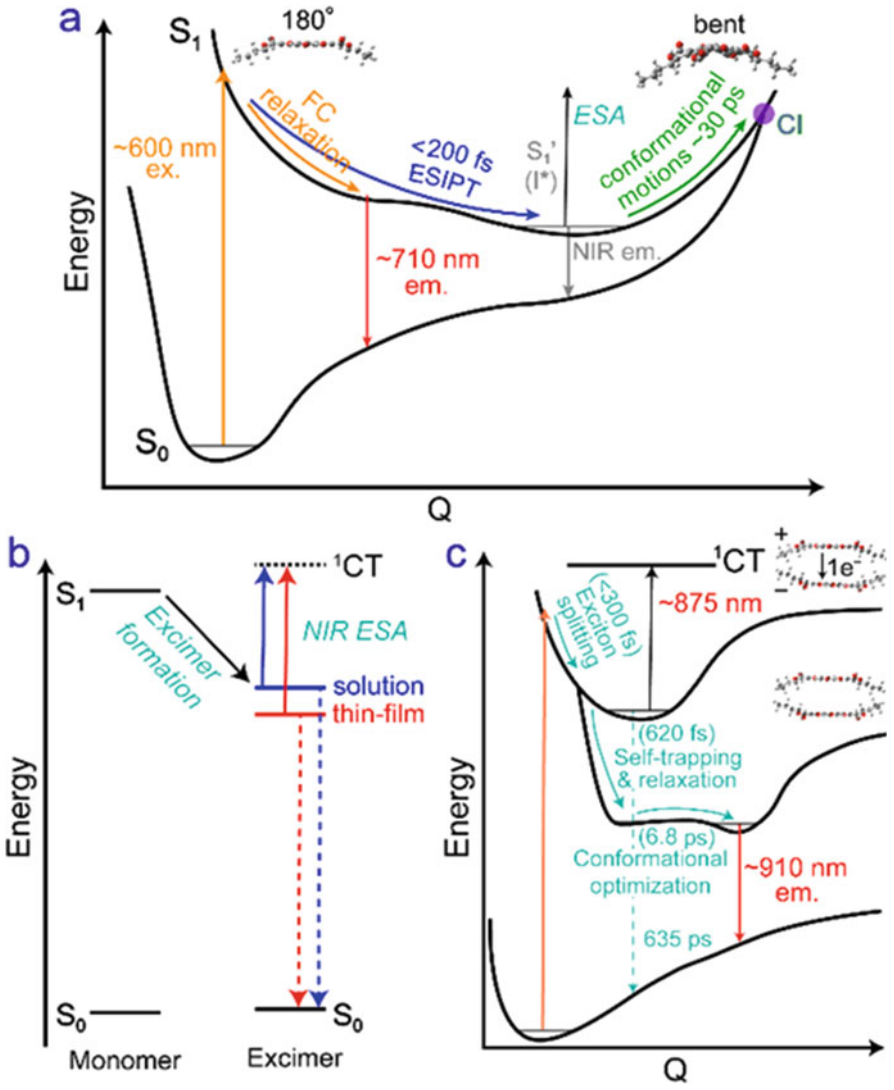


Fig. 12 Potential energy surfaces (PESs) of xylindein in solution and in thin film. (**a**) For xylindein monomer in solution, the insets show the decreasing ring-coplanarity due to conformational motions, which leads to a conical intersection (CI). (**b**) Energy levels for the monomer in solution and for the aggregated solution and thin-film excimers. The excimer to charge-transfer (^{1}CT) absorption band is denoted near-infrared excited-state absorption (NIR ESA). (**c**) PESs of the thin-film xylindein. The excimers following initial structural relaxation exhibit the NIR ESA band to a CT state (Xyl^+-Xyl^-). In (**a**) and (**c**), time constants are listed with their respective dynamic processes. From Krueger et al. (2021a)

xylindein derivatives, which has to be taken into account when optimizing these materials for applications in optoelectronic devices.

3.3 Effect of Morphology

Even though some aspects of pristine xylindein photophysics discussed above are unfavorable for optoelectronic applications, in order to obtain a more comprehensive picture of xylindein as an optoelectronic material, xylindein was investigated as a non-fullerene n-type acceptor material in donor-acceptor (D:A) blends for photovoltaics. This was motivated by the continuing search for more stable n-type organic materials, as current n-type organic semiconductors exhibit low stability with respect to environmental factors (Ostroverkhova 2018; Ostroverkhova 2016). Additionally, the most successful n-type organic materials serving as acceptors in D:A photovoltaics have traditionally been fullerene derivatives that have not only low-stability issues but other unfavorable features including relatively high costs.

In the D:A organic photovoltaics, light excites the donor (p-type organic semiconductor) and/or acceptor (n-type organic semiconductor) and creates a bound electron-hole pair (exciton). The exciton diffuses within the D and/or A domain (which have to have dimensions on the order of exciton diffusion length, typically within ~10 nm) to the D-A interface where it separates, via charge transfer, into free electron and hole charge carriers which then travel to the electrodes and generate the electric current. Initial studies explored potential D:A blends of xylindein with other materials, looking for signatures of charge transfer from the p-type donor molecule to the xylindein acceptor and from xylindein to another acceptor molecule. No signatures of charge transfer states were observed spectroscopically in blends with well-known p-type materials such as poly(3-hexylthiophene-2,5-diyl) (P3HT) and Poly([2,6′-4,8-di(5-ethylhexylthienyl)benzo[1,2-*b*;3,3-*b*]dithiophene]{3-fluoro-2 [(2-ethylhexyl)carbonyl]thieno[3,4-*b*]thiophenediyl})(PTB7-Th) polymers or diF TES-ADT small molecules (Ostroverkhova 2016), or n-type $PC_{60}BM$ fullerene molecule. Thin films of these blends were deposited onto glass substrates with patterned coplanar Au electrodes, excited with a 532 nm light, and the photoconductivity was measured under applied electric field. The blends of P3HT:xylindein and diF TES-ADT:xylindein were less photoconductive than pristine P3HT and diF TES-ADT films, respectively. A PTB7-Th:xylindein blend, however, had a higher photosensitivity (defined as the ratio of photocurrent to dark current) than either pristine PTB7-Th or pristine xylindein (Fig. 13a), thus potentially exhibiting enhanced charge separation at D-A interfaces. This blend was thus chosen to be further investigated in solar cell devices as the active layer, in both conventional (ITO/PEDOT:PSS/active layer/Al) and inverted (ITO/ZnO/active layer/Au) geometry (Giesbers 2021).

Figure 13b shows current-voltage characteristics of solar cells under illumination from a solar simulator with PTB7-Th-only, PTB7-Th:$PC_{60}BM$ and PTB7-Th: xylindein active layers. The control cell with $PC_{60}BM$ acceptor exhibited the

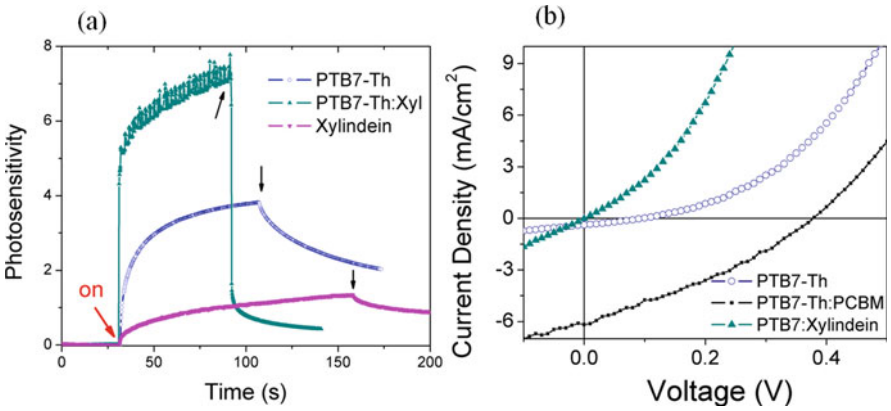


Fig. 13 (**a**) Photosensitivity of thin films of PTB7-Th, xylindein, and a 1:1 w/w PTB7-Th:xylindein blend drop-cast onto interdigitated Au electrodes deposited on glass. Voltage is applied, the white light is turned on at time t = 30 s, and the resulting photocurrent is measured. The arrows indicate the time when the light was turned off. The blend shows an increase in photosensitivity with respect to the pristine materials, indicating its potential as a D/A blend in solar cells. (**b**) I-V curves for organic solar cells under illumination from a solar simulator with PTB7-Th as a donor material. D:A blend of PTB7-Th:xylindein performs not only below benchmark D:A blend PTB7-Th:$PC_{60}BM$ but also below PTB7-Th donor-only solar cells, due to detrimental xylindein morphology for this device geometry

short-circuit current density $J_{SC} \sim 6$ mA/cm^2 and the open-circuit voltage $V_{OC} \sim 0.4$ V, and improvement as compared to pristine PTB7-Th, for which $J_{SC} < 1$ mA/cm^2 and $V_{OC} \sim 0.1$ V were observed. Solar cells with xylindein as an acceptor material, however, had a negligible short-circuit current and open-circuit voltage. In contrast to the enhanced photosensitivity observed in PTB7-Th:xylindein films as compared to both pristine PTB7-Th and xylindein in a planar geometry (i.e. when the film is deposited on the top of the substrate with pre-patterned coplanar electrodes, Fig. 13a), the same blend was not successful in the sandwich configuration (i.e. when the film is placed between top and bottom electrodes) under experimental conditions required by the solar cell operation. In order to investigate the cause of this poor performance in the solar cell with xylindein, ternary blend solar cells were fabricated with PTB7-Th and $PC_{60}BM$ and small amounts (0–5%) of xylindein. These investigations showed that addition of even small amounts of xylindein to the PTB7-Th:$PC_{60}BM$ blend result in a significant decrease in performance, reducing both J_{SC} and V_{OC} (Giesbers 2021).

One important difference between the planar and sandwich geometries and associated experimental conditions for photocurrent measurements is that the latter is considerably more demanding in terms of achieving the optimal film morphology for photoinduced charge separation. In particular, smooth films with the domains on the order of the exciton diffusion length (~10 nm in best-performing organic solar cells) are required. These are difficult to achieve with xylindein which is prone to forming porous structures (Fig. 14) and has a short exciton diffusion length $L_D =$

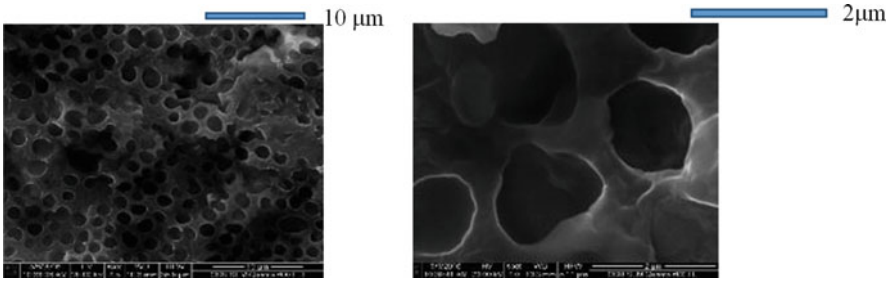


Fig. 14 Scanning electron microscopy images of a drop-cast xylindein film illustrating porous structure of the film (Giesbers et al. 2018)

$\sqrt{D\tau}$, where D is the diffusion coefficient and τ is the exciton lifetime, due to ultra-short exciton lifetimes (Fig. 12). Even though film roughness can be partially mitigated by blending xylindein with polymers (Giesbers et al. 2018), a relatively large polymer fraction is required to achieve film morphology that is suitable for electronic devices in a sandwich geometry such as solar cells (Giesbers et al. 2021), which is detrimental to the electronic properties. The methods for controlling film morphology in xylindein and its derivatives need to be further developed to achieve optimal morphology for applications in solar cells and organic field-effect transistors in which the film quality is critical in order to utilize enhanced electron transport characteristics of xylindein observed in less demanding (i.e. more forgiving with respect to film morphology) device geometries (Sect. 2.3) as compared to those of solar cells and transistors.

In particular, most organic thin-film transistors achieve their best performance when the organic active layer is crystalline (Ostroverkhova 2016). Attempts to apply standard methods of improving film crystallinity (such as substrate surface treatments, thermal and solvent vapor annealing, etc.) developed for conventional organic semiconductors to xylindein have proven unsuccessful, and all xylindein films were amorphous and porous (Fig. 14), regardless of the treatment. This is consistent with the literature (Saikawa et al. 2000) where xylindein crystals were obtained from hot aqueous phenol yielding 4 phenol molecules per xylindein molecule in the asymmetric unit (with the X-ray diffraction-revealed structure yielding a monoclinic space group $P2_1$ with the lattice parameters of a = 8.4 Å, b = 24.0 Å, c = 11.6 Å, and $\beta = 102.2^{\circ}$), but attempts to remove the phenol molecules from crystals resulted in a crystal collapse yielding an amorphous solid. Therefore, routes to xylindein device fabrication with a controlled film morphology and crystallinity require further investigation. Although porous film morphology of Fig. 14 is undesirable for organic solar cells or transistors, it can be beneficial for other applications, for example, energy storage (batteries) which specifically utilize porous materials (e.g. porous titania (Fischer et al. 2017)). Xylindein use in device geometry towards energy storage applications is discussed in Sect. 4.2.

4 Applications

The challenges discussed in the previous section motivated exploration of applications of xylindein in which film quality is less critical. It is known from the literature that hydrogen bonded pigments, exemplified by melanin (another hydroxyl containing naturally occurring pigment), may exhibit protonic conductivity (Vahidzadeh et al. 2018). One signature of this phenomenon is that the conductivity of melanin increases drastically with humidity. It was found that films of xylindein also experience an increase in conductivity in the presence of moisture, which indicates that xylindein too exhibits protonic conductivity, facilitated by the hydroxyl groups. This protonic conduction is a form of ionic conduction. Materials that have significant ionic and electronic conduction have a number of different applications (Surgailis et al. 2021), and only a few have so far been investigated for xylindein, which represents a promising area of research.

4.1 Xylindein for Organic Electrochemical and Water-Gated Transistors

A prominent device application for organic mixed conductors (i.e. materials exhibiting both electronic and ionic conductivity) is an organic electrochemical transistor (OECT) which has been considered to be a building block of biosensors, neuromorphic devices, and complementary circuits (Surgailis et al. 2021). Schematic of OECT geometry is shown in Fig. 15a. First, benchmark OECTs based on poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) were fabricated. PEDOT:PSS is a conductive polymer mixture that is widely used in the literature and is one of the best performing active materials in OECTs (Rivnay et al. 2018). As expected, the PEDOT:PSS OECT in a conventional geometry (using a 0.1 M NaCl solution as the electrolyte and an Ag/AgCl gate electrode) exhibited a switching behavior in the output characteristics, operating in depletion mode ("on" state with no gate bias and "off" state when positive voltage is applied) (Giesbers 2021). The switching behavior is due to ions from the electrolyte penetrating into the polymer film doping the film. In the case of PEDOT:PSS, which is already p-doped with sulfonate anions from the PSS, the applied gate voltage causes cations to penetrate into the film, negating the doping effects of PSS and decreasing the conductivity of the film (Rivnay et al. 2018). In contrast to well-studied p-type PEDOT:PSS OECTs, xylindein OECT was expected to be a n-type OECT, which are of considerable interest yet face critical challenges and specific requirements for the molecular design (Sun et al. 2018; Giovannitti et al. 2018). When xylindein was tested in the same OECT geometry as PEDOT:PSS, however, the output characteristics were not as expected from an OECT, either p- or n-type (Fig. 15b). Instead, the data exhibited features characteristic of redox activity typically observed using cyclic voltammetry (CV). In particular, the devices (e.g. Figure 15c) exhibited

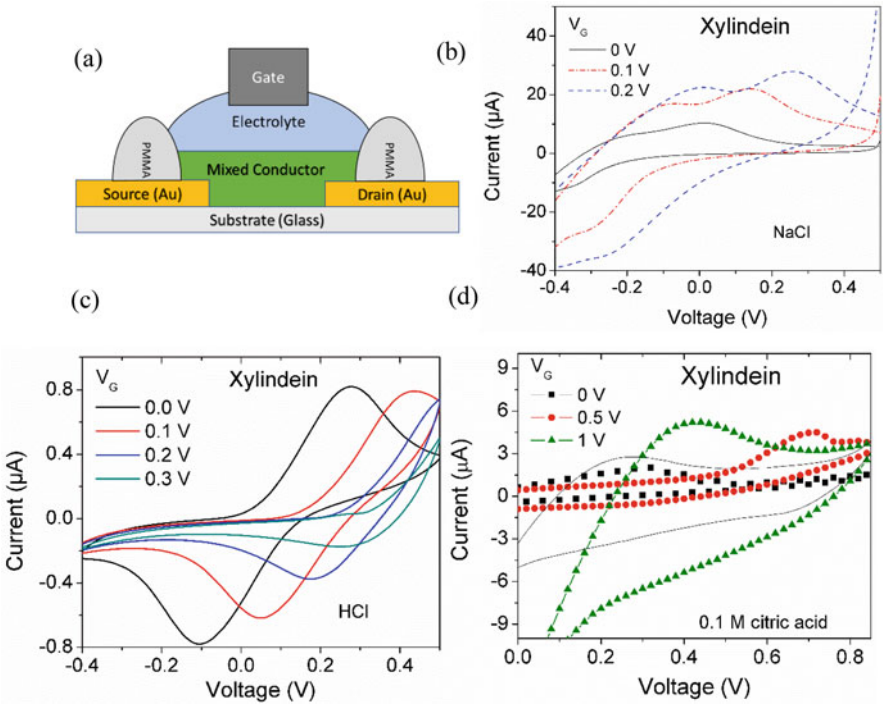


Fig. 15 (**a**) Schematics of OECT configuration. (**b–d**) Cyclic current-voltage sweeps for xylindein devices in an electrochemical transistor configuration with NaCl solution (**b**), 1 mM HCl solution (**c**), or 0.1 M citric acid (**d**) as the gating medium

curves with reduction and oxidation peaks in the classic "duck shape" of CV curves. Several different electrolyte solutions were tested for xylindein devices as a gating medium in a typical OECT configuration of Fig. 15a, each showing redox behavior (NaCl, HCl, and citric acid in Fig. 15b–d). The observed CV-like characteristics are indicative of promising energy storage potential, which will be the focus of Sect. 4.2.

Another class of transistors similar to the OECT is the water gated organic field effect transistor (WGOFET). The configuration of a WGOFET is essentially the same as an OECT, except that deionized (DI) water is used instead of an electrolyte solution in Fig. 15a. The principle of operation of such WGOFETs is that dissolved ions in the water from exposure to air form an electronic double layer when voltage is applied and a field effect occurs in the material (Nguy et al. 2019; Yaman et al. 2014; Porrazzo et al. 2017). Similar to OECTs, the WGOFETs have applications in biotechnology such as biosensors and neuromorphic devices (Rivnay et al. 2018; Cramer et al. 2013). Xylindein is particularly suited to these applications due to its non-toxicity (Almurshidi et al. 2021).

Output characteristics of a xylindein WGOFET are shown in Fig. 16a, exhibiting a n-type response and a relatively large hysteresis. Other xylindein-containing active layers have also been tested such as xylindein:PEIE (Fig. 16b) where PEIE

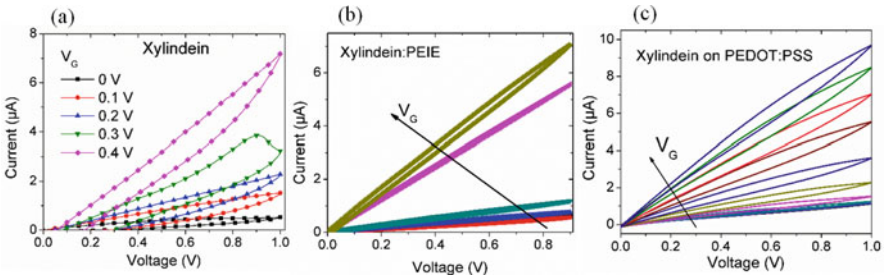


Fig. 16 Output curves from DI water-gated transistors with xylindein (**a**), xylindein:PEIE (**b**) and xylindein on a thin layer of PEDOT:PSS (**c**) as the active layer

(polyethylenimine ethoxylated) is a polymer commonly used as an interface layer material in organic solar cells which promotes charge collection. It also has been successful in blends (such as ZnO:PEIE (Yu et al. 2019)) in enhancing electron transport and serving as an electron transport layer in organic solar cells. Blending xylindein with PEIE almost eliminated the hysteresis seen in pristine xylindein devices, although the currents in both devices were comparable (Fig. 16a, b). Xylindein on PEDOT:PSS (a material widely used in OECTs discussed above) as an active layer in WGOFETs was also explored (Fig. 16c) and yielded higher currents than those in pristine xylindein devices. A figure of merit derived from I-V characteristics of Fig. 16 is transconductance, defined as a ratio of a change in the output current of the device and a change in the applied voltage. The transconductance values were on the order of μA/V, comparable to other WGOFET devices such as those based on a commonly used polymer P3HT (Yaman et al. 2014; Porrazzo et al. 2017). However, the response time for the observed switching behavior in the xylindein WGOFETs was notably slow, on the order of minutes. This would be unexpected for an electronic response such as a field effect, and still many times slower than the response of a typical OECT. Therefore, working mechanism of this device is potentially different from that in both an FET and an OECT which requires further investigation. The slow response in this geometry, however, could be related to an efficient charge storage, which would be advantageous for battery applications, as discussed next.

4.2 Xylindein for Energy Storage Applications

Quinone derivatives, including naturally derived pigments such as melanin and alizarin, have been studied extensively for use in battery applications (Kim et al. 2016; Tong et al. 2019; Luo et al. 2014; Han et al. 2019). There are several considerations that could make xylindein promising for these applications: (1) xylindein is also a quinone derivative; (2) xylindein forms porous films, which may allow ions to penetrate into the film and enhance the surface area for

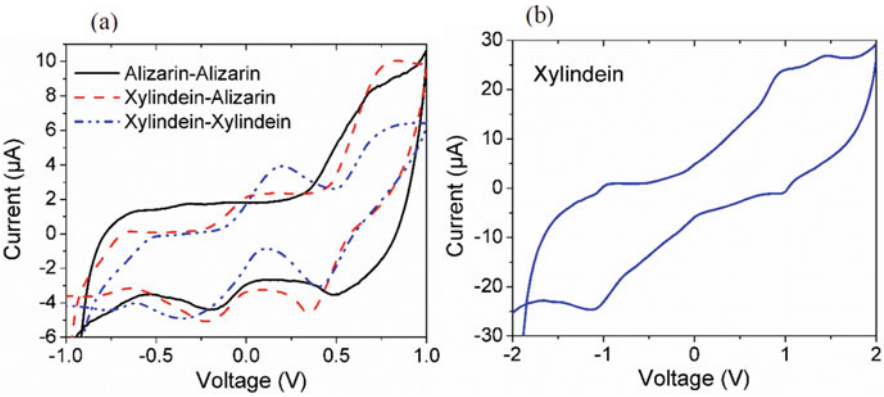


Fig. 17 Cyclic current voltage sweeps for pseudocapacitor devices. (**a**) Sweeps for devices with two alizarin coated electrodes, one alizarin and one xylindein, and two xylindein coated electrodes. Hysteresis indicates charge storage and bumps/dips indicate electrochemical reactions. (**b**) Sweep for the all xylindein device with an expanded voltage window

electrochemical interactions; (3) the redox behavior was observed in xylindein OECT devices, and (4) the slow response time was observed in xylindein WGOFETs discussed in the previous section. Therefore, initial investigation of xylindein-based electrochemical cells was performed in which xylindein was compared to alizarin, a naturally derived hydroxyl groups-containing quinone derivative which has already been demonstrated as a candidate for battery applications (Tong et al. 2019). In this investigation, pseudocapacitor structures were fabricated consisting of two carbon electrodes immersed in a 0.1 M H_2SO_4 solution. The electrodes were coated via drop-casting with either xylindein or alizarin. Figure 17 shows cyclic current-voltage sweeps for the alizarin-alizarin, xylindein-alizarin, and xylindein-xylindein pseudocapacitors. The symmetric alizarin pseudocapacitor (i.e. alizarin-alizarin, with both electrodes coated with alizarin) exhibited two sets of small features due to reduction and oxidation, similar to those reported in the literature (Tong et al. 2019). Similar levels of currents obtained from xylindein pseudocapacitors indicate similar specific capacitance of xylindein-based and alizarin-based devices, which encouraged further exploration. When the voltage window was increased on the symmetric xylindein device (Fig.17b), sets of redox peaks were observed approximately 2 V apart, which is an improvement upon reported alizarin devices (Tong et al. 2019). The wider potential window for xylindein compared to alizarin indicates that there are greater operational limits for the xylindein when compared to alizarin in the same electrolyte, which merits further investigation.

The ongoing research into the development of K^+-ion and Mg-ion battery technology, which are poised to replace Li-ion platforms due to their inherent stability and improved energy density, have not only shown the incorporation of blue pigments (e.g. Prussian blue in the first prototype K^+-ion battery (Eftekhari 2004)), but also the incorporation of quinone compounds as cathode materials within these

next-generation devices (Jian et al. 2016; Dong et al. 2019). The recent literature and promising results of Fig. 17 could position xylindein well as a sustainable option for batteries of the future.

5 Conclusions and Outlook

Investigation into properties of fungi-derived pigment xylindein has provided an insight into mechanisms behind its fascinating optical, photophysical, electrochemical, and electronic properties. Analysis of the optical spectra revealed the presence of two tautomers whose structures and properties were characterized using density functional theory and ultrafast spectroscopy, revealing considerable differences in excited-state dynamics. Excellent photostability was observed in xylindein solutions as compared to those of benchmark organic semiconductor molecules and other hydrogen-bonded pigments. Electron mobility of up to 0.4 cm^2/Vs was obtained in amorphous xylindein films. Xylindein blends with PMMA that feature an improved film morphology and processability, at optimized relative concentrations exhibited (opto)electronic performance comparable to that of pristine xylindein films.

Comparison between optical and (opto)electronic properties of xylindein and those of its methylated derivative, dimethylxylindein has been performed using comprehensive contributions from wood sciences, organic synthesis, ultrafast spectroscopy, quantum chemistry calculations, and device physics. The hydroxyl groups in xylindein, which are not present in dimethylxylindein, were found to play a critical role in optical absorption and photoluminescence properties, excited state dynamics, photostability, and (photo)conductivity. Enhanced photostability of xylindein is attributed to fast deactivation of its excited state; this fast deactivation channel is not as efficient in dimethylxylindein which exhibits a long-lived dark (triplet) state formation instead, which considerably reduces its photostability. At room temperature, amorphous xylindein films were found to be over four orders of magnitude more conductive than both amorphous and crystalline dimethylxylindein films, in part due to differences in charge trap characteristics. The observed large difference in electronic properties of xylindein and dimethylxylindein is partly attributed to the effects of H-bonding in xylindein which promotes morphology supportive of efficient conductive network in xylindein films, in addition to effects of π-π stacking. In contrast to lower electron mobility in dimethylxylindein, the photosensitivity of dimethylxylindein films is considerably higher than that of xylindein films, attributed to higher charge photogeneration efficiency in dimethylxylindein enabled by longer-lived excited states in dimethylxylindein.

Xylindein has proven to have many favorable properties, such as a high stability, relatively high charge carrier mobility in amorphous films, and non-toxicity, for device applications. However, challenges remain to fully utilize these properties. For example, fast excited state deactivation and rough film morphology of xylindein films were found to be detrimental for its use in organic solar cell; the latter is also an obstacle for its applications in organic thin-film transistors. Therefore, better control

of morphology and derivatization to produce xylindein derivatives with more favorable excited state dynamics for applications involving photoinduced charge generation is necessary to address these challenges. The former would require an approach that would counteract poor solubility of xylindein in organic solvents which is detrimental for solution deposition of high-quality films in organic electronic devices. The latter introduces a trade-off as the particular excited state dynamics of xylindein is behind its exceptional photostability, and so any required manipulation of the molecular structure and associated excited states photophysics would most likely decrease the photostability. These issues, however, are not an impediment for certain electrochemical applications (water gated transistors, electrochemical transistors, and batteries), where, for example, the porous morphology of xyindein films could be a feature. Results showing xylindein redox activity in electrochemical device configurations show promise for this type of devices. There are many other potential device applications that are yet to be explored. Similar molecules have been explored for use in fuel cell photocatalysis (Sridharan et al. 2020) and as molecular switches (Früchtl and Van Mourik 2021). Potential bioelectronic applications include biosensors, analytical/diagnostic devices, and neural interfaces (Malliaras 2013). Finally, xylindein is only one example of fungi-derived pigments with properties of interest for sustainable electronics, energy storage, and photonics. Further research is needed to fully understand and harness desirable properties of these fascinating nature-derived materials.

Acknowledgments We thank Profs. C. Fang and C. Beaudry, Drs. J. D. B. Van Schenck and S. Vega Gutierrez, and T. Krueger for their contributions to understanding various aspects of xylindein extraction, characterization, and properties. The authors acknowledge National Science Foundation (CBET-1705099) for financial support.

References

Al-Aqar R, Benniston AC, Harriman A, Perks T (2017) Structural dynamics and barrier crossing observed for a fluorescent o-doped polycyclic aromatic hydrocarbon. Chem Photo Chem 1:198–205

Almurshidi B, Van Court RC, Vega Gutierrez SM, Harper B, Harper S, Robinson SC (2021) Preliminary examination of the toxicity of spalting fungal pigments: a comparison between extraction methods. J, Fungi 7(2):155

Blackburn G, Neilson A, Todd A (1962) Structure of Xylindein. Proc Chem Soc:327–328

Cramer T, Campana A, Leonardi F, Casalini S, Kyndiah A, Murgia M, Biscarini F (2013) Water-gated organic field effect transistors-opportunities for biochemical sensing and extracellular signal transduction. J Mater Chem B 31:3728–3741

Day J, Platt A, Subramanian S, Anthony J, Ostroverkhova O (2009) Influence of organic semiconductor-metal interfaces on the photoresponse of functionalized anthradithiophene thin films. J Appl Phys 105:103703

Deng Y, Liu J, Wang J, Liu L, Li W, Tian H, Zhang X, Xie Z, Geng Y, Wang F (2014) Dithienocarbazole and Isoindigo based amorphous low bandgap conjugated polymers for efficient polymer solar cells. Adv Mater 26(3):471–476

Dong H, Liang Y, Tutusaus O, Mohtadi R, Zhang Y, Hao F, Ya Y (2019) Directing mg-storage chemistry in organic polymers toward high-energy mg batteries. Joule 3:782–793. https://doi.org/10.1016/j.joule.2018.11.022

Donner CD, Cuzzupe AN, Falzon CL, Gill M (2012) Investigations towards the synthesis of Xylindein, a blue-green pigment from the fungus *Chlorociboria aeruginosa*. Tetrahedron 68(13):2799–2805

Edwards RL, Kale N (1965) The structure of Xylindein. Tetrahedron 21(8):2095–2107

Eftekhari A (2004) Potassium secondary cell based on Prussian blue cathode. J Power Sources 126: 221–228. https://doi.org/10.1016/j.jpowsour.2003.08.007

Ehrenfreund E, Moses D, Heeger AJ (1992) Doped Beta-carotene films: Spinless charge storage stabilized by structural relaxation. Chem Phys Lett 196:84–90

Fischer M, Hua X, Wilts B, Gunkel I, Bennett T, Steiner U (2017) Mesoporous Titania microspheres with highly tunable pores as an anode material for lithium ion batteries. ACS Appl Mater Interfaces 9(27):22388–22397

Früchtl H, Van Mourik T (2021) A Quinone based single-molecule switch as building block for molecular electronics. Phys Chem Chem Phys 23(3):1811–1814. https://doi.org/10.1039/d0cp06250b

Giesbers G (2021) (Opto)electronic properties of Xylindein and organic (Opto)electronic devices. Ph. D. Thesis, Oregon State University

Giesbers G, Krueger T, Van Schenck J, Kim R, Van Court RC, Robinson SC, Beaudry C, Fang C, Ostroverkhova O (2021) Role of hydroxyl groups in the photophysics, photostability, and (opto)electronic properties of the fungi-derived pigment xylindein. J Phys Chem C 125:6534–6545

Giesbers G, Krueger T, Van Schenck J, Van Court R, Morré J, Fang C, Robinson SC, Ostroverkhova O (2019a) Fungi-derived xylindein: effect of purity on optical and electronic properties. MRS. Advances 4(31–32):1796–1777. https://doi.org/10.1557/adv.2019.269

Giesbers G, Van Schenck J, Van Court R, Vega Gutierrez SM, Robinson SC, Ostroverkhova O (2019b) Xylindein: naturally produced fungal compound for sustainable (Opto)electronics. ACS Omega 4(8):13309–13318. https://doi.org/10.1021/acsomega.9b01490

Giesbers G, Van Schenck J, Vega Gutierrez SM, Robinson SC, Ostroverkhova O (2018) Fungi-derived pigments for sustainable organic (opto)electronics. MRS Adv 3:3459–3464. https://doi.org/10.1557/adv.2018.446

Giovannitti A, Maria IP, Hanifi D, Donahue MJ, Bryant D, Barth KJ, Makdah BE, Savva A, Moia D, Zetek M, Barnes PRF, Reid OG, Inal S, Rumbles G, Malliaras GG, Nelson J, Rivnay J, McCulloch I (2018) The role of the side chain on the performance of N-type conjugated polymers in aqueous electrolytes. Chem Mater 30(9):2945–2953

Głowacki E, Voss G, Sariciftci N (2013b) 25th anniversary article: Progress in chemistry and applications of functional indigos for organic electronics. Adv Mater 25:6783–6800

Głowacki ED, Coskun H, Blood-Forsythe MA, Monkowius U, Leonat L, Grzybowski M, Gryko D, White MS, Aspuru-Guzik A, Sariciftci NS (2014) Hydrogen-bonded Diketopyrrolopyrrole (DPP) pigments as organic semiconductors. Org Electron 15(12):3521–3528

Głowacki ED, Irimia-Vladu M, Bauer S, Sariciftci NS (2013a) Hydrogen-bonds in molecular solids – from biological systems to organic electronics. J Mater Chem B1(31):3742

Głowacki ED, Leonat L, Irimia-Vladu M, Schwödiauer R, Ullah M, Sitter H, Bauer S, Sariciftci SN (2012) Intermolecular hydrogen-bonded organic semiconductors-quinacridone versus pentacene. Appl Phys Lett 101(2):023305

Głowacki ED, Romanazzi G, Yumusak C, Coskun H, Monkowius U, Voss G, Burian M, Lechner RT, Demitri N, Redhammer GJ, Sünger N (2015) Epindolidiones-versatile and stable hydrogen-bonded pigments for organic field-effect transistors and light-emitting diodes. Adv Funct Mater 25:776–787

Gospodinova N, Tomšík E (2015) Hydrogen-bonding versus π–π stacking in the Design of Organic Semiconductors: from dyes to oligomers. Prog Polym Sci 43:33–47

Han C, Li H, Shi R, Zhang T, Tong J, Li J, Li B (2019) Organic Quinones towards advanced electrochemical energy storage: recent advances and challenges. J Mater Chem A 7:23378–23415. https://doi.org/10.1039/c9ta05252f

Harrison R, Quinn A, Weber G, Johnson B, Rath J, Remcho V, Robinson SC, Ostroverkhova O (2017) Fungi-derived pigments as sustainable organic (opto)electronic materials. Proc SPIE 10101:101010U

He Y, Cao Y, Hwang HJ, Debarajb H, Vega Gutierrez SM, Chen HL, Robinson SC, Malhotra R, Chang CH (2021) Inkjet printing and in-situ crystallization of biopigments for eco-friendly and energy-efficient fabric coloration. In: International Journal of Precision Engineering and Manufacturing-Green Technology, pp 1–13

Hinsch E, Weber G, Chen H-L, Robinson SC (2015) Colorfastness of extracted wood-staining fungal pigments on fabrics –a new potential for textile dyes. J Text Appar Technol Manag 9(3): 1–11

Irimia-Vladu M (2014) Green electronics: biodegradable and biocompatible materials and devices for sustainable future. Chem Soc Rev 43(2):588–610

Irimia-Vladu M, Sariciftci NS, Bauer S (2011) Exotic materials for bio-organic electronics. J Mater Chem 21(5):1350–1361

Ito S, Saitou T, Imahori H, Uehara H, Hasegawa N (2010) Fabrication of dye-sensitized solar cells using natural dye for food pigment: Monascus yellow. Energy Environ Sci 3(7):905

Jian Z, Liang Y, Rodríguez-Pérez I, Yao Y, Ji X (2016) Poly(anthraquinonyl sulfide) cathode for potassium-ion batteries. Electrochem Commun 71:5–8. https://doi.org/10.1016/j.elecom.2016.07.011

Kamat PV, Chauvet JP, Fessenden RW (1986) Semiconductor with a chlorophyll analogue. J Phys Chem 90:1389–1394

Kim YJ, Khetan A, Wu W, Chun SE, Viswanathan V, Whitacre JF, Bettinger CJ (2016) Evidence of porphyrin-like structures in natural melanin pigments using electrochemical fingerprinting. Adv Mater 28(16):3173–3180. https://doi.org/10.1002/adma.201504650

Kobayashi N, Sasaki M, Nomoto K (2009) Stable peri-xanthenoxanthene thin-film transistors with efficient carrier injection. Chem Mater 21:552–556

Krueger T, Giesbers G, Van Court RC, Zhu L, Kim R, Beaudry C, Robinson SC, Ostroverkhova O, Fang C (2021a) Ultrafast dynamics and photoresponse of a fungi-derived pigment xylindein from solution to thin films. Chem Eur J 27:5627–5631. https://doi.org/10.1002/chem.202005155

Krueger T, Tang L, Giesbers G, Van Court RC, Zhu L, Robinson SC, Ostroverkhova O, Fang C (2021b) Ultrafast triplet state formation in a methylated fungi-derived pigment: towards rational molecular design for sustainable optoelectronics. J Phys Chem C 125(31):17565–17572. https://doi.org/10.1021/acs.jpcc.1c06260

Luo W, Allen M, Raju V, Ji X (2014) An organic pigment as a high-performance cathode for sodium-ion batteries. Adv Energy Mater 4(15):1400554

Malliaras GG (2013) Organic bioelectronics: a new era for organic electronics. Biochim Biophys Acta 1830:4286–4287. https://doi.org/10.1016/j.bbagen.2012.10.007

Nguy TP, Hayakawa R, Kilinc V, Petit M, Raimundo JM, Charrier A, Wakayama Y (2019) Stable operation of water-gated organic field-effect transistor depending on channel flatness, electrode metals and surface treatment. Jpn J Appl Phys 58:SDDH02. https://doi.org/10.7567/1347-4065/ab09d2

Ostroverkhova O (2016) Organic optoelectronic materials: mechanisms and applications. Chem Rev 116:13279–13412

Ostroverkhova O (ed) (2018) Handbook of organic materials for electronic and photonic devices (Woodhead publishing series in electronic and optical materials), 2nd edn. Woodhead Publishing

Porrazzo R, Luzio A, Bellani S, Bonacchini GE, Noh YY, Kim YH, Lanzani G, Antognazza MR, Caironi M (2017) Water-gated n-type organic field-effect transistors for complementary integrated circuits operating in an aqueous environment. ACS Omega 2(1):1–10

Richter DL, Glaeser JA (2015) Wood decay by *Chlorociboria aeruginascens* (Nyl.) Kanouse (Helotiales, Leotiaceae) and associated basidiomycete fungi. Int Biodeterior Biodegradation 105:239–244

Rivnay J, Inal S, Salleo A, Owens RM, Berggren M, Malliaras GG (2018) Organic electrochemical transistors. Nat Rev Mater 3:17086

Robinson SC, Vega Gutierrez SM, Cespedes Garcia RA, Iroume N, Vorland NR, Andersen C, de Oliveira Xaxa ID, Kramer OE, Huber ME (2018) Potential for fungal dyes as colorants in oil and acrylic paints. J Coat Technol Res 15(4):845–849

Robinson SC, Vega Gutierrez SM, Cespedes RA, Iroume N, Vorland NR, McClelland A, Huber M, Stanton S (2017) Potential for carrying pigments derived from spalting fungi in natural oils. J Coat Technol Res 14(5):1107–1113

Saikawa Y, Watanabe T, Hashimoto K, Nakata M (2000) Absolute configuration and tautomeric structure of xylindein, a blue–green pigment of *Chlorociboria* species. Phytochemistry 55:237–240

Shepherd W, Grollman R, Robertson A, Paudel K, Hallani R, Loth M, Anthony J, Ostroverkhova O (2015) Single-Molecule Imaging of Organic Semiconductors: toward Nanoscale Insights into Photophysics and Molecular Packing. Chem. Phys. Lett. 629:29–35

Shirakawa H, Louis E, MacDiarmid A, Chiang C, Heeger AJ (1977) Synthesis of Electrically Conducting Organic Polymers: Halogen Derivatives of Polyacetylene, (CH)x. Chem Comm 16: 578–580

Sridharan M, Kamaraj P, Vennila R, Huh YS, Arthanareeswari M (2020) Bio-inspired construction of melanin-like polydopamine-coated CeO_2as a high-performance visible-light-driven photocatalyst for hydrogen production. New J Chem 44(35):15223–15234

Stange S, Steudler S, Delenk H, Werner A, Walther T, Wagenfuhr A (2019) Influence of the nutrients on the biomass and pigment production of *Chlorociboria Aeruginascens*. J Fungi 5(2): 40

Sun H, Gerasimov J, Berggren M, Fabiano S (2018) N-Type organic electrochemical transistors: materials and challenges. J Mater Chem C 6:11778–11784

Surgailis J, Savva A, Druet V, Paulsen B, Wu R, Hamidi-Sakr A, Ohayon D, Nikiforidis G, Chen X, McCulloch I, Rivnay J, Inal S (2021) Mixed conduction in an N-type organic semiconductor in the absence of hydrophilic side-chains. Adv Funct Mater 31:2010165

Sytnyk M, Glowacki ED, Yakunin S, Voss G, Scho W, Kriegner D, Stangl J, Trotta R, Gollner C, Tollabimazraehno S et al (2014) Hydrogen-bonded organic semiconductor micro- and nanocrystals: from colloidal syntheses to (Opto-)electronic devices. J Am Chem Soc 136(47): 16522–16533

Tong L, Jing Y, Gordon RG, Aziz MJ (2019) Symmetric all-quinone aqueous battery. ACS Appl Energy Mater 2(6):4016–4021

Vahidzadeh E, Kalra AP, Shankar K (2018) Melanin-based electronics: from proton conductors to photovoltaics and beyond. Biosens Bioelectron 122:127–139

Van Court R, Giesbers G, Ostroverkhova O, Robinson SC (2020a) Optimizing xylindein from *Chlorociboria* spp. for (opto)electronic applications. *Processes* 8:1477. https://doi.org/10.3390/pr8111477

Van Court R, Vega Gutierrez P, Robinson SC (2020b) Exploration of Pigment Production by Spalting Fungi *Scytalidium ganodermophthorum* and potential for industry. In: IRG51 Scientific Conference on Wood Protection. International Research Group on Wood Protection. IRG Secretariat. IRG/WP 20-10957

Vega Gutierrez S, Robinson SC (2017) Microscopic analysis of pigments extracted from spalting fungi. J Fungi 3:15

Vega Gutierrez SM, Hazell KK, Simonsen J, Robinson SC (2018) Description of a novel naphthoquinonic crystal produced by the fungus *Scytalidium cuboideum*. Molecules 23(8):1905

Vega Gutierrez P, Almurshidi B, Huber M, Andersen C, Van Court RC, Robinson SC (2020) Expanding the spalting palette: developing yellow, purple, and green pigments from

Scytalidium ganodermophthorum for artistic applications. Int Wood Prod J. https://doi.org/10.1080/20426445.2020.1780543

Vega Gutierrez SM, Stone DW, He R, Vega Gutierrez PT, Walsh ZM, Robinson SC (2021) Red pigment from the fungus *Scytalidium cuboideum*helps prevent 'greying' in decking and other outdoor wood products. Coatings 11:511

Weber G, Chen HL, Hinsch E, Freitas S, Robinson SC (2014) Pigments extracted from the wood-staining fungi *Chlorociboria aeruginosa, Scytalidium cuboideum*, and *S. Ganodermophthorum*show potential for use as textile dyes. Color Technol 130(6):445–452

Weber G, Boonloed A, Naas K, Koesdjojo M, Remcho V, Robinson S (2016) A method to stimulate production of extracellular pigments from wood-degrading fungi using a water carrier. Curr Res Environ Appl Mycol 6(3):218–230

Wiesner C (2020) Optoelectronic properties of a novel fungi-derived pigment. B. Sc. thesis, Oregon State University

Yaman B, Terkesli I, Turksoy KM, Sanyal A, Mutlu S (2014) Fabrication of a planar water gated organic field effect transistor using a hydrophilic polythiophene for improved digital inverter performance. Org Electron 15(3):646–653. https://doi.org/10.1016/j.orgel.2013.12.024

Yamazaki S, Sobolewski A, Domcke W (2011) Molecular mechanisms of the photostability of indigo. Phys Chem Chem Phys 13:1618–1628

Yu H, Huang X, Huang C (2019) PEIE doped ZnO as a tunable cathode interlayer for efficient polymer solar cells. Appl Surf Sci 470:318–330

Zalas M, Gierczyk B, Bogacki H, Schroeder G (2015) The Cortinarius Fungi Dyes as Sensitizers in Dye-Sensitized Solar Cells. Int J Photoenerg 2015:653740