Environmental and Microbial Biotechnology

Shachi Shah
V. Venkatramanan
Ram Prasad *Editors*

Bio-valorization of Waste

Trends and Perspectives

Springer

Environmental and Microbial Biotechnology

Series Editor

Ram Prasad, School of Life Sciences, Mahatma Gandhi Central University, Motihari, Bihar, India

Innovative and novel advances in microbial biotechnology are providing great understandings in to the machineries of nature, presenting fascinating prospects to apply principles of biology to different arenas of science. Sustainable elucidations are emerging to address the concerns on improving crop productivity through microbes, depleting natural resources, environmental pollution, microbial degradation of pollutants, nanomaterials, nanotoxicity & safety issues, safety of food & agricultural products etc. Simultaneously, there is an increasing demand for natural bio-products of therapeutic and industrial significance (in the areas of healthcare, environmental remediation, microbial biotechnology). Growing awareness and an increased attention on environmental issues such as climate change, energy use, and loss of non-renewable resources have carried out a superior quality for research that provides potential solutions to these problems. Emerging microbiome approaches potentially can significantly increase agriculture productivity & human healthcare and henceforth can contribute to meet several sustainable development goals.

The main objectives have provided an impetus for research on plants and microorganisms that produce novel bio-products with variable properties and understanding their mechanisms of action at cellular and molecular level. Hence, research activities of the environmental and microbial Biotechnology are comprehensively focused up on major sectors viz., bioresources, biorefining, bioremediation of organic and inorganic pollutants, environmental risk analysis of microorganisms, environmental assessment using microbiological indicators, enzymes for environment, food & industrial applications, nanomaterials & nanotoxicity, sustainable ecobiotechnology, biofertilizer, biocontrol agents for agriculture improvement and natural products for healthcare applications.

This book series is a state-of-the-art for a wide range of scientists, researchers, students, policy makers and academician involve in understanding and implementing the knowledge on environmental and microbial biotechnology to develop biologics for proper health care to continue life in smooth and sustainable strategy without any adverse effect.

More information about this series at http://www.springer.com/series/16324

Shachi Shah • V. Venkatramanan •
Ram Prasad
Editors

Bio-valorization of Waste

Trends and Perspectives

Springer

Editors
Shachi Shah
Indira Gandhi National Open University
School of Interdisciplinary and Transdisciplinary Studies
New Delhi, Delhi, India

V. Venkatramanan
Indira Gandhi National Open University
School of Interdisciplinary and Transdisciplinary Studies
New Delhi, Delhi, India

Ram Prasad
Department of Botany
School of Life Sciences
Mahatma Gandhi Central University
Motihari, Bihar, India

ISSN 2662-1681 ISSN 2662-169X (electronic)
Environmental and Microbial Biotechnology
ISBN 978-981-15-9695-7 ISBN 978-981-15-9696-4 (eBook)
https://doi.org/10.1007/978-981-15-9696-4

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

Preface

Human population growth and waste generation go hand in hand. Population growth and concomitant growth in industry, urbanization, and manufacturing sector have resulted in waste generation on a mammoth scale. Nevertheless, waste is no more considered as valueless material but as a resource or as a raw material for value addition or as an opportunity to tap the huge potential that is embedded in the waste material. Waste valorization as a process endeavours to reduce, reuse, and recycle the waste into usable, value added, and environmental benign raw materials which can be a source of energy. Waste valorization imbibes the natural recycling principles of zero waste and loop closing and further underlines the importance of the treatment of waste and value addition. Indeed, waste valorization is construed as the last step in waste disposal hierarchy. Pertinent issues about waste valorization are the constant need for innovative and disruptive technologies and an urge to adapt and evolve the waste valorization activities that are amenable to valorize various wastes. Looking at the present need and future scenario, the book attempts to highlight the broad contours of waste valorization principles and waste valorization technologies for a diverse group of wastes including agricultural, municipal, and industrial waste. Further, the book reflects the emerging paradigms of waste valorization, waste biorefineries, and valorization technologies for energy, biofuel, and biochemical production. The book meets the growing need for a comprehensive and holistic outlook on waste valorization, imperatives and perspectives, underlying principles, and emerging technologies which are sustainable and environmentally friendly. The chapters focus on the valorization of wastes particularly agro-industrial waste, lignocellulosic waste, food waste, coal waste, mining waste, and tailings. Further, the book delves into the biovalorization of waste into valuable products like bioethanol, value added chemicals, polyhydroxyalkanoates, and bacterial cellulose. We are extremely honoured to receive chapters from leading scientists and professors with rich experience and expertise in the field of biovalorization, environmental microbiology and biotechnology, chemical and process engineering. The book targets scientists, researchers, academicians, graduates, and doctoral students working on biological sciences and waste management.

Our sincere gratitude goes to the contributors for their insights into biovalorization of wastes. We sincerely thank Dr. Naren Aggarwal, Editorial Director, Springer; Ms. Aakanksha Tyagi, Associate Editor; Mr. Ashok Kumar, and

Ms. Beauty Christobel, Production Editor for their generous assistance, constant support, and patience in finalizing this book.

New Delhi, Delhi, India — Shachi Shah
New Delhi, Delhi, India — V. Venkatramanan
Motihari, Bihar, India — Ram Prasad

Contents

Editors and Contributors

About the Editors

Shachi Shah, Ph.D., is an environmentalist with nearly two decades of teaching and research experience at various reputed universities and institutes. She is an Associate Professor (Environmental Studies) at the School of Interdisciplinary and Transdisciplinary Studies, IGNOU, New Delhi. Her research interests include green technologies for waste management and energy generation, bioremediation, waste valorization, plant growth promoting organisms, and biodiversity conservation. Moreover, she has authored more than 50 publications.

V. Venkatramanan, Ph.D., is an assistant professor at the School of Interdisciplinary and Transdisciplinary Studies, Indira Gandhi National Open University, New Delhi. His interests include climate smart agriculture, climate policy, biodegradation and green technologies for environmental management. He has published more than 20 research papers in peer-reviewed journals and book chapters.

Ram Prasad, Ph.D., is associated with the Department of Botany, Mahatma Gandhi Central University, Motihari, Bihar, India. His research interest includes applied and environmental microbiology, plant–microbe interactions, sustainable agriculture, and nanobiotechnology. Dr. Prasad has more than 175 publications to his credit, including research papers, review articles, and book chapters; five patents issued or pending; and edited or authored several books. Dr. Prasad has 12 years of teaching experience and has been awarded the Young Scientist Award and Prof. J.S. Datta Munshi Gold Medal by the International Society for Ecological Communications; FSAB fellowship by the Society for Applied Biotechnology; the American Cancer Society UICC International Fellowship for Beginning Investigators, USA; Outstanding Scientist Award in the field of Microbiology by Venus International Foundation; BRICPL Science Investigator Award; Research Excellence Award, etc. He has been serving as an editorial board member of *Frontiers in Microbiology*, *Frontiers in Nutrition*, *Archives of Phytopathology and Plant Protection*, *Phyton-International Journal of Experimental Botany*, *Academia Journal of Biotechnology*, *Journal of Renewable Materials*, and *Biocell* and Series editor of Nanotechnology in the Life Sciences, Springer Nature, USA. Previously, Dr. Prasad served as assistant professor at Amity University, Uttar Pradesh, India; visiting assistant professor, Whiting School of Engineering, Department of Mechanical Engineering at Johns Hopkins University, Baltimore, United States; and research associate professor at School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou, China.

Contributors

Siti Mahira Ahmad Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, Gambang, Pahang, Malaysia

Nicoletta Cascelli Department of Chemical Sciences, University Federico II, Naples, Italy

J. A. Cecilia Departamento de Química Inorgánica, Cristalografía y Mineralogía (Unidad Asociada al ICP-CSIC), Facultad de Ciencias, Universidad de Málaga, Málaga, Spain

G. K. Chua Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, Gambang, Kuantan, Malaysia

Iolanda Corrado Department of Chemical Sciences, University Federico II, Naples, Italy

Fabíola Fernandes Costa Institute of Biological Sciences, Federal University of Pará, Campus Universitário do Guamá, Belém, Pará, Brazil

Geraldo Narciso da Rocha Filho Institute of Biological Sciences, Federal University of Pará, Campus Universitário do Guamá, Belém, Pará, Brazil

Nur Fathin Shamirah Daud Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, Gambang, Pahang, Malaysia

Cristiano José de Andrade Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, SC, Brazil

Débora de Oliveira Department of Chemical Engineering and Food Engineering (EQA), Federal University of Santa Catarina (UFSC), Florianópolis, SC, Brazil

Deborah Terra de Oliveira Institute of Biological Sciences, Federal University of Pará, Campus Universitário do Guamá, Belém, Pará, Brazil

Luís Adriano Santos do Nascimento Institute of Biological Sciences, Federal University of Pará, Campus Universitário do Guamá, Belém, Pará, Brazil

Yrvana Pereira dos Santos Brito Institute of Biological Sciences, Federal University of Pará, Campus Universitário do Guamá, Belém, Pará, Brazil

Alcilene Rodrigues Monteiro Fritz Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, SC, Brazil

C. García-Sancho Departamento de Química Inorgánica, Cristalografía y Mineralogía (Unidad Asociada al ICP-CSIC), Facultad de Ciencias, Universidad de Málaga, Málaga, Spain

Asmita Gupta Daulat Ram College, University of Delhi, New Delhi, Delhi, India

Tomy J. Gutiérrez Grupo de Materiales Compuestos Termoplásticos (CoMP), Facultad de Ingeniería, Instituto de Investigaciones en Ciencia y Tecnología de Materiales (INTEMA), Universidad Nacional de Mar del Plata (UNMdP) y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Colón, Mar del Plata, Argentina

Nor Farhana Hamid Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, Gambang, Pahang, Malaysia

Igor Hernández Facultad de Farmacia, Universidad del País Vasco/Euskal Herriko Unibertsitatea, Vitoria-Gasteiz, Spain

Dachamir Hotza Department of Chemical Engineering and Food Engineering (EQA), Federal University of Santa Catarina (UFSC), Florianópolis, SC, Brazil

Jone Ibarruri Food Research Division, AZTI, Parque Tecnológico de Bizkaia, Derio, Bizkaia, Spain

Jaciane Lutz Ienczak Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, SC, Brazil

C. P. Jiménez-Gómez Departamento de Química Inorgánica, Cristalografía y Mineralogía (Unidad Asociada al ICP-CSIC), Facultad de Ciencias, Universidad de Málaga, Málaga, Spain

Amir Khan Department of Microbiology, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Madan Kumar Centre for Rural Development, Indian Institute of Technology, Delhi, New Delhi, Delhi, India

Érika Tallyta Leite Lima Institute of Biological Sciences, Federal University of Pará, Campus Universitário do Guamá, Belém, Pará, Brazil

Danielle Maass Institute of Science and Technology (ICT), Federal University of São Paulo (UNIFESP), São José dos Campos, SP, Brazil

N. I. F. Mahadi Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, Gambang, Kuantan, Malaysia

P. Maireles-Torres Departamento de Química Inorgánica, Cristalografía y Mineralogía (Unidad Asociada al ICP-CSIC), Facultad de Ciencias, Universidad de Málaga, Málaga, Spain

Tripti Malik Department of Microbiology, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun, Uttarakhand, India

Cinzia Pezzella Department of Agricultural Sciences, University Federico II, Naples, Italy

Patrícia Poletto Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, SC, Brazil

Ram Prasad Department of Botany, School of Life Sciences, Mahatma Gandhi Central University, Motihari, Bihar, India

Shiv Prasad Centre for Environment Science and Climate Resilient Agriculture, Indian Agricultural Research Institute, New Delhi, Delhi, India

Sarita Cândida Rabelo Department of Bioprocess and Biotechnology, College of Agricultural Sciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil

Dheeraj Rathore School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar, Gujarat, India

Seema Rawat Microbiology Lab, School of Life Sciences, Central University of Gujarat, Gandhinagar, Gujarat, India

Mohamad Al-Aamin Razali Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, Gambang, Pahang, Malaysia

Diogo Robl Department of Microbiology, Immunology and Parasitology, Federal University of Santa Catarina, Florianópolis, SC, Brazil

Farhan M. Said Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, Gambang, Pahang, Malaysia

Giovanni Sannia Department of Chemical Sciences, University Federico II, Naples, Italy

Mrinalini Shah Institute of Management Technology, Ghaziabad, Uttar Pradesh, India

Shachi Shah School of Interdisciplinary and Transdisciplinary Studies, Indira Gandhi National Open University, New Delhi, Delhi, India

Ajay Veer Singh Department of Microbiology, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Anoop Singh Department of Scientific and Industrial Research (DSIR), Ministry of Science and Technology, Government of India, Technology Bhawan, New Delhi, Delhi, India

Jyoti Singh Department of Microbiology, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Shaili Srivastava Amity School of Earth and Environmental Sciences, Amity University Haryana, Gurgaon, Haryana, India

F. H. Y. Tan Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, Gambang, Kuantan, Malaysia

Viabhav Kumar Upadhayay Department of Microbiology, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Germán Ayala Valencia Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, SC, Brazil

Alexsandra Valério Department of Chemical Engineering and Food Engineering (EQA), Federal University of Santa Catarina (UFSC), Florianópolis, SC, Brazil

Marco Vastano Department of Chemical Sciences, University Federico II, Naples, Italy

V. Venkatramanan School of Interdisciplinary and Transdisciplinary Studies, Indira Gandhi National Open University, New Delhi, Delhi, India

Microbial Valorization of Coir Pith for Development of Compost and Bioethanol Production

1

Tripti Malik and Seema Rawat

Abstract

Coco pith, an agricultural by-product of coir industry, is a dust left after the extraction of coir fibers from coconut husk. It is accumulated outside the coir industries as huge heaps, which usually becomes an environmental hazard. It is degraded very slowly due to its high lignin and cellulose content. The tannins and phenols are leached out from coir pith heaps by rains which enter the soil and aquatic ecosystems leading to loss of soil fertility and have adverse effect on soil and aquatic biodiversity. Thus, the safe disposal of coir pith is the need of hour which can be achieved by its conversion into value-added products like compost and bioethanol. Due to its rich nutrient content, it has a good prospective as compost. The microbial valorization of coir pith has been proved not only to enrich nutrients in the agricultural soil, but also to increase the pathogen resistance and will surely resolve the environmental pollution problems. The high cellulose and hemicellulose content of coco pith make it a suitable candidate for conversion into bioethanol. This chapter will outline the future prospects in the processing and conversion of coir pith into commercially viable compost and bioethanol.

Keywords

Agricultural waste · Coco pith · Biofertilizer · Bioethanol · *Saccharomyces*

T. Malik
Department of Microbiology, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun, Uttarakhand, India

S. Rawat (✉)
Microbiology Lab, School of Life Sciences, Central University of Gujarat, Gandhinagar, Gujarat, India
e-mail: seema.rawat@cug.ac.in

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_1

1.1 Introduction

Cocos nucifera L. is an important fruit tree in the world, providing food to the population throughout the world, especially in the tropical and subtropical regions (DebMandal and Mandal 2011). The people in the traditional coconut growing areas use all parts of the tree in different ways in their daily life. The various products which are derived from coconut are: Copra, tender coconut water, raw kernel, coconut oil, coconut cake, coconut toddy, coconut shell and wood-based products, coconut leaves, coir pith, etc. (DebMandal and Mandal 2011; Sangamithra et al. 2013). While the endosperm has edible uses, its leafy and hard parts are used in thatching and furniture purposes. Its oil is used in industries as an ingredient for the manufacture of soaps and detergents (Kempton 2006). Coconut oil can be used as a renewable and alternative to bio-diesel (Hossain et al. 2012). It is, therefore, often called as "tree of life" (Sudha et al. 2019) or "Kalpavriksha" in Indian classics (DebMandal and Mandal 2011). The coconut palm is distributed in tropical regions throughout the world, in islands and along coastal areas, such as Fiji, and Samoa, as well as in the humid tropics, such as India, the Philippines, Thailand, and Indonesia. Food and Agriculture Organization Corporate Statistical Database estimated that the total world production of coconuts in 2017 was 60,773,435 metric tons which was 3.0% higher than that in 2016 (FAOSTAT 2018). In India alone, the area under coconut plantation is about 1078 million hectares, producing 12,252 million nuts with a productivity of 6982 nuts per hectare (Theradimani et al. 2018).

The outer non-edible fibrous portion of the nuts (coconut husk) is used for extracting coconut fiber or coir, which is commercially utilized for making value-added products such as mats, geotextiles, biofuel pellets, etc. (Wang and Gu 2009; Stelte et al. 2018). In the husk, coconut fibers are tightly packed along with non-fibrous, fluffy and lightweight croaky material known as coir pith or coir dust or coco peat. It constitutes about 50–70% of the husk. A huge amount of coir pith is generated as a by-product of coir industry. 1.6 ton of coir pith is obtained as a by-product after the extraction of coir from the husk of 10,000 coconuts (Ravindranath and Radhakrishnan 2016). About one ton of each coir fiber and coir pith are generated from one hectare of land under cultivation of coconut, which annually produces approximately 10,600 nuts per hectare (Theradimani et al. 2018). It is estimated that, if all the coconut husks available in India are processed, about 2.25 million tons of coir pith would be obtained annually (Ravindranath and Radhakrishnan 2016). Coir pith is widely available, though it has a number of applications, yet it is not always fully utilized.

The coir pith is either disposed of by burning or is dumped in huge hillocks on roadside and along the vicinity of the industries (Paramanandham and Ross 2016). The burning of coir pith results in various environmental problems such as deposition of carbon deposits as well as results in the warming of the atmosphere (Seal et al. 2015; Ghosh et al. 2007). The accumulation of coir pith in the vicinity of coir processing units causes fire hazards and groundwater contamination due to the release of phenolics compounds (Ravindranath and Radhakrishnan 2016). The tannins and phenols of coir pith gets leached into the agricultural soils and irrigation

canals. It is recalcitrant to degradation, has a high salt content, clogs the drains and canals and gets accumulated in the soil, making the agricultural lands unproductive. It can also enter the food chain from soil and water thereby undergoes biomagnification which has serious impacts on wildlife and human beings (Nandhini et al. 2016). Hence, it is imperative to address the issue of safe disposal of coir waste and the best approach could be recycling of coir pith into valuable products.

1.2 Chemical Composition of Coir Pith

The chemical composition of coir pith is similar to the wood fibers. It has three complex polysaccharides: cellulose, hemicellulose, and lignin. Lignin content is quite high (30–31%), while cellulose and hemicellulose account for 26.8%. The chemical composition of coco husk fibers shows that water-soluble substances (in % of dry weight) has the value of 26% in old nut, 29% in young nut, and 38.50% in very young nuts (Ravindranath and Radhakrishnan 2016). Coir pith is highly rich in carbon but has nitrogen in very less amounts. There is also wide variation in C:N ratio from 58:1 to 112:1 (Savithri and Khan 1994). Nandhini et al. (2016) determined the total dissolved solids, lignin content (acid soluble and acid insoluble), cellulose content (alpha, beta, gamma) of coir pith at 1, 7, 14, and 21 days of degradation. Raw coir pith was found to contain high content of both acid soluble and acid insoluble lignin. The content of all three forms of cellulose were found to decline as a result of degradation (Nandhini et al. 2016).

Pectins and substances which are soluble in boiling water are present in 14.25, 14.85, and 15.25%, respectively in old, young, and very young nuts. Being less hydrophilic than cellulose and hemicellulose, the absorption of water by these polysaccharides is prevented in plant cell walls and the efficient transport of water in the vascular tissues is also prevented (Ravindranath and Radhakrishnan 2016). It also contains phenolics (8–12%), has fixed carbon, low sulfur and phosphorus, fats and ash. Phosphorus and micronutrients such as calcium, copper, magnesium, boron, chlorine, copper, iron, manganese, molybdenum, and zinc are also present in trace amounts (Ravindranath and Radhakrishnan 2016). It contains a high concentration of sodium and chlorine as soil in which they grow is often bathed by the sea water (Silva et al. 2018). The composition and properties of coir pith show variation depending upon the maturity of coconut, method used for extraction of coir fibers, method used for its disposal, time period between extraction and its use, and the environmental factors. The nutrient content of coir pith also varies with the place of cultivation, method of retting, rate of decomposition, and storage method (Fernando and Amarasinghe 2017). The mechanical processing of coir husk yields higher nutrients as compared to the methods which included the retting of the fiber. The amount of lignin and cellulose also depends on the level of maturation. The fully matured nuts have higher amounts of lignin and cellulose and lesser amount of water-soluble salts as compared to the younger ones (Prabhu and Thomas 2002).

1.3 Compost

The excessive use of chemical fertilizers for increasing the fertility of soil has led to loss of soil organic matter. Chemical fertilizers also have adverse effects on the environment and serious impacts upon the health of animals and human beings. Therefore, the use of organic amendments like compost and manure, which being the source of nutrients and organic matter, is an economic and eco-friendly approach. As compared to the plant residues and manures, composts release nutrients more slowly to the soil and have longer-lasting effects. Compost is the "black gold" which acts as a soil conditioner.

Since ancient times, composting has been the simplest method of the management and stabilization of organic wastes throughout the world. There are historical references of composting by Greeks and Romans, and it is also mentioned in "The Bible." The organic wastes obtained from different sources such as agricultural waste, market waste, kitchen waste, urban solid wastes, and municipal solid wastes account for a large amount of solid wastes. The organic biomass is transformed into humic substances which formulate the humus which is biologically stable and easily absorbed by the plants (Lee et al. 2004; Adunga 2016). These organic materials can be deliberately converted to compost by the action of mesophilic and thermophilic microorganisms (Raza and Ahmad 2016). The first scientific approach to composting can be credited to Sir Albert Howard, an agricultural scientist, who devised the "Indoor method" of composting (Cooperband 2000) in which the decomposition of organic materials occurs under controlled aerobic condition at a very rapid rate as compared to outdoor composting (Zhu 2006; Paulin and O'Malley 2008; Gonawala and Jardosh 2018).The process of composting is a complex and dynamic process which is carried out by a consortium of indigenous mixed microbial communities. The composting process is initiated by the saprophytes and thereafter the microbial community succession occurs with the change in the nutrient content. The process of composting reduces the volume of waste and thus the most effective mean of management of lignocellulosic wastes (Tiquia and Tam 2000; Zhu 2006; Aziz et al. 2018). It is being carried out at commercial level also and products are sold in the market and thus can serve as a mean of revenue generation from waste (Raza and Ahmad 2016).

1.3.1 Characteristics of Raw Material for Compost Formation

The most important factor which influences the composting process and quality of compost is C/N ratio (Golueke 1991; Tripetchkul et al. 2012). The nature of raw material determines C/N ratio which can vary from 11 to 105 (Eiland et al. 2001; Ghosh et al. 2007). Lower C/N ratio of organic matter is, higher will be the degradation rates. Shafawati et al. (2014) reported that the low initial C/N ratio resulted into high microbial activity due to which 40% to 80% of both cellulose and hemicelluloses present in the raw materials were degraded to produce compost (Shafawati et al. 2014). Eiland et al. (2001) reported that the high C/N ratio resulted

into lower microbial activity due to which only 10% to 20% degradation of raw materials was achieved.

The ratio of C:N lower than 20:1 will lead to complete utilization of carbon and production of a large amount of ammonia. If C:N ratio exceeds 40:1, then the decomposition of organic compounds gets retarded due to the insufficient nitrogen (Venglovsky et al. 2005; Garg and Tothill 2009; Long et al. 2017). The optimum C:N ratio has been reported to be 30:1 at the start of composting process which should be decreased to 20:1 at the end of the process (Kavitha and Subramanian 2007; Raza and Ahmad 2016). Several studies have shown that if the raw material has low or high concentration of any of C and N, then it can be appropriately supplemented with other materials to yield better compost (Huang et al. 2004; Garg and Tothill 2009).

1.4 Coir Pith as a Component of Growing Media for Plants

Coir pith can be used as a good potting medium as it is a rich source of nutrients, retains water and provides support to the plant (Prasad and Roeber 1997). It has characteristically large number of pores with large pore space which ensures sufficient amount of air and available water for the plant growth. Lodolini et al. (2016) evaluated the suitability of three formulations of coir pith (100% coir pith, 70% coir pith with 30% coco fiber, and 40% coir pith with 60% coco fiber) for using them as alternative growing medium. No potential toxicity was shown by coir pith and pH was found to be sub-neutral. Water holding capacity was also found to be high for all the mixes of coir pith and coco fiber.

The pH of coco peat has been found to be optimum for many plants, whereas the traditional peat is highly acidic. Hence, it reduces the cost of cultivation as the cost of liming gets eliminated (Cresswell 2011). Coco peat, being lighter in weight than traditional peat is a better alternative to commercial peat. The humic substances present in it make it suitable for use in plant propagation and culture of plants, as humic substances are known to have hormone-like activity which stimulates plant growth (Prabha et al. 2013). It has also been found good for the reclamation of soils contaminated with toxic substances and heavy metals like chromium (Santiago and Santhamani 2010). Owing to its peat like properties, coir pith is now being used as a supplement or substitute for the commercial peat (Ghosh et al. 2007). It is gaining increased popularity for soil conditioning in developed countries (Sarma and Das 1993). It is now widely used for the germination of seeds, rising of the nursery plants, propagation for root cuttings and vegetative propagation methods, cultivation of glass house plants and in soil conditioning (Bavappa and de Gurusinghe 1978; Rao 1999; Mahanta 2017). Like other organic materials, coir pith can also be converted to the compost which will resolve the problem of safe disposal of coir waste.

Coir pith is rich in lignin and polyphenols and thus has high C:N ratio which makes it resistant to decomposition (Abad et al. 2002). Different composting techniques have been developed which can enhance the degradation by reducing the amount of lignin and hence C:N ratio (Alkoaik 2019; Jain et al. 2019). Some

composting techniques not only stabilize and increase decomposition but also increase the content of N, P, K, and other micronutrients. The nutritive value of coir pith, for the purpose of composting, can be increased either by adding specific nutrients or by pre-inoculation of beneficial microorganisms to it. The inoculation of beneficial microorganisms, such as *Azotobacter*, *Trichoderma* and some phosphate solubilizing microorganisms, will not only make compost an effective biofertilizer but also biopesticide (Moorthy and Rao 1997). Composted coir pith is rich in aromatic polyphenols and tannins which can also suppress phytopathogenic nematodes (Mian and Rodriguez-Kabana 1982).

The rate of composting can be enhanced by the addition of easily digestible organic wastes such as leguminous weeds, green manures, cow dung, oil cakes, and biogas slurry during compost preparation (Prabhu and Thomas 2002; Elfstrand et al. 2007). Rock phosphates and pyrites can be added to coir pith which get solubilized during the composting process, thereby enriching the compost with phosphates and prevents the loss of nitrogen (Bangar et al. 1988; Thomas et al. 2013). The organic acids and sulfur which is produced by biological or chemical oxidation during the decomposition of organic matter help in the dissolution of rock phosphates (Singh and Singh 1991; Biddappa et al. 1998). Nitrogen can be enriched by using green manures, weeds, etc. (Anand et al. 1999).

Lime ($CaCO_3$) enhances the process of decomposition of recalcitrant plant materials which are rich in lignin. The humification process is increased in plant residues by weakening of lignin structure and by increasing microbial population. The quality of humus is also improved as the ratio of humic to fulvic acids gets changed, the amount of humin gets decreased, which otherwise interferes with the decomposition process (Thomas et al. 2013). The addition of micronutrients at the beginning of the composting process fortifies the compost as the trace metals get chelated with natural ligands like humic acids and fulvic acids, synthesized during decomposition of coir dust (Kadalli et al. 2001). Marimuthu and Nagarajau (1993) reported an increase in the yield of rice due to compost prepared by addition of zinc sulfate (@ 4 kg/750 kg of raw coir pith) as zinc gets chelated and thus enriched the compost. The addition of manganese during composting will increase the rate of process as several white rot fungi require it for the degradation of lignin (Gold and Alic 1993).

1.5 Microbial Valorization of Coir Pith

A formulation of biofertilizer consists of a mixture of an active ingredient (which enhances the productivity of agricultural soil) with inert (inactive) ingredients. The inert material, which helps in the growth of active ingredient or living cells and assures their easy establishment in and around the root microcosm of the plant, is called a carrier (Mahanta 2017). The carrier for biofertilizer also increases the likelihood of enhancing plant growth or killing target pests. It would be beneficial to use by-product of agricultural practices, such as coir pith, coffee husk, tea waste, areca nut leaf sheath and dried husk, cocoa pod husk and bean shell, as carrier. These

wastes are cheap as well as easily available and hence are found to be suitable carriers for microbial inoculants (Kousalya and Jeyarajan 1990; Ponmurugan and Baby 2005). The first implication of using coir pith as a carrier for effective microorganisms date back to 1956; *Rhizobium* inoculated coir pith was applied in the legume cover crops of rubber plantations in the Rubber Research Institute of Malaysia (Prabhu and Thomas 2002).

A number of microorganisms, like lignin degrading fungi, actinomycetes, nitrogen fixing bacteria, phosphate solubilizers, *Azospirillum* and fluorescent pseudomonads, have been used to carry out valorization of coir pith (Theradimani and Marimuthu 1992; Thomas et al. 2013). Wood rotting basidiomycetes that cause white rot in wood have been found to be the most efficient lignin degraders amongst various fungi (Kirk and Farrell 1987). Some other fungi such as *Fusarium proliferatum*, *Phanerochaete chrysosporium*, *Pleurotus eryngii*, *Streptomyces viridosporus*, and *Trametes trogii* have also been found to be involved in the biodegradation of lignin (Ravindranath and Radhakrishnan 2016). All lignin degrading microorganisms excrete enzymes such as phenol oxidases into the substrate, classified as lignin peroxidase and manganese peroxidases (Kirk and Farrell 1987).

Espiritu (2010) inoculated cultures of *Azotobacter* sp. and *Trichoderma* sp. into a sterile coir dust and chicken manure (60/409, w/w). The mixture was composted for 28 days under three conditions. In the first condition, composting was performed in a flask having coir dust and chicken manure mixture without any combined nitrogen and in the second condition 1% ammonium sulfate was added to the mixture in the flask. The third condition was carried out in the actual compost heaps of coir dust and chicken manure mixture, inoculated with *Azotobacter* alone, which was present in a net bag. The loss of carbon, increase in nitrogen and decrease in C:N ratio was observed in the first two conditions, however the maximum degradation of raw material was observed in the third condition. Muthurayar and Dhanarajan (2013) reported decrease in the content of cellulose and lignin along with increase in the concentration of N, P, and K and neutral pH when coir pith was composted by adding cow dung, vegetable market waste, poultry waste *Pleurotus sajor-caju* and *Trichoderma viride*. Thomas et al. (2013) carried out composting of coir pith by adding 10% (w/w) poultry manure and also by adding a combination of poultry manure, lime, and rock phosphate. The poultry manure improved the physico-chemical characteristics of coir pith compost. The composted coir pith was of superior quality, with near-neutral pH, a C:N ratio in the range nearly 20–27% N and % K ranging above 1.0 and stable CO_2 evolution after 60–65 days of composting, compared to the non-decomposed control coir pith. The coir pith amended with poultry manure and rock phosphate exhibited higher counts of plant growth promoting bacteria such as P-solubilizers, *Azospirillum* spp., and fluorescent pseudomonads. The composted coir pith exhibited a positive effect upon the growth of cowpea plants as indicated by shoot length, root length, number of leaves, and dry weight of the plant. Prabhu and Thomas (2002) reported enhancement in the growth when coir pith compost prepared by pre-inoculation of *Rhizobium* was applied in the legume cover crops of rubber plantations in the Rubber Research Institute of

Malaysia. Baliah and Rajalakshmi (2015) used various carriers viz., coir pith, lignite, organic manure, vermicompost, and vermiculite for the multiplication of *Azospirillum* strains which were used in the field of *Okra* plants. Coir pith formulation was found to be the best carrier for the *Azospirillum* inoculants. Coir pith not only improved nutritive value of the soil, but also enhanced the growth of the plants.

Pleurotus fungus is well known for coir pith degrading ability. Different species such as *Pleurotus citrinopileatus*, *P. platypus, P. florida*, *P. sapidus*, and *Pleurotus sajor-caju* (oyster mushroom) have been used for complete degradation of coir pith. *P. platypus* was found to be the most efficient degrader of coir pith. The inoculation of *P. platypus* was found to result in 58.6% reduction of cellulose and 78% reduction of lignin after 35 days of inoculation while C:N ratio was reduced to 18:1 from 104:1 (Theradimani and Marimuthu 1992). Dharani and Sarojini (2014) carried out composting in which the pre-inoculation of *Pleurotus* and addition of urea into coir waste resulted into compost with reduced C:N ratio and increased nutrient content. This compost was used as a fertilizer at different concentrations for sugarcane plant. The application of this coir compost was found to increase the growth as indicated by shoot and root length and also the productivity of sugar cane. The cellulolytic bacteria and fungi recovered from coir waste compost were identified as *Aspergillus niger, A. flavus*, *Pseudomonas, Rhizopus,* and *Streptococcus*. Kanan et al. (2013) used the compost, prepared from coir pith supplemented with urea after pre-inoculation of *Pleurotus* fungus, in the rain-fed agricultural field. The moisture content of soil was increased. Plant height, length of the cob and stover, and yield of maize were found to be more due to the use of coir pith compost as compared to the control plot. Seventeen percent increase in the yield of maize was observed. Theradimani et al. (2018) inoculated coir pith with different white rot fungi including *Pleurotus djamor, Pleurotus eous, Pleurotus sajor-caju, Pleurotus florida* and other basidiomycetes fungi, *Calocybe indica, Hypsizygus ulmarius,* antagonist's organism viz., *Pseudomonas fluorescens*, *Trichoderma viride,* nitrogen fixing bacteria viz., *Azospirillum* and Phosphobacteria. *P. djamor* was found to be the best degrader as it brought 37.53% reduction in the organic carbon content of coconut coir pith. The content of nitrogen, phosphorus, potash, and calcium was found to be more in the decomposed coir pith. Ramamoorthy et al. (1999) reported reduction in the carbon content of coir pith from 28.97% to 23.14% by *Pleurotus djamor*. The nitrogen content was found to be increased from 0.28% to 1.5%, C:N was reduced from 103:1 to 20:1 (Ramamoorthy et al. 1999).

The composting properties of coir pith were found to be improved when it was mixed with a bioformulation PITHPLUS, derived from *Pleurotus sajor-caju,* an oyster mushroom. PITHPLUS bioformulation along with urea has been reported to decrease lignin and cellulose content and increase the total nitrogen and other nutrients in a period of 30 days. Das et al. (2005) carried out the composting of coir pith in a six layered structure. At the bottom, two layers of coir were interspersed with a layer of PITHPLUS, fourth, fifth, and sixth layer were of urea, coir pith, and PITHPLUS, respectively. These six layered arrangements were continued till the height of heap reached a maximum of 1 m. The heaps were sprinkled with water so as to maintain 200% humidity. In this manner, in about 30–45 days the coir pith got

composted to the organic manure, which was found to be rich in nitrogen, phosphorus, and potassium. This multilayered heap structure method was although quite effective but had the limitations of both space and time. The time taken for composting was more than a month and large land area was also utilized for the purpose. The absence of proper aeration system in the heaped structure of coir pith slowed down the composting process. Ghosh et al. (2007) suggested another design to overcome the problem of aeration. In pilot-scale design, a number of perforated polyvinyl chloride (PVC) pipes of 100 mm diameter were embedded into the heap, which was equally spaced at a distance of 60 cm from each other. The perforations allowed the air to flow inside the heap uninterruptedly, an outlet for carbon dioxide and also allowed the heat generated during composting to dissipate. In real field design, a raised platform was constructed with bricks laid with spaces in between and covered with a sheet of plastic fabric or PVC sheet. The raised platform had a minimum ground clearance of 15–20 cm; the fabric on the platform was coir matting having a large number of circular holes over its surface. In both the pilot-scale and real field, composting was completed in 21 days. C/N ratio was reduced from 112:1 to 20:1. The volume was reduced to 42%, and pH was found to increase from 5.5 to 6.5. These setups have been employed by many small coir-fiber manufacturers to convert their agricultural waste (coir pith) into useful manure. Central Coir Research Institute (CCRI) has recommended this technique for coir pith sites in Kerala (Ghosh et al. 2007).

Seal et al. (2015) prepared a Novcom solution for the composting of coir pith. The solution is potentially biologically activated and contains the extracts of Doob grass (*Cynodon dactylon*), Bel (*Sida cordifolia* L), and common basil (*Ocimum basilicum*). The compost samples were analyzed for physico-chemical properties, microbial population, maturity, and phytotoxicity parameters. The population count of total bacteria, fungi, and actinomycetes increased in exponential manner during composting. It attained maturity within 21 days as total nitrogen ranged in between 1.34 and 1.64 which is above the Indian standards. C:N ratio was also found to be greater than 20. Lavanya and Padmaja (2018) inoculated coir pith with an inoculant Effective Microorganism (EM), which is a liquid concentrate and consists of aerobic and anaerobic mixed culture of beneficial microorganisms like photosynthetic bacteria, *Lactobacillus*, *Streptomyces*, actinomycetes, etc. Cellulose content was found to reduce from 34.71% to 10.93%, C:N ratio of raw coir pith was also drastically reduced after 90 days of decomposition by EM. The reduction in the phenolic content was due to the detoxification of phenolic compounds by microorganisms. Ningshen and Daniel (2013) isolated bacteria, fungi, and actinomycetes from the samples of old coir pith dumped around the coir industry. Seven bacterial and five fungal isolates which showed predominant growth were selected for further studies. Four different consortiums were prepared by mixing *Aspergillus niger, Pleurotus sajor-caju,* and *Pseudomonas* spp. which were inoculated in fresh coir pith and aged old coir pith. The pH of coir pith subjected to pre-decomposition, using the four types of microbial consortia decreased with the increase in the number of days of decomposition. The temperature increased steadily from 29 °C to 37 °C up to 15th day and thereafter it declined to an average of 29 °C on the 30th day. After 30 days of

pre-decomposition, the resultant compost was found to be suitable for vermicomposting. Motha et al. (2018) pretreated the coconut waste with urea, lime, rock phosphate, cow dung, and green manure. It was then inoculated with the lignocellulose degrading microorganisms such as *Pleurotus sajor-caju* and *Trichoderma viride.* The biodegradation and decomposition of coir waste with earthworms *Eudrilus eugeniae* significantly reduced the time of composting and increased the amount of compost as compared to the treatment using microbial composting only (Motha et al. 2018).

1.6 Bioethanol Production from Coir Pith

Bioethanol is the promising fuel of the future generations as the supplies of fossil fuels are decreasing at an alarming rate (Venkatramanan et al. 2021). The characteristics of bioethanol viz., high-octane level, ability to reduce the level of particle emission, and similarity to gasoline allow it to be used directly without any modification of engine. It is also regarded as renewable fuel which has low-cost production and does not have CO_2 emissions (Muhaji and Sutjahjo 2018). The first-generation bioethanol is produced from carbohydrates, lipids and oils or agro-industrial wastes using conventional technologies (Ingale et al. 2014; Ahorsu et al. 2018; Shah and Venkatramanan 2019; Shah et al. 2019). The second generation (2G) bioethanol is generally derived from lignocellulosic biomass including cellulosic plant biomass such as the stalks, stems, and wood (Ramos and Valdivia 2016; Khuong et al. 2017; Shah and Venkatramanan 2019; Prasad et al. 2019). The production of 2G bioethanol involves various steps viz., pretreatment (separation of cellulose and hemicellulose from lignin), hydrolysis (breakdown of cellulose and hemicellulose into fermentable sugars), and fermentation (conversion of sugars to ethanol). It is the latest implication in the production of bioethanol, as lignocellulosic biomasses are cheap, easily available and are also sustainable. The lignocellulosic agricultural residues such as cassava bagasse, coconut coir, corn stover, sugar cane bagasse, and wheat straw have been used for bioethanol production (Goncalves et al. 2011; Agbro and Ogie 2012; Prasad et al. 2019). The coir fibers are potential substrate for bioethanol production as they are cheap, abundantly available and rich in cellulose and hemicelluloses.

The first crucial step in second generation bioethanol production is the pretreatment of biomass which reduces the rigid structure and converts lignin to the fermentable sugars (Rabelo et al. 2008). The objective of the pretreatment process is to increase the enzymatic digestibility of lignocellulosic materials (Chang et al. 2001). Cellulose, hemicellulose, and lignin which are the main components of lignocellulosic biomass are strongly intermeshed and bonded through covalent or non-covalent bonds forming the lignocellulosic matrix. The pretreatment will cause changes in microstructure, macrostructure, and chemical composition of lignocellulose (Chen et al. 2017). It removes lignin and hemicellulose, avoids the degradation or loss of carbohydrate and also avoids the formation of by-products which are inhibitory to the subsequent hydrolysis and fermentation

processes. The lignin content is reduced to release the fermentable sugars, thereby preparing the biomass ready for the enzymatic conversion (Cabral et al. 2016).

The pretreatment methods include physical (comminution), chemical (acid or alkaline treatment), or a combination of both methods (thermal treatment and microwave-assisted-alkaline treatment) (Mood et al. 2013). The most common physical pretreatment is the comminution process in which the particle size of lignocellulosic materials is reduced which can be achieved by a combination of chipping, grinding, or milling depending on required particle size of the material (Sun and Cheng 2002). The chemical pretreatment techniques used in bioethanol conversion from lignocellulosic biomass involve the use of acid (usually sulfuric acid or hydrochloric acid) and alkaline (sodium hydroxide). The most studied and widely used pretreatment strategy is acid hydrolysis using dilute sulfuric acid (Satimanont et al. 2012). Other acids such as phosphoric acid, acetic acid, and nitric acid are now more used because these acids are less aggressive as compared to sulfuric acid. The fermentable hydrolyzate which is produced using these acids has a lower concentration of microbial growth inhibitors such as furfural and hydroxyl methyl furfural (HMF) (Nantapipat et al. 2013). Amenaghawon et al. (2015) used dilute nitric and acetic acid for pretreatment of coconut coir for bioethanol production. The amount of fermentable sugar produced was increased by 54% when both of these acids were used together. The alkaline pretreatment method involves lower temperature and pressure. This method involves lesser costs as compared to acid pretreatment and also break the lignin bonding without obstructing the hydrolysis process (Zhang et al. 2012). In the alkaline pretreatment method, the dried coconut fibers are treated with 3% NaOH (w/v) in autoclave at 121 °C for 90 min. The pretreated materials are washed with water until a neutral pH is obtained. The pretreated solids are then dried in the oven at 85 °C for 4 h and stored in desiccators until their use. The treatment of lignocellulosic materials with dilute sodium hydroxide leads to the swelling of the coir material, which increases the internal surface area, decreases the degree of polymerization, decreases the crystallinity, the structural linkages between lignin and carbohydrates are separated, and the lignin structure is disrupted (Sun and Cheng 2002; Jannah and Asip 2015).

The thermal pretreatment method exposes the lignocellulosic raw material to pressurized steam in a vessel for some time period and thereafter it is depressurized. The autohydrolysis of acetyl groups present in hemicellulose occurs at the high temperature because of which acetic acid is formed, which can further catalyze the degradation of lignocelluloses (Alvira et al. 2010). Microwave-assisted alkaline pretreatment is a physico-chemical method in which the microwave treatment is done along with addition of alkaline solution. The reaction between microwave radiation and the polar molecules in the solution creates thermal and non-thermal effects on the raw materials which causes delignification (Fernández et al. 2011). In the pretreatment methods of coir, the pretreated coconut husk is washed to extract inhibitors from the biomass but this practice cannot be considered as best as it increases the number of process steps and also causes the loss of high content of sugars (Albuquerque et al. 2016).

The efficiency of bioethanol production is calculated as substrate to cell conversion (YX/S, g g^{-1}), cell to ethanol conversion (YE/X g g^{-1}), substrate to ethanol conversion (YE/S, g g^{-1}), maximum specific growth rate (μ_{max}), correctness of linear fit of the ln (X/X_0) vs. time curves, productivity of cells (PX, g L^{-1} h^{-1}), ethanol (PE, g L^{-1} h^{-1}), ratio between the maximum change of cellular concentration (g L^{-1}) and fermentation time (h). Further, efficiency of sugar to ethanol conversion (η, %) is determined as the ratio between YE/S (g g^{-1}) and the theoretical value (0.511 g g^{-1}) of this parameter (Hahn-Hagerdal et al. 1994). Cabral et al. (2016) reported less cell growth but large substrate consumption (81%) and production of ethanol with a growth rate (0.76 day^{-1}) which is characteristic of anaerobic processes, fermentative metabolism, and low concentration of sugars. The kinetics of fermentation process showed decrease in the consumption of substrate during the fermentation process, while the ethanol production was observed to be directly proportional to the cell growth (X). An overall yield of 0.078 g ethanol g^{-1} was obtained from the untreated coconut fiber. The initial amount of total sugar content of the coconut fiber was 36.96% while only 15.74% of sugars remained in the pretreated fiber. Ninety seven percent of the theoretical yield was obtained which confirmed the viability of using the green coconut husk as a feedstock for the production of 2G ethanol. Nogueira et al. (2018) reported that the hydrothermal, dilute alkaline pretreatment (2.0% (w/v) NaOH, 121 °C, 10 min and acid treatment (H_2SO_4) increased the conversion from 48.7% to 56.1% into glucose when coconut fiber was enzymatically hydrolyzed and Tween-80 was added to the green coconut fiber. Araújo et al. (2017) also found an increase in conversion on using rhamnolipids produced by *Pseudomonas aeruginosa* during enzymatic hydrolysis of coconut husk.

The second step is called saccharification in which the biomass is converted to fermentable sugars by hydrolysis that unlocks and saccharifies the polysaccharides which are present in the biomass. In the acid hydrolysis method, either dilute acid or concentrated acid is added which converts the cellulose to pentoses or hexoses (Champagne 2006). This step involves either acid or cellulases for the production of fermentable sugars. The dilute acids (1–9%v/v) are usually used which yield 50% sugar conversion while the concentrated acids yield 40–70% sugars (Yoswathana 2010). Enzyme cocktails are used to catalyze this step of saccharification in which simple sugars such as glucose and mannose are obtained, which are further subjected to fermentation by microorganisms. This process is very crucial, since it requires not a single enzyme but complexes of enzymes which act synergistically and therefore it is a costly process. Depending on the enzyme used, celluloses can be hydrolyzed into glucose and hemicelluloses can be hydrolyzed to release xylose, arabinose, galactose, glucose, and mannose. The challenging part is to bring down the cost in order to make this step economically feasible (Mohanram et al. 2013). Cabral et al. (2016) reported that the alkaline pretreatment promoted an efficient solubilization of lignin (80%), converting the coconut fibers into a feasible raw material for 2G ethanol production. They also found that enzymatic hydrolysis converted 87% of the sugars and the ethanolic fermentation consumed 81% of the substrate in the hydrolyzate, leading to a sugar to ethanol conversion efficiency of 59.6%.

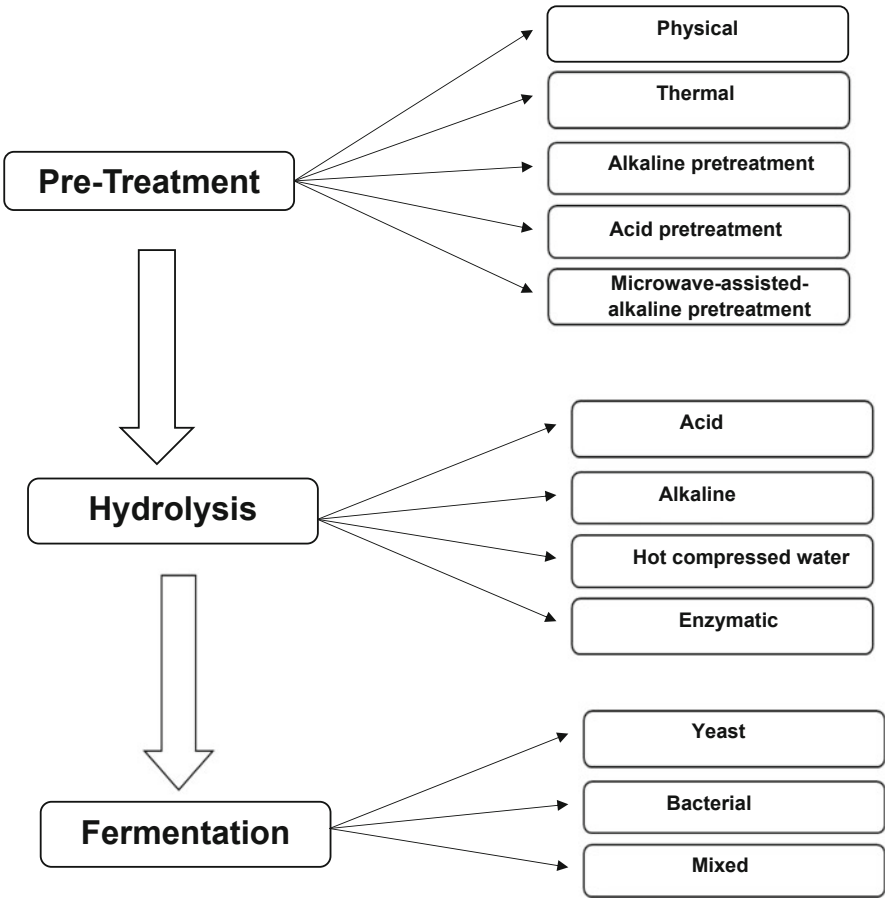


Fig. 1.1 Steps involved in microbial valorization of coir pith into bioethanol

In the third phase, sugars obtained from the treated lignocellulosic biomass is converted to bioethanol. It involves the fermentation of mixed sugars (hexose and pentose) to produce bioethanol (Champagne 2006). The microorganisms of primary interest in fermentation of ethanol include *Pichia stipites* (ferments xylose), *Saccharomyces cerevisiae* (ferments mostly hexoses), *Kluyveromyces* sp. and *Schwanniomyces alluvius* (hydrolyzes starch), and *Zymomonas mobilis* (ferments lactose) (Ding et al. 2012; Goncalves et al. 2016) (Fig. 1.1).

Albuquerque et al. (2016) used fresh and rotting coconut husk for the isolation of fungi which converted the coco husk to bioethanol. *Penicillium variabile* and *Trichoderma* sp. were found to be the best fungi with high specificity submerged fermentation. Higher efficiency in ethanol production, utilization of a variety of hexoses, and high tolerance to ethanol are the advantages of *S. cerevisiae* over other yeast strains (Classen et al. 1999). The genetically engineered yeast *S. cerevisiae* expressing cellulase gene has been reported to be more capable in fermenting

pentoses and hexoses. The recombinant strains with the overexpression of genes encoding enzymes involved in the degradation pathway of xylose; the genes related to the pentose phosphate pathway (such as TKL1 and TAL1) were used for ethanol production (Jeffries and Jin 2004).The yield of ethanol was found to be 13% (using 4% NaOH pretreated banana pseudo stem), 6% (using 2% NaOH pretreated coir pith), and 8% (using 4% HCl pretreated sugarcane bagasse) in different pretreated feedstocks when recombinant yeast was used (Immanuel 2016). Not only *Saccharomyces* but nowadays other yeasts and bacteria are also being used for bioethanol production. A combination of microorganisms exhibits co-fermentation in which *Candida shehatae*, *Pachysolen tannophilus* and *Pichia (Scheffersomyces) stipitis*, and/or bacteria such as *Zymomonas mobilis* carry out fermentation under anaerobic conditions and collectively exhibit high ethanol tolerance, and high ethanol-producing capacity (Fu and Peiris 2008; Cho et al. 2014).

The studies on the production of bioethanol from coconut husk have been made only on a small scale till date. It has been used as a substrate in biorefinery (Goncalves et al. 2014). The ethanol production from coco husk has also been used to obtain other substances. Sugars, acetic acid, phenolic compounds, and lignin found in the coconut hydrolyzate can be further processed. The phenolic compounds obtained during pretreatment and autohydrolysis are antioxidants which can be used as food additives. Lignin can be used to produce pharmaceutical and veterinarian bioactive compounds and thermoplastic polymers. After gasification or pyrolysis of lignin, energy can also be produced. Xylans which are obtained from hemicellulose are further hydrolyzed to obtain xylooligosaccharide which can be employed in food and animal feed (Goncalves et al. 2014). Pre-hydrolysis and simultaneous saccharification and fermentation abbreviated as PSSF at high solid loading is the recent approach which has been used for ethanol production from cotton stalks (Dimos et al. 2019).

1.7 Conclusion

Coir pith, a conspicuous agricultural waste which is still an untapped source, could be used and conversion of this waste into manure will also result in its fruitful disposal. The practical application of coir pith bioinoculums or compost in agricultural fields is quite possible in near future. The formulation of coir pith as carriers for biofertilizers is quite promising but its development in the current scenario requires rigorous collaborative research efforts of microbiologists and technologists. Some pilot-scale and real field studies have already showed methods for composting of coir pith which can be effectively implemented in the coir mass heaps. For the commercial purposes, co-composting the coir pith with different additives such as poultry manure, cow dung, farmyard manure, etc. should be carried out so that the rate of decomposition is high. A well-formulated technology has to be adopted for converting the coir pith wastes to a quality compost product. The practice of co-composting coir compost with the earthworms can be further implemented on the commercial scale as the microbial load and nutritive value of coir compost has

been found to be enhanced. The coconut husks have a high availability of sugars, which can also be effectively harnessed. Shortage of fuels is another global problem which needs immediate solution. Considering the trend of green technologies, the carbohydrates locked inside coconut husk could be subsequently converted to eco-friendly bioethanol. Coir pith can be a promising feedstock for bioethanol production, and it will also preferably suit the biorefinery approach due to its high carbohydrate content, cheap, and wide abundance. The production of bioethanol should be carried out at a commercial scale. The process should be optimized so as to obtain maximum yield with low cost so that it becomes economically viable. The economical viability of a process for bioethanol is determined by calculating minimum ethanol selling prices (MESP) which would include the cost of feedstock, pretreatment costs, and enzymatic hydrolysis costs. Till now, the studies on the use of coir pith for bioethanol production and as a substrate for a biorefinery have been performed only on a small scale and therefore further studies are required which can check the economical viability and practical feasibility of using coir pith for bioethanol production. Thus, if coir pith is effectively harnessed by both the approaches, not only the disposal and pollution related issues will be resolved but also it could become a money spinner in near future.

References

Abad M, Noguera P, Puchades R, Maquieira A, Noguera V (2002) Physicochemical and chemical properties of some coconut coir dust for use as a peat substitute for containerized ornamental plants. Bioresour Technol 82:241–245

Adunga G (2016) A review on impact of compost on soil properties, water use and crop productivity. Acad Res J Agric Sci Res 4(3):93–104

Agbro EB, Ogie NA (2012) A comprehensive review of biomass resources and biofuel production potential in Nigeria. Res J Eng Appl Sci 1(3):149–155

Ahorsu R, Medina F, Constanti M (2018) Significance and challenges of biomass as a suitable feedstock for bioenergy and biochemical production: a review. Energies 11:366

Albuquerque ED, Torres FAG, Fernandes AAR, Fernandes PMB (2016) Combined effects of high hydrostatic pressure and specific fungal cellulase improve coconut husk hydrolysis. Process Biochem 51(11):1767–1775

Alkoaik F (2019) Integrating aeration and rotation processes to accelerate composting of agricultural residues. PLoS One 14:e0220343. https://doi.org/10.1371/journal.pone.0220343

Alvira P, Tomas Pejo E, Ballesteros M, Negro MJ (2010) Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour Technol 101 (13):4851–4861

Amenaghawon NA, Osayuki-aguebor W, Egharevba IP (2015) Effect of acid mixtures on the hydrolysis of coconut coir for recovery of fermentable sugars. J Appl Sci Environ Manag 19 (3):345–351

Anand HS, Devi LS, Kadalli GG, Tharamani GH (1999) Lime pretreatment for faster degradation and humification of coir pith. J Trop Agric 37:73–74

Araújo CKC, Campos AO, Araújo CE, de Araújo P (2017) Enhancing enzymatic hydrolysis of coconut husk through *Pseudomonas aeruginosa* AP 029/GLVIIA rhamnolipid preparation. Bioresour Technol 237:20–26

Aziz SQ, Omar IA, Mustafa JS (2018) Design and study for composting process site. Int J Eng Invent 7(9):09–18

Baliah NT, Rajalakshmi V (2015) Isolation and characterization of azospirillum strains isolated from different agroclimatic zones of Virudhunagar district, Tamil Nadu. Indian J Appl Res 5(12)
Bangar KC, Kapoor KK, Mishra MM (1988) Effect of pyrite on conservation of nitrogen during composting. Biol Wastes 3(25):227–231
Bavappa KVA, de Gurusinghe PS (1978) Rapid multiplication of black pepper for commercial planting. J Plant Crop 6:92–95
Biddappa CC, Palnniswami C, Upadhyay AK, Ramanujam B (1998) Comparison of techniques for organic manure production from plantation wastes. J Plant Crop 26:120–126
Cabral MMS, Abud AKS, Silva CEF, Rosas RM, Almeida G (2016) Bioethanol production from coconut husk fiber. Ciência Rural 46(10):1872–1877
Champagne P (2006) Feasibility of producing bioethanol from waste residues: a Canadian perspective. Resour Conserv Recycl 50(3):211–230
Chang VS, Nagwani M, Kim CH, Holtzapple MT (2001) Oxidative lime pretreatment of high-lignin biomass: poplar wood and newspaper. Appl Biochem Biotechnol 94:1–28
Chen H, Liu J, Chang X, Chen D, Xue Y, Liu P (2017) A review on the pretreatment of lignocellulose for high-value chemicals. Fuel Process Technol 160:196–206
Cho SH, Lei R, Henninger TD, Contreras LM (2014) Discovery of ethanol-responsive small RNAs in *Zymomonas mobilis*. Appl Environ Microbiol 80(14):4189–4198
Classen PAM, van Lier JB, Lopez Contreras AM, van Niel EWJ, Sijtsma L, Stams AJM, de Vries SS, Weusthuis RA (1999) Utilisation of biomass for the supply of energy carriers. Appl Microbiol Biotechnol 52(6):741–755
Cooperband LR (2000) Composting: art and science of organic waste conversion to a valuable soil resource. Lab Med 31(6):283–290
Cresswell G (2011) Coir dust a proven alternative to peat. Cresswell Horticultural Services, pp 1–13. (report)
Das AR, Sarma US, Radhakrishnan S (2005) Coir fibre extraction and coir pith composting technology. National Workshop on women friendly technologies; Kochi, India, Jan 20–22, 2005
DebMandal M, Mandal S (2011) Coconut (*Cocos nucifera* L.: Arecaceae): in health promotion and disease prevention. Asian Pac J Trop Med 4:241–247
Dharani A, Sarojini G (2014) Composting of coir waste using bacteria (*Pseudomonas* and streptococci) and fungi (*Aspergillus* and *Rhizopus*) and their effect on sugar cane plant growth. Int J Adv Res Biol Sci 5:71–75
Dimos K, Paschos T, Louloudi A, Kalogiannis K, Lappas A, Papayannakos N, Kekos D, Mamma D (2019) Effect of various pretreatment methods on bioethanol production from cotton stalks. Fermentation 5:5. https://doi.org/10.3390/fermentation5010005
Ding TY, Hii SL, Ong LGA (2012) Comparison of pretreatment strategies for conversion of coconut husk fiber to fermentable sugars. Bioresources 7(2):1540–1547
Eiland F, Klamer M, Lind AM, Leth M, Bååth E (2001) Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw. Microb Ecol 41(3):272–280
Elfstrand S, Båth B, Martensson A (2007) Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. Appl Soil Ecol 36(1):70–82
Espiritu BM (2010) Composting and microbial inoculation of coconut coir dust-chicken manure mixture for organic fertilizer use. Int Syst Agric Sci Technol 35(2):47–56
FAOSTAT (2018) Food and agriculture Organization of the United Nations. FAOSTAT statistical database. FAO, Rome
Fernández Y, Arenillas A, Menendez JA (2011) Microwave heating applied to pyrolysis. In: Stanislaw G (ed) Advances in induction and microwave heating of mineral and organic materials. InTech, Rijeka, Croatia, pp 723–751
Fernando JAKM, Amarasinghe ADUS (2017) Effects of retting and drying on quality of coir pith and coco discs. Natl Sci Found 45(1):3–12

Fu N, Peiris P (2008) Co-fermentation of a mixture of glucose and xylose to ethanol by *Zymomonas mobilis* and *Pachysolen tannophilus*. World J Microbiol Biotechnol 24(7):1091–1097

Garg A, Tothill IE (2009) A review of solid waste composting process—dynamic soil, dynamic plant. Global Science Books, The UK Perspective

Ghosh PK, Sarma US, Ravindranath AD, Radhakrishnan S, Ghosh P (2007) A novel method for accelerated composting of coir pith. Energy Fuel 21:822–827

Gold MH, Alic M (1993) Molecular biology of the lignin degrading basidiomycete Phaneroc Fwele chrysosporiwn. Microbiol Rev 51:605–622

Golueke CG (1991) Principles of composting. In: The Staff of Bio Cycle (ed) The Art and Science of Composting, Journal of Waste Recycling. The JG Press, Pennsylvania, pp 14–27

Gonawala SS, Jardosh H (2018) Organic waste in composting: a brief review. Int J Curr Eng Technol 8(1):36–38

Goncalves FA, Sanjinez-Argandoña EJ, Fonseca GG (2011) Utilization of agro-industrial residues and municipal waste of plant origin for cellulosic ethanol production. J Environ Prot 2:1303–1309

Goncalves FA, Ruiz HA, Nogueira CDC, Santos ESD, Teixeira JA, Macedo GR (2014) Comparison of delignified coconuts waste and cactus for fuel-ethanol production by the simultaneous and semi-simultaneous saccharification and fermentation strategies. Fuel 131:66–76

Goncalves FA, Ruiz HA, dos Santos ES, Teixeira JA, de Macedo GR (2016) Bioethanol production by *Saccharomyces cerevisiae, Pichia stipitis* and *Zymomonas mobilis* from delignified coconut fibre mature and lignin extraction according to biorefinery concept. J Renew Energy 94:353–365

Hahn-Hagerdal B, Jeppsson BH, Skoog K, Prior BA (1994) Biochemistry and physiology of xylose fermentation by yeasts. Enzyme Microb Technol 16:933–943

Hossain A, Chowdhury SM, Rekhu Y, Khandakar SF, Monzur F (2012) Biodiesel from coconut oil: a renewable alternative fuel for diesel engine. World Acad Sci Eng Technol 68:524–528

Huang GF, Wong J, Wu Q, Nagar B (2004) Effect of C/N on composting of pig manure with sawdust. Waste Manag 24(8):805–813

Immanuel SR (2016) Bioethanol productions from banana pseudo stem by recombinant yeast *Saccharomyces cerevisiae* as sustainable energy. J Pharmaceut Care Health Syst 3(Suppl):2. https://www.longdom.org/conference-abstracts-files/2376-0419.C1.014_026.pdf

Ingale S, Joshi SJ, Gupte A (2014) The characteristics of bioethanol fuel made of vegetable raw materials. Braz J Microbiol 45(3):885–892

Jain MS, Daga M, Kalamdhad AS (2019) Variation in the key indicators during composting of municipal solid organic wastes. Sustain Environ Res 29:9

Jannah AF, Asip F (2015) Bioethanol production from coconut fiber using alkaline pretreatment and acid hydrolysis method. Int J Adv Sci Eng Inform Technol 5(5):320–322

Jeffries TW, Jin Y-S (2004) Metabolic engineering for improved fermentation of pentoses by yeasts. Appl Microbiol Biotechnol 63(5):495–509

Kadalli GG, Suseela Dev L, Siddaramappa R, John E (2001) Characterization of humic fractions extracted from coir dust-based composts. J Indian Soc Soil Sci 48:51–55

Kanan K, Selvi V, Singh DV, Khola OPS, Mohanraj R, Murugesan A (2013) Coir pith composting-an alternate source of organic manure for rainfed maize. Central Soil &Water Conservation Research &Training Institute, Research Centre (Indian Council of Agricultural Research)

Kavitha R, Subramanian P (2007) Bioactive compost—a value added compost with microbial inoculants and organic additives. J Appl Sci 7:2514–2518

Kempton TJ (2006) Value-added coconut co-products. In: Adkins SW, Foale M, Samosir YMS (eds) Coconut revival—new possibilities for the 'tree of life'. Proc. Int. coconut forum, Cairns, Australia, 22–24 November 2005. ACIAR Proceedings No 125

Khuong LS, Masjuki HH, Zulkifli NWM, Mohamad EN, Kalam MA (2017) Effect of gasoline–bioethanol blends on the properties and lubrication characteristics of commercial engine oil. R Soc Chem Adv 7:15005–15019

Kirk TK, Farrell RL (1987) Enzymatic "combustion": the microbial degradation of lignin. Annu Rev Microbiol 41:465–501
Kousalya G, Jeyarajan R (1990) Mass multiplication of *Trichoderma* spp. J Biol Control 4:70–72
Lavanya DL, Padmaja CK (2018) Impact of effective microorganism on the decomposition of coir pith. Int Res J Environ Sci 7(8):21–23
Lee JJ, Park RD, Kim YW, Shim JH, Chae DH, Rim YS, Sohn BK, Kim TH, Kim KY (2004) Effect of food waste compost on microbial population, soil enzyme activity and lettuce growth. Bioresour Technol 9:21–28
Lodolini EM, Pica F, Massetani F, Neri D (2016) Physical, chemical and biological properties of some alternative growing substrates. Int J Sci 12:32–38
Long Y, Liu W, Yang Y, Shen D (2017) Effect of C/N on water state during composting of kitchen waste and vegetable waste mixture. J Chem 2017:1–10. https://doi.org/10.1155/2017/9409145
Mahanta B (2017) Efficacy of coir pith as a carrier for microbial formulations in agriculture: a review. Int J Recent Sci Res 8(8):19328–19334
Marimuthu T, Nagarajau R (1993) Coir pith: a useful crop residue. Biol Educ 32(Sept):161–164
Mian IH, Rodriguez-Kabana R (1982) Organic amendments with high tannin and phenolic contents for the control of *Meloidogyne arenaria* in infested soil. Nematropica 2:221–234
Mohanram S, Amat D, Choudhary J, Arora A, Nain L (2013) Novel perspectives for evolving enzyme cocktails for lignocellulose hydrolysis in biorefineries. Sustain Chem Process 1(1):15
Mood SH, Golfeshan AH, Tabatabaei M, Jouzani GS, Ardjmand M (2013) Lignocellulosic biomass to bioethanol, a comprehensive review with a focus to pretreatment. Renew Sustain Energy Rev 27:77–93
Moorthy VK, Rao KB (1997) Recycling of coirpith- a successful VRF experience. Coir News 26 (9):21–24
Motha K, Dorajerao AVD, Vishala S (2018) Comparative performance of microbial cultural and earthworm in composting of tender coconut waste into high quality organic manure. Int J Curr Microbiol App Sci 7(5):3076–3086S
Muhaji, Sutjahjo D (2018) The characteristics of bioethanol fuel made of vegetable raw materials. IOP Conf Ser Mater Sci Eng 296:012019. https://doi.org/10.1088/1757-899x/296/1/012019
Muthurayar T, Dhanarajan MS (2013) Biochemical changes during composting of coir pith waste as influenced by different agro industrial wastes. Agric Sci 4(5B):28–33
Nandhini PR, Kalaiselvi G, Saravanan N, Ramesh Babu NG (2016) Chemical analysis of agro-pollutant coir pith—a byproduct of coir industry. Int J Environ Agric Res 2(11):12–20
Nantapipat J, Luengnaruemichai A, Wongkasemjit S (2013) A comparison of dilute sulfuric and phosphoric acid pretreatments in biofuel production from corn cobs. World Acad Sci Eng Technol 7:477–481
Ningshen L, Daniel T (2013) A study on biodegradation of coir pith using microbial consortium. IOSR J Environ Sci Toxicol Food Technol 5(6):01–06
Nogueira CC, Padilha EC, Leitão AL, Maria RP, Macedo RG, Santos SE (2018) Enhancing enzymatic hydrolysis of green coconut fiber—pretreatment assisted by tween 80 and water effect on the post-washing. Ind Crop Prod 112:734–740
Paramanandham J, Ross PR (2016) Enumeration and characterization of microorganisms in raw coir pith and coir pith dumped soil. Int Lett Nat Sci 53:34–39
Paulin B, O'Malley P (2008) Compost production and use in horticulture. Department of Agriculture and Food, Western Australia, Perth. Bulletin 4746
Ponmurugan P, Baby UI (2005) Management of Phomopsis canker of tea with fungicides and biocontrol agents. J Plant Crop 33:175–178
Prabha ML, Priya MS, Don S (2013) Composting of coir pith using biopond effluent from effluent treatment plant of petrochemical division. Int J Adv Pharm Med Bioallied Sci 2(1):173–178
Prabhu SR, Thomas GV (2002) Biological conversion of coir pith into a value-added organic resource and its application in Agri-horticulture: current status, prospects and perspective. J Plant Crop 30(1):1–17

Prasad M, Roeber RU (1997) Physical, chemical and biological properties of coir dust. Acta Hortic 456:21–29

Prasad S, Venkatramanan V, Kumar S, Sheetal K (2019) Biofuels: a clean technology for environment management. In: Sustainable green technologies for environmental management, pp 219–240. https://doi.org/10.1007/978-981-13-2772-8_11

Rabelo SC, Maciel Filho R, Costa AC (2008) Lime pretreatment of sugarcane bagasse for bioethanol production. Appl Biochem Biotechnol 153(1–3):139–150

Ramamoorthy V, Meena B, Muthusamy M, Seetharaman K, Alice D (1999) Composting coir pith using lignocellulosic fungi for the management of root rot of black gram. Mushroom Res 8:13–17

Ramos JL, Valdivia M (2016) Benefits and perspectives on the use of biofuels. J Microbial Biotechnol 9(4):436–440

Rao KSV (1999) Application of coco peat (pith) to horticulture. Coir News 28(5):33–35

Ravindranath DA, Radhakrishnan S (2016) Coir pith—wealth from waste: a reference. Coir Board, Ministry of Micro, Small and Medium Enterprise, Government of India, Cochin. http://coirboard.gov.in/wp-content/uploads/2016/07/Coir-Pith.pdf

Raza S, Ahmad J (2016) Composting process: a review. Int J Biol Res 4(2):102–104

Sangamithra A, John SG, Sorna Prema R, Chandrasekar V, Sasikala S, Hasker E (2013) Coconut: an extensive review on value added products. Indian Food Ind Mag 32(6):29–36

Santiago M, Santhamani S (2010) Remediation of chromium contaminated soils: potential for phyto and bioremediation. In: World Congress of soil science, soil solutions for a changing world, 1–6 August 2010, Brisbane, Australia

Sarma US, Das AR (1993) Extraction of lignosulphonates from coirpiths—an agrowaste. In: Proceedings of the workshop on utilization of CoirPith; Coimbatore, India, pp 11–13. http://www.ccriindia.org/pdf/ExtractionofLignosulphonatefromcoirpithAnAgrowaste.pdf

Satimanont S, Luengnaruemitchai A, Wongkasemjit S (2012) Effect of temperature and time on dilute acid pretreatment of corn cobs. Int J Chem Biol Eng 6:333–337

Savithri P, Khan HH (1994) Chemical analysis of agro-pollutant coir pith—a byproduct of coir industry. Int J Environ Agric Res 2(1):1–18

Seal A, Bera R, Datta A, Saha S, Chatterjee AK, Barik AK, Mazumdar D (2015) Successful biodegradation of coir pith waste using novcom composting method: a case study from Vaniampara rubber estate, India. J Pharmaceut Sci Innov 4(1):72–77

Shafawati SN, Siddiquee S, Wong CMVL, Kumar SV (2014) Lignocellulolytic activities among *Trichoderma* isolates from Lahad Datu Sabah and Deception Island, Antarctic. J Microb Biochem Technol 6(5):295–302

Shah S, Venkatramanan V (2019) Advances in microbial technology for upscaling sustainable biofuel production. In: New and future developments in microbial biotechnology and bioengineering, pp 69–76. https://doi.org/10.1016/b978-0-444-63504-4.00005-0

Shah S, Venkatramanan V, Prasad R (eds) (2019) Sustainable green technologies for environmental management. Springer Nature, Singapore. https://doi.org/10.1007/978-981-13-2772-8

Silva ARA, Bezerra FML, Lacerda LM, Miranda RS, Marques EC (2018) Ion accumulation in young plants of the 'green dwarf' coconut under water and salt stress. Rev Ciênc Agron 49 (2):249–258

Singh TA, Singh GR (1991) Evaluation of partially acidulated phosphate rocks and liquid ammonium polyphosphate as phosphorus sources for wheat. J lndian Soc Soil Sci 39:191–193

Stelte W, Barsberg ST, Clemons C, Morais JPS, Rosa MF, Sanadi AR (2018) Coir fibers as valuable raw material for biofuel pellet production. Waste Biomass Valor 60(1):44–53

Sudha R, Niral V, Hebbar KB, Samsudeen K (2019) Coconut inflorescence sap. Curr Sci 116 (11):1809–1817

Sun T, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83(1):1–11

Theradimani M, Marimuthu T (1992) Ulilization of *Pleurotus* spp. for decomposing coconut coir pith. Mushroom Res 1:49–5.1

Theradimani M, Thangeshwari S, Parthasarathy S (2018) Biological decomposition of coconut coirpith waste. Plant Dis Res 33:142–147

Thomas GV, Palaniswami C, Prabhu SR, Gopal M, Gupta A (2013) Co-composting of coconut coir pith with solid poultry manure. Curr Sci 104(2):245–249

Tiquia SM, Tam NYF (2000) Co-composting of spent pig litter and sludge with forced-aeration. Bioresour Technol 72:1–7

Tripetchkul S, Pundee K, Koonsrisuk S, Akeprathumchai S (2012) Co-composting of coir pith and cow manure: initial C/N ratio vs physico-chemical changes. Int J Recycl Org Waste Agric 1:15

Venglovsky J, Sasakova N, Vargova M (2005) Evolution of temperature and chemical parameters during composting of the pig slurry solid fraction amended with natural zeolite. Bioresour Technol 96(2):181–189

Venkatramanan V, Shah S, Prasad R (eds) (2021) Sustainable bioeconomy: pathways to sustainable development goals. Springer Nature, Singapore. https://doi.org/10.1007/978-981-15-7321-7

Wang W, Gu H (2009) Characterization and utilization of natural coconut fibres composites. Mater Des 30(7):2741–2744

Yoswathana N (2010) Bioethanol production from rice straw. Energy Res J 1(1):26–31

Zhang Q, De Vigier KO, Royer S, Jérôme F (2012) Deep eutectic solvents: syntheses, properties and applications. Chem Soc Rev 41:7108–7146

Zhu N (2006) Composting of high moisture content swine manure with corncob in pilot scale aerated static bin system. Bioresour Technol 97:1870–1875

Transforming the Lignocellulosic Biomass into High Value-Added Bioproducts

2

Jaciane Lutz Ienczak, Patrícia Poletto, Diogo Robl, and Sarita Cândida Rabelo

Abstract

Lignocellulosic biomass comprehends the most abundant and renewable material in the world, being its efficient fractionation crucial to develop economically viable biorefineries. Chemical, physical, physical-chemical, biological, or enzymatic conversion can be used as strategies to produce important bioproducts as carbohydrates, bioactive compounds, and lignin derivatives. Carbohydrates as xylose and glucose can be used for food, chemical blocks, materials, and biofuels production by microorganisms like the yeast *Spathaspora passalidarum* for the production of xylitol, ethanol, acetoin, and 2,3-butanediol. Besides that, the lignocellulosic biomass is an important substrate for the production of several enzymes such as glycohydrolases (cellulases and hemicellulases) and oxidoreductases (laccase, peroxidases, and polysaccharide monooxygenases). Hemicellulases are necessary enzymes to achieve the required degree of polymerization of xylooligosaccharides, a new class of prebiotics extracted from the hemicelluloses fraction. Chemicals derived from lignin have found applications in various industries including nanoparticles, composites, antioxidants, polymer, among others. The focus of this chapter is to review the state of the art with regard of the characterization and valorization of lignocellulosic feedstock, as well as the

J. L. Ienczak (✉) · P. Poletto
Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, SC, Brazil
e-mail: jaciane.ienczak@ufsc.br

D. Robl
Department of Microbiology, Immunology and Parasitology, Federal University of Santa Catarina, Florianópolis, SC, Brazil

S. C. Rabelo
Department of Bioprocess and Biotechnology, College of Agricultural Sciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_2

process involving in the biomass fractionation, bioproducts recovery, and production.

Keywords

Biomass · Xylooligosaccharides · Enzymes · *Spathaspora passalidarum* · Lignin

2.1 Introduction

Lignocellulosic biomass comprehends the most abundant and renewable material in the world, which is mainly composed of cellulose, hemicelluloses, and lignin (Nanda et al. 2013; Shah and Venkatramanan 2019). Although agriculture, forest, and industrial sectors are known to be the main sources of lignocellulosic feedstock, agricultural wastes and forest residues are the most promising sources due to their abundance and low cost (Rubio et al. 2015; Venkatramanan et al. 2021). These biomasses can be converted into numerous compounds by chemical and/or biotechnological routes, leading to the production of fuels (Venkatramanan et al. 2021) or high value-added biochemical products such as enzymes, oligomers, lignin compounds, glycerol, xylitol, acetoin, and 2,3-butanediol produced by yeast *Spathaspora passalidarum*. This chapter reviews the state of the art with regard to the transformation of lignocellulosic biomass into high value-added bioproducts. Biotechnological routes are discussed starting with pretreatment of lignocellulosic biomass in order to break the compact structure of the plant cells, followed by a hydrolysis step (by enzymes application) to depolymerize its polysaccharides into sugars (oligosaccharides or monomers) and lignin-rich residues as depicted in Fig. 2.1.

2.2 Structure and Chemical Composition of Lignocellulosic Biomass

Lignocellulosic biomass refers to the dry mass of the plant, and it is also called lignocellulose. When compared to petroleum, lignocellulosic biomasses are sustainable, more abundant and homogeneously distributed on the planet, which makes it inexpensive and susceptible to technological bioconversion (Shah and Venkatramanan 2019). However, biomass recalcitrance, related to the physical, chemical, and morphological characteristics of the material, constitute physical barriers that protect, especially the cellulose from degradation by microorganisms or enzymes, thus representing a huge techno-scientific challenge for efficient biomass fractionation (Sun and Cheng 2002).

Natural features of plants, which are responsible for biomass recalcitrance, are described from a macrostructural perspective (Himmel et al. 2007): (1) the epidermal tissue of the plant body, particularly the cuticular and epicuticular waxes; (2) the arrangement and density of vascular bundles; (3) the relative amount of

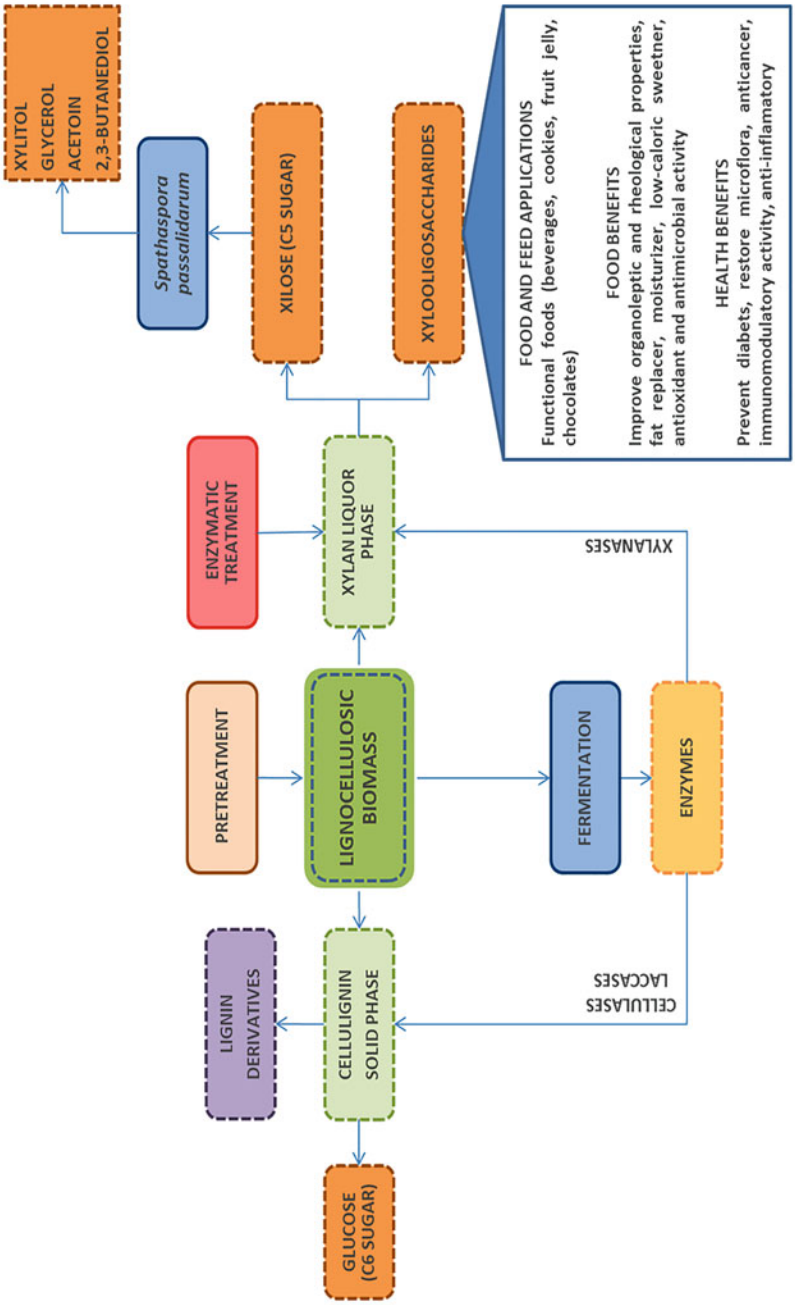


Fig. 2.1 Co-production of high value-added bioproducts from lignocellulosic biomasses

Table 2.1 Typical chemical compositions of the main components of lignocellulosic biomass

Lignocellulosic biomasses	Cellulose (%)	Hemicelluloses (%)	Lignin (%)
Dicots	45–50	20–30	7–10
Grasses	35–45	40–50	20
Softwoods	25–50	20–30	25–35
Hardwoods	40–55	20–35	18–25

(Source: Vogel 2008; Abramson et al. 2013; Marriott et al. 2016)

sclerenchyma (thick wall); (4) the lignification degree; and (5) the structural heterogeneity and complexity of the components of the cell wall, such as microfibrils and polymer matrices.

The main source of lignocellulose is the secondary cell walls of plants, and the thick, strengthening layer of the cell wall that is laid down inside the primary wall after cell elongation has terminated. Lignocellulosic biomasses are formed by the complex interaction of three components: two polysaccharides (cellulose and hemicelluloses) and one aromatic macromolecule (lignin); and, in minor amounts, structural proteins, lipids, and ashes (Rabelo et al. 2011), and these are present in varying proportions in different feedstock (Table 2.1). Approximately 75% of lignocellulose is comprised of polysaccharides, which can potentially be converted into oligosaccharides and/or monosaccharides for several applications.

Cellulose is a homopolysaccharide with linear chains, resulting from the linkage of several anhydroglucopyranose (β-D-glucopyranose) units liked by β (1→4) bonds. Cellobiose is defined as the minimum conformational unit of cellulose, whereas glucose represents the fundamental unit of the homopolymer chains (Fengel and Wegener 1989).

Also known as polyoses, hemicelluloses are heteropolysaccharides with shorter chains than cellulose and branched. Sugar moieties in hemicelluloses may be subdivided into groups, such as pentoses, hexoses, hexouronic acids, and deoxyhexoses. Hydroxyl groups from some sugars may be partially substituted by acetyl groups. The degree of acetylation varies according to the type of biomass and the amount of acetyl groups are between 1 and 6 wt% (dry basis) of total biomass (Peng et al. 2011).

Lignin is a phenolic macromolecule synthesized in plants by oxidative coupling of three major C9 (phenylpropanic) units: syringyl alcohol (S), guaiacyl alcohol (G), and p-coumaryl alcohol (H) that together form a random structure in a 3D arrangement in the cell wall. The main bond type between the units is aryl-aryl ether, i.e., linkages between phenolic units. After cellulose, lignin is the most abundant organic macromolecule in plants. It is mainly present in the middle lamella and the secondary wall and provides rigidity to the cell wall. It also plays an important role in the transport of water, nutrients, and metabolites, being responsible for the mechanical resistance of plants and protecting tissues against microorganisms (Fengel and Wegener 1989).

2.3 Importance and Types of Lignocellulosic Biomass Pretreatment for High Value-Added Bioproducts Production

As mentioned before, due to the natural recalcitrance of the biomass, pretreatment processes are necessary to render biomass more available to chemical or enzymatic attack, looking for an efficient product generation. An ideal pretreatment should meet the following requirements (El-Naggar et al. 2014; Hassan et al. 2018): (1) economically and operationally simple; (2) minimum energy, chemicals and process water requirements; (3) use whole and raw (non-comminuted) biomass; (4) cause minimal corrosion (including mineral impurities); (5) alter the structure of lignocellulosic materials; (6) selectivity for loss of polysaccharides; (7) high quality of the hydrolysates, in the case to be applied in fermentations processes (if possible without detoxification steps); (8) minimum degradation product formation from cellulose and hemicelluloses; (9) production of substrates with high cellulose content and accessibility to apply in other processes; (10) high-quality lignin or lignin-derived products.

There is still no consensus on the best pretreatment to be applied in industrial processes from a techno-economic point of view. This is because pretreatment must be chosen according to the desired end product, and, besides, the variability in biomass composition is also one of the main culprits for the lack of a universal pretreatment for lignocellulosic biomass.

Table 2.2 shows a comparison, in terms of advantages and disadvantages, of most important and commonly pretreatments listed in literature (Jørgensen et al. 2007; Rabelo et al. 2011; Martins et al. 2015; Santucci et al. 2015; Jönsson and Martín 2016; Kim et al. 2016; Jin and Dale 2018; Pin et al. 2019). The mentioned items were short descriptions and showed the main effects of each pretreatment, as well as difficulties related to each process.

Dilute acid pretreatment has been studied for many years with a particular focus on biofuel and chemical production. This process can promote the recovery of up to 90% of hemicelluloses. The basic mechanism consists of the breakage of glycosidic bonds and very sensitive to the presence of the hydronium ion (H_3O^+) in the medium. Although it presents several advantages, such process is quite severe due to the low pH and high temperatures and requires special steel alloys to withstand such conditions, alkaline reagents for pH adjustment, and also the formation of degradation products from carbohydrates and lignin that impact negatively on the following steps (Kiran Kumar and Sharma 2017; Wan et al. 2019). Hydrothermal pretreatment in its different forms—hot liquid water or steam injection—does not require the addition of acid catalysts and is a more environmentally friendly alternative. The high temperatures of the process promote the formation of H_3O^+ ions by autocatalytic processes (Garrote et al. 1999); therefore, the pretreatment mechanism is also ruled by acid catalysis. However, there is less recovery of the solubilized hemicelluloses and greater solubilization of the lignin compared to the diluted acid, which makes the downstream of the hemicellulosic hydrolysate more complex, mainly because the hemicelluloses are in the oligomeric form in the hydrolysate,

Table 2.2 Comparison between some types of pretreatment of lignocellulosic biomass (Jørgensen et al. 2007; Rabelo et al. 2011; Martins et al. 2015; Santucci et al. 2015; Jönsson and Martín 2016; Kim et al. 2016; Jin and Dale 2018; Pin et al. 2019)

Main objective	Pretreatments	Chemicals	Advantages	Disadvantages
Hemicelluloses solubilization	Dilute acid	Brønsted acids like H_2SO_4, H_3PO_4, HNO_3, and SO_2	Good digestibility of pretreated material, high solubilization of hemicelluloses in monomeric form, short reaction times	Abrasive/ corrosive, generation of degradation products (furfural, HMF, phenols, etc.)
	Hydrothermal	Water (liquid or in the vapor form)	Environmentally friendly (no reagents other than water), low sugar degradation	Downstream of hemicellulosic hydrolysate, conversions lower than dilute acid
	Steam explosion	Water vapor, SO_2	Environmentally friendly, low sugar degradation	Downstream of hemicellulosic hydrolysate, conversions lower than dilute acid
Delignification and hemicelluloses solubilization	Alkaline	Arrhenius bases like NaOH, $Ca(OH)_2$ and NH_3	High digestibility of pretreated material, low sugar degradation	Recycle/ recovery of chemicals (NH_3), long reaction times, high energy input
	Organosolv	Aqueous organic solvents such as ethanol, methanol, ethylene glycol, acetone, with or without a catalyst such as H_2SO_4, magnesium chloride, or NaOH	Lower water, energy, and reagent consumption compared to other pretreatment methods, easy solvent recovery	High solvent and energy consumption, security attention against fire and explosion risks, due to volatile materials
	Ammonia fiber expansion (AFEX)	Ammonia (NH_3)	Decrystallizes the cellulose, removes and depolymerizes lignin, increases the size and number of micropores in the cell wall	High energy demand, NH_3 consumption, post-hydrolysis of the hemicellulosic hydrolysate rich in oligomers is necessary

(continued)

Table 2.2 (continued)

Main objective	Pretreatments	Chemicals	Advantages	Disadvantages
Lignin and/or hemicellulose solubilization	Aprotic ionic liquids (AIL)	Wide combination of cations and anions, e.g., 1-ethyl-3-methyl imidazolium chloride	High digestibility of pretreated material, selective solubilization of lignin/ hemicelluloses/ lignin	Recovery and recycling of LI, recovery of dissolved sugars in LI, high cost of reagents
	Protic ionic liquids (PILs)	Wide combination of cations and anions, e.g., bis (2-hydroxyethyl) ethanol ammonium acetate	High digestibility of pretreated material, selective solubilization of lignin/ hemicelluloses/ lignin	Recovery and recycling of LI, recovery of dissolved sugars in LI, high cost of reagents

requiring a post-hydrolysis of these sugars, when the goal is its application in monomeric form (Garrote et al. 2001; Zhang et al. 2015; Nakasu et al. 2016).

Steam explosion pretreatment consists of pressurization of the reactor with water vapor and rapid decompression for physical disruption of the fiber. The main goal of this pretreatment is to remove hemicelluloses, which are also solubilized into the liquid fraction in the oligomeric form. It is also common to add catalysts like SO_2 to increase the medium's acidity and promote higher solubilization of the hemicelluloses (Hendriks and Zeeman 2009; Rabelo et al. 2012).

An alkaline medium also favors the occurrence of structural modifications at macroscopic and microscopic levels in the biomass. By means of epimerization reactions and breakdown of the glycosidic bonds (which are more stable in alkaline medium), carbohydrates are depolymerized by reactions of primary—hydrolysis of the reducing terminals—and secondary peeling—hydrolysis of the inner bonds in the sugar chains (Rabelo et al. 2013).

Ammonia fiber expansion pretreatment (AFEX) promotes up to 90% conversion of cellulose and hemicelluloses into sugars for a number of biomasses such as wheat straw, barley, sugarcane bagasse, and corn stover. For most of these materials, considerable cellulose conversions have been achieved in the enzymatic saccharification (Yang and Wyman 2008).

Organosolv pretreatment removes mainly lignin and hemicelluloses, while simultaneously making cellulose more easily digestible. The organic solvent and water are mixed to give a solvent concentration of 35%–70% (w/w) and, in general, the operating temperature and time range is between 120 and 200 °C and 30 and 90 min, respectively. This pretreated solid must be washed with an organic solvent to prevent precipitation of lignin (Borand and Karaosmanoğlu 2018).

Ionic liquid (IL) pretreatment is a recent alternative that has been gaining attention for its potential to be environmentally friendly. ILs can act as catalysts

and alter the structure of lignocellulosic biomass under certain conditions. However, since both kinetics and thermodynamics of the IL-promoted are not well understood, there is no way to predict the reaction behavior of ILs (Earle and Seddon 2000). The main disadvantages of the process arise mainly from the recent use of these compounds. In fact, interest in biomass pretreatment with ILs has been increasing since the last decade (Greaves and Drummond 2008; Reddy 2015), but little is known about some downstream stages such as IL recovery after pretreatment (Negi and Pandey 2015).

2.4 Lignocellulosic Biomass Degradation Enzymes for the Production of High Value-Added Bioproducts

Nowadays, several enzymes are produced and applied in large scale in many industrial areas such as paper/cellulose, food, animal feed, pharmaceuticals, and water treatment. Glycohydrolases (GH) are enzymes able to break down the glycosidic bonds of lignocellulosic biomasses to obtain high amounts of reducing sugar for second ethanol production and high value-added bioproducts.

An efficient hydrolysis of lignocellulosic materials depends on a set of enzymes that are able to break it down into fermentable sugars or directly into products of commercial interest. Combined enzymatic extracts rich in cellulases and hemicellulases increased expressively biomass hydrolysis yields (Kumar and Wyman 2009; Robl et al. 2015a). One of the main reasons is that xylobiose and higher xylooligosaccharides (XOS) inhibit enzymatic hydrolysis of xylan, glucan, and cellulose (Kumar and Wyman 2009; Qing et al. 2010). Nowadays the partial hydrolysis of hemicelluloses becomes a potential opportunity market for XOS production once that these molecules can be applied as prebiotics and to enhance beneficial human colon bacteria (Deutschmann and Dekker 2012).

2.4.1 Production of Lignocellulose Degrading Enzymes

The main enzymes used for the degradation of lignocellulosic materials are from fungi kingdom and actinobacteria members. It has become clear that in nature each microorganism is specialized in the decomposition of a part of cell wall and produces a set of enzymes that acts in synergy for the entire degradation of lignocellulosic materials. In fungi the most explored species are from *Trichoderma* genera for cellulases, *Aspergillus* for hemicellulases and pectinases, and white-rot fungi for lignin oxidative enzymes. Filamentous bacteria, especially in *Streptomyces* genera, have shown capability to produce cellulases and hemicellulases (Robl et al. 2019). However, bacterial cellulase production is lower when compared with fungi strains.

In order to reduce enzyme production costs, agro-waste is broadly used. The agro-waste depends on the region and availability. Highest xylanase production was achieved with wheat bran combined with other wastes, corn cobs, and malt sprout (996.3 U/mL) (Dobrev et al. 2007). Other wastes rich in xylose and xylooligomers

also presented promising results, such as the liquor of the hydrothermal pretreatment of sugarcane bagasse (458 U/mL) (Robl et al. 2015a). Even though agro-wastes are potential in culture media formulation, they present challenges to overcome, such as the presence of inhibitors. Most of the wastes used in hemicellulase and cellulase production are from lignocellulosic materials. In this way, lignin and furfurals are the principal inhibitors produced during feedstock processing that impact negatively in microbial metabolism and enzymatic production (Robl et al. 2015a).

The statistical tools are extensively applied in enzyme production, especially the design of experiments (DOE) which allows a culture media optimization approach (Robl et al. 2015b; Costa et al. 2016, 2017). Several authors have seen that the culture media play a role in cellulase and hemicellulase production mainly according to the nature of the carbon source. Costa et al. (2016) rationally modulated the glycoside hydrolase (GH) activity of the enzymatic complex secreted by *Penicillium echinulatum* using adjustment of the culture medium composition. Robl et al. (2013) showed that endophytic fungi produced different set of GHs according to the main carbon source used in the media.

Genetic modification of microorganisms used in the production of lignocellulolytic enzymes has been conducted in the recent years. Classical random mutagenesis methods (UV and chemical) were used to obtain hypercellulolytic strains, such as *T. reesei* RUT-C30 (Peterson and Nevalainen 2012), *P. echinulatum* 9A02S1 (Dillon et al. 2006), and *Acremonium cellulolyticus* CF-2612 (Fang et al. 2009). Heterologous expression of GH encoding genes has been widely explored and can be a tool for GH platform construction. Bauer et al. (2006) cloned 72 genes encoding polysaccharide-degrading enzymes from *A. nidulans* and expressed as secreted proteins in *Pichia pastoris*. Further, Tramontina et al. (2016) used these clones to understand the cooperation of several GH involved in the degradation of xylan, glucan, xyloglucan, and crude plant biomass.

Two possible ways of lignocellulolytic enzyme production can be performed, viz. the submerse cultivation (SmF) and solid-state cultivation (SSF). In SmF the microorganism grows in high free water medium, which allows higher homogenization grade. This characteristic gives notable advantages for this bioprocess in mass/heat transfer (mixing, oxygen transfer, and cooling) and process control (temperature, dissolve oxygen, and pH) (Soccol et al. 2017).

The SSF simulates the natural environment of several microorganisms, principally the filamentous fungi and several authors report the production of biomass degrading enzymes by this type of fermentation (Farinas 2015). Besides, once that less water is available, and the mixing is limited, lower energy is required, and less water wastes are produced during the process (Viniegra-González et al. 2003; Soccol et al. 2017). In GH production, it seems that SSF achieves higher production parameters when compared to SmF. Viniegra-González et al. (2003) developed a general approach to compare productivity of three fungal enzymes (invertase, pectinase, tannase) and verified that in these three cases the productivity using an SSF system was higher than in SmF. Even though several publications on SSF have been done during the last decades, a major part of them focused on simple lab scale

experiments. According to Arora et al. (2018), the lack of efficient bioreactor design, lack of mathematical models and the lack of effective process monitoring and control are factors that challenge SSF industrialization.

2.4.1.1 Fungal GH Physiology

The culture media influences directly the GH production. Fungal genes encoding carbohydrate-active enzymes are under a regulatory mechanism to save energy during life cycle and direct the metabolism to the available carbon sources at any given time (Kowalczyk et al. 2014). One of these mechanisms is the carbon catabolic repression (CCR) that consists of the production of a repressor in the presence of glucose and consequently inhibition of the expression of enzyme genes related with polysaccharide hydrolysis and metabolism (Ruijter et al. 1997). Other saccharides are able to induce the expression of this repressor in higher concentration such as fructose, xylose, and sucrose (Graaff et al. 1994). CCR regulation was described in several fungi species; in *T. ressei* is regulated by the protein Cre1, in *A. niger* and *A. nidulans* by CreA, and *Neurospora crassa* by Cre1 (Brown et al. 2014). In order to increase lignocellulolytic enzyme production, genetic modifications have been performed in ascomycetes strains. Disruption of CreA or similar proteins in *A. cellulolyticus* (Fujii et al. 2013), *T. reesei* (Nakari-Setälä et al. 2009), *Humicola insolens* (Xu et al. 2019b), and *A. niger* (de Vries et al. 1999) led to an improvement of cellulase and hemicellulase production. However, these studies revealed that those modifications affected negatively the strain growth rate, which is an undesirable physiological characteristic for a bioprocess strain.

Besides that, many ascomycetes present another regulatory mechanism for hemicellulase production in transcriptional and translation level. These mechanisms are based on a network of transcriptional regulators of GH genes and proteins. *A. niger* has several hemicellulolytic transcriptional activators that have been described like XlnR, AraR, InuR, GalR, RhaR, and ManR (Kowalczyk et al. 2014). The xylanolytic activator (XlnR) is a protein constitutively present among *Aspergilli* that is produced in the presence of xylose (Battaglia et al. 2011). Also in *A. niger,* xylose presence is necessary for XlnR phosphorylation and increase its affinity to target genes promoters (Hasper 2004). Genetic modifications based on CCR of an XlnR were used as strategy to improve fungi hemicellulase production. Robl et al. (2018) developed an *A. niger* strain that expresses constitutively an active version of XlnR combined with a deficiency in CCR by deletion of the *CreA* gene and were able to double the productivity of β-xylosidase. Xia et al. (2019) expressed xylanase transcriptional regulators of *T. reesei* (Xyr1) and *N. crassa* (XLR-1) in *P. oxalicum* with their corresponding target cellulase genes and were able to increase cellulase production in 2.8-folds.

2.4.2 Lignin Oxidative Enzymes as Tools for Bioproducts

Lignin is a heterogeneous polymer that provides strength and rigidity to wood and protects cellulose and hemicelluloses from microbial attack (Floudas et al. 2012). It

is a phenylpropane polymer that connects the cell wall polysaccharides mainly by hydroxycinnamic acids, like p-coumaric acid and ferulic acid. These acids are found principally in ester bonds with xylan arabinose, and glucuronoyl residues (Sun et al. 2001). This bond impacts negatively in plant cell wall hydrolysis, and this removal results in better GH accessibility to cellulose fiber (Taherzadeh and Karimi 2008).

Several oxidases such as laccases and peroxidases can be applied for lignin removal, which is an important step for paper pulping and ethanol industry. This polymer used to be considered as a waste being burned for energy supply. However, lignin could be used as a feed stock for aromatic chemicals and polymers of high value-added products (Belgacem and Gandini 2008).

Ligninolytic fungi and their enzymes show promiscuity in the oxidation of aromatic compounds. This ability seems to be associated with an unspecific extracellular system capable to act on a heterogeneous polymer, the lignin (Ruiz-Dueñas and Martínez 2009). Laccases (EC 1.10.3.2) are a multi-copper oxidase that catalyzes phenol oxidation molecules and oxygen reduction into water. This enzyme can act on diverse molecules including benzenediols, aminophenols, polyphenols, polyamines, and lignin-related molecule (Couto and Herrera 2006). The oxidation of ferulic acid by laccase from *Myceliophthora thermophila* produced yellow-colored products with potential for food application (Mustafa et al. 2005).

In contrast with laccases, the ligninolytic peroxidases do not require mediators to degrade high-redox-potential compounds (Ruiz-Dueñas and Martínez 2009). The lignin degradation is an oxidative process, where the extracellular hydrogen peroxide oxidizes the polymer in a reaction catalyzed by high redox-potential hemeperoxidases (Martínez et al. 2009). This fungal class II peroxidases (PODs) are classified into four major groups, including three ligninolytic forms—lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP)—and a fourth POD type defined here as "generic peroxidase" (GP) (Floudas et al. 2012). Several microorganisms have been studied for ligninase production such as a white-rot and brown-rot fungi. In the last decade, genomes of wood decay fungi were obtained, and large amount of information regarding ligninolytic models were obtained, e.g., *Phanerochaete chrysosporium* (Martinez et al. 2004). However, Floudas et al. (2012) brought out that only a small part of the biodiversity of white-rot fungi and its lignin decomposition enzymes were explored.

2.5 Xylooligosaccharides: Emerging Bioproduct from Hemicelluloses Fraction

The hemicelluloses fraction from lignocellulosic biomass is an important raw material that has been used in oligosaccharide production. The xylan polysaccharide extracted from the biomass is hydrolyzed to the suitable polymerization degree containing from 2 to 10 xylose units. The xylooligosaccharides (XOS) produced are non-digestible carbohydrate, showing prebiotic effect leading to a cascade of beneficial effects to the host health. In addition, the branched structure of XOS is decorated with substituents such as acetyl group, uronic acids, and arabinose units

that contributed with their solubility and their physiological effects (Mhetras et al. 2019).

The most known oligosaccharides in the prebiotic market are the fructooligosaccharides (FOS) and galactooligosaccharides (GOS), which are produced by enzymatic synthesis from sucrose (Nobre et al. 2015) and lactose (Panesar et al. 2018), respectively. XOS is already industrially produced by some companies and the forecast to the worldwide market expected an increase from 86 to 120 million US$ between 2019 and 2024. Besides the growing market, there are other motivators for XOS production, which are mentioned below: (1) favored competitiveness when compared with other oligosaccharides since the price per recommended dose to achieve the expected prebiotic effects is lower (Amorim et al. 2019b); (2) high selectivity for beneficial bacteria—no pathogenic or harmful bacteria grow in XOS, while other oligosaccharides can be assimilated by them (Saville and Saville 2018); (3) better heat and acidity resistance than FOS, which make it more interesting from the technical point of view as a food ingredient (Mano et al. 2018). Despite the advantages cited, there are still some challenges with regard to XOS production such as the yield and costs of the final product.

2.5.1 From Xylan Structure to Xylooligosaccharides

The lignocellulosic material used to produce XOS must be rich in xylan, the most abundant polysaccharide that composes the hemicelluloses. Xylan consists of β-D-xylopyranosyl (xylose) residues linked via β-1,4 glycosidic bonds (Naidu et al. 2018). The most common lignocellulosic materials reported in the literature for XOS production are the agro-wastes such as sugarcane bagasse (Goldbeck et al. 2016; Li et al. 2019), corn wastes (Boonchuay et al. 2018; Arai et al. 2019), wheat straw (Álvarez et al. 2017; Xu et al. 2019a), and several types of hardwoods (Azelee et al. 2016; Nieto-Domínguez et al. 2017; Gullón et al. 2018). Despite the great availability of agro-wastes, the process faces some challenges regarding the XOS production and costs.

Direct hydrolysis of lignocellulosic biomass is a costly effort because hemicelluloses is not readily accessible to enzymes. Therefore, to overcome this problem, the enzymatically produced XOS is commonly divided into two stages (Fig. 2.1): (1) extraction of xylan from the lignocellulosic material applying chemical and/or hydrothermal methods and (2) enzymatic hydrolysis of xylan to obtain hydrolyzed oligomers with a degree of polymerization between 2 and 7.

First, the recovery of xylan from the three-dimensional structure of lignocellulosic biomass requires the use of pretreatments, since hemicelluloses is strongly bound to lignin and cellulose (de Freitas et al. 2019). The common pretreatment methods for xylan extraction are dilute acid, alkaline, and hydrothermal (combined or not combined). These methods are used under conditions optimized for high recovery of xylan chains from the biomass, avoiding the degradation of the polysaccharide and the formation of undesirable compounds. Second, although the enzymatic hydrolysis is the preferred method for the hydrolysis of xylan in XOS, its yield

is influenced by the type of enzyme, xylan composition, pretreatment applied to the xylan extraction, and other parameters of enzymatic reaction (Carvalho et al. 2013). In addition, xylanolytic enzyme cocktails should have specific enzymes to prevent xylose production and promote efficient production of short-chain XOS.

2.5.2 Lignocellulosic Biomass Pretreatment for XOS Production

Pretreatment of lignocellulosic biomass plays an important role in the production of XOS, in which the hemicelluloses can be removed from the material and depolymerized into water-soluble saccharides. The choice of the pretreatment is governed by the characteristics of each lignocellulosic material and aims to preserve hemicelluloses fraction. The researches have focused on improving the efficiency of xylan extraction and reducing the chemicals used, and consequently, reducing the total production costs (Naidu et al. 2018).

In addition to the costs, the pretreatment of lignocellulosic biomass faces some challenges that directly affect the efficiency of the process. The control of xylan extraction with minimal xylose formation depends on the pretreatment conditions and how the biomass responds to the pretreatment. The formation of toxic compounds, such as furfural, and hydroxymethylfurfural (HMF) are especially encountered when treatment with acid and high temperatures are used (Otieno and Ahring 2012; Amorim et al. 2019b). The production of xylose and toxic compounds during the pretreatment is undesired, which demands more efforts in the purification step. The presence of toxic compounds can affect the performance of enzymes by inhibitory effects during the stage of enzymatic hydrolysis (Romaní et al. 2014; Álvarez et al. 2017) as well as may have an inhibitory effect on bacteria of the gut microbiota (Hong et al. 2019).

Other biomasses such as brewer's spent grain (Sajib et al. 2018), vine shoots (Dávila et al. 2016, 2019), wheat bran (Morgan et al. 2017), and chestnut shell (Gullón et al. 2018) have also been studied as raw materials for XOS. Some challenges are faced when the waste contains compounds that can affect the yield and purity of XOS. The brewer's spent grain and wheat bran, for example, have a significant amount of starch, which needs to be removed prior to the extraction of xylan to provide a product with improved yield and purity (Mathew et al. 2017; Morgan et al. 2017; Sajib et al. 2018). Large amounts of phenolic compounds can also be found in lignocellulosic materials. A high selectivity method was reported to isolate the antioxidant compounds and XOS extracted in the same step during the hydrothermal treatment from the vine shoot (Gullón et al. 2017). Wheat straw has large amounts of ash which affect the efficiency of pretreatment and enzymatic hydrolysis (Huang et al. 2017). The purification of XOS is an important step because food and pharmaceutical ingredients require high purity ranging from 75 to 99%, and this should be taken into account in the process downstream.

2.5.3 Enzymatic Hydrolysis for XOS Production

After the pretreatment of lignocellulosic biomass, the liquor containing xylan and XOS (high degree of polymerization) are submitted to enzymatic hydrolysis by hemicellulase or xylanases (Basit et al. 2018). The key enzymes present in xylanolytic cocktails are endo-xylanase and β-xylosidase. Endo-xylanase acts on the xylan backbone producing xylooligomers of short chain, while β-xylosidase acts on these xylooligomers releasing xylose (Juturu and Wu 2012). For this reason, engineered microorganisms have been used to produce recombinant xylanases, mainly endo-xylanases with high substrate specificity (Liu et al. 2017; Amorim et al. 2019a; Katsimpouras et al. 2019). The presence of β-xylosidase is not desirable because the released xylose reduces the purity degree of XOS and can cause an inhibitory effect on endo-xylanase.

Xylanases from families GH10 and GH11 are the most studied enzymes because they act as endo-xylanases. The families of xylanases have been differentiated according to the end products released from the hydrolysis of xylan (e.g., xylose, xylobiose, xylotriose, and arabinose). Enzymes have different binding interactions at the active sites, a consistently different substituent pattern in the XOS and arabino-XOS produced by GH10 and GH11 (Mathew et al. 2017). Thus, xylanases may be classified as non-debranching (arabinose non-liberating) or debranching (arabinose liberating) enzymes (Juturu and Wu 2012). Besides that, the enzymes have specificities with respect to the xylan chain decorated with acetyl groups and 4-O-methyl-D-glucuronic acids also releasing different pattern products (Puchart et al. 2018). The type and conditions of pretreatment carried out for the extraction of xylan directly influence chain deacetylation and, consequently, affect the efficiency of these enzymes. GH11 endo-xylanases are more strongly influenced by the content of acetyl groups than GH10 endo-xylanases. These groups limit the accessibility of enzymes to the xylan backbone (Hu and Saddler 2018). However, the presence of accessory enzymes such as acetyl esterase and feruloyl esterase contributes to the generation of much simpler XOS patterns (Puchart et al. 2018).

Table 2.3 summarizes the XOS yield obtained in the most recent studies for enzymatically produced XOS preceded by a pretreatment. Enzymatic hydrolysis was applied in the liquor or in the solid from the pretreatment and the yield is presented as $Y_{XOS/xylan}$ (mg/g). A high XOS yield was obtained by the hydrolysis of the mixture of water-soluble fraction and a water-insoluble fraction of corncob treated by an acidified water-assisted steam explosion (Liu et al. 2018). The novel recombinant xylanase (PbXyn10A) acted as an endo-type manner and the content of XOS (xylobiose, xylotriose, and xylotetraose) was high up to 90%. The performance of this enzyme was considered very promising since the yield obtained is one of the highest reported in the literature (750 mg/g xylan).

The direct hydrolysis of lignocellulosic material in XOS is more difficult due to the natural recalcitrance of the biomass. The production of XOS by this method also requires a pretreatment to enhance the enzymatic digestibility of the solids, which tends to reduce the enzyme dosages and, finally, increase the xylan recovery yield (Huang et al. 2017). The recalcitrance of the material can be reduced by the removal

Table 2.3 Conditions of pretreatment and enzymatic hydrolysis in the production of XOS. Enzyme tested in the respective substrate and the corresponding yield

Substrate/ enzyme	Conditions of pretreatment	Enzymatic hydrolysis condition	Y_{XOS} (mg/g xylan) hydrolysis result	Reference
Corncob Xylanase (PbXyn10A) from *Paenibacillus barengoltzii*	Steam explosion, 165 °C, 35 min, SL 1:12	50 U/mL, 50 °C, 12 h, 150 rpm	750 X2–X5	Liu et al. (2018)
Sugarcane bagasse Endo-xylanase from *Kitasatospora* sp.	Sodium hypochlorite 1% (w/v) followed by sodium hydroxide 15% (w/v), 24 h	Enzyme combination GH10 and GH11, substrate concentration 1% (w/v), 50 °C for 72 h, 190 rpm	350 X2–X6, no release of xylose	Rahmani et al. (2019)
Arecanut husk Endo-xylanase M1 (*Trichoderma viridea*, Megazyme)	Sodium hydroxide 10% (w/v), S/L 1:10, 65 °C, 8 h	10 U/mL, substrate concentration 2% (w/v), 50 °C, 12 h	351 X1–X4	Singh et al. (2018)
Mahogany wood Mango wood Xylanase from *Clostridium* sp. BOH3	Sodium hydroxide 15%, (w/v), S/L 1:6, 24 h	1 U/mL, substrate concentration 5% (w/v), 50 °C, 24 h, 75 rpm	572 504 X2–X3	Rajagopalan et al. (2017)
Wheat straw Endo-xylanase from *Trichoderma reesei*	De-ashing followed by hydrothermal S: L 1:10, 180 °C, 40 min	0.06 mg protein per mL hydrolizated, 50 °C, 48 h, 150 rpm	478 X2–X6	Huang et al. (2017)
Enzymatic hydrolysis of pretreated solid				
Sugarcane bagasse Xylanases (SUNSON industry group)	Alkaline oxidation pretreatment (sodium hydroxide 1%, hydrogen peroxide 1.5%), SL 1:20, 70 °C, 6 h	300 U/g, substrate concentration 5% (w/v), 50 °C, pH 5, 800 rpm	361 X2	Li et al. (2019)
Reed scraps Xylanase (Qingdao blue biological technology)	Hydrothermal (1:8 w/v), 170 °C, 0.5 h	66 U/g, substrate concentration 5% (w/v), 8 h, 120 rpm	681	Chen et al. (2019)
Rice straw β-Xylosidase from *Weissella cibaria*	Ammonium hydroxide 27% (w/v), (1:12 w/v), 60 °C for 7 days	50 U/g pretreated substrate, substrate concentration 2% (w/v), 37 °C, 10 h, 150 rpm	542 46.8% X1, 15.9% X2, 13.6% X3–X5, 27.3% > X5	Le and Yang (2019)

(continued)

Table 2.3 (continued)

Substrate/ enzyme	Conditions of pretreatment	Enzymatic hydrolysis condition	Y_{XOS} (mg/g xylan) hydrolysis result	Reference
Oil palm frond bagasse Cellic Htec2 (Novozymes)	Diluted nitric acid 0.1% (w/v), 60 °C, S/L 1:10, 12 h	50 U/mL, substrate concentration 1% (w/v), 55 °C, 4 h	555 X2	Mazlan et al. (2019)
Wheat chaff Endo-xylanase NS 22083 (Novozymes)	Ultrasonic waves, 25 kHz, 540 W for 10 min, 3% (w/v) in water	0.3 U/g pretreated substrate, substrate concentration 5% (w/v), 50 °C, 9 h	376 X2–X5	Antov and Đorđević (2017)

SL solid–liquid ratio; Yields were calculated from the results informed in the text and converted in mg XOS per g xylan

of lignin and enhance the enzymatic digestibility of the pretreated solid, mainly for the biomass with high amounts of lignin (Rajagopalan et al. 2017). However, the use of commercial cocktails, which usually contain cellulase activity, does not appear to be advantageous, since other monomers can be released from the cellulose, including glucose and cellobiose by-products (Li et al. 2019).

Biomass such as wheat bran (Wu et al. 2017; Mathew et al. 2018) has lower amounts of lignin compared to hardwoods or sugarcane bagasse, presenting good yield of XOS when xylanases are applied directly on the biomass. Wu et al. (2017) showed that XOS yield increased with synergistic action of two xylan-degrading enzymes, xylanase, and feruloyl esterase. The removal of ferulic acid from the xylan backbone causes the opening of the substrate structure, improving the action of xylanases.

Production of XOS by direct fermentation using brewer's spent grain was also proposed (Amorim et al. 2018, 2019a, c). In this type of production, the enzymes are produced directly from the biomass releasing the oligosaccharides. However, the success of this method is governed by the type of biomass and microorganism used (Amorim et al. 2019c). Low content of xylose is obtained in the final product, due to the microorganism preference for readily available sugars, which are first consumed before XOS degradation.

2.6 *Spathaspora passalidarum*: Deciphering Physiological and Processual Parameters for Ethanol and Chemicals Production by Lignocellulosic Biomass Hydrolysates

As previously described, lignocellulosic biomass is a magnificent renewable source of polysaccharides. The cell wall, which is the main component of lignocellulosic biomass, is a recalcitrant structure constituted by polymers, composed primarily of

fibrous cellulose intertwined with hemicelluloses and lignin (Rubio et al. 2015). Regarded as a prospective feedstock for value-added products generation, this raw material can be converted into biofuels and chemicals, supporting the worldwide aimed sustainable production process (Arevalo-Gallegos et al. 2017).

In contrast to single sugar use, mixed sugar bioprocesses, which are often obtained from hydrolysis of lignocellulosic biomass (pretreatment and enzymatic hydrolysis), present significant challenges for cost-effective ethanol and chemicals production (Zhang et al. 2015). Considering techno-economic reasons, it depends on the complete and efficient conversion of all carbohydrates, including those from the cellulosic and hemicellulosic fractions (co-fermenting glucose and non-glucose sugars, mainly xylose—C5).

Thus, robust microorganisms that are able to efficiently ferment all sugars (C5 and C6 fractions) present in lignocellulosic hydrolysates are one of the key factors for cost-effective lignocellulosic ethanol and chemical production (Cadete et al. 2016). Despite substantial genetic modification strategies in this field, there are still metabolic bottlenecks that need to be addressed, such as xylose uptake and transport, catalytic efficiency of xylose metabolism enzymes, and increased specific growth in xylose. Moreover, the use of genetically modified microorganisms in the industry is subject to strict biosafety regulations that may itself prevent their large-scale application, in some cases (Moysés et al. 2016).

Some yeast species as *Scheffersomyces stipitis* (previously known as *Pichia stipitis*), *Scheffersomyces shehatae* (previously known as *Candida shehatae*), *Candida tenuis*, *Debaryomyces hansenii*, *Pachysolen tannophilus* and *Spathaspora passalidarum*, are able to naturally consume and ferment xylose (present in hemicelluloses) as well as glucose (present in cellulose) (Urbina et al. 2013).

Despite the number of studies being carried out in order to fully cover and understand the metabolism of pentoses in those species, the same is not observed with *S. passalidarum*, in spite of its ability to consume xylose at higher rate in comparison with glucose when both sugars are in the same medium (Santos et al. 2016; Nakanishi et al. 2017), as well as co-ferment xylose and cellobiose in the presence of glucose, proving its advantages over *S. stipitis* (Long et al. 2012).

S. passalidarum is a fungi of the phylum Ascomycota, first found on the guts of woodboring beetles in the state of Louisiana (USA) by Nguyen et al. (2006). Due to earlier findings and literature reports on xylose-fermenter yeast associations with woodboring beetles, tests have been run to prove the hypothesis that new yeasts with the ability to ferment pentoses would be found in association with those insects. Probable woodboring beetle hosts were collected and disinfected, and their guts were removed aseptically, then crushed in saline solution and streaked on acidified yeast medium agar. Cultures were incubated at 25 °C and purified twice. After the screening of 300 isolates regarding grouping by D1/D2 sequencing of the LSU *rRNA* gene (rDNA) and morphological and physiological observations, the sequenced and newly isolated *Candida jeffriesii* and *S. passalidarum* were compared with a variety of yeasts previously sequenced.

S. passalidarum has the ability to produce different chemical blocks of interest such as D-ribitol, D-ribulose, D-xylitol, glycerol, acetic acid, 2,3-butanediol, and

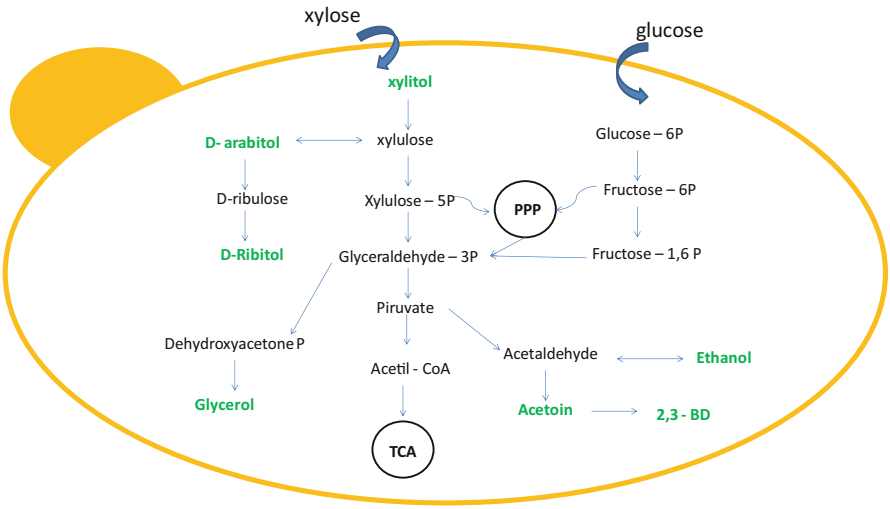


Fig. 2.2 Proposed metabolic pathway for xylose and glucose consumption by *Spathaspora passalidarum* for chemical production. *TCA* tricarboxylic acid cycle, *PPP* pentose phosphate pathway. (Source: Modified from Su et al. 2015)

ethanol, among others (Su et al. 2015). In this section, we make a review of the most relevant chemical blocks produced by *S. passalidarum* and its commercial interest, including ethanol, xylitol, glycerol, acetoin, and 2,3-butanediol (2,3-BD) (Fig. 2.2). Studies on *S. passalidarum* aim at improving the production of those compounds using different types of substrates and operational strategies.

2.6.1 Ethanol

Since isolation of novel xylose-fermenting yeasts *S. passalidarum* (Nguyen et al. 2006), most researches have been focusing on understanding and enhancing production of ethanol from different raw materials, mostly from lignocellulosic biomasses, due to the capacity of this microorganism to ferment a wide range of sugars present on lignocellulosic materials (Nguyen et al. 2006) and the commercial interest of ethanol as a more sustainable biofuel (Garcia-Ochoa and Gomez 2009). Many approaches have been adopted throughout the years to improve ethanol production.

Su et al. (2015) investigated the effect of aeration on fermentation of three different culture media (15% xylose, 15% glucose, or 12% xylose plus 3% glucose), employing different oxygen-limiting conditions on *S. passalidarum* NRRL Y-27907. When using 150 g/L of xylose, the maximum specific fermentation rate was observed (0.153 g/L h), with an ethanol yield of 0.448 g/g, at an oxygen transfer rate of 2.47 $mmol_{O2}$/L h. When increasing the OTR (oxygen transfer rate) to 4.27 $mmol_{O2}$/L h, ethanol yield decreased from 0.46 to 0.42 g/g while increasing

volumetric ethanol productivity from 0.52 to 0.8 g/L h, when xylose was the sole carbon source. Thus, fermentation time decreased as aeration increased under the microaerobic range evaluated.

By applying a strategy of high initial cell concentration, cell recycle, operation under fed-batch and temperature decrease between cycles, Nakanishi et al. (2017) achieved an increase on volumetric productivity from 0.38 to 0.8 g/L h for *S. passalidarum* NRRL Y-27907, from first to fourth cycle, using sugarcane bagasse hydrolysate as substrate. This strategy is widely applied in the first-generation ethanol mills and showed to be beneficial for the second-generation ethanol production.

With a different approach, Hou and Yao (2012) used a combination of UV mutagenesis and protoplast fusion to construct strains with enhanced performance, improving the tolerance of *S. passalidarum* NRRL Y-27907 for inhibitors from lignocellulosic materials. Hou and Yao (2012) constructed a strain that produced 50% more ethanol than the wild-type strain in a synthetic xylose medium containing 2 g/L of the inhibitory compound furfural.

Therefore, different strategies have been evaluated in the past few years and proved to be beneficial to improve fermentation performance of *S. passalidarum* strains.

2.6.2 Xylitol

Xylitol ($C_5H_{12}O_5$) is a polyol with a sweetening power similar to sucrose, with 40% less calories. Xylitol was discovered in 1891 by chemist Emil Fischer and his team, where from the reaction of xylose with sodium amalgam, it was obtained in syrup form (Molinary and Quinlan 2012). Xylitol has anticariogenicity, the ability to inhibit the growth of oral bacteria, especially *Streptococcus mutans*, reducing plaque (Molinary and Quinlan 2012), because the bacteria do not use xylitol, (Cai et al. 2009). Xylitol does not contribute to the Maillard reaction because it does not have ketone or aldehyde groups, as these are responsible for the darkening and reduction of the nutritional value of proteins, making it suitable for use in the food industry.

Some yeast has the ability to convert xylose to D-xylulose via an oxido-reductive pathway consisting of two sequence reactions. The first, xylose reductase (XR), in the presence of NADH and/or NADPH converts D-xylose to xylitol. Subsequently, xylitol is transformed into D-xylulose by the enzyme NAD + -linked xylitol dehydrogenase (XDH) as well as by NADP+. Thus, xylulose can be phosphorylated to xylulose-5-phosphate and can be converted by non-oxidative reactions of the phosphopentose pathways (Hahn-Hägerdal et al. 1994). According to Yokoyama et al. (1995), microorganisms with NADH-dependent XR enzyme are better ethanol producers, whereas those with NADPH-dependent xylose reductase produce more xylitol. In addition, the availability of oxygen influences the requirement of enzyme cofactors. Under anaerobic conditions and oxygen limited conditions, they cause redox imbalance, interfering with the production of xylitol and by-products, ethanol and/or glycerol. Some authors have investigated xylitol production by

S. passalidarum. Su et al. (2015) evaluated ethanol production together with polyol accumulation in three different fermentations, the first being 15% glucose, followed by 15% xylose and a mixture of both containing 12% xylose and 3% of glucose. According to Su et al. (2015), there was no accumulation of xylitol when the fermentation was by glucose alone (15%). However the fermentation by xylose alone (15%) had an accumulation of 0.8–1.4 g/L of xylitol. In contrast, the mixture of glucose and xylose had a higher accumulation of xylitol, being 2.9–3.8 g/L, demonstrating that this yeast has its metabolism diverted to xylitol production (Su et al. 2015).

2.6.3 Glycerol

Glycerol is an important building block for the synthesis of many products, including cosmetics, automotive, pharmaceutical, and food industries (Semkiv et al. 2017). It can be recovered as a by-product of soap manufacturing or produced from polypropylene. As a more environmentally friendly process, glycerol can also be obtained through microbial fermentation (Wang et al. 2001), with the use of a wide range of microorganisms, but mostly yeasts (Overkamp et al. 2002). In yeasts, glycerol is produced by the reduction of DHAP (dihydroxyacetone phosphate) to G3P (glycerol-3-phosphate), with G3P-dehydrogenase as catalyst. G3P is then dephosphorylated by a glycerol-3-phosphatase.

Concerning yeast *S. passalidarum*, glycerol can be produced from lignocellulosic sugars xylose or glucose. However, its production is smaller in comparison to ethanol, which was observed in a fermentation with synthetic medium composed of glucose and xylose (1.72 and 2.29 g/L, respectively), where the final concentration of glycerol was less than 0.03 g/L in a non-continuous process. It is important to highlight that this study used adapted *S. passalidarum* strains that obtained the same glycerol productivity in comparison to the native strain (Hou and Yao 2012).

Four strains of *S. passalidarum* (UFMG-HMD-1.1; UFMG-HMD-14.1; UFMG-XMD-16.2; and UFMG-XDM-23.2) were also individually investigated in batch fermentation using sugarcane bagasse hydrolysates as substrate (Cadete et al. 2012). After 96 h, glycerol concentration reached 0.05 g/L, 0.1 g/L, 0.2 g/L, and 0.6 g/L, respectively, values that were inferior than the concentration of ethanol. Using the yeast *S. passalidarum* NRRL Y-27907, Morales et al. (2017) obtained glycerol concentrations below 2 g/L when *Eucalyptus globulus* hydrolysate was fermented under a non-continuous process. However, they also observed that, after dilution of the hydrolysate with distilled water (25%) and using *S. passalidarum* mutated by UV, the final concentration of glycerol increased to 22 g/L. Su et al. (2015) observed that *S. passalidarum* NRRL Y-27907 accumulated more glycerol when cultivated on glucose or on a mixture of glucose and xylose than when cultivated only on xylose, reaching a maximum concentration of 1.9 g/L.

Another study performed by Nakanishi et al. (2017) with the application of cell recycle technology showed that the decrease of temperature between the cycles (from 30 °C to 27 °C) during a fed-batch fermentation, using *S. passalidarum*

NRRL Y-27907 and sugarcane bagasse hydrolysates as substrate (42.9 g/L of glucose and 14.9 g/L of xylose), decreased the final concentration of glycerol in the fermentation. The highest glycerol concentration obtained was on the first cycle at 30 °C (approximately 0.7 g/L). The formation of glycerol by *S. passalidarum* is low or sometimes even null, thus, this microorganism has low glycerol productivity. The low glycerol productivity presents an advantage to the process of ethanol production, once consumption of carbon is directed to ethanol and cell biomass (Wohlbach et al. 2011; Silva et al. 2012).

2.6.4 Acetoin and 2,3-Butanediol

As one of the by-products of interest that can be produced by *Spathaspora passalidarum*, 3-hydroxy-2-butanone, commonly known as acetoin, is an important flavor compound. Due to its natural cream aroma flavor, it can be applied in food and beverage industry as a flood flavor enhancer (Chen et al. 2013; Jia et al. 2017). Acetoin is mainly produced from fossil feedstocks through chemical conversion of 2,3-butanediol, butanone, or diacetyl, which are not environmentally friendly chemical processes (Jia et al. 2017). Therefore, researches on the obtainment of this compound by biotechnological methods are increasing, including microbial fermentation, which not only displays advantages in environmental-friendly sources of raw materials but also has benefits in lowering production costs.

In the metabolism pathway of yeast *S. passalidarum*, acetoin can be converted to 2,3-butanediol (2,3-BD) by 2,3-butanediol dehydrogenase (also known as acetoin reductase) using NADH as a cofactor. Therefore, to accumulate acetoin, 2,3-butanediol production should be inhibited, which can be achieved by two different strategies: disruption of 2,3-butanediol dehydrogenase and overexpression of NADH oxidase.

On the other hand, the compound 2,3-butanediol is also a platform chemical applied in the synthesis of valuable products in cosmetic, pharmaceutical, food, and solvent industry. 2,3-BD can be converted to 1,3-butadiene, a precursor for synthetic rubber, which has increased the demand of 2,3-BD due to the growth of 1,3-butadiene market. The use of low-cost lignocellulosic biomass for obtainment of this product is interesting to make this process sustainably and economically feasible (Kim et al. 2014).

Although great efforts have been made to obtain acetoin and 2,3-BD by genetically modified bacteria (Chen et al. 2013; Jia et al. 2017; Kim et al. 2017) and *S. cerevisiae*, the accumulation of these products in native pentose-fermenting yeasts was recently investigated (Su et al. 2015). In comparison with *S. stipitis*, authors reported that both yeasts produced acetoin, meso-2,3-BD, and R,R-2,3-BD during fermentation. Regarding yeast *S. passalidarum*, higher concentrations were obtained by increasing the aeration, accumulating four times more R,R-2,3-BD than *S. stipitis*, being the most important by-product after xylitol.

2.7 Conclusion

Recently, the IPCC (Intergovernmental Panel on Climate Change) published a scientific report (Climate Change and Land) pointing out that environmental problems linked to global warming are not related only to the use of fossil sources but also the misuse of the land. Associated with these problems, population estimates are expected to reach 9.7 billion people by 2050. Thus, many efforts have been made to achieve the total reuse of the lignocellulosic biomass fractions, and its advances were demonstrated by the several bioproducts discussed in this chapter. The development of new strategies and new process for biomass pretreatment is focused to achieve an adequately depolymerization of polymers that constitute the cell wall biomass (mainly cellulose and hemicelluloses), allowing high yields of carbohydrates without release of toxic compounds. Despite the development of new classes of enzymes and genetically modified microorganisms, it is not yet possible to achieve high yields of biomass deconstruction due to its recalcitrance. More attention has recently been paid to the hemicelluloses fraction due to the valuable by-products obtained from it. XOS are a new class of prebiotic produced industrially mainly by corncob, i.e., new opportunities can be generated from other biomasses. Important bioproducts produced from xylose can be a promising alternative when this sugar is metabolized by specific strains such as *S. passalidarum*.

Acknowledgments The authors would like to thank the Federal University of Santa Catarina (UFSC) and College of Agricultural Sciences, São Paulo State University (UNESP) for their support.

Conflict of Interests: The author declares no conflict of interest.

References

Abramson M, Shoseyov O, Hirsch S, Shani Z (2013) Genetic modifications of plant cell walls to increase biomass and bioethanol production. In: Lee J (ed) Advanced biofuels and bioproducts. Springer, New York. https://doi.org/10.1007/978-1-4614-3348-4_18

Álvarez C, González A, Negro M, Ballesteros I, Oliva J, Sáez F (2017) Optimized use of hemicellulose within a biorefinery for processing high value-added xylooligosaccharides. Ind Crop Prod 99:41–48. https://doi.org/10.1016/j.indcrop.2017.01.034

Amorim C, Silvério S, Silva S, Coelho E, Coimbra M, Prather K, Rodrigues L (2018) Single-step production of arabino-xylooligosaccharides by recombinant Bacillus subtilis 3610 cultivated in brewers' spent grain. Carbohydr Polym 199:546–554. https://doi.org/10.1016/j.carbpol.2018.07.017

Amorim C, Silvério S, Gonçalves R, Pinheiro A, Silva S, Coelho E, Coimbra M, Prather K, Rodrigues L (2019a) Downscale fermentation for xylooligosaccharides production by recombinant Bacillus subtilis 3610. Carbohydr Polym 205:176–183. https://doi.org/10.1016/j.carbpol.2018.09.088

Amorim C, Silvério S, Prather K, Rodrigues L (2019b) From lignocellulosic residues to market: production and commercial potential of xylooligosaccharides. Biotechnol Adv 37:107397. https://doi.org/10.1016/j.biotechadv.2019.05.003

Amorim C, Silvério SC, Rodrigues LR (2019c) One-step process for producing prebiotic arabino-xylooligosaccharides from brewer's spent grain employing Trichoderma species. In: Food

chemistry, vol 270. Elsevier, Amsterdam, pp 86–94. https://doi.org/10.1016/J.FOODCHEM.2018.07.080

Antov MG, Đorđević TR (2017) Environmental-friendly technologies for the production of antioxidant xylooligosaccharides from wheat chaff. In: Food chemistry, vol 235. Elsevier, Amsterdam, pp 175–180. https://doi.org/10.1016/J.FOODCHEM.2017.05.058

Arai T, Biely P, Uhliariková I, Sato N, Makishima S, Mizuno M, Nozaki K, Kaneko S, Amano Y (2019) Structural characterization of hemicellulose released from corn cob in continuous flow type hydrothermal reactor. J Biosci Bioeng 127:222–230. https://doi.org/10.1016/j.jbiosc.2018.07.016

Arevalo-Gallegos A, Ahmad Z, Asgher M, Parra-Saldivar R, Iqbal H (2017) Lignocellulose: a sustainable material to produce value-added products with a zero waste approach-a review. Int J Biol Macromol 99:308–318. https://doi.org/10.1016/j.ijbiomac.2017.02.097

Arora S, Rani R, Ghosh S (2018) Bioreactors in solid state fermentation technology: design, applications and engineering aspects. J Biotechnol 269:16–34. https://doi.org/10.1016/j.jbiotec.2018.01.010

Azelee NIW, Jahim J, Ismail A, Fuzi S, Rahman R, Md Illias R (2016) High xylooligosaccharides (XOS) production from pretreated kenaf stem by enzyme mixture hydrolysis. Ind Crop Prod 81:11–19. https://doi.org/10.1016/j.indcrop.2015.11.038

Basit A, Liu J, Rahim K, Jiang W, Lou H (2018) Thermophilic xylanases: from bench to bottle. Crit Rev Biotechnol 38:989–1002. https://doi.org/10.1080/07388551.2018.1425662

Battaglia E, Hansen S, Leendertse A, Madrid S, Mulder H, Nikolaev I, de Vries R (2011) Regulation of pentose utilisation by AraR, but not XlnR, differs in *Aspergillus nidulans* and *Aspergillus niger*. Appl Microbiol Biotechnol 91:387–397. https://doi.org/10.1007/s00253-011-3242-2

Bauer S, Vasu P, Persson S, Mort A, Somerville C (2006) Development and application of a suite of polysaccharide-degrading enzymes for analyzing plant cell walls. Proc Natl Acad Sci 103:11417–11422. https://doi.org/10.1073/pnas.0604632103

Belgacem MN, Gandini A (2008) Monomers, polymers and composites from renewable resources, monomers, polymers and composites, 1st edn. Elsevier Science, Amsterdam

Boonchuay P, Techapun C, Leksawasdi N, Seesuriyachan P, Hanmoungjai P, Watanabe M, Takenaka S, Chaiyaso T (2018) An integrated process for xylooligosaccharide and bioethanol production from corncob. Bioresour Technol 256:399–407. https://doi.org/10.1016/j.biortech.2018.02.004

Borand MN, Karaosmanoğlu F (2018) Effects of organosolv pretreatment conditions for lignocellulosic biomass in biorefinery applications: a review. J Renew Sustain Energy 10(3). https://doi.org/10.1063/1.5025876

Brown NA, Ries LNA, Goldman GH (2014) How nutritional status signalling coordinates metabolism and lignocellulolytic enzyme secretion. Fungal Genet Biol 72:48–63. https://doi.org/10.1016/j.fgb.2014.06.012

Cadete R, Melo M, Dussán K, Rodrigues R, Silva S, Zilli J, Vital M, Gomes F, Lachance M, Rosa C (2012) Diversity and physiological characterization of D-xylose-fermenting yeasts isolated from the Brazilian Amazonian Forest. PLoS One 7:e43135. https://doi.org/10.1371/journal.pone.0043135

Cadete R, de las Heras A, Sandström A, Ferreira C, Gírio F, Gorwa-Grauslund M, Rosa C, Fonseca C (2016) Exploring xylose metabolism in Spathaspora species: XYL1.2 from Spathaspora passalidarum as the key for efficient anaerobic xylose fermentation in metabolic engineered Saccharomyces cerevisiae. Biotechnol Biofuels 9:167. https://doi.org/10.1186/s13068-016-0570-6

Cai F, Shen P, Walker G, Reynolds C, Yuan Y, Reynolds E (2009) Remineralization of enamel subsurface lesions by chewing gum with added calcium. J Dent 37:763–768. https://doi.org/10.1016/j.jdent.2009.06.003

Carvalho A, Neto P, da Silva D, Pastore G (2013) Xylo-oligosaccharides from lignocellulosic materials: chemical structure, health benefits and production by chemical and enzymatic hydrolysis. Food Res Int 51:75–85. https://doi.org/10.1016/j.foodres.2012.11.021

Chen T, Liu W, Fu J, Zhang B, Tang Y (2013) Engineering Bacillus subtilis for acetoin production from glucose and xylose mixtures. J Biotechnol 168:499–505. https://doi.org/10.1016/j.jbiotec.2013.09.020

Chen M, Lu J, Cheng Y, Li Q, Wang H (2019) Novel process for the coproduction of xylo-oligosaccharide and glucose from reed scraps of reed pulp mill. Carbohydr Polym 215:82–89. https://doi.org/10.1016/j.carbpol.2019.03.068

Costa PS, Büchli F, Robl D, da Silva DP, Rabelo S, da Cruz PJ (2016) Enhancement of Penicillium echinulatum glycoside hydrolase enzyme complex. J Ind Microbiol Biotechnol 43:627–639. https://doi.org/10.1007/s10295-016-1746-6

Costa P, Robl D, Costa I, Lima D, Costa A, Pradella J (2017) Potassium biphthalate buffer for pH control to optimize glycosyl hydrolase production in shake flasks using filamentous fungi. Braz J Chem Eng 34:439–450. https://doi.org/10.1590/0104-6632.20170342s20150522

Couto SR, Herrera JLT (2006) Industrial and biotechnological applications of laccases: a review. Biotechnol Adv 24(5):500–513. https://doi.org/10.1016/j.biotechadv.2006.04.003

Dávila I, Gordobil O, Labidi J, Gullón P (2016) Assessment of suitability of vine shoots for hemicellulosic oligosaccharides production through aqueous processing. Bioresour Technol 211:636–644. https://doi.org/10.1016/j.biortech.2016.03.153

Dávila I, Gullón B, Alonso J, Labidi J, Gullón P (2019) Vine shoots as new source for the manufacture of prebiotic oligosaccharides. Carbohydr Polym 207:34–43. https://doi.org/10.1016/j.carbpol.2018.11.065

de Freitas C, Carmona E, Brienzo M (2019) Xylooligosaccharides production process from lignocellulosic biomass and bioactive effects. In: Bioactive carbohydrates and dietary fibre, vol 18. Elsevier, Amsterdam, p 100184. https://doi.org/10.1016/J.BCDF.2019.100184

de Vries RP, Visser J, de Graaff LH (1999) CreA modulates the XlnR-induced expression on xylose of Aspergillus Niger genes involved in xylan degradation. Res Microbiol 150(4):281–285. https://doi.org/10.1016/S0923-2508(99)80053-9

Deutschmann R, Dekker RFH (2012) From plant biomass to bio-based chemicals: latest developments in xylan research. Biotechnol Adv 30(6):1627–1640. https://doi.org/10.1016/j.biotechadv.2012.07.001

Dillon A, Zorgi C, Camassola M, Henriques J (2006) Use of 2-deoxyglucose in liquid media for the selection of mutant strains of Penicillium echinulatum producing increased cellulase and β-glucosidase activities. Appl Microbiol Biotechnol 70:740–746. https://doi.org/10.1007/s00253-005-0122-7

Dobrev G, Pishtiyski I, Stanchev V, Mircheva R (2007) Optimization of nutrient medium containing agricultural wastes for xylanase production by Aspergillus Niger B03 using optimal composite experimental design. Bioresour Technol 98:2671–2678. https://doi.org/10.1016/j.biortech.2006.09.022

Earle MJ, Seddon KR (2000) Ionic liquids. Green solvents for the future. Pure Appl Chem 72 (7):1391–1398. https://doi.org/10.1351/pac200072071391

El-Naggar NEA, Deraz S, Khalil A (2014) Bioethanol production from lignocellulosic feedstocks based on enzymatic hydrolysis: current status and recent developments. Biotechnology 13 (1):1–21. https://doi.org/10.3923/biotech.2014.1.21

Fang X, Yano S, Inoue H, Sawayama S (2009) Strain improvement of Acremonium cellulolyticus for cellulase production by mutation. J Biosci Bioeng 107:256–261. https://doi.org/10.1016/j.jbiosc.2008.11.022

Farinas CS (2015) Developments in solid-state fermentation for the production of biomass-degrading enzymes for the bioenergy sector. Renew Sustain Energy Rev 52:179–188. https://doi.org/10.1016/j.rser.2015.07.092

Fengel D, Wegener G (1989) Wood-chemistry, ultrastructure, reactions. De Gruyter, Berlin

Floudas D, Binder M, Riley R, Barry K, Blanchette R, Henrissat B, Martínez A, Otillar R, Spatafora J, Yadav J, Aerts A, Benoit I, Boyd A, Carlson A, Copeland A, Coutinho P, de Vries R, Ferreira P, Findley K, Foster B, Gaskell J, Glotzer D, Górecki P, Heitman J, Hesse C, Hori C, Igarashi K, Jurgens J, Kallen N, Kersten P, Kohler A, Kües U, Kumar T, Kuo A, LaButti K, Larrondo L, Lindquist E, Ling A, Lombard V, Lucas S, Lundell T, Martin R, McLaughlin D, Morgenstern I, Morin E, Murat C, Nagy L, Nolan M, Ohm R, Patyshakuliyeva A, Rokas A, Ruiz-Dueñas F, Sabat G, Salamov A, Samejima M, Schmutz J, Slot J, St. John F, Stenlid J, Sun H, Sun S, Syed K, Tsang A, Wiebenga A, Young D, Pisabarro A, Eastwood D, Martin F, Cullen D, Grigoriev I, Hibbett D (2012) The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336:1715–1719. https://doi.org/10.1126/science.1221748

Fujii T, Inoue H, Ishikawa K (2013) Enhancing cellulase and hemicellulase production by genetic modification of the carbon catabolite repressor gene, creA, in Acremonium cellulolyticus. AMB Expr 3(1):73. https://doi.org/10.1186/2191-0855-3-73

Garcia-Ochoa F, Gomez E (2009) Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview. Biotechnol Adv 27(2):153–176. https://doi.org/10.1016/j.biotechadv.2008.10.006

Garrote G, Domínguez H, Parajó JC (1999) Hydrothermal processing of lignocellulosic materials. J Wood Wood Prod 57:191–202

Garrote G, Dominguez H, Parajó JC (2001) Generation of xylose solutions from Eucalyptus globulus wood by autohydrolysis–posthydrolysis processes: posthydrolysis kinetics. Bioresour Technol 79(2):155–164. https://doi.org/10.1016/S0960-8524(01)00044-X

Goldbeck R, Gonçalves T, Damásio A, Brenelli L, Wolf L, Paixão D, Rocha G, Squina F (2016) Effect of hemicellulolytic enzymes to improve sugarcane bagasse saccharification and xylooligosaccharides production. J Mol Catal B: Enzym 131:36–46. https://doi.org/10.1016/j.molcatb.2016.05.013

Graaff L, Broeck H, Ooijen A, Visser J (1994) Regulation of the xylanase-encoding xlnA gene of Aspergilius tubigensis. Mol Microbiol 12:479–490. https://doi.org/10.1111/j.1365-2958.1994.tb01036.x

Greaves TL, Drummond CJ (2008) Protic ionic liquids: properties and applications. Chem Rev 108 (1):206–237. https://doi.org/10.1021/cr068040u

Gullón B, Eibes G, Moreira M, Dávila I, Labidi J, Gullón P (2017) Antioxidant and antimicrobial activities of extracts obtained from the refining of autohydrolysis liquors of vine shoots. Ind Crop Prod 107:105–113. https://doi.org/10.1016/j.indcrop.2017.05.034

Gullón B, Eibes G, Dávila I, Moreira M, Labidi J, Gullón P (2018) Hydrothermal treatment of chestnut shells (Castanea sativa) to produce oligosaccharides and antioxidant compounds. Carbohydr Polym 192:75–83. https://doi.org/10.1016/j.carbpol.2018.03.051

Hahn-Hägerdal B, Jeppsson H, Skoog K, Prior B (1994) Biochemistry and physiology of xylose fermentation by yeasts. Enzyme Microb Technol 16:933–943. https://doi.org/10.1016/0141-0229(94)90002-7

Hasper AA (2004) Functional analysis of the transcriptional activator XlnR from Aspergillus Niger. Microbiology 150(5):1367–1375. https://doi.org/10.1099/mic.0.26557-0

Hassan SS, Williams GA, Jaiswal AK (2018) Emerging technologies for the pretreatment of lignocellulosic biomass. Bioresour Technol 262(March 2019):310–318. https://doi.org/10.1016/j.biortech.2018.04.099

Hendriks ATWM, Zeeman G (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 100(1):10–18. https://doi.org/10.1016/j.biortech.2008.05.027

Himmel M, Ding S, Johnson D, Adney W, Nimlos M, Brady J, Foust T (2007) Biomass recalcitrance: engineering plants and enzymes for biofuels production. Science 315:804–807. https://doi.org/10.1126/science.1137016

Hong C, Corbett D, Venditti R, Jameel H, Park S (2019) Xylooligosaccharides as prebiotics from biomass autohydrolyzate. LWT 111:703–710. https://doi.org/10.1016/j.lwt.2019.05.098

Hou X, Yao S (2012) Improved inhibitor tolerance in xylose-fermenting yeast Spathaspora passalidarum by mutagenesis and protoplast fusion. Appl Microbiol Biotechnol 93 (6):2591–2601. https://doi.org/10.1007/s00253-011-3693-5

Hu J, Saddler JN (2018) Why does GH10 xylanase have better performance than GH11 xylanase for the deconstruction of pretreated biomass? Biomass Bioenergy 110:13–16. https://doi.org/10.1016/J.BIOMBIOE.2018.01.007

Huang C, Lai C, Wu X, Huang Y, He J, Huang C, Li X, Yong Q (2017) An integrated process to produce bio-ethanol and xylooligosaccharides rich in xylobiose and xylotriose from high ash content waste wheat straw. Bioresour Technol 241:228–235. https://doi.org/10.1016/j.biortech.2017.05.109

Jia X, Peng X, Liu Y, Han Y (2017) Conversion of cellulose and hemicellulose of biomass simultaneously to acetoin by thermophilic simultaneous saccharification and fermentation. Biotechnol Biofuels 10(1):1–11. https://doi.org/10.1186/s13068-017-0924-8

Jin M, Dale BE (2018) AFEX™ pretreatment-based biorefinery technologies. In: Handbook of biorefinery research and technology. Springer, Dordrecht, pp 1–16. https://doi.org/10.1007/978-94-007-6724-9_2-1

Jönsson LJ, Martín C (2016) Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. Bioresour Technol 199:103–112. https://doi.org/10.1016/j.biortech.2015.10.009

Jørgensen H, Kristensen JB, Felby C (2007) Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. Biofuels Bioprod Biorefin 1(2):119–134. https://doi.org/10.1002/bbb.4

Juturu V, Wu JC (2012) Microbial xylanases: engineering, production and industrial applications. Biotechnol Adv 30(6):1219–1227. https://doi.org/10.1016/J.BIOTECHADV.2011.11.006

Katsimpouras C, Dedes G, Thomaidis N, Topakas E (2019) A novel fungal GH30 xylanase with xylobiohydrolase auxiliary activity. Biotechnol Biofuels 12(1):1–14. https://doi.org/10.1186/s13068-019-1455-2

Kim Y, Kim S, Han T, Park Y, Watanabe C (2014) Pyrolysis reaction characteristics of Korean pine (Pinus Koraiensis) nut shell. J Anal Appl Pyrolysis 110:435–441. https://doi.org/10.1016/j.jaap.2014.10.013

Kim JS, Lee YY, Kim TH (2016) A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. Bioresour Technol 199:42–48. https://doi.org/10.1016/j.biortech.2015.08.085

Kim S, Sim H, Kim J, Lee Y, Park Y, Seo J (2017) Enhanced production of 2,3-butanediol from xylose by combinatorial engineering of xylose metabolic pathway and cofactor regeneration in pyruvate decarboxylase-deficient Saccharomyces cerevisiae. Bioresour Technol 245:1551–1557. https://doi.org/10.1016/j.biortech.2017.06.034

Kiran Kumar A, Sharma S (2017) Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. Bioprocess 4:7. https://doi.org/10.1186/s40643-017-0137-9

Kowalczyk JE, Benoit I, de Vries RP (2014) Regulation of plant biomass utilization in Aspergillus. Adv Appl Microbiol 88:31–56. https://doi.org/10.1016/B978-0-12-800260-5.00002-4

Kumar R, Wyman CE (2009) Effect of xylanase supplementation of cellulase on digestion of corn Stover solids prepared by leading pretreatment technologies. Bioresour Technol 100 (18):4203–4213. https://doi.org/10.1016/j.biortech.2008.11.057

Le B, Yang SH (2019) Production of prebiotic xylooligosaccharide from aqueous ammonia-pretreated rice straw by β-xylosidase of Weissella cibaria. J Appl Microbiol 126:1861–1868. https://doi.org/10.1111/jam.14255

Li H, Chen X, Xiong L, Luo M, Chen X, Wang C, Huang C, Chen X (2019) Stepwise enzymatic hydrolysis of alkaline oxidation treated sugarcane bagasse for the co-production of functional xylo-oligosaccharides and fermentable sugars. Bioresour Technol 275:345–351. https://doi.org/10.1016/j.biortech.2018.12.063

Liu M, Huo W, Xu X, Weng X (2017) Recombinant Bacillus amyloliquefaciens xylanase a expressed in Pichia pastoris and generation of xylooligosaccharides from xylans and wheat bran. Int J Biol Macromol 105:656–663. https://doi.org/10.1016/j.ijbiomac.2017.07.073

Liu X, Liu Y, Jiang Z, Liu H, Yang S, Yan Q (2018) Biochemical characterization of a novel xylanase from Paenibacillus barengoltzii and its application in xylooligosaccharides production from corncobs. Food Chem 264:310–318. https://doi.org/10.1016/j.foodchem.2018.05.023

Long T, Su Y, Headman J, Higbee A, Willis L, Jeffries T (2012) Cofermentation of glucose, xylose, and Cellobiose by the beetle-associated yeast Spathaspora passalidarum. Appl Environ Microbiol 78:5492–5500. https://doi.org/10.1128/aem.00374-12

Marriott PE, Gómez LD, Mcqueen-Mason SJ (2016) Unlocking the potential of lignocellulosic biomass through plant science. New Phytol 209(4):1366–1381. https://doi.org/10.1111/nph.13684

Martinez D, Larrondo L, Putnam N, Gelpke M, Huang K, Chapman J, Helfenbein K, Ramaiya P, Detter J, Larimer F, Coutinho P, Henrissat B, Berka R, Cullen D, Rokhsar D (2004) Genome sequence of the lignocellulose degrading fungus Phanerochaete chrysosporium strain RP78. Nat Biotechnol 22:695–700. https://doi.org/10.1038/nbt967

Martínez Á, Ruiz-Dueñas F, Martínez M, del Río J, Gutiérrez A (2009) Enzymatic delignification of plant cell wall: from nature to mill. Curr Opin Biotechnol 20:348–357. https://doi.org/10.1016/j.copbio.2009.05.002

Martins LH, Rabelo SC, da Costa AC (2015) Effects of the pretreatment method on high solids enzymatic hydrolysis and ethanol fermentation of the cellulosic fraction of sugarcane bagasse. Bioresour Technol 191:312–321. https://doi.org/10.1016/j.biortech.2015.05.024

Mathew S, Karlsson EN, Adlercreutz P (2017) Extraction of soluble arabinoxylan from enzymatically pretreated wheat bran and production of short xylo-oligosaccharides and arabinoxylo-oligosaccharides from arabinoxylan by glycoside hydrolase family 10 and 11 endoxylanases. J Biotechnol 260:53–61. https://doi.org/10.1016/J.JBIOTEC.2017.09.006

Mathew S, Aronsson A, Karlsson E, Adlercreutz P (2018) Xylo- and arabinoxylooligosaccharides from wheat bran by endoxylanases, utilisation by probiotic bacteria, and structural studies of the enzymes. Appl Microbiol Biotechnol 102:3105–3120. https://doi.org/10.1007/s00253-018-8823-x

Mano MCR, Neri-Numa IA, da Silva JB, Paulino BN, Pessoa MG, Pastore GM (2018) Oligosaccharide biotechnology: an approach of prebiotic revolution on the industry. Appl Microbiol Biotechnol 102(1):17–37. https://doi.org/10.1007/s00253-017-8564-2

Mazlan N, Samad K, Wan Yussof H, Saufi S, Jahim J (2019) Xylooligosaccharides from potential agricultural waste: characterization and screening on the enzymatic hydrolysis factors. Ind Crop Prod 129:575–584. https://doi.org/10.1016/j.indcrop.2018.12.042

Mhetras N, Mapre V, Gokhale D (2019) Xylooligosaccharides (XOS) as emerging prebiotics: its production from Lignocellulosic material. Adv Microbiol 09(01):14–20. https://doi.org/10.4236/aim.2019.91002

Mustafa R, Muniglia L, Rovel B, Girardin M (2005) Phenolic colorants obtained by enzymatic synthesis using a fungal laccase in a hydro-organic biphasic system. Food Res Int 38:995–1000. https://doi.org/10.1016/j.foodres.2005.02.019

Molinary SV, Quinlan ME (2012) Sucralose. In: Donnell K, Kearsley MW (eds) Sweeteners and sugar alternatives in food technology, 2nd edn. Wiley-Blackwell, Hoboken, NJ, pp 167–184

Morales P, Gentina J, Aroca G, Mussatto S (2017) Development of an acetic acid tolerant Spathaspora passalidarum strain through evolutionary engineering with resistance to inhibitors compounds of autohydrolysate of Eucalyptus globulus. Ind Crop Prod 106:5–11. https://doi.org/10.1016/j.indcrop.2016.12.023

Morgan N, Wallace A, Bedford M, Choct M (2017) Efficiency of xylanases from families 10 and 11 in production of xylo-oligosaccharides from wheat arabinoxylans. Carbohydr Polym 167:290–296. https://doi.org/10.1016/j.carbpol.2017.03.063

Moysés D, Reis V, Almeida J, Moraes L, Torres F (2016) Xylose fermentation by Saccharomyces cerevisiae: challenges and prospects. Int J Mol Sci 17:207. https://doi.org/10.3390/ijms17030207

Naidu DS, Hlangothi SP, John MJ (2018) Bio-based products from xylan: a review. Carbohydr Polym 179:28–41. https://doi.org/10.1016/J.CARBPOL.2017.09.064

Nakanishi S, Soares L, Biazi L, Nascimento V, Costa A, Rocha G, Ienczak J (2017) Fermentation strategy for second generation ethanol production from sugarcane bagasse hydrolyzate by Spathaspora passalidarum and Scheffersomyces stipitis. Biotechnol Bioeng 114:2211–2221. https://doi.org/10.1002/bit.26357

Nakari-Setälä T, Paloheimo M, Kallio J, Vehmaanperä J, Penttilä M, Saloheimo M (2009) Genetic modification of carbon catabolite repression in Trichoderma reesei for improved protein production. Appl Environ Microbiol 75:4853–4860. https://doi.org/10.1128/aem.00282-09

Nakasu P, Ienczak L, Costa A, Rabelo S (2016) Acid post-hydrolysis of xylooligosaccharides from hydrothermal pretreatment for pentose ethanol production. Fuel 185:73–84. https://doi.org/10.1016/j.fuel.2016.07.069

Nanda S, Kozinski JA, Dalai A (2013) Biomass-an overview on classification, composition and characterization. In: Zhang B, Wang Y (eds) Biomass processing, conversion and biorefinery. Nova Science, Hauppauge, NY, pp 1–35

Negi S, Pandey AK (2015) Ionic liquid pretreatment. In: Pretreatment of biomass, pp 137–155. https://doi.org/10.1016/B978-0-12-800080-9.00008-6

Nguyen N, Suh S, Marshall C, Blackwell M (2006) Morphological and ecological similarities: wood-boring beetles associated with novel xylose-fermenting yeasts, Spathaspora passalidarum gen. Sp. nov. and Candida jeffriesii sp. nov. Mycol Res 110:1232–1241. https://doi.org/10.1016/j.mycres.2006.07.002

Nieto-Domínguez M, de Eugenio L, York-Durán M, Rodríguez-Colinas B, Plou F, Chenoll E, Pardo E, Codoñer F, Jesús Martínez M (2017) Prebiotic effect of xylooligosaccharides produced from birchwood xylan by a novel fungal GH11 xylanase. Food Chem 232:105–113. https://doi.org/10.1016/j.foodchem.2017.03.149

Nobre C, Teixeira JA, Rodrigues LR (2015) New trends and technological challenges in the industrial production and purification of fructo-oligosaccharides. Crit Rev Food Sci Nutr 55 (10):1444–1455. https://doi.org/10.1080/10408398.2012.697082

Otieno DO, Ahring BK (2012) The potential for oligosaccharide production from the hemicelluloses fraction of biomasses through pretreatment processes: xylooligosaccharides (XOS), arabinooligosaccharides (AOS), and mannooligosaccharides (MOS). Carbohydr Res 360:84–92. https://doi.org/10.1016/J.CARRES.2012.07.017

Overkamp K, Bakker B, Kötter P, Luttik M, van Dijken J, Pronk J (2002) Metabolic engineering of glycerol production in *Saccharomyces cerevisiae*. Appl Environ Microbiol 68:2814–2821. https://doi.org/10.1128/aem.68.6.2814-2821.2002

Panesar P, Kaur R, Singh R, Kennedy J (2018) Biocatalytic strategies in the production of galacto-oligosaccharides and its global status. Int J Biol Macromol 111:667–679. https://doi.org/10.1016/j.ijbiomac.2018.01.062

Peng F, Ren J, Xu F, Sun R (2011) Chemicals from hemicelluloses: a review. In: ACS symposium series, pp 219–259. https://doi.org/10.1021/bk-2011-1067.ch009

Peterson R, Nevalainen H (2012) Trichoderma reesei RUT-C30—thirty years of strain improvement. Microbiology 158(1):58–68. https://doi.org/10.1099/mic.0.054031-0

Pin T, Nakasu P, Mattedi S, Rabelo S, Costa A (2019) Screening of protic ionic liquids for sugarcane bagasse pretreatment. Fuel 235:1506–1514. https://doi.org/10.1016/j.fuel.2018.08.122

Puchart V, Fraňová L, Mørkeberg Krogh K, Hoff T, Biely P (2018) Action of different types of endoxylanases on eucalyptus xylan in situ. Appl Microbiol Biotechnol 102:1725–1736. https://doi.org/10.1007/s00253-017-8722-6

Qing Q, Yang B, Wyman CE (2010) Xylooligomers are strong inhibitors of cellulose hydrolysis by enzymes. Bioresour Technol 101(24):9624–9630. https://doi.org/10.1016/j.biortech.2010.06.137

Rabelo S, Carrere H, Maciel Filho R, Costa A (2011) Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept. Bioresour Technol 102:7887–7895. https://doi.org/10.1016/j.biortech.2011.05.081

Rabelo S, Vaz Rossell C, de Moraes RG, Zacchi G (2012) Enhancement of the enzymatic digestibility of sugarcane bagasse by steam pretreatment impregnated with hydrogen peroxide. Biotechnol Prog 28:1207–1217. https://doi.org/10.1002/btpr.1593

Rabelo SC, Maciel Filho R, Costa AC (2013) Lime pretreatment and fermentation of enzymatically hydrolyzed sugarcane bagasse. Appl Biochem Biotechnol 169(5):1696–1712. https://doi.org/10.1007/s12010-013-0097-2

Rahmani N, Kahar P, Lisdiyanti P, Lee J, Yopi, Prasetya B, Ogino C, Kondo A (2019) GH-10 and GH-11 Endo-1,4-β-xylanase enzymes from Kitasatospora sp. produce xylose and xylooligosaccharides from sugarcane bagasse with no xylose inhibition. Bioresour Technol 272:315–325. https://doi.org/10.1016/j.biortech.2018.10.007

Rajagopalan G, Shanmugavelu K, Yang K-L (2017) Production of prebiotic-xylooligosaccharides from alkali pretreated mahogany and mango wood sawdust by using purified xylanase of clostridium strain BOH3. Carbohydr Polym 167:158–166. https://doi.org/10.1016/J.CARBPOL.2017.03.021

Reddy P (2015) A critical review of ionic liquids for the pretreatment of lignocellulosic biomass. S Afr J Sci 111(11–12):1–9. https://doi.org/10.17159/sajs.2015/20150083

Robl D, Delabona P, Mergel C, Rojas J, Costa P, Pimentel I, Vicente V, da Cruz PJ, Padilla G (2013) The capability of endophytic fungi for production of hemicellulases and related enzymes. BMC Biotechnol 13:94. https://doi.org/10.1186/1472-6750-13-94

Robl D, dos Santos CP, Büchli F, da Silva LD, da Silva DP, Squina F, Pimentel I, Padilla G, da Cruz PJ (2015a) Enhancing of sugar cane bagasse hydrolysis by Annulohypoxylon stygium glycohydrolases. Bioresour Technol 177:247–254. https://doi.org/10.1016/j.biortech.2014.11.082

Robl D, da Silva DP, dos Santos CP, da Silva LD, Rabelo S, Pimentel I, Büchli F, Squina F, Padilla G, da Cruz PJ (2015b) Xylanase production by endophytic Aspergillus Niger using pentose-rich hydrothermal liquor from sugarcane bagasse. Biocatal Biotransformation 33:175–187. https://doi.org/10.3109/10242422.2015.1084296

Robl D, Benoit I, Padilla G, da Cruz PJ, de Vries R (2018) Improved Hemicellulase production by genetic modification of carbon Catabolite repression and Xylanolitic activation in Aspergillus Niger. Curr Biotechnol 7:10–18. https://doi.org/10.2174/2211550105666160722120556

Robl D, Mergel C, Costa P, Pradella J, Padilla G (2019) Endophytic Actinomycetes as potential producers of Hemicellulases and related enzymes for plant biomass degradation. Braz Arch Biol Technol 62. https://doi.org/10.1590/1678-4324-2019180337

Romaní A, Ruiz HA, Pereira FB (2014) Effect of hemicelluloses liquid phase on the enzymatic hydrolysis of autohydrolyzed Eucalyptus globulus wood. Biomass Convers Biorefin 2:77–86. https://doi.org/10.1007/s13399-013-0093-3

Rubio MV et al (2015) Heterologous expression of carbohydrate-active enzymes in filamentous fungi. In: Ventura Rubio M et al (eds) Mycology: current and future developments, vol 1. Bentham Science, Sharjah, pp 148–201. https://doi.org/10.2174/9781681080741115010009

Ruijter G, Vanhanen S, Gielkens M, van de Vondervoort P, Visser J (1997) Isolation of Aspergillus Niger creA mutants and effects of the mutations on expression of arabinases and L-arabinose catabolic enzymes. Microbiology 143:2991–2998. https://doi.org/10.1099/00221287-143-9-2991

Ruiz-Dueñas FJ, Martínez ÁT (2009) Microbial degradation of lignin: how a bulky recalcitrant polymer is efficiently recycled in nature and how we can take advantage of this. J Microbial Biotechnol 2(2):164–177. https://doi.org/10.1111/j.1751-7915.2008.00078.x

Saville BA, Saville S (2018) Xylooligosaccharidesand arabinoxylanoligosaccharides and their application as prebiotics. Appl Food Biotechnol 5(3):121–130. https://doi.org/10.22037/afb.v%vi%i.20212

Sajib M, Falck P, Sardari R, Mathew S, Grey C, Karlsson E, Adlercreutz P (2018) Valorization of Brewer's spent grain to prebiotic oligosaccharide: production, xylanase catalyzed hydrolysis, in-vitro evaluation with probiotic strains and in a batch human fecal fermentation model. J Biotechnol 268:61–70. https://doi.org/10.1016/j.jbiotec.2018.01.005

Santos S, de Sousa A, Dionísio S, Tramontina R, Ruller R, Squina F, Vaz Rossell C, da Costa A, Ienczak J (2016) Bioethanol production by recycled Scheffersomyces stipitis in sequential batch fermentations with high cell density using xylose and glucose mixture. Bioresour Technol 219:319–329. https://doi.org/10.1016/j.biortech.2016.07.102

Santucci B, Maziero P, Rabelo S, Curvelo A, Pimenta M (2015) Autohydrolysis of hemicelluloses from sugarcane bagasse during hydrothermal pretreatment: a kinetic assessment. Bioenergy Res 8:1778–1787. https://doi.org/10.1007/s12155-015-9632-z

Semkiv M, Dmytruk K, Abbas C, Sibirny A (2017) Metabolic engineering for high glycerol production by the anaerobic cultures of Saccharomyces cerevisiae. Appl Microbiol Biotechnol 101:4403–4416. https://doi.org/10.1007/s00253-017-8202-z

Shah S, Venkatramanan V (2019) Advances in microbial technology for upscaling sustainable biofuel production. In: New and future developments in microbial biotechnology and bioengineering, pp 69–76. https://doi.org/10.1016/b978-0-444-63504-4.00005-0

Silva J, Mussatto S, Roberto I, Teixeira J (2012) Fermentation medium and oxygen transfer conditions that maximize the xylose conversion to ethanol by Pichia stipitis. Renew Energy 37:259–265. https://doi.org/10.1016/j.renene.2011.06.032

Singh R, Banerjee J, Sasmal S, Muir J, Arora A (2018) High xylan recovery using two stage alkali pre-treatment process from high lignin biomass and its valorisation to xylooligosaccharides of low degree of polymerisation. Bioresour Technol 256:110–117. https://doi.org/10.1016/j.biortech.2018.02.009

Soccol C, Costa E, Letti L, Karp S, Woiciechowski A, Vandenberghe L (2017) Recent developments and innovations in solid state fermentation. Biotechnol Res Innov 1:52–71. https://doi.org/10.1016/j.biori.2017.01.002

Su YK, Willis LB, Jeffries TW (2015) Effects of aeration on growth, ethanol and polyol accumulation by Spathaspora passalidarum NRRL Y-27907 and Scheffersomyces stipitis NRRL Y-7124. Biotechnol Bioeng 112(3):457–469. https://doi.org/10.1002/bit.25445

Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83(1):1–11. https://doi.org/10.1016/S0960-8524(01)00212-7

Sun R-C, Sun X-F, Zhang S-H (2001) Quantitative determination of hydroxycinnamic acids in wheat, rice, rye, and barley straws, maize stems, oil palm frond fiber, and fast-growing poplar wood. J Agric Food Chem 49(11):5122–5129. https://doi.org/10.1021/jf010500r

Taherzadeh M, Karimi K (2008) Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. Int J Mol Sci 9(9):1621–1651. https://doi.org/10.3390/ijms9091621

Tramontina R, Robl D, Maitan-Alfenas G, de Vries R (2016) Cooperation of Aspergillus nidulans enzymes increases plant polysaccharide saccharification. Biotechnol J 11:988–992. https://doi.org/10.1002/biot.201500116

Urbina H, Schuster J, Blackwell M (2013) The gut of Guatemalan passalid beetles: a habitat colonized bycellobiose- and xylose-fermenting yeasts. Fungal Ecol 6(5):339–355. https://doi.org/10.1016/j.funeco.2013.06.005

Venkatramanan V, Shah S, Prasad R (eds) (2021) Sustainable bioeconomy: pathways to sustainable development goals. Springer Nature, Singapore. https://doi.org/10.1007/978-981-15-7321-7

Viniegra-González G, Favela-Torres E, Aguilar C, Rómero-Gomez S, Diaz-Godinez G, Augur C (2003) Advantages of fungal enzyme production in solid state over liquid fermentation systems. Biochem Eng J 13:157–167. https://doi.org/10.1016/s1369-703x(02)00128-6

Vogel J (2008) Unique aspects of the grass cell wall. Curr Opin Plant Biol 11(3):301–307. https://doi.org/10.1016/j.pbi.2008.03.002

Wan G, Zhang Q, Li M, Jia Z, Guo C, Luo B, Wang S, Min D (2019) How pseudo-lignin is generated during dilute sulfuric acid pretreatment. J Agric Food Chem 67:10116–10125. https://doi.org/10.1021/acs.jafc.9b02851

Wang Z, Zhuge J, Fang H, Prior B (2001) Glycerol production by microbial fermentation. Biotechnol Adv 19:201–223. https://doi.org/10.1016/s0734-9750(01)00060-x

Wohlbach D, Kuo A, Sato T, Potts K, Salamov A, LaButti K, Sun H, Clum A, Pangilinan J, Lindquist E, Lucas S, Lapidus A, Jin M, Gunawan C, Balan V, Dale B, Jeffries T, Zinkel R, Barry K, Grigoriev I, Gasch A (2011) Comparative genomics of xylose-fermenting fungi for enhanced biofuel production. Proc Natl Acad Sci 108:13212–13217. https://doi.org/10.1073/pnas.1103039108

Wu H, Li H, Xue Y, Luo G, Gan L, Liu J, Mao L, Long M (2017) High efficiency co-production of ferulic acid and xylooligosaccharides from wheat bran by recombinant xylanase and feruloyl esterase. Biochem Eng J 120:41–48. https://doi.org/10.1016/j.bej.2017.01.001

Xia C, Li Z, Xu Y, Yang P, Gao L, Yan Q, Li S, Wang Y, Qu Y, Song X (2019) Introduction of heterologous transcription factors and their target genes into Penicillium oxalicum leads to increased lignocellulolytic enzyme production. Appl Microbiol Biotechnol 103:2675–2687. https://doi.org/10.1007/s00253-018-09612-y

Xu J, Liu B, Wu L, Hu J, Hou H, Yang J (2019a) A waste-minimized biorefinery scenario for the hierarchical conversion of agricultural straw into prebiotic xylooligosaccharides, fermentable sugars and lithium-sulfur batteries. Ind Crop Prod 129:269–280. https://doi.org/10.1016/j.indcrop.2018.12.002

Xu X, Fan C, Song L, Li J, Chen Y, Zhang Y, Liu B, Zhang W (2019b) A novel CreA-mediated regulation mechanism of cellulase expression in the thermophilic fungus Humicola insolens. Int J Mol Sci 20:3693. https://doi.org/10.3390/ijms20153693

Yang B, Wyman CE (2008) 'Pretreatment: the key to unlocking low-cost cellulosic ethanol. Biofuels Bioprod Biorefin 2(1):26–40. https://doi.org/10.1002/bbb.49

Yokoyama S, Suzuki T, Kawai K, Horitsu H, Takamizawa K (1995) Purification, characterization and structure analysis of NADPH-dependent d-xylose reductases from *Candida tropicalis*. J Ferment Bioeng 79:217–223. https://doi.org/10.1016/0922-338x(95)90606-z

Zhang G, Liu J, Kong I, Kwak S, Jin Y (2015) Combining C6 and C5 sugar metabolism for enhancing microbial bioconversion. Curr Opin Chem Biol 29:49–57. https://doi.org/10.1016/j.cbpa.2015.09.008

3 Microbial Mediated Valorization of Lignocellulose: A Green Technology for Bioethanol Production

Viabhav Kumar Upadhayay, Amir Khan, Jyoti Singh, and Ajay Veer Singh

Abstract

In the modern world, the attention is raised for the development of newer technologies for the transformation of biological wastes into biofuels as an alternative option of exhaustible petroleum or other sources. The organic parts of agricultural wastes, forest residues, food wastes, and municipal and industrial wastes contain an unlimited source of lignocellulosic biomass which could potentially be used for generating second-generation biofuels such as "bioethanol." Microorganisms play an important role in all probable steps intended for lignocelluloses hydrolysis. The greener technological approach for green fuel production through application of microorganisms is a sustainable and renewable approach which is carried out in three steps such as (a) hydrolysis of lignin; (b) hydrolysis of cellulose and hemicelluloses; (c) fermentation of glucose to ethanol. The high production of ethanol is the need of the cotemporary world and therefore it becomes necessary to explore different microorganisms having a high potential for ethanol yield. Moreover, introducing metabolic engineering techniques is the current advancement for development of modified microbial cells for enhanced production of ethanol from lignocellulosic biomass. The present chapter focuses on the valorization of lignocelluloses waste through microorganisms and their mechanisms required for bioethanol synthesis from lignocellulosic biomass.

Keywords

Lignocellulose · Bioethanol · Valorization · Fermentation · Hemicellulose

V. K. Upadhayay · A. Khan · J. Singh · A. V. Singh (✉)
Department of Microbiology, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_3

3.1 Introduction

From the past few years, the global researches endeavored to find alternative sources of energy. Main reasons behind searching sustainable energy supply are (1) increase of atmospheric CO_2 and concern for global climate change (Venkatramanan et al. 2020; 2021a), (2) depletion of non-renewable energy source, (3) rising energy demand, (4) energy security, (5) rural economic development, (6) rapid urbanization, (7) development of power driven technology, and (8) transportation (Baños et al. 2011; De Bhowmick et al. 2018; Prasad et al. 2019; Shah et al. 2019). It has been anticipated that in near future (approximately by 2025) around 50% increment in the energy demand will be appeared from a number of both developed and developing countries (Tong et al. 2012). Therefore, harvesting energy from plant biomass through sustainable, environmentally friendly and cost-effective approach is an important substitute of petroleum and non-renewable energy source (Prasad et al. 2021; Venkatramanan et al. 2021b). Plants and related waste materials contain cellulosic properties so they can be utilized to produce bioethanol (Prasad et al. 2019). In present world, the liquid biofuel in the form of "bioethanol" is being used having various benefits over fossil fuels. It can diminish the emission of greenhouse gases and reduce the particulate materials in the atmosphere (up to 50%) (Riccio et al. 2017; Donato et al. 2019). Various food crops such as "maize," "sugarcane," and "sugar beet" have become prominent source of carrying out fermentation process for bioethanol production, and such type of production is also described as "first-generation technology" which is anticipated to attain a level of approx. 100 billion liters (in 2022) (Saini et al. 2015). The maize and sugarcane are rich source of starch and sugars (sucrose) as raw materials and exhibit inadequacy to meet emergent requirement of bioethanol. Moreover, the cultivation of these crops for energy production has shown negative impact on issue of biodiversity and food chain, and considered as probable means for deforestation as huge farmland would be needed. Keeping such issues about risks associated with first-generation bioethanol, the research focus has been moved towards "second-generation technologies," where the exploitation of non-food-based crops (with no-food parts) and wastes originated from wood or food-based industries represent most plentiful renewable organic constituents in the biosphere (Zucaro et al. 2016; Donato et al. 2019). Therefore, the second-generation bioethanol is derived from "lignocellulosic biomass" which is generated by agricultural practices, wood-based industries, municipal solid wastes, and dedicated energy crops cultivating on trivial lands (Nair et al. 2017). The biomass in form of lignocelluloses represents an economically feasible and renewable/inexhaustible reservoir for the production of eminent fuel in form of "bioethanol" (Donato et al. 2019; Prasad et al. 2019). "The lignocellulosic crop residues have huge potential to be used as feedstock for biofuel production" (Venkatramanan et al. 2021c). Although the fixing of lignocellulosic material into bioethanol production has been attributed to give numerous advantages in terms of environmental impact and sustainability, the "2G" or second-generation technology for bioethanol production is under infancy and all the concerning researches are going on all aspects (from biomass treatment to hydrolysis and fermentation). In this

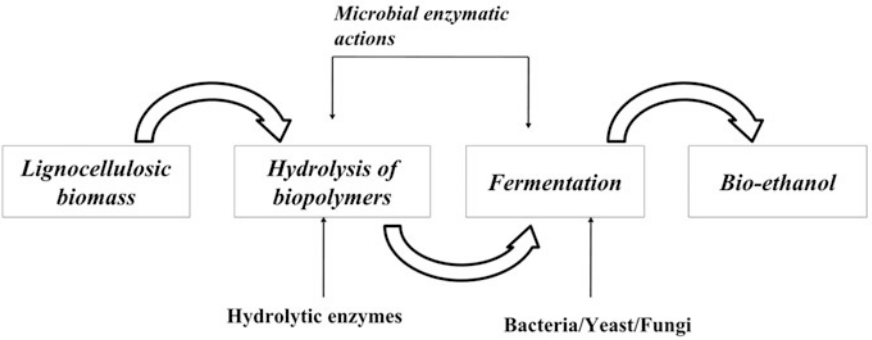


Fig. 3.1 Schematic representation for valorization of lignocellulosic biomass

context, microorganisms (such as bacteria and fungi) and their enzymes have provided reasonable and cost-competitive strategy for switching the lignocellulosic biomass into bioethanol (Prasad et al. 2019). After pretreatment of raw materials (lignocellulosic biomass), the next step includes hydrolysis of biopolymers (cellulose and hemicelluloses) through hydrolytic enzymes into simpler sugars and their use in process of fermentation for bioethanol production (Fig. 3.1). Present chapter outlined the concise introduction of role of microorganisms and their enzymes in valorization of lignocellulosic materials for production of second-generation biofuel in more economically feasible and sustainable manner with considering the associated facts of less detrimental impacts on the environment.

3.2 Source of Lignocellulosic Biomass

Plant and agricultural residues (such as barley straw, corn stover, wheat, rice, husk of coconut, sugarcane bagasse, wood, sorghum stalks), forest residues, and municipal organic wastes are the key sources for the lignocellulosic biomasses (Shah and Venkatramanan 2019) (Fig. 3.2). Most of the countries produce considerable number of sources for deriving lignocellulosic material, for example corn stover is produced at high level by the USA; however, wood and large quantity of residues (agricultural and forest residues) produced by New Zealand and China (Zhu and Pan 2010). India, after China supplies approximately 0.2 billion tons of agriculture-based residues annually (De Bhowmick et al. 2018). Additionally, overall huge amount (approximately 180 million tons) of cellulosic biomass each year is derived from agricultural resources (Kurian et al. 2013; De Bhowmick et al. 2018). Lignocellulosic material in the form of either as crop or residues is chiefly produced from perennial herbaceous plants and woody plants, and such plant materials are abundantly presented on earth. Besides agricultural and forest residues, the municipal organic wastes are another main source of lignocelluloses (FitzPatrick et al. 2010; De Bhowmick et al. 2018). Structurally the "lignocellulosic biomass" is comprised of three most important biopolymers which are widely referred as cellulose,

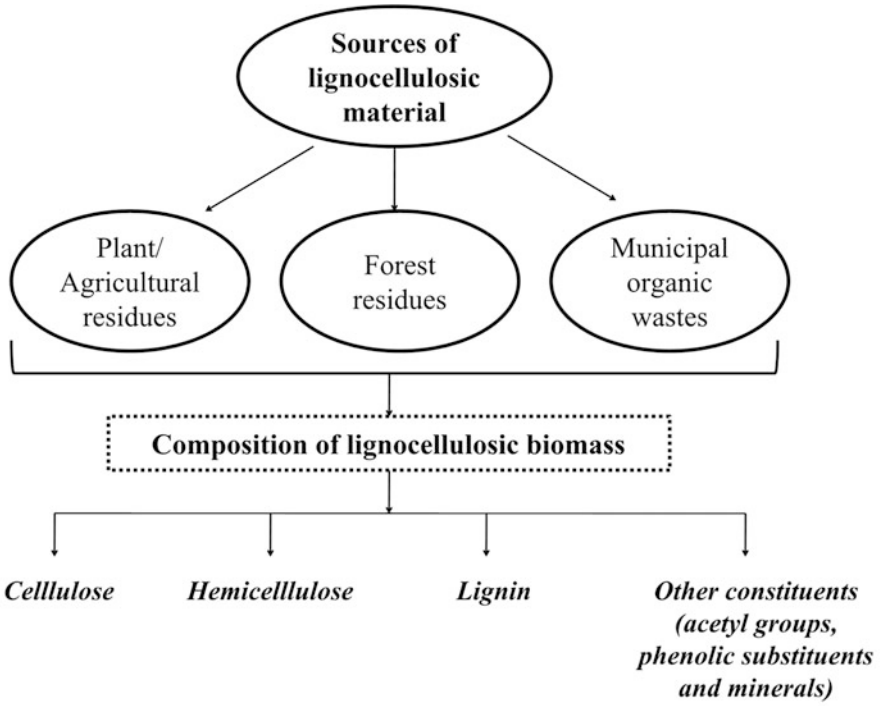


Fig. 3.2 Important sources and constituents of lignocellulosic biomass

hemicellulose, and lignin. Other constituents in small quantity (such as acetyl groups, phenolic substituents, and minerals) are also present in lignocellulosic biomass (Fig. 3.2). Biopolymers involved in synthesis of lignocellulosic biomass are organized in intricate and inhomogeneous three-dimensional structures to provide varying degrees of relative composition depending on type of lignocelluloses.

3.3 Importance of Pretreatment Technologies

The three important biological materials such as cellulose, lignin, and hemicelluloses participate in the formation of lignocellulosic biomass, where cellulose and lignin as a matrix are bounded with chains of hemicelluloses. The main motto of the pretreatment process is to breakdown the lignocellulosic material which results in reduced crystallinity of cellulose and also augments the part of amorphous form of cellulose. Such cellulose form is actually exerting suitability for enzymatic activity (Sánchez and Cardona 2008). Moreover, the pretreatment is necessary to make lignocellulosic waste liable for fast hydrolysis with augmented monomeric sugars (Mosier et al. 2005), and features of pretreatment must be proficient and effective for the production of biofuel (Lu and Mosier 2008; Saxena et al. 2009; Gupta and Verma

2015). However, the important goals of pretreatment methods can be summarized in brief as (a) production of sugars through the hydrolysis, (b) avoiding the degradation of sugars, (c) avoiding the maximum formation of products having inhibitory properties, (d) to lessen the energy demand, and (e) decreasing the costs. The basic structure of plants such as "cell wall" hampers the entry of various pathogens. Number of pathogens actually produced certain hydrolytic enzymes which disrupt the internal parts of plants, but tough cell wall restricts the enzymatic activity to perform its action of degradation (Kim 2013). For the effective production of sugars required for fermentation from the cellulosic material, there is the necessity to further modify the physical and chemical characteristics of the cell wall structure of the plants. The factors involved in increment of pretreatment step are: (a) very less amount of lignocellulosic biomass (less than 20%) undergoes incomplete/partial digestion in its native state; (b) complex or mixed composition; (c) recalcitrant nature of the cellulose; (c) elevated crystallinity of cellulose fiber; and (d) enhancement in the accessibility of the enzymes (Kim 2013; De Bhowmick et al. 2018). Furthermore, it is also apparent that the preferable pretreatment processes have revealed an incredible impact on the physicochemical properties of the treated lignocellulosic biomass. Such properties influence the downstream processes including preconditioning, selection of microbes, utilization of by-products, and waste management along with the recuperation of the aimed product, concentration and purification of the product (da Costa Sousa et al. 2009). In addition, grasses and woods (both soft and hardwood) present the wide arrays of lignocellulosic material bearing different chemical and physical properties which necessitates various approaches. As a result, the suitable pretreatment processing means should be used for a particular substrate, and such aspects of interdependence between pretreatment processing and substrates make the pretreatment step as fundamental unit operational division in "lignocellulosic biorefinery" (De Bhowmick et al. 2018).

3.4 Pretreatment of Lignocellulosic Substrates

The pretreatment is an important process in valorization of lignocellulosic material and the production of second-generation biofuel, namely "bioethanol." There are various ways of pretreatment such as physical or physio-chemical or chemical or biological or combinations of all these (Fig. 3.3). The critical step for pretreatment of biomass results the alteration of complex lignocellulosic material into amorphous and crystalline cellulose and such form of cellulose exhibits suitability for its further digestion (Saini et al. 2015; Furusato et al. 2018). Thus, it is a noteworthy step to attain elevated yield of ethanol from lignocellulosic material. One of the critical ways of pretreatment is "physical treatment" which involves certain important steps such as fragmentation, grinding, milling/shearing of the biomaterial/biomass. These all steps assist in lessening the level of polymerization and particle size, and on another side provide lignocellulosic material with increased bulk density, and surface area (Maurya et al. 2015; Amin et al. 2017). Physical treatment is considered as an ordinary step for enzymatic accessibility and effective bioconversion

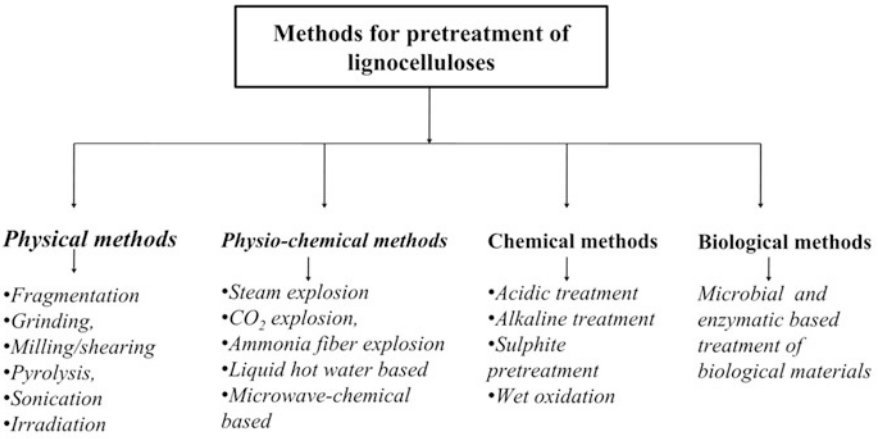


Fig. 3.3 Schematic representation of various methods used in pretreatment process of lignocellulosic biomass

competence to the distorted particles (Barakat et al. 2014; Kumar and Sharma 2017). Pyrolysis, sonication, and irradiation (particularly with gamma radiation) are other methods of physical treatments (Isikgor and Becer 2015). Physio-chemical treatment is another important way of pretreatment method which involves chemical reactions for the distortion of the structure of lignocellulosic material. Physio-chemical treatment involves (a) steam explosion (also referred as hydrolysis), (b) CO_2 explosion, (c) ammonia fiber explosion, (d) steam explosion with addition of sulfur dioxide (SO_2), (e) liquid hot water-based pretreatment, and (f) microwave-chemical pretreatment (Brodeur et al. 2011; Isikgor and Becer 2015). Chemical treatment also played a significant role in process of pretreatment and there are number of foremost chemical treatment methods such as acidic treatment and alkaline treatment. Besides these ionic liquids (also known as green solvents), sulfite pretreatment and wet oxidation are other important methods of chemical-based pretreatment (Bensah and Mensah 2013; Amin et al. 2017). Next method of treatment is widely known as "biological treatment" process which has been illustrated as microbial mediated step to treat the biological material. As compared to other two methods of pretreatments (physical and chemical), the biological way of pretreatment is determined as an inexpensive and eco-friendly approach for the valorization of lignocelluloses (Wan and Li 2012; Maurya et al. 2015). In biological pretreatment process, the enzymes secreted by microorganisms (both bacteria and fungi) involve in degradation of the substrate. A range of bacteria such as "*Actinomycetes*" have been determined to produce lignocellulose degrading enzymes, and these enzymes are prominently efficient in degradation of grasses (as grasses possess huge cellulosic biomass) (Amin et al. 2017). However, biological pretreatment process of lignocelluloses is relatively economically feasible and proficient. Moreover, it is an eco-friendly source of wide arrays of enzymes for degrading complex biomass, and in industrial application enzymes hold huge potential.

3.5 Microbial Mediated Biological Pretreatment

In the modern era, the production of high-quality biofuel (such as ethanol) from least useful biomass through fermentation has given a new trend (Mohd Azhar et al. 2017). The bioethanol production is the green synthesis of renewable biofuels and may assist in reducing the need of precious fossils fuels. Moreover, it will be attributed to sustain future generation in respect of fuel-based energy. After illustrating few modern various pretreatment strategies in previous section, the greener approach in form of biological pretreatment has been assigned as most effective and eco-friendly approach causing lesser pollution. Biological approach for the pretreatment involves numerous enzymes which indirectly exhibit the role of microorganisms producing the particular enzyme. Conventional approach or the physio-chemical method for lignocelluloses degradation needs huge energy input and also determined as an important factor to cause pollution. Therefore, biological based pretreatment process of lignocelluloses could be an excellent instance of environment friendly and inexpensive strategy (Maurya et al. 2015). The conversion or transformation of the biomass/raw materials to the biofuel by using the preeminent microorganism could provide better productivity in most efficient way with less investment of money. The conversion of raw biomass might be improved by having appropriate understanding of the microorganisms participated in different steps of pretreatment. Biological pretreatment is essential because it enhances fermentation rate. This approach particularly uses the cellulose or hemicelluloses degrading microorganism for carrying out pretreatment of substrate such as lignocelluloses. Earlier studies reported the vital role of bacteria including *Bacillus* to degrade organic materials (Poszytek et al. 2016), and such organisms have important place in the biological pretreatments of raw materials. Bacteria are profoundly beneficial for secreting enzymes (both industrial and biotechnological important enzymes) (Singh et al. 2012). The combination of more than two microorganisms (also known as microbial consortia) aids in enhanced degradation of complex biomaterials. Microbial consortia comprising of cellulolytic bacteria (*Bacillus* and *Streptomyces*), and fungi (*Candida* and *Aspergillus*) showed wide-spectrum biodegradation (Nikiema et al. 2017). Biomolecules with complex structure such as the polysaccharides are degraded to the simpler sugars through the involvement of wide arrays of enzymes like amylase, cellobiase, cellulase, and xylanase. Moreover, protease plays a pivotal role for the degradation of protein into the amino acids and lipase breaks the lipids into two subsequent main products (such as glycerol and long-chain fatty acids) (Indrasith et al. 1988; Lass et al. 2011). However, the lignin shows extremely resistive nature against degradation, but few fungi degrade lignin too. Modification in conventional steps also required for improving the bioethanol synthesis from the biomass, and it is also reported that simultaneous "saccharification" and "fermentation" through the association of fungi can improve bioethanol productivity (Cheng et al. 2017). White-rot fungi were examined to being an effective candidate to bring out pretreatment process of most of the available lignocellulosic biomass (Kumar and Wyman 2009). Numerous white-rot fungi (*Ceriporia lacerate*, *Cyathus stercolerus*, *P. chrysosporium*, *Pleurotus ostreatus*,

Phanerochaete chrysosporium, and *Pycnoporus cinnabarinus*) have the trait to produce lignin peroxidases (lignin-degrading enzymes) and manganese- dependent peroxidases, and these enzymes were reported to exhibit higher delignification efficacy on different lignocellulosic biomasses (Shi et al. 2008; Kumar and Wyman 2009; Maurya et al. 2015; Ummalyma et al. 2019). An effectual delignification of different biomass was reported by fungus, namely *Ceriporiopsis subvermispora* in the mutual action of two enzymes such as laccase and manganese peroxidase (Wan and Li 2012). Assessment of mild alkali and also the steam pretreatment of "wet-milled corn fiber" are done with using fungi, namely *Gloeophyllum trabeum*, *P. chrysosporium*, and *Trichoderma reesei,* which resulted into the instant hydrolysates fermentation to ethanol. This phenomenon illustrates that the yields of ethanol are 75% superior as compared to a commercially accessible cellulase enzyme utilized in instantaneous saccharification and fermentation process (Brahmachari et al. 2016). Microorganisms which had been isolated from diverse ecological niches or regions (such as soil, manure/compost, agriculture-based residues, and rumen of animals) are potential consortia having capacity for carrying out efficient degradation process of lignocelluloses (Poszytek et al. 2016). It became important to comprehend the specific microorganism involved in making a particular microbial consortium for the relevant lignocellulosic biomass to be treated, and this understanding could direct to an insightful modification in the eminent production rate of bioethanol. Consortia (mixture of pure strains of yeast and cellulolytic bacteria) screened from natural environment were also employed for successful pretreatment of lignocellulosic matter in process of biological pretreatment (Zhang et al. 2011).

3.6 Hydrolysis: A Process Involves Microbial Enzymes

Both celluloses and hemicelluloses undergo the enzymatic hydrolysis which is regulated by numerous factors such as temperature, pH, quality of substrate, incubation period, and ratio of enzyme-substrate (Achinas and Euverink 2016). Though, the use of either diluted or concentrated acid such as sulfuric acid for the acid hydrolysis is a common practice to degrade the celluloses. But, to hydrolyze the cellulosic polymers through the "acid hydrolysis" has limitations and shows unsuitability for efficient ethanol fermentation due to synthesis of toxic components such as phenols (Sun and Cheng 2002; Moe et al. 2012; Achinas and Euverink 2016). Moreover, this method of acid hydrolysis is not economically feasible as it involves high consumption of acids (Moe et al. 2012) and requires specialized reactors because of higher degree of corrosion and high toxicity rate (Wijaya et al. 2014). Therefore, it is required to use microbial based enzymes for solving the purpose of hydrolysis of celluloses and hemicelluloses in more effective manner. Plenty of researches have been performed on microbes (both bacteria and fungi) bearing cellulolytic/lignocellulolytic nature and the respective hydrolytic enzymes for efficient hydrolysis of sugars and their conversion into the ethanol (Jessen et al. 2015; Prasad et al. 2019).

Each step in the hydrolysis of polysaccharide matrix of plant cell wall is a complex phenomenon and needs a suitable treatment. The method of pretreatment of lignocellulosic material as substrate is connected with enzymatic hydrolysis, and such practices further help in enhanced porosity and enzyme accessibility to the substrate (lignocellulosic biomass) (Limayem and Ricke 2012; Prasad et al. 2019). In pretreatment process, the separation of lignin moiety from the lignocellulosic material is necessary as it interferes with the hydrolysis step through blocking the access of cellulose degrading enzyme "cellulases." Therefore, the separation of the lignin can dramatically result into the increased hydrolysis rate of celluloses (McMillan 1994). Enzymes mediated hydrolysis have exhibited benefits over acid-based hydrolysis, as the method of enzyme hydrolysis is very mild process and potentially provides high yields with low cost. Moreover, it doesn't have corrosion problems so it can be proposed as the preferable method for "wood-to-ethanol processes" in future (Menon and Rao 2012).

3.6.1 Cellulases

Enzymes hydrolysis coupled with activities of various kinds of hydrolytic enzymes which converts complex carbohydrate molecules into the simple monomeric sugars. In comparison with acid hydrolysis, the enzymatic hydrolysis needs less input of energy and mild conditions (Ferreira et al. 2009). Cellulase is the most significant enzyme present in various cellulolytic bacteria (*Acetovibrio*, *Bacillus*, *Bacteroides*, *Cellulomonas*, *Clostridium*, *Erwinia*, *Ruminococcus*, *Streptomyces*, *Thermomonospora*) and cellulolytic fungi (*Fusarium*, *Penicillium*, *Phanerochaete*, *Schizophyllum* sp., and *Trichoderma*). Cellulases possess the ability to convert cellulose into simplest sugars (e.g., glucose or galactose monomer) (Gupta and Verma 2015). Cellulase enzymes are comprised of a catalytic unit and a non-catalytic carbohydrate-binding unit and also associated with other accessory domains (Herve et al. 2010; Chatterjee et al. 2015). The enzyme "cellulases" belong to glycoside hydrolases family with three different classes of enzymes: (a) Endo-1,4-β-endoglucanase (cleave the glucosidic linkages randomly on the complex molecule of polysaccharide), (b) Exo-1,4- β-exoglucanase (binds to crystalline region of the cellulose and randomly cleaves the cellulose molecules), and (c) β-glucosidase or cellobiase (these enzymes specifically cleave the cellobiose molecule) (Willis et al. 2010; Chatterjee et al. 2015). Cellulose degrading microorganisms are widely known as cellulolytic microorganisms and possess the capability to degrade recalcitrant plant cell wall. The cellulolytic microorganisms, for instances thermophilic and mesophilic anaerobes, fungi, and bacteria are robustly capable to hydrolyze extremely crystalline insoluble cellulose (Shaw et al. 2008; Himmel et al. 2010). Lamed and Bayer (1988) stated that there is huge attention towards thermophiles as these microorganisms have the ability to secrete "thermo-stable cellulose" mainly under higher temperature (more than 90 °C temperature too). In case of anaerobic bacteria, the degradation of cellulose is carried out by a particular multienzyme complex, termed as "cellulosomes," which either found in free or associated to the

cell surface (Chatterjee et al. 2015). Mitchell (1998) illustrated the cellulolytic activity of *Clostridium* (a thermophilic anaerobe bacteria) for the degradation of cellulosic plant materials and also showed adaptable fermentable ability. For the last some years, *T. reesei* based cellulases have drawn attraction for the research, and are extensively employed in the laboratory and pilot-scale study for ethanol application (Gray et al. 2006). Cellulases from two prominent fungi such as *Aspergillus niger* and *Trichoderma viride* are also used for the hydrolysis of biomass (Passos et al. 2009). The majority of commercially available enzymes for hydrolysis of biomass are in fact blends of cellulases from fungi (*Aspergillus* or *Trichoderma*) supplemented with β-glucosidases. Other potent cellulases producing microorganisms are *Cellulomonas* sp., *Clostridium* sp., *Thermomonospora* sp., *Aspergillus* sp., and *Trichoderma* sp. (Kuhad et al. 2011).

3.6.2 Hemicellulases

"Hemicellulase" is a unique factor for plant biomass degradation and particularly acts on hemicelluloses. This enzyme is main constituent for carbon flow in nature. The main substrate of this enzyme is hemicelluloses which can be represented as an assemblage of branched and linear polysaccharides connected through hydrogen bonds to the cellulose microfibrils. Hemicellulose is comprised of a combination of glucose and sugar monomers (Ummalyma et al. 2019). Xylan is the most copious hemicelluloses which contain pentose sugars (such as xylose), and the enzyme namely "xylanase" catalyzes the hydrolysis of xylan. The catalytic or functional unit of hemicellulases can be described either as glycosidic hydrolases (which hydrolyze glycosidic bonds) or carbohydrate esterases (which catalyze the degradation of ferulic acid and acetate). Multiple xylanases with varied specificities and functions perform the action of xylan hydrolysis. There are numerous microorganisms (*A. niger*, *Bacillus* sp., *Humicola insolens,* and *T. reesei*) from which xylanases are produced on a commercial basis. The action of endoxylanases and exoxylanases commence the process of hydrolysis of hemicelluloses (Binod et al. 2011; Ummalyma et al. 2019). The redundant by-product of hemicelluloses hydrolysis is L-arabinitol which affects the diminution of D-xylose to xylitol. However, xylose reductase has the ability to reduce the L-arabinose to "L-arabinitol." Nair and Zhao (2010) engineered a strain of *Escherichia coli* with a "xylose reductase mutant" which resulted into elimination of L-arabinitol production to synthesize xylitol from a combination of hemicelluloses sugars (such as L-arabinose, D-glucose, and D-xylose). Sakamoto et al. (2012) and his group designed *Saccharomyces cerevisiae* through genetic engineering intervention which showed the ability to degrade hemicelluloses through co-presenting the enzymes from different microorganisms such as endoxylanase (from *Trichoderma reesei*), β-xylosidase (from *Aspergillus oryzae*), β-glucosidase (from *Aspergillus aculeatus*), expression of xylulokinase (from *S. cerevisiae*) and xylose reductase and xylitol dehydrogenase (from *Pichia stipitis*) with the inclusion of xylose. The genetically engineered microorganisms also have the ability to produce bioethanol using rice straw, as the

rice straw also provide suitable hemicelluloses (cellooligosaccharides, xylooligosaccharides, and xylan) substrate. Su et al. (2015) engineered a bacterial strain, namely *E. coli* W3110 to secrete xylitol to display xylose reductase from *Neurospora crassa* at elevated temperature without inclusion bodies. The genes of xylose isomerase ("xylA") and xylulose kinase ("xylB") liable for "D-xylose catabolism" were eradicated. This engineered bacterial strain can abolish catabolite repression, therefore permits the simultaneous uptaking of sugars including glucose and xylose, which is reliant on "phosphoenolpyruvate-dependent glucose phosphotransferase system (ptsG)."

3.6.3 Ligninases

Lignin is considered as second major abundant organic polymer which provides a rigidity to plant cell wall structure and also inhibits hydrolysis of hemicelluloses and celluloses. The valorization of huge biomass such as "lignocelluloses" is performed for producing green fuel "bioethanol" (Kawaguchi et al. 2016; Ragauskas et al. 2014), but degradation of lignin is prime task for efficient utilization of biomass in biorefineries. Structurally lignin is determined as a cross-linked polymer of "4-hydroxyphenylpropanoid monomers/monolignols" having various carbon(C)-carbon(C) and ether bonds. p-hydroxyphenyl, guaiacyl, and syringyl groups are the phenolic moieties of monomeric units and their proportion varied with the plant species. Generally, the most common linkages present in lignin are β-β, β-0-4, and β-5 bonds (Vanholme et al. 2010). Highly lignin selective enzyme "ligninases" is the current demand for lignin degradation. There are few fungi which produce ligninases, and among these specifically white-rot fungi synthesize some particular enzymes including MnP (Mn peroxidases), LiP (lignin peroxidases), and laccases which all arrive in category of "ligninases." Various microorganisms produce different combinations of lignin-degrading enzymes displaying varying mechanisms of lignin degradation (Sahadevan et al. 2013). The term "enzymatic combustion" has been described in case of degradation of lignin by lignin-degrading microorganisms, where the oxidizing potential of hydrogen peroxide/molecular oxygen by two enzymes, namely "ligninolytic peroxidase" or "laccase" are subjected to oxidize aromatic units (Kirk and Farrell 1987; Bugg and Rahmanpour 2015). White-rot fungi *Phanerochaete chrysosporium* has been extensively studied to produce extracellular enzymes (Mn peroxidases, lignin peroxidases, and laccases) for biodegradation of lignin (Bugg and Rahmanpour 2015). Several researchers have reported MnPs production from wide range of microorganisms (bacteria, fungi, and algae) (Zhang et al. 2018; Bugg and Rahmanpour 2015). MnPs are the broadly distributed extracellular and potential peroxidases produced by fungi, especially white-rot fungi (*C. subvermispora, Dichomeris squalens, P. sordida, P. chrysosporium, P. radiate,* and *P. rivulosu*) (Hakala et al. 2006). Laccases and LiPs also show vibrant role in the course of lignin de-polymerization (Hammel and Cullen 2008; Bugg and Rahmanpour 2015). Besides aforementioned three important enzymes (cellulases, hemicellulases, and ligninases), some other enzymes including "xyloglucanase"

have been employed for degradation of those secondary polysaccharides which are unable to be transformed into simple sugars through the action of "cellulases" (Stickel et al. 2014). The process of enzymatic hydrolysis carried out at elevated solid loadings is considered to be inexpensive approach due to the accumulation of higher concentration of sugar at the end phase of hydrolysis. And this plentiful amount of sugar is converted into elevated level of ethanol which exhibits low-priced approach with less energy requirement for distillation process (Modenbach and Nokes 2013). Another saccharification method is termed as simultaneous saccharification and fermentation (SSF) in which fermentative microbes are used for simultaneous SSF of hemicelluloses and celluloses (Mosier et al. 2005).

3.7 Fermentation

After hydrolysis, the next imperative step is "fermentation," where the molecules of sugar are taken up by the enzymes synthesized by bacteria or yeasts for producing variety of organic acids and alcohols (Mussatto and Teixeira 2010; Bhagchandanii et al. 2020). The efficiency of the fermentation depends upon two main factors: (1) effective hydrolysis and (2) selection of correct microbial strains to diminish the formation of inhibitory toxic compounds to attain elevated yield of ethanol (Achinas and Euverink 2016). "SHF (Separated Hydrolysis and Fermentation)" is determined as the conventional method in which the process of hydrolysis is performed at earliest to produce monosaccharide sugar as the fermentation proceeds (Dahnum et al. 2015; Devarapalli and Atiyeh 2015; Prasad et al. 2019). One more and important method of hydrolysis and fermentation is known as "SSF (Simultaneous Saccharification and Fermentation)" where the process of cellulose hydrolysis and the process of fermentation of hexose take place in a same reactor by using yeast and enzyme together, so glucose is quickly transformed into ethanol (Cantarella et al. 2004; Dahnum et al. 2015; Prasad et al. 2019). Wyman et al. (1992) described SSF as the better process for providing high ethanol yield in comparison of SHF. Besides the better ethanol yields, the SSF process helps in elimination of end product inhibition, and eradicates the requirement for separate reactors. *Saccharomyces cerevisiae* is the common yeast which plentifully used in the ethanol fermentation. Moreover, *Saccharomyces* is also used as food additive and "generally recognized as safe (GRAS)," and as a result it became best candidate for manufacturing alcoholic beverages. Generally, *S. cerevisiae* has been characterized to carry out glucose fermentation to ethanol very effectively. But on contrary, the fermentation of xylose is exigent as very few conventional ethanol-producing microorganisms depict the ability to readily ferment xylose, although a lot of microorganisms consume "xylose" as a carbon ("C") source (Lin and Tanaka 2006). Biofuel-based industries use different biomass or substrate and specific microbial strain for ethanol production, and are seeking various approaches for the modifications for huge level production of green fuel in more economical manner. In sugar-based and corn-based biofuel industries, the extensive preference has been given to *Saccharomyces cerevisiae* for carrying out fermentation (Achinas and Euverink 2016; Prasad et al.

2019). The role of bacteria in fermentation cannot be avoided as it is very economically feasible and easier strategy for ethanol production (Senthilkumar and Gunasekaran 2005). The common bacterial examples are *Corynebacterium glutamicum* and *Zymomonas mobilis*, which are extensively exploited in industry for ethanol production (Senthilkumar and Gunasekaran 2005; Tsuchida et al. 2007; Kang et al. 2014). Enhancement in ethanol yield is the main task for the researchers (Rai et al. 2010; Jessen et al. 2015), therefore the approach of genetic engineering accepted challenges and resulted in high ethanol production through genetically modified microorganisms. The application of first metabolic engineering surprisingly resulted into the construction of *E. coli* strains which selectively produce ethanol, and *E. coli* presents numerous benefits as a biocatalyst for the ethanol production, as well as the capacity to ferment wide ranges of sugars with no need of complex growth factors (Lin and Tanaka 2006).

3.8 Advancement in Ligocellulosic Valorization: A Biotechnological Mediated Reform

Current development in biotechnology brought a boom in excellent solubility of lignocelluloses.

Modification in genetic program depicted alterations in either microorganisms for efficient production of cellulose degrading enzymes or developing plants having nature of easy solubility of residues for improved fermentation practices. Biotechnological advances have been resulted into the development of genetically modified microorganisms for synthesizing modified cellulosome (cellulose degrading machinery). Cloned and over-expressed man5K gene in *Clostridium cellulolyticum* confirmed 20-fold higher activities of altered/modified form of cellulosomes on substrate "galactomannan" in comparison with control with promising cellulase activities (Perret et al. 2004). Ethanol yields and its titer can be improved by inhibition of by-products, and for accomplishing such task the three respective genes, namely lactate dehydrogenase (ldh), hygromycin phosphotransferase, and phosphotransacetylase (pta) in *C. thermocellum* were knocked out. Deleting only pta gene did not increase ethanol yield, but knocking out of all three genes resulted into a fourfold enhancement in production of ethanol (Argyros et al. 2011). Research on trifunctional cellulosomal complex has represented cellulosome chimera amid cellulases and hemicellulase from several microbes exhibited improved hydrolytic action on complex substrates (Fierobe et al. 2005).

3.9 Conclusion

Valorization of widely available lignocellulosic biomass and the synthesis of bioethanol is the prime need for the present world for lessening the dependency on non-renewable sources such as fossil/petroleum-based fuels. Lignocellulosic material is generated from different sources including plant materials, agricultural and

forest residues, and wastes originating from wood and food-based industries. However, the practice of microbial mediated lignocellulosic waste valorization gave new trends for efficient pretreatment process for increasing the accessibility of cellulose-hemicellulose matrix. Microorganisms particularly bacteria and fungi secrete wide range of hydrolytic enzymes which assist in hydrolysis of large biopolymers such as cellulose, hemicelluloses, and lignin which eventually results in formation of fermentable sugars. Crucial step of fermentation requires activity of numerous microorganisms for utilizing various sugars and their transformation into bioethanol. The microbial mediated steps for lignocelluloses valorization is considered to be economically feasible, and provide environment friendly hub for higher yield of bioethanol. Strategies such as pretreatment and hydrolysis of lignocelluloses and subsequent fermentation step are using microorganisms and their enzymes in current era for the green production of "second-generation biofuel" at efficient level.

3.10 Future Prospects

Application of microorganisms in valorization of lignocellulosic waste is wider, but existing challenges must be addressed to further improvement in generation of second-generation biofuel. The future research is required to employ strategies for elimination of inhibitory by-products with more efficiency. Construction of genetically and metabolically engineered microbial strains should be the prime topic for research in scientific world for improving cellular machinery for many folds higher production of bioethanol with less cost. Therefore, future research needs to be intended for developing strategies through microbial strains which could reduce the duration of pretreatment period and other steps required for bioethanol production.

References

Achinas S, Euverink GJW (2016) Consolidated briefing of biochemical ethanol production from lignocellulosic biomass. Electron J Biotechnol 23:44–53. https://doi.org/10.1016/j.ejbt.2016.07.006

Amin FR, Khalid H, Zhang H, Rahman SU, Zhang R, Liu G, Chen C (2017) Pretreatment methods of lignocellulosic biomass for anaerobic digestion. AMB Express 7(1):72. https://doi.org/10.1186/s13568-017-0375-4

Argyros D, Tripathi S, Barrett T, Rogers S, Feinberg L, Olson D, Foden J, Miller B, Lynd L, Hogsett D, Caiazza N (2011) High ethanol titers from cellulose by using metabolically engineered thermophilic, anaerobic microbes. Appl Environ Microbiol 77:8288–8294. https://doi.org/10.1128/aem.00646-11

Baños R, Manzano-Agugliaro F, Montoya F, Gil C, Alcayde A, Gómez J (2011) Optimization methods applied to renewable and sustainable energy: a review. Renew Sustain Energy Rev 15 (4):1753–1766. https://doi.org/10.1016/j.rser.2010.12.008

Barakat A, Mayer-Laigle C, Solhy A, Arancon RA, De Vries H, Luque R (2014) Mechanical pretreatments of lignocellulosic biomass: towards facile and environmentally sound

technologies for biofuels production. RSC Adv 4(89):48109–48127. https://doi.org/10.1039/c4ra07568d

Bensah EC, Mensah M (2013) Chemical pretreatment methods for the production of cellulosic ethanol: technologies and innovations. Int J Chem Eng 2013:1–21. https://doi.org/10.1155/2013/719607

Bhagchandanii DD, Babu RP, Sonawane JM, Khanna N, Pandit S, Jadhav D, Khilari S, Prasad R (2020) A comprehensive understanding of electro-fermentation. Fermentation 6(3):92. https://doi.org/10.3390/fermentation6030092

Binod P, Janu KU, Sindhu R, Pandey A (2011) Hydrolysis of lignocellulosic biomass for bioethanol production. In: Biofuels. Elsevier, Amsterdam, pp 229–250. https://doi.org/10.1016/B978-0-12-385099-7.00010-3

Brahmachari G, Demain AL, Adrio JL (2016) Biotechnology of microbial enzymes: production, biocatalysis and industrial applications. In: Biotechnology of microbial enzymes: production, biocatalysis and industrial applications. Elsevier, Amsterdam, pp 1–608

Brodeur G, Yau E, Badal K, Collier J, Ramachandran KB, Ramakrishnan S (2011) Chemical and physicochemical pretreatment of lignocellulosic biomass: a review. Enzyme Res 2011:787532. https://doi.org/10.4061/2011/787532

Bugg TD, Rahmanpour R (2015) Enzymatic conversion of lignin into renewable chemicals. Curr Opin Chem Biol 29:10–17. https://doi.org/10.1016/j.cbpa.2015.06.009

Cantarella M, Cantarella L, Gallifuoco A, Spera A, Alfani F (2004) Comparison of different detoxification methods for steam-exploded poplar wood as a substrate for the bioproduction of ethanol in SHF and SSF. Process Biochem 39(11):1533–1542

Chatterjee S, Sharma S, Prasad RK, Datta S, Dubey D, Meghvansi MK, Vairale MG, Veer V (2015) Cellulase enzyme based biodegradation of cellulosic materials: an overview. South Asian J Exp Biol 5(6):271–282

Cheng N, Koda K, Tamai Y, Yamamoto Y, Takasuka TE, Uraki Y (2017) Optimization of simultaneous saccharification and fermentation conditions with amphipathic lignin derivatives for concentrated bioethanol production. Bioresour Technol 232:126–132. https://doi.org/10.1016/j.biortech.2017.02.018

da Costa Sousa L, Chundawat SP, Balan V, Dale BE (2009) "Cradle-to-grave" assessment of existing lignocellulose pretreatment technologies. Curr Opin Biotechnol 20:339. https://doi.org/10.1016/j.copbio.2009.05.003

Dahnum D, Tasum SO, Triwahyuni E, Nurdin M, Abimanyu H (2015) Comparison of SHF and SSF processes using enzyme and dry yeast for optimization of bioethanol production from empty fruit bunch. Energy Procedia 68:107–116. https://doi.org/10.1016/j.egypro.2015.03.238

De Bhowmick G, Sarmah AK, Sen R (2018) Lignocellulosic biorefinery as a model for sustainable development of biofuels and value added products. Bioresour Technol 247:1144–1154. https://doi.org/10.1016/j.biortech.2017.09.163

Devarapalli M, Atiyeh HK (2015) A review of conversion processes for bioethanol production with a focus on syngas fermentation. Biofuel Res J 7:268–280. https://doi.org/10.18331/brj2015.2.3.5

Donato PD, Finore I, Poli A, Nicolaus B, Lama L (2019) The production of second generation bioethanol: the biotechnology potential of thermophilic bacteria. J Clean Prod 233:1410–1417. https://doi.org/10.1016/j.jclepro.2019.06.152

Ferreira S, Duarte AP, Ribeiro MHL, Queiroz JA, Domingues FC (2009) Response surface optimization of enzymatic hydrolysis of *Cistus ladanifer* and *Cytisus striatus* for bioethanol production. Biochem Eng J 45(3):192–200. https://doi.org/10.1016/j.bej.2009.03.012

Fierobe H, Mingardon F, Mechaly A, Bélaïch A, Rincon MT, Pagès S et al (2005) Action of designer cellulosomes on homogeneous versus complex substrates. J Biol Chem 280(16):16325–16334. https://doi.org/10.1074/jbc.m414449200

FitzPatrick M, Champagne P, Cunningham MF, Whitney RA (2010) A biorefinery processing perspective: treatment of lignocellulosic materials for the production of value-added products. Bioresour Technol 101(23):8915–8922. https://doi.org/10.1016/j.biortech.2010.06.125

Furusato S, Takagaki A, Hayashi S, Miyazato A, Kikuchi R, Oyama ST (2018) Mechanochemical decomposition of crystalline cellulose in the presence of protonated layered niobium Molybdate solid acid catalyst. ChemSusChem 11(5):888–896. https://doi.org/10.1002/cssc.201702305

Gray KA, Zhao L, Emptage M (2006) Bioethanol. Curr Opin Chem Biol 10(2):141–146. https://doi.org/10.1016/j.cbpa.2006.02.035

Gupta A, Verma JP (2015) Sustainable bio-ethanol production from agro-residues: a review. Renew Sustain Energy Rev 41:550–567. https://doi.org/10.1016/j.rser.2014.08.032

Hakala TK, Hildén K, Maijala P, Olsson C, Hatakka A (2006) Differential regulation of manganese peroxidases and characterization of two variable MnP encoding genes in the white-rot fungus *Physisporinus rivulosus*. Appl Microbiol Biotechnol 73(4):839–849. https://doi.org/10.1007/s00253-006-0541-0

Hammel KE, Cullen D (2008) Role of fungal peroxidases in biological ligninolysis. Curr Opin Plant Biol 11:349–355. https://doi.org/10.1016/j.pbi.2008.02.003

Herve C, Rogowski A, Blake AW, Marcus SE, Gilbert HJ, Knox JP (2010) Carbohydrate-binding modules promote the enzymatic deconstruction of intact plant cell walls by targeting and proximity effects. Proc Natl Acad Sci 107(34):15293–15298. https://doi.org/10.1073/pnas.1005732107

Himmel ME, Xu Q, Luo Y, Ding S, Lamed R, Bayer EA (2010) Microbial enzyme systems for biomass conversion: emerging paradigms. Biofuels 1(2):323–341. https://doi.org/10.4155/bfs.09.25

Indrasith LS, Sasaki T, Yamashita O (1988) A unique protease responsible for selective degradation of a yolk protein in *Bombyx mori*. Purification, characterization, and cleavage profile. J Biol Chem 263(2):1045–1051

Isikgor FH, Becer CR (2015) Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. Polym Chem 6(25):4497–4559. https://doi.org/10.1039/c5py00263j

Jessen JE, Sveinsson T, Scully SM, Orlygsson J (2015) Ethanol production by a *Paenibacillus* species isolated from an Icelandic hot spring—production yields from complex biomass. Icel Agric Sci 28(1):15–24. https://doi.org/10.16886/IAS.2015.02

Kang Q, Appels L, Tan T, Dewil R (2014) Bioethanol from lignocellulosic biomass: current findings determine research priorities. Sci World J 2014:298153. https://doi.org/10.1155/2014/298153

Kawaguchi H, Hasunuma T, Ogino C, Kondo A (2016) Bioprocessing of bio-based chemicals produced from lignocellulosic feedstocks. Curr Opin Biotechnol 42:30–39. https://doi.org/10.1016/j.copbio.2016.02.031

Kim TH (2013) Pretreatment of lignocellulosic biomass. In: Yang S, El-Enshasy HA, Thongchul N (eds) Bioprocessing technologies in biorefinery for sustainable production of fuels, chemicals, and polymers. Wiley, Hoboken, NJ, pp 91–105

Kirk TK, Farrell RL (1987) Enzymatic "combustion": the microbial degradation of lignin. Annu Rev Microbiol 41:465–501. https://doi.org/10.1146/annurev.mi.41.100187.002341

Kuhad RC, Gupta R, Singh A (2011) Microbial cellulases and their industrial applications. Enzyme Res 2011:280696. https://doi.org/10.4061/2011/280696

Kumar AK, Sharma S (2017) Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. Bioresour Bioprocess 4(1):7. https://doi.org/10.1186/s40643-017-0137-9

Kumar R, Wyman CE (2009) Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. Biotechnol Prog 25(2):302–314. https://doi.org/10.1002/btpr.102

Kurian JK, Raveendran Nair G, Hussain A, Vijaya Raghavan G (2013) Feedstocks, logistics and pre-treatment processes for sustainable lignocellulosic biorefineries: a comprehensive review. Renew Sustain Energy Rev 25:205–219. https://doi.org/10.1016/j.rser.2013.04.019

Lamed R, Bayer EA (1988) The cellulosome of *Clostridium thermocellum*. Adv Appl Microbiol 33:1–46. https://doi.org/10.1016/s0065-2164(08)70203-x

Lass A, Zimmermann R, Oberer M, Zechner R (2011) Lipolysis—a highly regulated multi-enzyme complex mediates the catabolism of cellular fat stores. Prog Lipid Res 50(1):14–27. https://doi.org/10.1016/j.plipres.2010.10.004

Limayem A, Ricke SC (2012) Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects. Prog Energy Combust Sci 38(4):449–467. https://doi.org/10.1016/j.pecs.2012.03.002

Lin Y, Tanaka S (2006) Ethanol fermentation from biomass resources: current state and prospects. Appl Microbiol Biotechnol 69(6):627–642. https://doi.org/10.1007/s00253-005-0229-x

Lu Y, Mosier NS (2008) Current technologies for fuel ethanol production from lignocellulosic plant biomass. In: Vermerris W (ed) Genetic improvement of bioenergy crops. Springer, New York

Maurya DP, Singla A, Negi S (2015) An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. 3 Biotech 5(5):597–609. https://doi.org/10.1007/s13205-015-0279-4

McMillan JD (1994) Pretreatment of lignocellulosic biomass. In: Himmel ME, Baker JO, Overend RP (eds) Enzymatic conversion of biomass for fuels pro-duction. American Chemical Society, Washington, DC, pp 292–324

Menon V, Rao M (2012) Trends in bioconversion of lignocellulose: biofuels, platform chemicals & biorefinery concept. Prog Energy Combust Sci 38(4):522–550. https://doi.org/10.1016/j.pecs.2012.02.002

Mitchell WJ (1998) Physiology of carbohydrate to solvent conversion by clostridia. Adv Microb Physiol 39:31–130. https://doi.org/10.1016/s0065-2911(08)60015-6

Modenbach AA, Nokes SE (2013) Enzymatic hydrolysis of biomass at high-solids loadings—a review. Biomass Bioenergy 56:526–544. https://doi.org/10.1016/j.biombioe.2013.05.031

Moe ST, Janga KK, Hertzberg T, Hägg M, Øyaas K, Dyrset N (2012) Saccharification of lignocellulosic biomass for biofuel and biorefinery applications—a renaissance for the concentrated acid hydrolysis? Energy Procedia 20:50–58. https://doi.org/10.1016/j.egypro.2012.03.007

Mohd Azhar SH, Abdulla R, Jambo SA, Marbawi H, Gansau JA, Mohd Faik AA, Rodrigues KF (2017) Yeasts in sustainable bioethanol production: a review. Biochem Biophys Rep 10:52–61. https://doi.org/10.1016/j.bbrep.2017.03.003

Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96(6):673–686. https://doi.org/10.1016/j.biortech.2004.06.025

Mussatto S, Teixeira J (2010) Lignocellulose as raw material in fermentation processes. Appl Microbiol Microb Biotechnol 2:897–907. https://doi.org/10.1016/j.jrras.2014.02.003

Nair NU, Zhao H (2010) Selective reduction of xylose to xylitol from a mixture of hemicellulosic sugars. Metab Eng 12(5):462–468. https://doi.org/10.1016/j.ymben.2010.04.005

Nair RB, Lennartsson PR, Taherzadeh MJ (2017) Bioethanol production from agricultural and municipal wastes. In: Current Developments in biotechnology and bioengineering: solid waste management. Elsevier, Amsterdam, pp 157–190. https://doi.org/10.1016/B978-0-444-63664-5.00008-3

Nikiema M, Somda MK, Adeoti K, Traore D, Baba-Moussa F, Toukourou F, Dianou D, Traore AS (2017) Production of efficient microbial complex for organic fraction of municipal organic solid waste pretreatment upstream anaerobic digestion. Int J Environ Bioremediat Biodegrad 5(3):77–85. https://doi.org/10.12691/ijebb-5-3-1

Passos CP, Yilmaz S, Silva CM, Coimbra MA (2009) Enhancement of grape seed oil extraction using a cell wall degrading enzyme cocktail. Food Chem 115(1):48–53. https://doi.org/10.1016/j.foodchem.2008.11.064

Perret S, Bélaich A, Fierobe HP, Bélaich JP, Tardif C (2004) Towards designer cellulosomes in clostridia: mannanase enrichment of the cellulosomes produced by *Clostridium cellulolyticum*. J Bacteriol 186(19):6544–6552. https://doi.org/10.1128/JB.186.19.6544-6552.2004

Poszytek K, Ciezkowska M, Sklodowska A, Drewniak L (2016) Microbial consortium with high cellulolytic activity (MCHCA) for enhanced biogas production. Front Microbiol 7:324. https://doi.org/10.3389/fmicb.2016.00324

Prasad R, Chatterjee S, Mazumder P, Gupta S, Sharma S, Vairale M, Datta S, Dwivedi S, Gupta D (2019) Bioethanol production from waste lignocelluloses: a review on microbial degradation potential. Chemosphere 231:588–606. https://doi.org/10.1016/j.chemosphere.2019.05.142

Prasad S, Venkatramanan V, Singh A (2021) Renewable energy for a low-carbon future: policy perspectives. In: Venkatramanan V, Shah S, Prasad R (eds) Sustainable bioeconomy. Springer, Singapore. https://doi.org/10.1007/978-981-15-7321-7_12

Ragauskas A, Beckham G, Biddy M, Chandra R, Chen F, Davis M, Davison B, Dixon R, Gilna P, Keller M, Langan P, Naskar A, Saddler J, Tschaplinski T, Tuskan G, Wyman C (2014) Lignin valorization: improving lignin processing in the biorefinery. Science 344:1246843–1246843. https://doi.org/10.1126/science.1246843

Rai SK, Roy JK, Mukherjee AK (2010) Characterisation of a detergent-stable alkaline protease from a novel thermophilic strain *Paenibacillus tezpurensis* sp. nov. AS-S24-II. Appl Microbiol Biotechnol 85(5):1437–1450. https://doi.org/10.1007/s00253-009-2145-y

Riccio A, Chianese E, Tirimberio G, Prati MV (2017) Emission factors of inorganic ions from road traffic: a case study from the city of Naples (Italy). Transp Res Pt D Transp Environ 54:239–249. https://doi.org/10.1016/j.trd.2017.05.008

Sahadevan L, Misra C, Thankamani V (2013) Ligninolytic enzymes for application in treatment of effluent from pulp and paper industries. Univ J Environ Res Technol 3(1):14–26

Saini JK, Saini R, Tewari L (2015) Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. 3 Biotech 5 (4):337–353. https://doi.org/10.1007/s13205-014-0246-5

Sakai S, Tsuchida Y, Nakamoto H, Okino S, Ichihashi O, Kawaguchi H et al (2007) Effect of lignocellulose-derived inhibitors on growth of and ethanol production by growth-arrested *Corynebacterium glutamicum* R. Appl Environ Microbiol 73(7):2349–2353. https://doi.org/10.1128/AEM.02880-06

Sakamoto T, Hasunuma T, Hori Y, Yamada R, Kondo A (2012) Direct ethanol production from hemicellulosic materials of rice straw by use of an engineered yeast strain codisplaying three types of hemicellulolytic enzymes on the surface of xylose-utilizing *Saccharomyces cerevisiae* cells. J Biotechnol 158(4):203–210. https://doi.org/10.1016/j.jbiotec.2011.06.025

Sánchez ÓJ, Cardona CA (2008) Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour Technol 99:5270. https://doi.org/10.1016/j.biortech.2007.11.013

Saxena RC, Adhikari DK, Goyal HB (2009) Biomass-based energy fuel through biochemical routes: a review. Renew Sustain Energy Rev 13:167. https://doi.org/10.1016/j.rser.2007.07.011

Senthilkumar V, Gunasekaran P (2005) Bioethanol production from cellulosic substrates: engineered bacteria and process integration challenges. J Sci Ind Res 64:845–853

Shah S, Venkatramanan V (2019) Advances in microbial technology for upscaling sustainable biofuel production. In: New and future developments in microbial biotechnology and bioengineering, pp 69–76. https://doi.org/10.1016/b978-0-444-63504-4.00005-0

Shah S, Venkatramanan V, Prasad R (eds) (2019) Sustainable green technologies for environmental management. Springer Nature, Singapore. https://doi.org/10.1007/978-981-13-2772-8

Shaw A, Podkaminer K, Desai S, Bardsley J, Rogers S, Thorne P, Hogsett D, Lynd L (2008) Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield. Proc Natl Acad Sci 105:13769–13774. https://doi.org/10.1073/pnas.0801266105

Shi J, Chinn MS, Sharma-Shivappa RR (2008) Microbial pretreatment of cotton stalks by solid state cultivation of *Phanerochaete chrysosporium*. Bioresour Technol 99(14):6556–6564. https://doi.org/10.1016/j.biortech.2007.11.069

Singh AV, Sharma A, Johri BN (2012) Phylogenetic profiling of culturable bacteria associated with early phase of mushroom composting assessed by amplified rDNA restriction analysis. Ann Microbiol 62:675–682. https://doi.org/10.1007/s13213-011-0304-8

Stickel JJ, Elander RT, McMillan JD, Brunecky R (2014) Enzymatic hydrolysis of lignocellulosic biomass. In: Bioprocessing of renewable resources to commodity bioproducts, pp 77–103. https://doi.org/10.1002/9781118845394.ch4

Su B, Wu M, Zhang Z, Lin J, Yang L (2015) Efficient production of xylitol from hemicellulosic hydrolysate using engineered Escherichia coli. Metab Eng 31:112–122. https://doi.org/10.1016/j.ymben.2015.07.003

Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83:1–11. https://doi.org/10.1016/S0960-8524(01)00212-7

Tong Z, Pullammanappallil P, Teixeira AA (2012) How ethanol is made from cellulosic biomass 1 constituents of cellulosic biomass. UF IFAS Extension, Nov, 2–5. http://edis.ifas.ufl.edu

Ummalyma SB, Supriya RD, Sindhu R, Binod P, Nair RB, Pandey A, Gnansounou E (2019) Biological pretreatment of lignocellulosic biomass—current trends and future perspectives. In: Second and third generation of feedstocks. Elsevier, Amsterdam, pp 197–212. https://doi.org/10.1016/b978-0-12-815162-4.00007-0

Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W (2010) Lignin biosynthesis and structure. Plant Physiol 153(3):895–905. https://doi.org/10.1104/pp.110.155119

Venkatramanan V, Shah S, Prasad R (eds) (2020) Global climate change and environmental policy: agriculture perspectives. Springer Nature, Singapore. https://doi.org/10.1007/978-981-13-9570-3

Venkatramanan V, Shah S, Prasad R (eds) (2021a) Exploring synergies and trade-offs between climate change and the sustainable development goals. Springer, Singapore. https://doi.org/10.1007/978-981-15-7301-9

Venkatramanan V, Shah S, Prasad R (eds) (2021b) Sustainable bioeconomy: pathways to sustainable development goals. Springer Nature, Singapore. https://doi.org/10.1007/978-981-15-7321-7

Venkatramanan V, Shah S, Rai AK, Prasad R (2021c) Nexus between crop residue burning, bioeconomy and sustainable development goals over North-Western India. Front Energy Res 8:614212. https://doi.org/10.3389/fenrg.2020.614212

Wan C, Li Y (2012) Fungal pretreatment of lignocellulosic biomass. Biotechnol Adv 30 (6):1447–1457. https://doi.org/10.1016/j.biotechadv.2012.03.003

Wijaya YP, Putra RD, Widyaya VT, Ha J, Suh DJ, Kim CS (2014) Comparative study on two-step concentrated acid hydrolysis for the extraction of sugars from lignocellulosic biomass. Bioresour Technol 164:221–231. https://doi.org/10.1016/j.biortech.2014.04.084

Willis JD, Oppert C, Jurat-Fuentes JL (2010) Methods for discovery and characterization of cellulolytic enzymes from insects. Insect Sci 17(3):184–198. https://doi.org/10.1111/j.1744-7917.2010.01322.x

Wyman CE, Spindler DD, Grohmann K (1992) Simultaneous saccharification and fermentation of several lignocellulosic feedstocks to fuel ethanol. Biomass Bioenergy 3(5):301–307. https://doi.org/10.1016/0961-9534(92)90001-7

Zhang Q, He J, Tian M, Mao Z, Tang L, Zhang J, Zhang H (2011) Enhancement of methane production from cassava residues by biological pretreatment using a constructed microbial consortium. Bioresour Technol 102(19):8899–8906. https://doi.org/10.1016/j.biortech.2011.06.06

Zhang H, Zhang J, Zhang X, Geng A (2018) Purification and characterization of a novel manganese peroxidase from white-rot fungus *Cerrena unicolor* BBP6 and its application in dye decolorization and denim bleaching. Process Biochem 66:222–229. https://doi.org/10.1016/j.procbio.2017.12.011

Zhu J, Pan X (2010) Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation. Bioresour Technol 101(13):4992–5002. https://doi.org/10.1016/j.biortech.2009.11.007

Zucaro A, Forte A, Basosi R, Fagnano M, Fierro A (2016) Life cycle assessment of second generation bioethanol produced from low-input dedicated crops of *Arundo donax* L. Bioresour Technol 219:589–599. https://doi.org/10.1016/j.biortech.2016.08.022

4 Microbial Valorization: Strategies for Agro-Industry Waste Minimization and Value-Added Product Generation

Jone Ibarruri and Igor Hernández

Abstract

It is estimated that between 20 and 30% of the total food produced in Europe is wasted, generating associated costs of 143 billion euros per year. These wastes include the non-eaten fraction and food chain by-products, including fish and poultry processing by-products, chitinous bioresources, agricultural, dairy, bakery, winery, and brewery by-products. Many of these wastes are rich in nutrients, even so, their high content in humidity and variability and due fundamentally to the nonexistence of an integral and efficient recovery activity causes their elimination without valorization.

The Waste Directive 2018/851 establishes a series of priorities, starting with reuse, recycling, and recovery, and only eliminating them as a last resort, in order to reduce the environmental and economic impact generated. In this context, valorization via fermentation can be an attractive technology. The objective of the chapter is to review recent developments in microbial valorization of by-products, mainly focused on their revalorization as food, feed, and added-value products. Along the chapter, the possible technologies and drawbacks are exposed, describing the bioconversion agents, possible substrates, and resulting products. As general conclusion, biotransformation is a technology with a huge potential for diverse food by-products' valorization when the process is correctly designed and optimized.

J. Ibarruri (✉)
AZTI Food Research, Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Spain
e-mail: jibarruri@azti.es

I. Hernández
Facultad de Farmacia, Universidad del País Vasco/Euskal Herriko Unibertsitatea, Vitoria-Gasteiz, Spain

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_4

Keywords

Microbial valorization · Sustainable bioeconomy · Agro-industry waste · Waste minimization · Added-value products

4.1 Introduction

"The existential environmental challenges and overshoot of planetary boundaries urged humanity to revisit the paradigms of sustainable development, self-discipline and sustainable consumption, Earth's lifesupport system, and carrying capacity" (Venkatramanan et al. 2021a). One such challenge is the global increase in waste generation. According to the World Bank, in 2016, each person produced 0.74 kg of wastes per day, which represent a global amount of 2.01 billion tons of urban solid wastes (SW) approximately. Furthermore, this footprint is expected to increase to 70% by the year 2050 due to rapid population growth and urbanization.

In this context, SW management is a universal issue that affects everyone worldwide. Poorly managed SW have negative environmental and economic impact and requires urgent action at each level of society. A total of 2.01 billion tons of municipal SW is generated, and at least, 33% is not adequately managed. Waste composition depends on where it is generated; for example, in high-income countries, food and green waste represents 32% of total waste, while in low-income countries green and food waste represents 56%. The management of the generated waste also depends on incomes, being the percentage of dumped waste

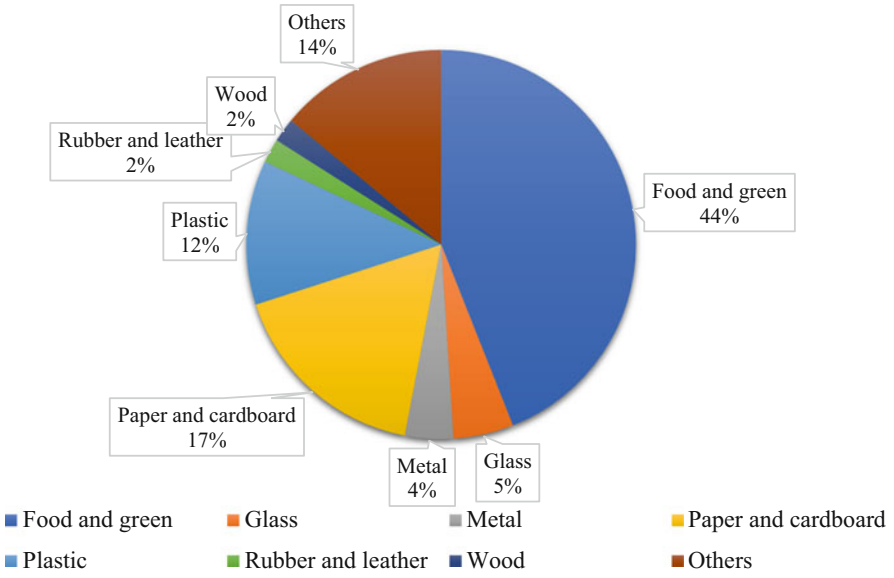


Fig. 4.1 Global waste composition (percent). (Source: Data obtained from Kaza et al. (2018))

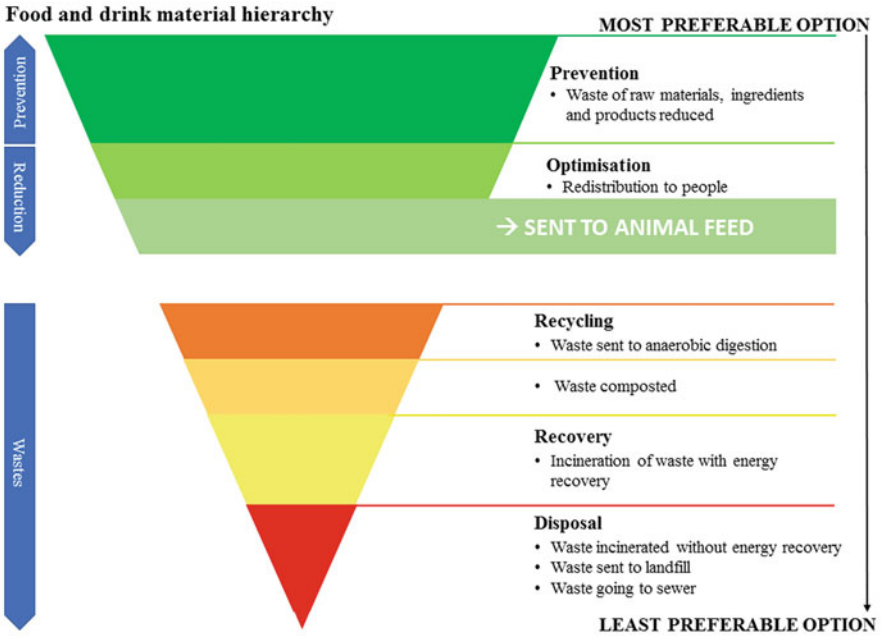


Fig. 4.2 Food and drink material hierarchy developed by the WRAP (Waste and Resources Action Program, United Kingdom). (Source: WRAP (2019). http://www.wrap.org.uk/content/why-take-action-legalpolicy-case)

of 93% in low-income countries, and just of 2% in high-income countries (Kaza et al. 2018). As represented in the Fig. 4.1 on global waste composition, food and green waste represents 44% of total waste.

A very important part of the food produced is not consumed, either because it does not reach the consumer or because the consumer does not use it. However, before addressing the problem, we must do a semantic exercise in order to understand the published data and the existing regulation and clarify the terminology in this field. To unify the definition of what is a by-product, perhaps the most graphic form is to use the "Food and drink material hierarchy" (Fig. 4.2) developed within the WRAP (Waste and Resources Action Program, United Kingdom) as a "roadmap" when considering the possible uses of discards or waste from the food and drink industry depending on the value of the generated new products (WRAP 2019).

According to this classification, the material that is generated due to the production process of a food or a drink, that does not reach the consumers, or that they do not use it, is called "discarding or by-product," while the fraction of the discards that cannot enter the food chain again is called "waste," and it is defined as any substance or object from which its holder has the intention or obligation to dispose of. Although it may seem a mere semantic difference, it is of great importance,

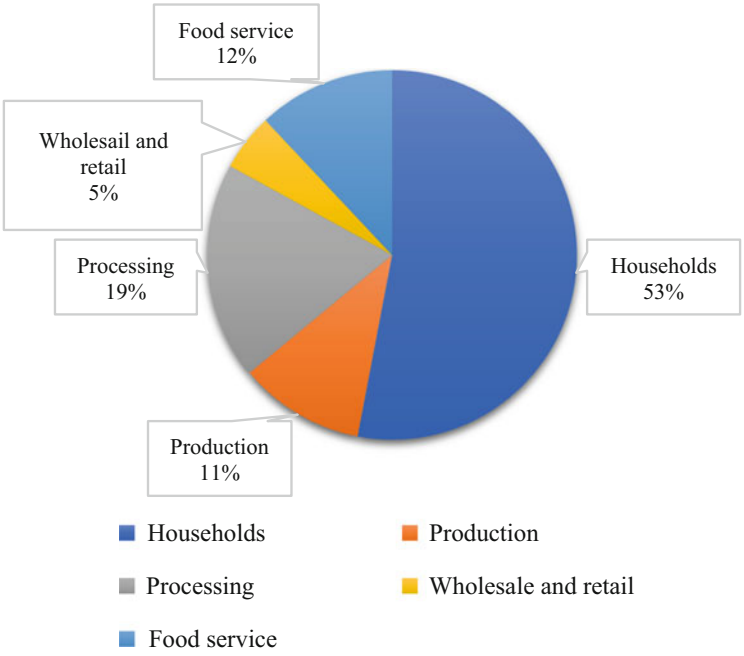


Fig. 4.3 EU-28 food waste in 2012 by sector. (Source: Data obtained from Stenmarck et al. (2016))

since it has important legal and economic repercussions for companies, regulators, and control agencies.

Food waste is a global problem, and across the world, there are several initiatives with the aim of estimating, reducing and valorizing it. As an example, we can consider the European FUSIONS project (Food Use for Social Innovation by Optimizing Waste Prevention Strategies) (Stenmarck et al. 2016) and the world initiative "SAVE FOOD: Global initiative on the reduction of food waste" (Gustavsson et al. 2011).

Both projects estimate that between 20 and 30% of the total food produced in Europe is lost or wasted, generating negative impacts in the entire food supply chain, and that there is a lack of coordination between the components of the food chain and a need for awareness of industries. The FUSIONS project concluded that of the 865 kg of food per person per year produced in the EU-28, 173 kg of food were ended up wasted. However, not all actors in the food chain have the same loss rate, with household waste being the most problematic point. More than half of the food waste in the EU-28 corresponds to the consumer, while only 5% corresponds to the wholesale and retail trade (Fig. 4.3). The costs associated with the waste of food for the EU-28 in 2012 were estimated around 143 billion euros, and two-thirds come from household food waste; due to the high costs associated with processing, packaging, retail costs, etc. (Stenmarck et al. 2016).

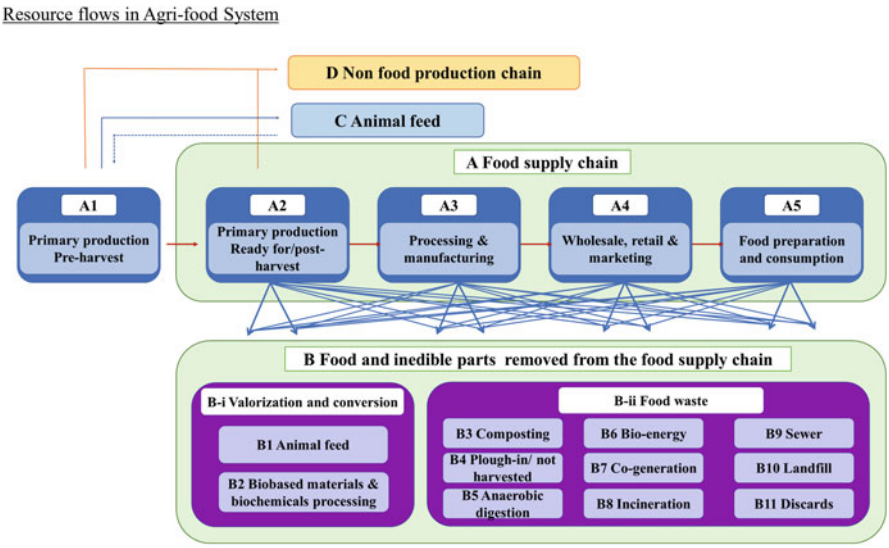


Fig. 4.4 Technical framework in the food supply chain. (Source: Adapted from Östergren et al. (2014))

There are also differences between countries. In developing countries, 40% of losses take place in the post-harvest and processing stages, while in industrialized countries, more than 40% of losses take place at the consumer and retail level. In this last group, large quantities of food are wasted due to quality standards that overestimate the appearance.

Currently, operators have worked on the supply chain and the losses that occur during the food processing are smaller, reducing its margin for improvement (Konstantas et al. 2019; Parajuli et al. 2019). At the same time, care for the environment begins to be a relevant factor in the overall management of companies. Many of them have successfully incorporated the environmental factor into their daily management, taking advantage of the opportunities that the inclusion of environmental criteria entails in the company policy, the improvement of processes, the development of new products, the opening of new markets, new eco-packaging, etc. (Ramos et al. 2016). In last years, new initiatives are being launched to develop comprehensive systems for the recovery of these by-products and waste and to solve the lack of recovery processes in many unprocessed by-products. For example, the EU FUSIONS project defined a Technical Framework to analyze discards resulting from the food supply chain (Östergren et al. 2014) (Fig. 4.4).

When prevention or reduction in the production of a by-product is not possible (Fig. 4.2), current legislation in Europe (Directive EU 2018/851 on waste) establishes the order of priority of different options: reuse, recycling, valorization and disposal. The third option, "valorization", an integral part of sustainable bioeconomy (Venkatramanan et al. 2021b) is considered when the by-product serves

a useful purpose by replacing other materials that would otherwise have been used to fulfill that function. Existing studies show that recovery and conversion (Fig. 4.4) is a common and preferred alternative for many sectors, avoiding treatment costs for their elimination and, in turn, being able to generate a financial return (San Martin et al. 2016). For example, within this option, the use of discards as animal feed can reach 50–80% of the total discard (Stenmarck et al. 2016).

Likewise, from the European institutions it is encouraged to transform these organic discards into products that generate an economic and environmental benefit, being the technological development the key to obtain the maximum potential of some of these by-products.

4.2 Biotransformation Processes for Added-Value Product Generation

Food by-products are usually rich in nutrients, composed mainly of polysaccharides (usually starch 30–60%), proteins (5–10%) and lipids (10–40%) (Kwan et al. 2015). They can be an essential and cost-effective feedstock in biorefineries to produce added-value products while reducing the amount of organic waste that needs to be treated. Biorefinery concept could be related to several industrial sectors, such as feed and/or food, pharmaceutical, cosmetics and energy sector.

Related to food and feed industry, it is estimated that by the year 2050 the world will need to feed 2 billion additional people and produce 70% more meat and milk. The growing future demand for livestock products, related to the increase in income, population and urbanization, will lead to a great demand for food and resources. The sustainability of feed production system is being challenged due to factors such as scarcity of land, soil and water, competition for resources between crops for food or biofuel, climate change and competition for non-renewable resources.

One of the keys to sustainable livestock development is the efficient use of available resources, including the reduction of food waste and the application of new resources for animal feed that do not compete with human food. The EFFPA (European Former Foodstuff Processor Association) states that five million tons of raw materials are used in animal feed (EFFPA 2018) and that there is an upward trend to seven million tons until 2025. In general, the demand for animal feed in livestock is increasing in most of the developing countries, which means that many of these countries cannot produce enough feed, having to import a large part of the ingredients, such as soybeans.

In this scenario, the appearance of alternative raw materials, as is the case of food industry by-products after microbial valorization, would reduce dependence on international markets and costs, and therefore, improve the competitiveness of the sector. In fact, most of the studies based on food by-product biorefineries highlight the economic benefits as one of the major advantages. However, it is necessary to develop economically feasible processes (Kwan et al. 2015). In this sense, there are numerous experiences and studies that demonstrate the high content of valuable

compounds in most of the by-products discarded by the agri-food industry (Brar et al. 2014; Liu et al. 2016; Mamma et al. 2009).

Apart from the direct extraction of valuable compounds (fibers, antioxidants, antimicrobials, pectins, pigments, etc.), an alternative that has generated increasing interest is the development of bioprocesses, which allow the transformation of by-products into high added-value compounds or products (Laufenberg et al. 2003; Nigam and Pandey 2009; Sadh et al. 2018). Bioprocesses, based on both submerged fermentation (SmF) and solid-state fermentation (SSF), have a wide range of applications in valorization processes (Kosseva 2013; Parveen et al. 2007). Depending on the cases, the product to be obtained will be the biomass of microorganisms, enzymes, metabolites, pigments, or other high-value products, the fermented medium or compounds present in said medium and that can be purified by downstream processes (filtration, centrifugation, flocculation, precipitation, absorption, etc.). In addition, a reduction in total COD (chemical oxygen demand) or BOD (biological oxygen demand) in the effluent is usually obtained.

Bioconversion defined as "*the use of biological processes or materials to change organic substances into a new form*" (Collins English Dictionary) has four axes or variables that have to be defined: the first one is defined by the initial by-product (the Substrate), the second one is our high-value product (the Product), a third axis would be the biotransformation agent (Agent) and the last one would be the selected technology for the fermentation (Technology). Along this chapter we will do an extensive review about the first two variables (substrate and product) and describe the main highlights of the third one (agents). About the last one (technology), we present a slight introduction, but authors encourage to the interested readers to consult detailed information before defining their own biotransformation process.

In the field of industrial fermentation, two types of fermentation are clearly distinguished: SSF and SmF. The optimization and scaling up of this type of processes can suppose the development of new activities of biotechnological base, that will allow an advantage of the by-products and will supply new interesting products to varied sectors.

4.2.1 Solid State Fermentation (SSF)

The SSF is defined as the fermentation process carried out in solid medium at low water activity (Aw 0.6–0.9) but always above the necessary to allow the growth and metabolic activity of the microorganism. The solid matrix can be the source of carbon and nutrients or also a support material for the growth of the microorganism to which a growth broth is added (Robinson and Nigam 2003). During the SSF, microorganisms grow in an environment close to their natural habitat, usually resulting in high yields of target compounds, especially when fungi are used as transforming agents (Thomas et al. 2013). It is also a fermentation process especially appropriate for filamentous fungi, due to their special ability to penetrate the mass of solids and because it allows high aeration (Ugwuanyi et al. 2009).

Table 4.1 Advantages and disadvantages related to SSF and SmF

Parameters	SSF	SmF
Free water	– Lower reactor volume – Lower risk of contamination – No foam formation – Lower cost for treatment of effluents	– Higher reactor volume – Highly susceptible to bacterial contamination – High foam formation and higher cost for treatment of effluents
Fermentation medium	– Usually, no supplementation needed	– Sometimes supplementation is needed
Adaptation	– Natural environment of fungi	– Natural environment of bacteria and algae
Productivity	– Higher productivity with fungi	– Lower productivity with fungi
Downstream process	– Highly concentrated product	– Higher purification and concentration costs
Scale-up and process control	– Expensive and complex	– Relatively simple and cheap

This process is increasingly linked to the concept of valorization of discards or food by-products, using them as raw material in SSF processes increases the economic value while avoiding the expense and the environmental impact of having to eliminate them (Lizardi-Jimenez and Hernandez-Martinez 2017; Soccol et al. 2017). There is also a growing trend toward using this technology in the field of biotechnology due to its simplicity and ability to perform the bioconversion of low-cost solid substrates with higher productivities in many cases in comparison with the SmF technology (Lincoln and More 2018). In addition, compared with SmF, the absence of liquid phase in SSF relax the strictly sterile processes requirement.

The SSF requires few reagents and/or additional nutrients since the product itself normally contains the necessary nutrients for the process, and usually requires low-energy consumption since agitation and sterilization are not always necessary (Table 4.1), being a process with numerous environmental advantages (Nigam and Pandey 2009).

Despite all the mentioned advantages, the fermentation equipment presents a series of complexities as it is a technology in continuous study with challenges to solve, such as mass and heat transfer, scaling and operational control, the low reproducibility and heterogeneity of natural substrates (Arora et al. 2018) and the problem to determine the biomass produced, especially when filamentous fungi are the transforming agent, which generate a close interaction between mycelium and substrate (Abu Yazid et al. 2017). The study of heat and mass transfer is the most critical aspect of the process and pose a challenge when designing and scaling processes in the solid state (Ali and Zulkali 2011; Pandey 2003).

The number of type of bioreactors used in the SSF at the pilot and/or industrial level is reduced due to various reasons: the elimination of heat generated in large volumes of substrate, control of process parameters (agitation, oxygen and temperature), nature of the substrate, the need for a pretreatment (sterilization, inoculation) and the control of the management factors such as filling, emptying and cleaning the reactor (Durand 2003).

In the field of valorization, SSF has been used in diversity of applications to transform a high variety of by-products into several end products, such as enzymes, organic acids, phenolic compounds, agents of biological control, lipids, bioethanol, animal feed, digestibility improvement, hydrolysis, mycotoxin reduction, antibiotics, pigments and so on (Brar et al. 2014; Kusumaningtyas et al. 2006; Kwan et al. 2015; Lizardi-Jimenez and Hernandez-Martinez 2017). In most cases, the microorganisms selected for this technology are filamentous fungi or yeast as explained above.

4.2.2 Submerged Fermentation (SmF)

SmF technology uses liquid media for the growth of microorganisms and/or the production of compounds of interest. In comparison with the SSF, the possibility of controlling environmental factors such as temperature, aeration and pH is simpler, keeping the growth medium more stable (Table 4.1) (Abu Yazid et al. 2017). Even so, there are several complications when working with filamentous fungi in SmF due to their morphology and respiratory requirements (Gibbs et al. 2000).

The use of microorganisms during the SmF as a valorizing agent within the sustainability program of the food industry, that generates large volumes of by-products with a high organic load that therefore are necessary to treat before being discarded, is an emerging field. These by-products can be used as growth medium of the microorganism to reduce the COD and BOD and in turn to obtain added-value products. The use of these by-products as the main source of carbon and energy for the production of single cell protein (SCP) and several end-products such as organic acids, enzymes, pigments ethanol, biogas, chitosan and so on has received much attention in recent years (Hernández-García et al. 2019; Khan et al. 2016; Nair et al. 2018; Pleissner and Rumpold 2018; Smetana et al. 2017; Ugwuanyi et al. 2009). As can be observed along this chapter, this practice has been studied with various microorganism such as fungi, bacteria and algae and a large number of food industry by-products.

In both situations (SSF and SmF), since the two main components of the process (the microorganism and the substrate) have been selected, the physiology of the microorganism and the physicochemical factors, necessary for its optimal development, must be studied, including initial humidity, particle size, pH, temperature, composition, sterilization, water activity, inoculum density, agitation, aeration and the treatment of the final product. They should be optimized based on factorial design and response surface experiments to identify critical factors and their interactions (Ashok and Kumar 2017).

4.2.3 The Transforming Agent

When designing a biovalorization process, the decision about the fermentation agent seems critical. In the process of valorization, there are different organisms that can act as "bio-converters," including fungi, yeasts, bacteria and algae.

Bacteria have been used widely in valorization processes, resulting in both high-value products and/or SCP. Their high metabolic diversity, few nutritional requirements and high cultivation plasticity make them a very convenient option for several by-products valorization, including mixed and spontaneous fermentation. In the other way, their low protein content, relatively high minimal Aw and difficulties to drive the metabolic fluxes would require intense selection, adaptation, and, if required, metabolic modification works. Used bacteria included *Brevibacterium*, *Methylophilus, Aeromonas*, *Bacillus, Lactobacillus*, *Pseudomonas*, *Rhodopseudomonas*, and *Flavobacterium* (Anupama and Ravindra 2000).

Yeasts are the other main group of organisms used for waste valorization, including *Candida*, *Kluyveromyces* or *Saccharomyces* (Gervais and Molin 2003). These unicellular organisms have a well-known metabolism and can be easily selected. Well-known yeast used in SCP production are *Candida, Hansenula, Pichia, Torulopsis*, and *Saccharomyces* (Anupama and Ravindra 2000).

Within the molds used for biotransformation, two main division are considered: *Zygomycota* and *Dikarya*. The first one present the main advantages in high metabolic plasticity and high growth rate, while the second ones resist better the mechanical shaking procedures. In the processes of biovalorization, we can find both types, but the first ones (*Zygomycota*) have been widely used for food and feed preparation. Classical examples of *Zygomycota* used in human food are *Rhizopus* (used for tempeh preparation) (Nout and Aidoo 2011). Examples of *Dikarya* utilization are the works developed with *Aspergillus* (used during koji preparation) (Jin et al. 2019) and *Yarrowia lipolytica* or with *Ceriporiopsis subvermispora* for oil and lignin-based by-product transformation (Do et al. 2019; Osman et al. 2019). Filamentous fungi are rich in B vitamins and provide a high percentage of protein (30–70%) while nucleic acids are not very high (9.7%) compared to other microorganisms (Anupama and Ravindra 2000).

Algae is a relatively newly introduced group of possible biotransformation agents. Despite their potential for biofuel production, *Spirulina* and *Chlorella* have emerged as good candidates for high-value compounds production from agro-industrial by-products (Markou et al. 2018), especially for pigments and SCP production, sectors with positive market expectatives (Cordero et al. 2011). To the best of our knowledge, all fermentation processes published are made in liquid medium with controlled pH and high oxygen supply, which reduces the range of possible substrates and increases the required technological equipment. In most of the cases, bioconversion processes use the heterotrophic metabolism of these algae, but in some examples, autotrophic metabolism is required in order to produce pigments. Table 4.2 shows the metabolic characteristics of molds, yeasts, algae and bacteria.

Table 4.2 Metabolic characteristics of molds, yeast, algae and bacteria

	Fungi	Yeast	Algae	Bacteria
Growth rate	Lower than bacteria and yeast	Quite high	Low	Highest
Respiration	Aerobic	Aerobic Anaerobic	Aerobic	Aerobic Anaerobic
Minimal Aw[a]	>0.70	>0.80	>0.85	>0.85
pH	2–10	3–10	6–7.5	2–10
Fermentation	SSF-SmF	SSF-SmF	SmF	SSF-SmF
Toxin	Mycotoxins in many species		Endotoxins form Gram-negative bacteria	
QPS	No	Yes	No	Yes

[a]As general recommendation. (Source: Data obtained from Van Alfen (2014))

About the water activity (Aw), and among the extremophiles and especially resistant yeast, most authors considered molds as the only organism able to grow below Aw = 0.8, and usually, optimal growth is much higher than this value. This effect and the possible variation along the bioprocess can justify the selection of these microorganisms. In addition, the effect of Aw on metabolism has to be considered along the bioprocess, because fungal and bacterial metabolisms are highly influenced by the available free water, as has been described in detail by different authors (Gervais and Molin 2003).

The pH range is also a variable to take into consideration during the process design. When possible, microorganisms with broad range of pH are selected because pH modifications are usual during fermentation processes as a result of metabolism. These changes can inhibit growth or reduce the metabolism and can occur locally in the substrate mass, especially in SSF. On the other hand, SmF usually allows to control pH in a narrower range.

Finally, one of the main concerns about fermentation is the safety of the obtained products. In Europe, the QPS approach (Qualified Presumption of Safety approach) was introduced in 2007 as a pre-assessment approach to identify safe microorganism that can be used deliberately in the food and feed chains (EFSA 2007). This list has been revised, and the update version included 74 Gram-positive bacteria, 3 Gram-negative bacteria, and 16 yeast (Ricci et al. 2017). No algae or filamentous fungi are included in the list. Bacteria and yeast present in this list are considered safe under most of the fermentation conditions, but the absence does not mean that the products are unsafe. In addition, the European Food Safety Authority (EFSA) have a "precautionary principle", where the foods that have been significantly consumed within the EU territory prior to May of 1997 are considered safe, and if not, are labelled as "novel food" and must undergo the novel food regulation stated by the EFSA before being marketed. Many of the bacteria used in food industry are not listed and are part of food and feed generally considered as safe (i.e., *Acetobacter aceti* and vinegar). In these cases, substrate and product characteristics, elaboration process, and metabolism should be considered case-by-case, in an approach similar to the GRAS

(generally recognized as safe) classification, the standard in the USA, or the equivalent in other countries. In this case, the "safe" label is a consequence of the absence of toxins or pathogenicity during the product elaboration and storage and does not depend only on the microorganisms' potential pathogenicity.

Beyond the generalities listed in Table 4.2, the medium composition will determine the suitability of each microorganism for a specific process. For example, ethanol has a significative inhibitory effect on most bacterial growth, but most of the *S. cerevisiae* strains have a good ethanol fermentation up to 15% of total volume. In this case, the yeast would be the logical selected "bio-converter" in industrial ethanol plants, but, in fact, many plants would use *Zymomonas mobilis* because of its very high yield and conversion rate (up to 1.9 mol of ethanol from 1 mol glucose) (Rogers et al. 2007). In a similar way, *A. aceti* has an intense oxidative fermentation, transforming glucose into CO_2, but, in the absence of glucose and the presence of alcohol, this microorganism will transform ethanol into acetic acid rapidly, with a subsequent pH decrease in the product (Mamlouk and Gullo 2013). Thus, microorganism population, dynamics and cross metabolism should be considered from a global point of view, especially in non-sterile or spontaneous fermentations.

In addition, the production of antibacterial or antifungal compounds have to be considered. A very well-known compound is the killer factor produced by *S. cerevisiae* or the antibacterial compounds produced by certain plants. Metabolites present in the substrate or produced during fermentation can boost or inhibit microorganism growth and modify the population. In conclusion, the biotransformation agents selected for bioprocesses require an exhaustive analysis to comply with technological, safety, and metabolic lines.

4.3 By-products

At European food and feed system, cereals are the group of food with the highest production, with almost 500 million tons/annum, followed by dairy products with 220 million tons and fruits and vegetables with 170 million tons (Fig. 4.5). The losses associated within fruits and vegetables are greater than in the rest of the groups and achieve almost 7% of their production. The use in animal feed, on the other hand, is much higher in cereals, which accounts for half of their production. In the case of legumes, roots and dairy products the percentage of product destined for animal feed is of 42, 18 and 15% respectively. In fruits and vegetables however, it is considerably lower with 4.5%, due to their high humidity and much lower protein content (FAOSTAT 2018).

4.3.1 Horticultural By-products

A large number of unconsumed fractions of horticultural products (fruits, vegetables, roots and cereals) and the by-products generated from their processing industries are available throughout the world. Figure 4.5 shows that, of the total not consumed

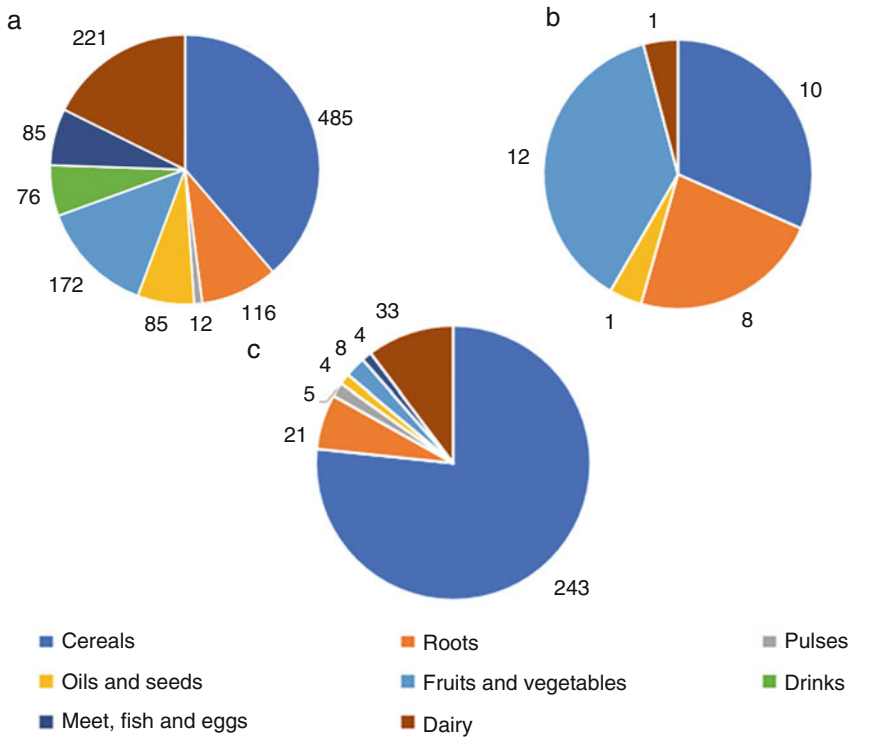


Fig. 4.5 Quantity (million tons) of (**a**) production, (**b**) discard, and (**c**) use as animal feed of cereals; oils and seeds; meet, fish, and eggs; roots; fruits and vegetables; dairy; pulses; and drinks in the European Union (2013). (Data obtained from FAOSTAT)

food in Europe in 2013, the highest discard rates are concentrated in this sector with a total of 30 million tons per year.

The quantities destined to animal feeding are minimum, 7 and 0.3 million tons in fruit and vegetables respectively, and much higher in roots and cereals with a total of 20 and 243 million tons per year, respectively. Most horticultural by-products, mainly fruits, vegetables and roots are highly fermentable and perishable, due to their high humidity (80–90%). Therefore, it is necessary to adopt measures to be able to value, stabilize and store these by-products in order to be able to use them throughout the year in a competitive and sustainable manner (Wadhwa and Bakshi 2013). The main impediment to use them in quality animal feed is the low nutritional value of some of these by-products (low protein content), as well as the low digestibility or the presence of possible undesirable substances like antinutritional agents or mycotoxins.

At present, these discards are mainly managed as waste, due to the lack of an integral and efficient recovery activity, thus causing environmental risks. Even in the case of the by-products collected by managers, the transformation that takes place with a large part of them is limited to the production of compost or silage with a low

economic value in the market. Other valuation alternatives that are applied to fruit and vegetable by-products are obtaining value-added products, such as extraction of essential oils, polyphenols, anticancer compounds, pigments, enzymes and SCP (Wadhwa and Bakshi 2013).

In the case of cereals, the half of the total production in Europe, 250 million tons per year, is used for animal feeding purposes (Fig. 4.5). The high quantity of cereals destined to animal feed sector is related to their higher energy and protein and their lower humidity proportion. Cereals' incidence on feed prices is very high and manufacturers need a continuous and guaranteed supply that allows them to cover the needs of livestock feed. According to the FEFAC (European Feed Manufacturers Federation), despite the large variation in the price of raw materials in recent years, the proportion of raw materials by categories remains relatively stable (48% cereal, 28% flours of oilseeds) (FEFAC 2012). Issues such as the quality of the raw material, the continuity in the offer, the guarantee that they are free of mycotoxins, or the logistical costs, impact on the competitiveness of the feed sector.

In this scenario, one of the alternatives proposed, is the use of food industry by-products in animal feed sector. This strategy can offer food producers a constant and sustainable market to their food losses and, at the same time, can be a source of alternative raw materials for feed manufacturers. However, most of the generated horticultural by-products have high humidity and low protein content, making them not suitable for direct use as animal feed. As a strategy, these horticultural discards, mainly composed of lignocelluloses, soluble sugars, starch (in case of root crops), fibers and phenols (Tengerdy and Szakacs 2003), can be metabolized by a wide range of microorganisms into nutritionally enriched and/or value-added products. Table 4.3 summarizes the by-products, microorganisms and the end-products obtained by biovalorization processes. Fungi are the most used microorganisms due to, as explained above, their higher yields in SSF, which is the best adapted process for horticultural by-products. Considering the obtained end-products, we can make four main groups: food and feed, enzymes, organic acids and others, where we can find antioxidants, aromatic compounds, and biofuels.

Enzyme production via biotechnological SSF processes has gained attention in the last years with the main objective of replacing previously existing chemical processes which have potential negative impact on the environment (El-Bakry et al. 2015). *Aspergillus* and *Rhizopus* genus are the most used microorganisms for enzyme production, and cereal and legume by-products are generally fermented, such as wheat, rice, corn, oat, sugarcane and soybean by-products (Table 4.3). Among the different enzymes, the most interesting ones produced by SSF of horticultural by-products are amylases, cellulases, and pectinases. For example, Kumar et al. (2013) used mango kernel (up to 60% starch) for alpha-amylase production via *Fusarium solani* fermentation (pH 4, 30 °C, and 9 days). In the same way, Suresh and Radha (2016) defined the best conditions for phytase production by *Rhizopus oligosporus* using as substrate different rice bran varieties and Central Composite Design (CCD) for process optimization.

Plant oil-extracted seed cakes are good fermentation substrates for producing lipases. In this context, Venkatesagowda et al. (2015) studied the production of

Table 4.3 Microbial valorization of horticultural by-products

Product	Substrate	Microorganism	Reference	Product	Substrate	Microorganism	Reference
Food-feed							
Animal feed	Apple, pear, potato starch	*Rhizopus oryzae, Candida tropicalis, Saccharomycopsis fibuligera, Geotrichum candidum*	Ibarruri and Hernández (2018) and Lei et al. (2012)	SCP	Orange molasses, starch processing wastewater, pea-processing industry by-product, custard apple, watermelon, and sweet lime, potato protein liquor	*Rhizopus* sp., *Aspergillus oryzae, Rhizopus arrhizus, Fusarium venenatum, Monascus purpureus, Neurospora intermedia, Rhizopus oryzae, Lactobacillus acidophilus, Streptococcus thermophilus, Bacillus coagulans*	Ibarruri and Hernández (2019), Potnis et al. (2016), Souza Filho et al. (2018a) and Souza Filho et al. (2017)
Organic acids							
Fumaric acid	Apple industry by-products, starchy material	*Rhizopus oryzae*	Das et al. (2015) and Deng et al. (2012)	Lactic acid	Sapota, banana, papaya, potato, corn cob	*Rhizopus oryzae*	Kumar and Shivakumar (2014) and Saito et al. (2012)
Citric acid	Apple pomace	*Aspergillus niger*	Dhillon et al. (2013a)				
Enzymes							
Amylases	Wheat flour, cassava bagasse, mango kernel,	*Rhizopus oryzae, Rhizopus microsporus var.*	Afrisham et al. (2016), Cerda et al. (2016),	Phytase	Agro-residues of wheat bran, mustard oilcake,	*Rhizopus oligosporus*	McKinney et al. (2015) and

(continued)

Table 4.3 (continued)

Product	Substrate	Microorganism	Reference	Product	Substrate	Microorganism	Reference
	soy, bread, wheat bran	*oligosporus, Fusarium solani*, *Thermomyces* sp., *Bacillus licheniformis*	Freitas et al. (2014) and Kumar et al. (2013)		rice bran, corn bran, oat bran and sugarcane bagasse		Suresh and Radha (2015)
Lipase	Coconut kernel-cake, wheat bran	***Lasiodiplodia theobromae*** VBE-1, *Penicillium camemberti*	Malilas et al. (2013) and Venkatesagowda et al. (2015)	Protease	Wheat bran and soybean meal, wheat bran, soybean bran, soy fiber	*Bacillus subtilis, Aspergillus niger, Aspergillus flavipes, Aspergillus brasiliensis, Aspergillus oryzae, Penicillium roqueforti, Thermus* sp.	El-Bakry et al. (2016), Novelli et al. (2016) and Saba et al. (2013)
Cellulase	Rice hull, banana by-product, wheat bran, cotton husks, alfalfa hay, oat straw, *Ilex paraguariensis*	*Rhizopus oryzae, Trichoderma reesei, Pleurotus* sp.	Kupski et al. (2015), Ortiz et al. (2015) and Reddy et al. (2003)	Tannase	Sugarcane, bagasse, corn, coconut husk, candelilla stalks	*Aspergillus niger*	Buenrostro-Figueroa et al. (2014)
Pectinase	Citrus by-products, sugarcane bagasse	*Aspergillus oryzae*	Biz et al. (2016)				

Others							
Polyphenols	*Lablab purpureus, Oryza sativa,* black rice bran	*Aspergillus awamori*, *Aspergillus oryzae*	Sadh et al. (2017b), Sadh et al. (2017c) and Shin et al. (2019)	γ-Oryzanol	Rice bran	*Rhizopus oryzae*	Massarolo et al. (2017)
Minerals	Peanut oil cakes	*Aspergillus oryzae*	Sadh et al. (2017a)	Biogas	Wheat straw	*Neurospora intermedia*	Nair et al. (2018)
Bioethanol	Sorghum stover, paddy straw	*Rhizopus oryzae, Trichoderma reesei*	Pandey et al. (2016) and Srujana et al. (2015)	Aromatic compounds	Cassava bagasse, apple, pomace, soybean, amaranth grain, orange peel	*Rhizopus* sp., *Saccharomyces cerevisiae, Hanseniaspora* sp., *Ceratocystis fimbriata, Penicillium digitatum*	Badee et al. (2011), Christen et al. (2000), Mantzouridou et al. (2015) and Rodríguez Madrera et al. (2015)

lipases on eight plant oil seed cakes by SSF with five lipolytic fungi (*Aspergillus niger, Chalaropsis thielavioides, Colletotrichum gloeosporioides, Lasiodiplodia theobromae,* and *Phoma glomerata*). Highest lipase production was obtained with *Lasiodiplodia theobromae* VBE-1 grown on coconut kernel cake, and key variables were selected with response surface methodology (RSM), obtaining a final yield of 698 U/g dry matter (DM). The aim of the investigation of Ortiz et al. (2015) was to find a promising wild-type *Trichoderma* strain for cellulases production. With this purpose, they screened 20 *Trichoderma* strains in cellulase-agar plate, and the selected one was used for six different agroindustry by-products' fermentation, obtaining highest activities with rice bran. Pectinases can be used for D-galacturonic acid production. Biz et al. (2016) used *Aspergillus oryzae* in a pilot-scale packed bed reactor for citrus pulp (51.6%) and sugarcane bagasse (48.4%) fermentation. They demonstrated that dried fermented solids added to a pectin solution gave a similar D-galacturonic release profile as that obtained with commercial enzyme.

Horticultural by-products have also been fermented to obtain nutritionally enriched products with animal feeding and human food production purposes. In this case, fruit and vegetable by-products are mainly used as substrates due to their higher easily digestible sugars content and lower protein content, what leave a higher room for nutritional enrichment. Apart from solid substrates, wastewaters, orange molasses, starch processing wastewaters and pea-processing industry by-products, derived from food processing industries, have also been valorized by SmF for SCP production (Ibarruri and Hernández 2019; Souza Filho et al. 2018a, b). Co-production of enzymes with interest in animal nutrition and nutritionally enriched biomass could be an exceptional combination in order to increase the final product value.

Another end-product with industrial interest are organic acids, with a wide range of applications as acidulants, preservatives, flavorings, emulsifiers, sequestrants and buffering in food, beverage, pharmaceutical, nutraceutical and cosmetic products (Ciriminna et al. 2017; Magnuson and Lasure 2004). Main substrates are also fruit by-products and starchy material due to their higher sugar availability, which could provide higher yields. Main lactic and fumaric acid producer is *Rhizopus* fungi while main citric acid producer is *Aspergillus niger* (Table 4.3). For example, Das et al. (2015) used apple pomace ultrafiltration sludge and apple pomace for fumaric acid production through *Rhizopus oryzae* submerged and solid fermentation. Dhillon et al. (2013b) also fermented apple processing by-products but with the aim to produce citric acid and to explore the possibility of extracting chitosan from fungal biomass as co-product by *Aspergillus niger*.

Finally, horticultural by-products have also been used for the production of polyphenols, minerals, biofuels and aromatic compounds. The latest research have been conducted using *Aspergillus* and *Rhizopus* as valorization agents and authors concluded that during SSF fungi release carbohydrate-cleaving enzymes (β-glucosidase), releasing free aglycones with antioxidant activity (Ajila et al. 2012). In other example, Shin et al. (2019) fermented black rice bran with *Aspergillus awamori* and *Aspergillus oryzae* and examined the phenolic acid composition and the antioxidant activity, concluding that SSF was an effective process to increase

phenolic acid content and antioxidant activity of the extract. In the case of bioethanol and biogas production, lignocellulosic materials are the most used substrates, such as wheat straw, or sorghum stover, generally fermented by fungi. Nair et al. (2018) integrated wheat straw fermentation for ethanol and biogas production while producing protein rich filamentous fungi biomass, suitable for feed purposes. Used bioagent was *Neurospora intermedia,* an *Ascomycete* used for food purposes. Some researcher also used SSF of cassava bagasse, apple pomace and orange peel, among others, for aroma compound production. During the fermentation process, terpineol, "fruity" esters and alcoholic notes were detected (Table 4.3).

4.3.2 Fish Processing By-products

Fish industry has several organic by-products that can be processed for biological valorization, like processing waters, viscera, head and bones. Some authors consider that waste can be up to 45% of live weight, resulting in over 60 million metric tons of waste per year (Rai et al. 2015). These by-products are rich in essential fatty acids, high-nutritional value proteins, minerals and active compounds.

Probably, the older fermentation technology for fish by-products is silage formation. Despite the ancient "garum" preparation method, to the best of our knowledge, the first scientific review of fish by-products' fermentation was published in 1982 (Raa and Gildberg 1982). Since then, many researchers had described the advantages of this technology to increase the nutritional value for feed. Sachindra et al. (2007) used silage as pre-treatment for carotenoids extraction from shrimp by-product, and Marcelino Oliveira Cavalheiro et al. (2007) used the shrimp-head silage powder for tilapia with positive results. In many cases, silage is a non-controlled SSF process, but other authors used selected starters, like Reyes-Becerril et al. (2012), who inoculated two fish sileages with *Lactobacillus sakei* and resulted in better growth performance in fishes infected with pathogenic *Aeromonas,* and Libonatti et al. (2019), who observed a fast pH decrease in the silage when encapsulated *Weissella* was inoculated. Fishes silage has also been used as nutrient substitute for bacterial growth medium. Vázquez et al. (2008) tested different by-products of fish species for lactic acid bacteria growth, resulting in excellent substrate for promoting growth and lactic and acetic acid production.

Different fishery by-products have been used for enzyme production or high value product generation. A recent review published by Ben Rebah and Miled (2013), reported numerous examples of proteases, lipases, chitinolytic enzymes, and ligninolytic enzymes in mediums derived or containing fish by-products. Recently, Ruthu et al. (2014) obtained protein (degree of hydrolysis up to 36%) and fish oil (up to 64% of total oil) from fish head inoculated with lactic acid bacteria (LAB) for 72 h. Best results were obtained with *Enterococcus faecium* as fermentation agent, including several antibacterial and antioxidant activities.

In many cases, fish-derived by-products require a pretreatment before the biotransformation process. In a combined strategy, Vázquez et al. (2019) obtained fish protein hydrolysate after alcalase hydrolysis and fermented this by-product with

P. acidilactici to obtain bacteriocins (pediocin SA-1) and bacterial biomass. These by-products have a great potential as peptone substitute in other bacterial culture medium for biomass, enzymes or bioplastic production.

Finally, fish by-products have been also used for polyhydroxybutyrate (PHB) production using *B. subtillis* as biotransformation agent (Mohapatra et al. 2017) in an attempt to produce biological compatible plastic.

4.3.3 Poultry By-products

Poultry production has an undoubtable social and economic importance. In European Union, more than 15 million tons of poultry meat is produced, with an accumulative rise of three million tons in last 10 years (EUROSTAT 2019). Scientific community has been interested in poultry waste management for years. The poultry development review of FAO proposed to re-use the poultry manure and litter for animal refeeding, as well as for bioenergy or land application as crop nutrients, but several risk situations have been described related to these practices (Adeoye et al. 2015; Akanni and Benson 2014). Waste transformation in order to obtain energy has been explored in various works, mainly with positive results (Dornelas et al. 2017). In addition, significant efforts have been done to improve the bacterial digestion of poultry wastes, but the possibility of obtaining high value products is relatively new. Poultry by-products' valorization in high-value products have two principal axes: keratinaceous wastes and the wastewater wastes' valorization.

In poultry, keratinaceous by-products are related with feathers. These are rich in nitrogen, minerals, and organic nutrients and would support the growth of bacteria and *actinomycetes* (Singh Ningthoujam et al. 2018). Different reviews have been published recently about the production and use of keratinases using feathers as substrate (Iijima et al. 2004; Verma et al. 2017). Łaba et al. (2018) used *Kocuria rhizophila* as keratolytic agent resulting in a supernatant rich in amino acids using SmF for 4 days and with diluted chicken feathers as substrate. In other cases, SSF is the best option for feather degradation, like demonstrated by Mazotto et al. (2013) in a novel application of *Aspergillus niger* as biotransformation agent.

Poultry wastewaters are rich in nitrogen, phosphorous, and other minerals. Oliveira et al. (2018) used the poultry wastewater for algae production (*Scenedesmus obliquus*) and generating high-value biomass for feed. Also, this nutrient richness has been exploited in slaughterhouse wastewater, a substrate that can be used as culture medium for probiotics or other bacteria production (Ashayerizadeh et al. 2017). Probably, future works will consolidate the poultry wastewaters as adequate substrate for bacteria or fungi production and will explore the ability of this bacteria to remove the contaminants eluted in these waters in order to increase the sustainability of the poultry production process.

4.3.4 Chitinous Bioresource

Chitin is a polysaccharide (β-(1,4)-2-acetamido-2-deoxy-D-glucopyranose) where each individual residue is N-acetyl-D-glucosamine and is used as a coagulating agent in water treatment, agents for coating seeds of plants in agriculture and for the formation of biomaterials in biomedicine (Liao et al. 2008).

Chitosan is a linear hydrophilic polysaccharide composed of β-1,4-D-glucosamine chains with a lower degree of acetylation than chitin (<30–40%). It is produced by deacetylation of the chitin found in the exoskeleton of some crustaceans and insects, although it can also be isolated from the cell wall of fungi *Zygomycetes* (Ghormade et al. 2017) since it has protective and support functions in the cell walls of them (Goksungur 2004). Chitosan has many more applications in the food, cosmetic, and pharmaceutical industries due to its biodegradability, biocompatibility, film-forming properties, and chelating agents, along with its antimicrobial activity (Tai et al. 2010).

The major source of industrial chitin comes from sea food processing by-products, mainly crustacean shells (e.g., prawn, shrimp, crab, krill shells), where it is found incrusted with proteins and calcium carbonate; therefore, chitin extraction from shellfish involves several extraction steps (Halder and Mondal 2018). Traditionally, chemical extraction has been applied, using alkali and acidic treatments. In contrast, biological methods are comparatively safe, low cost, and environmentally friendly where enzymes and microorganism are used for chitin extraction (Younes and Rinaudo 2015).

The main objective of microbial fermentation in crustacean shells is the deproteinization and liquefaction of the protein fraction by proteolytic enzymes, biosynthesized by endogenous microbes or by inoculating selected microbial strains. There are basically two kind of fermentations, lactic acid and non-lactic acid fermentation.

Lactic acid fermentation has been mainly performed by *Lactobacillus* strains (Arbia et al. 2012), generally in combination with dilute chemical treatments or commercial proteases to improve the efficiency of the process (Cira et al. 2002). In this process, *Lactobacillus* strains produce, in addition to proteases, lactic acid, which reduces the pH and in consequence the possibility of growth of spoilage bacteria. Using shrimp shell as substrate, *Alcaligenes faecalis* is able to deproteinized up to 86.5%, generating a protease with high activity in organic solvent and releasing an hydrolysate with significant antioxidant activity (Maruthiah et al. 2016). Non-lactic fermentation has been performed using several bacteria and fungi, such as *Bacillus* sp. (Ghorbel bellaaj et al. 2012; Sini et al. 2007; Yang et al. 2000), *Pseudomonas* sp. (Ghorbel-Bellaaj et al. 2011; Oh et al. 2007), *Serratia* sp. (Jo et al. 2008) and *Aspergillus* sp. (Mahmoud et al. 2007).

Co-fermentation has also been applied with success for chitin extraction of crustacean shells. For example, red crab shell was co-fermented with *Lactobacillus paracasei* spp. *tolerans* KCTC-3074 with protease producing *Serratia marcescens* FS-3, which resulted in the highest level of demineralization (97.2%) and moderate deproteinization (52.6%) (Jung et al. 2005). Two proteolytic *Bacillus licheniformis*

strains were also employed for fermentation of shrimp shells and resulted in 99% deproteinization and 98.8% demineralization (Waldeck et al. 2006). Another co-fermentation process describes the use of *Lactobacillus brevis* and *Rhizopus oligosporus* in shrimp waste with high deproteinization level (96%) (Aranday-García et al. 2017). As mentioned above, biological methods have several advantages compared to chemical processes; however, they take longer processing time and poorer protease accessibility, what reduces the yield of chitin.

Another alternative to obtain chitin and chitosan is filamentous fungi biomass production. The use of filamentous fungi for the production of chitosan by SmF of various agro-industrial by-products has been widely studied. Among the by-products, we can find cheese whey (Chatterjee et al. 2008; Chatterjee and Guha 2014), corn straw (Tai et al. 2010), apple processing waste (Dhillon et al. 2013b) and citrus waste (Satari et al. 2016).

4.3.5 Dairy Industry By-products

The dairy and cheese processing industries generate large volumes of liquid by-products, where cheese whey is the most studied one. Cheese whey is the liquid portion produced after the coagulation of milk casein. The total world production of whey is estimated at 180–190 million tons per year (Mollea et al. 2013), and most of its production occurs in the EU and the USA, approximately 70% of the total (Yadav et al. 2015).

The whey generates important environmental problems due to its high volume of production and its high organic content, with a COD of approximately 50–102 g/L (Carvalho et al. 2013). Therefore, proper management, with treatment or reuse, is mandatory before its elimination.

The factors responsible for the high organic load (and its contaminating capacity) are residual nutrients such as lactose (46.0–52.0 g/L), proteins (6.0–10.0 g/L), lipids (5.0 g/L) and vitamins.

The whey has a large amount of both, organic and inorganic nutrients, considered a potential resource for obtaining added-value products. Several technologies are used for their valorization, and nowadays, approximately 70% of it is valued in the form of whey powder; however, a considerable amount of whey remains unvalued. This valorization can be done through direct and/or biotechnological processing (Yadav et al. 2015).

Direct processing, by either thermal or physical treatment, is used to obtain whey powder, whey protein concentrate, whey protein isolate, permeated whey, lactose, and other fractions. In biotechnological processing, whey is used as a substrate for various microbial processes to obtain high added-value final products, such as SCP, probiotics, organic acids, enzymes, exopolysaccharides and bioplastics (Carvalho et al. 2013; Yadav et al. 2015). SCP is produced by fermentation with microorganisms that consume lactose and the protein produced can be used as animal feed or as a source of protein for human consumption. Yadav et al. (2014) used mixed culture of *Kluyveromyces marxianus* and *Candida krusei* to enhance

COD removal and to obtain SCP using cheese whey as substrate. They concluded that mixed culture can contribute to produce SCP and simultaneous COD removal under extreme operating conditions. Later, Yadav et al. (2016) also used mono (*Kluyveromyces marxianus)* and mixed culture (*K. marxianus* and *Saccharomyces cerevisiae*) for whey fermentation and obtained higher protein recovery (92%) when mixed culture was used. Other authors selected *Rhizopus* filamentous fungi to produce SCP and chitosan from deproteinized cheese whey (Chatterjee et al. 2008; Chatterjee and Guha 2014). Ibarruri and Hernández (2019) concluded that deproteinized cheese whey fermentation by *Rhizopus* sp. could contribute to promote a sustainable feed industry while reducing cheese whey treatment, obtaining a 76% of reducing sugar consumption and fungal biomass production with 49% of essential amino acids.

Turner et al. (2017) converted lactose into lactic acid from acid cheese whey using an engineered *S. cerevisiae.* The engineered yeast was capable of producing lactic acid (0.358 g/g lactose) from purified lactose, store-bought milk and whey. Aroma compounds have also been produced by *Wickerhamomyces pijperi* yeast with whey (Izawa et al. 2015). Twelve aroma compounds were identified in the fermented broth, in which ethyl acetate, acetaldehyde, and isoamyl alcohol are the major components. Authors concluded that *W. pijperi* could be used as a novel microorganism for the production of aroma compounds from whey.

Other dairy products, such as cream, crème fraiche, sour milk, mild yoghurt and sour milk, have also been used for microbial fermentation of fungi (*Aspergillus oryzae* and *Neurospora intermedia*) with the aim to convert lactose and fat to protein-rich fungal biomass, ethanol, fatty acids, and glycerol (Mahboubi et al. 2017a, b).

4.3.6 Bakery By-products

Bakery by-products, where we can include bread, are generated in huge quantities and, therefore, require a special attention in terms of recycling and reuse. Indeed, bread is the most popular baked cereal product widely consumed throughout the world. The percentage of waste generated by the bakery industry exceeds 7% of the total production in the European Union which may be approximately 1.3 million tons annually (Mena et al. 2011). In general, bakery by-products have homogenous composition, containing nutrients that can act as substrate in microbial valorization processes such as starch, which is the main constituent of the bread in dry weight (59.8% w/w) (Leung et al. 2012). Carbohydrates and nitrogen from bakery by-products can be valorized into desired end-products by microorganisms; however, in some cases mainly in bread, large molecules have to be broken into metabolizable sugars and amino acids, and in these cases, a previous step of hydrolysis is usually developed (Kawa-Rygielska et al. 2012).

In this context, several investigations were performed for the reuse of bakery by-products, such as the one developed by Adessi et al. (2018) with the purpose of energy production by lactic acid fermentation of bread discards. Other researchers

focused on organic acid production, like succinic acid production using *Actinobacillus succinogenes* (Lam et al. 2014; Zhang et al. 2013) and lactic acid production using fungal enzymes and *Chlorella pyrenoidosa* in mixed restaurant food and bakery discards (Pleissner et al. 2015) or by *Thermoanaerobacterium* sp. (Yang et al. 2015). Pigment production by heterotrophic microalga *Galdieria sulphuraria* on substrates made of food discards from restaurants and bakeries was investigated by Sloth et al. (2017) and bio-colorant production by *Monascus purpureus* previous hydrolysis by *Aspergillus awamori* and *Aspergillus oryzae* by Haque et al. (2016). Another study, focused on the use of bread discards for baker yeast growth as substitute of sugarcane molasses after enzymatic hydrolysis using amylase, amyloglucosidase, and proteases (Benabda et al. 2018). Other authors also used it for ethanol production using *Saccharomyces cerevisiae* (Kim et al. 2011; Yan et al. 2011) and biohydrogen production through fermentation of bread factory discards by microflora of rice rhizosphere origin (Doi et al. 2009). In addition, utilization of bakery by-products as growth substrate for biotechnological processes could lead to the generation of new end-products, which can be used as feedstock in the industry and could reduce the amount of generated bakery wastes (Lin et al. 2013).

4.3.7 Brewery By-products

The EU produces 416 million hl of beer per year in its more than 6500 breweries. The largest volume of waste corresponds to brewers spent grain (BSG) (80% of the total solid by-products), followed by the brewer's yeast (BY) (10%). Given the EU beer production in 2016, we can estimate that more than seven million tons of BSG were generated (15–20 kg of wet BSG per 1 hl of beer) (Steiner et al. 2015; Vieira et al. 2014) and more than 0.8 million of tons of BY (1.5–3 kg of BY per 1 hl of beer).

Brewing process consists of six stages: malting, milling, mashing, brewing, cooling and fermentation. During mashing, known as the enzymatic conversion, the starch of the barley is transformed to fermentable (maltose and maltotriose) and non-fermentable (dextrins) sugars and the proteins are also partially degraded to polypeptides and amino acids. Once this process is completed, a filtration stage is carried out, where the wort is obtained, composed of those fermentable sugars, and the fermentation to ethanol is carried out. The solid fraction obtained after filtration is the BSG (Xiros and Christakopoulos 2012).

Despite being readily available, BSG receives little attention in the industry and is traditionally used as animal feed (Vieira et al. 2016). However, due to the fact that it is available at low cost or no cost during the year and presents interesting nutritional values (20% protein and 70% fiber in dry matter basis), it is considered as a promising raw material in biotechnology, food, and pharmaceutical industry (Ikram et al. 2017).

However, its complex composition and its high moisture content (70%) make it difficult to store and transport, so an economically viable technology is necessary to

promote its recovery. In order to increase the digestibility of its proteins, the release of functional peptides, and the release of phenolic compounds, the use of enzymes has been applied for their hydrolysis (McCarthy et al. 2013; Vieira et al. 2016) or the application of SSF for its nutritional and functional improvement (Martins et al. 2011).

There are few examples related to fermentation as valorization process of BSG, and in general, they are focused on protein enrichment and phenolic compound liberation. In all cases, protein duplicates the initial value and antioxidant compounds and vitamins are released enhancing its nutritional value. SSF has been usually applied using *Rhizopus oryzae* and *Rhizopus oligosporus* fungal species (Canedo et al. 2016; Cooray and Chen 2018; Ibarruri et al. 2019). Gupta et al. (2013) otherwise, fermented BSG by SmF using *lactobacillus plantarum* for the production of a nutraceutical rich product and Das and Brar (2014) by *Rhizopus oryzae* for fumaric acid production.

Another valorization alternative is the use of microbial enzymes to hydrolyze the protein fractions and release functional peptides. For example, *Bacillus cereus* sp. extracellular peptidase has been applied in order to produce functional antimicrobial compounds (Kotlar et al. 2013) and BY proteases for antioxidant compound production (Vieira et al. 2017).

The direct supply of BSG for animal feed without any treatment depends on many factors, which can limit its viability and, in many cases, can make it unsustainable. The high moisture content together with its high microbial load and the high temperature at which they are generated means that its useful life does not exceed 48 h, and on the other hand, although the use of these by-products for human consumption or even for pharmaceutical purposes and cosmetics could be a valuable option, a reliable solution is needed to deal with the non-used fractions generated after the extraction of the compounds of interest (Xiros and Christakopoulos 2012). Maintaining the quality and requirements for use in the food and feed industries is complicated, and its dehydration is economically costly. However, there is increasing pressure to ensure an economically and environmentally viable solution.

4.3.8 Winery By-products

World wine production has fluctuated between 26 and 30 million tons in the last 10 years, and around the 75% of the harvested grape is used in wine industry (FAOSTAT). The grape pomace or grape marc is the main by-product, which represents 20% (in weight) of total grape used (García-Lomillo and González-SanJosé 2017). This solid by-product is generated after the extraction of the juice for white wine fermentation and after fermentation in the case of red wine (Muhlack et al. 2018). It is composed of mainly grape seeds, stalks and skins (García-Lomillo and González-SanJosé 2017) and it still requires of valorization processes. Main components of marc are organic acids, polyphenols, and residual sugars, and red marc also contains small quantities of alcohol (Hixson et al. 2014). Grape seeds have been valorized for oil recovery (Boso et al. 2018; Hosseini et al. 2018) due to

benefits related to human health as the extracted oil has high content of unsaturated fatty acids and antioxidant compounds (Coelho et al. 2018; Rombaut et al. 2015). Grape meal after oil recovery is still a valuable source of organic compounds (Peixoto et al. 2018); however, it is mainly used as substrate for combustion (Beres et al. 2017).

As regards the microbial valorization strategy, grape pomace is mainly used for alcoholic beverage production as its soluble sugars can be used for alcoholic fermentation. The final product is known with different names depending on the region, marc in France, grappa in Italy, tsipouro in Greece, and aguardiente/aguardente in Spain and Portugal (Muhlack et al. 2018). Apart from alcoholic fermentation, biogas and bioethanol production are another studied biovalorization option by many researchers. Corbin et al. (2015) analyzed red and white marc as substrate for biofuel production, and they obtained 270 L/t yield from soluble carbohydrates and another polyphenol-rich fraction which could be used as animal feed or fertilizer. Martinez et al. (2016) used red marc after polyphenol extraction for biogas production (113 L CH_4/kg) during 30 days of batch process. More recent studies (El Achkar et al. 2016; Eleutheria et al. 2016) also used grape marc with positive results, demonstrating that marc can be used as feedstock in anaerobic digestion.

Grape marc biovalorization, as happens with other horticultural wastes, has also been focused on hydrolytic enzyme production by SSF (Díaz et al. 2013). Díaz et al. (2012) produced exo-polygalacturonase (exo-PG), xylanase and cellulase by *Aspergillus awamori* and showed an effective application of produced enzymes for juice clarification.

Another interesting end-product of valorization has been biosurfactant production. Paradelo et al. (2009) studied the production of biosurfactant by hydrolysate grape marc fermentation with *Lactobacillus pentosus* achieving in most of the cases better results than using chemical surfactants. Portilla-Rivera et al. (2008) also used *L. pentosus* for biosurfactant production from agricultural wastes. Authors found that the surfactants produced from distilled grape marc hydrolysates were the ones with the highest capacity to maintain the emulsion.

Biovalorization has also been related to feed and food production. In this context, Sánchez et al. (2002) used vineyard pruning and grape pomace as substrate for SSF by *Pleurotus* spp. and concluded that the best substrate for mycelial growth and mushroom yield were the mixtures with higher vineyard pruning content. Campanella et al. (2017) used grape pomace as substrate for lactic acid bacteria and bifidobacteria (*Lactobacillus plantarum*, *Lactobacillus paracasei* and *Bifidobacterium breve*) with the objective of producing functional ingredient with antioxidant activity. Compared with the control, fermented grape pomace exhibited an in vitro antioxidant activity, and the increased antioxidant activity was confirmed using Caco-2 cell line showing a lowest pro-oxidant effect induced by fermented grape pomace. Finally, Tominaga et al. (2010) investigated the inhibitory effect of grape pomace fermented by *Lactobacillus plantarum* on type-I allergic responses in mice. They concluded that the oral administration of the fermented grape pomace could suppress both the phases of type-I allergic responses.

4.4 Conclusion

It is evident that the fermentation technologies have great potential in the valorization of agro-industry wastes. Applied correctly, with robust processes and solid bases, the procedures can generate value from large variety of by-products, contributing greatly to the sustainability of the food chain. A correct selection of the substrate and the target compounds, the fermentation technology, and the bioconversion agent could lead to a broad variety of processes that could have positive impact from the economic, social and environmental point of view.

References

Abu Yazid N, Barrena R, Komilis D, Sánchez A (2017) Solid-state fermentation as a novel paradigm for organic waste valorization: a review. Sustainability 9:224. https://doi.org/10.3390/su9020224

Adeoye P, Che Man H, Amin M, Mohammed Thamer A, Akinbile C (2015) Poultry waste generation, management and the environment: a case of Minna, North Central Nigeria. J Solid Waste Technol Manag 41:146–156. https://doi.org/10.5276/jswtm.2015.146

Adessi A, Venturi M, Candeliere F, Galli V, Granchi L, De Philippis R (2018) Bread wastes to energy: sequential lactic and photo-fermentation for hydrogen production. Int J Hydrogen Energy 43:9569–9576. https://doi.org/10.1016/j.ijhydene.2018.04.053

Afrisham S, Badoei-Dalfard A, Namaki-Shoushtari A, Karami Z (2016) Characterization of a thermostable, CaCl2-activated and raw-starch hydrolyzing alpha-amylase from *Bacillus licheniformis* AT70: production under solid state fermentation by utilizing agricultural wastes. J Mol Catal B: Enzym 132:98–106. https://doi.org/10.1016/j.molcatb.2016.07.002

Ajila CM, Gassara F, Brar SK, Verma M, Tyagi RD, Valéro JR (2012) Polyphenolic antioxidant mobilization in apple pomace by different methods of solid-state fermentation and evaluation of its antioxidant activity. Food Bioproc Tech 5:2697–2707. https://doi.org/10.1007/s11947-011-0582-y

Akanni KA, Benson OB (2014) Poultry wastes management strategies and environmental implications on human health in Ogun State of Nigeria. Adv Econ Business 24:161–171. https://doi.org/10.13189/aeb.2014.020402

Ali HKQ, Zulkali MMD (2011) Design aspects of bioreactors for solid-state fermentation: a review. Chem Biochem Eng Q 25:255–266

Anupama, Ravindra P (2000) Value-added food: single cell protein. Biotechnol Adv 18:459–479. https://doi.org/10.1016/S0734-9750(00)00045-8

Aranday-García R, Román Guerrero A, Ifuku S, Shirai K (2017) Successive inoculation of *Lactobacillus brevis* and *Rhizopus oligosporus* on shrimp wastes for recovery of chitin and added-value products. Process Biochem 58:17–24. https://doi.org/10.1016/j.procbio.2017.04.036

Arbia W, Arbia L, Adour L, Amrane A (2012) Chitin extraction from Crustacean shells using biological methods—a review. Food Technol Biotechnol 51:12–25

Arora S, Rani R, Ghosh S (2018) Bioreactors in solid state fermentation technology: design, applications and engineering aspects. J Biotechnol 269:16–34. https://doi.org/10.1016/j.jbiotec.2018.01.010

Ashayerizadeh O, Dastar B, Samadi F, Khomeiri M, Yamchi A, Zerehdaran S (2017) Study on the chemical and microbial composition and probiotic characteristics of dominant lactic acid bacteria in fermented poultry slaughterhouse waste. Waste Manag 65:178–185. https://doi.org/10.1016/j.wasman.2017.04.017

Ashok A, Kumar DS (2017) Different methodologies for sustainability of optimization techniques used in submerged and solid state fermentation. 3 Biotech 7:301. https://doi.org/10.1007/s13205-017-0934-z

Badee AZM, Helmy SA, Morsy NFS (2011) Utilisation of orange peel in the production of α-terpineol by *Penicillium digitatum* (NRRL 1202). Food Chem 126:849–854. https://doi.org/10.1016/j.foodchem.2010.11.046

Ben Rebah F, Miled N (2013) Fish processing wastes for microbial enzyme production: a review. 3 Biotech 3:255–265. https://doi.org/10.1007/s13205-012-0099-8

Benabda O, Kasmi M, Kachouri F, Hamdi M (2018) Valorization of the powdered bread waste hydrolysate as growth medium for baker yeast. Food Bioprod Process 109:1–8. https://doi.org/10.1016/j.fbp.2018.02.007

Beres C, Costa G, Cabezudo I, da Silva-James N, Teles A, Cruz A, Mellinger-Silva C, Tonon R, Cabral L, Freitas S (2017) Towards integral utilization of grape pomace from winemaking process: a review. Waste Manag 68:581–594. https://doi.org/10.1016/j.wasman.2017.07.017

Biz A, Finkler ATJ, Pitol LO, Medina BS, Krieger N, Mitchell DA (2016) Production of pectinases by solid-state fermentation of a mixture of citrus waste and sugarcane bagasse in a pilot-scale packed-bed bioreactor. Biochem Eng J 111:54–62. https://doi.org/10.1016/j.bej.2016.03.007

Boso S, Gago P, Santiago J-L, Rodríguez-Canas E, Martínez M-C (2018) New monovarietal grape seed oils derived from white grape bagasse generated on an industrial scale at a winemaking plant. LWT 92:388–394. https://doi.org/10.1016/j.lwt.2018.02.055

Brar SK, Dhillon GS, Soccol CR (2014) Biotransformation of waste biomass into high value biochemicals. Springer, New York. https://doi.org/10.1007/978-1-4614-8005-1

Buenrostro-Figueroa J et al (2014) Potential use of different agroindustrial by-products as supports for fungal ellagitannase production under solid-state fermentation. Food Bioprod Process 92:376–382. https://doi.org/10.1016/j.fbp.2013.08.010

Campanella D, Rizzello C, Fasciano C, Gambacorta G, Pinto D, Marzani B, Scarano N, De Angelis M, Gobbetti M (2017) Exploitation of grape marc as functional substrate for lactic acid bacteria and bifidobacteria growth and enhanced antioxidant activity. Food Microbiol 65:25–35. https://doi.org/10.1016/j.fm.2017.01.019

Canedo MS, de Paula FG, da Silva FA, Vendruscolo F (2016) Protein enrichment of brewery spent grain from *Rhizopus oligosporus* by solid-state fermentation. Bioprocess Biosyst Eng 39:1105–1113. https://doi.org/10.1007/s00449-016-1587-8

Carvalho F, Prazeres AR, Rivas J (2013) Cheese whey wastewater: characterization and treatment. Sci Total Environ 445–446:385–396. https://doi.org/10.1016/j.scitotenv.2012.12.038

Cerda A, El-Bakry M, Gea T, Sánchez A (2016) Long term enhanced solid-state fermentation: inoculation strategies for amylase production from soy and bread wastes by *Thermomyces* sp. in a sequential batch operation. J Environ Chem Eng 4:2394–2401. https://doi.org/10.1016/j.jece.2016.04.022

Chatterjee S, Guha AK (2014) A study on biochemical changes during cultivation of *Rhizopus oryzae* in deproteinized whey medium in relation to chitosan production. Lett Appl Microbiol 59:155–160. https://doi.org/10.1111/lam.12259

Chatterjee S, Chatterjee BP, Guha AK (2008) Enhancement of growth and chitosan production by *Rhizopus oryzae* in whey medium by plant growth hormones. Int J Biol Macromol 42:120–126. https://doi.org/10.1016/j.ijbiomac.2007.10.006

Christen P, Bramorski A, Revah S, Soccol CR (2000) Characterization of volatile compounds produced by *Rhizopus* strains grown on agro-industrial solid wastes. Bioresour Technol 71:211–215. https://doi.org/10.1016/s0960-8524(99)00084-x

Cira LA, Huerta S, Hall GM, Shirai K (2002) Pilot scale lactic acid fermentation of shrimp wastes for chitin recovery. Process Biochem 37:1359–1366. https://doi.org/10.1016/S0032-9592(02)00008-0

Ciriminna R, Meneguzzo F, Delisi R, Pagliaro M (2017) Citric acid: emerging applications of key biotechnology industrial product. Chem Cent J 11:22. https://doi.org/10.1186/s13065-017-0251-y

Coelho JP, Filipe RM, Robalo MP, Stateva RP (2018) Recovering value from organic waste materials: Supercritical fluid extraction of oil from industrial grape seeds. J Supercrit Fluids 141:68–77. https://doi.org/10.1016/j.supflu.2017.12.008

Cooray ST, Chen WN (2018) Valorization of brewer's spent grain using fungi solid-state fermentation to enhance nutritional value. J Funct Foods 42:85–94. https://doi.org/10.1016/j.jff.2017.12.027

Corbin K, Hsieh Y, Betts N, Byrt C, Henderson M, Stork J, DeBolt S, Fincher G, Burton R (2015) Grape marc as a source of carbohydrates for bioethanol: chemical composition, pre-treatment and saccharification. Bioresour Technol 193:76–83. https://doi.org/10.1016/j.biortech.2015.06.030

Cordero BF, Obraztsova I, Couso I, Leon R, Vargas MA, Rodriguez H (2011) Enhancement of lutein production in *Chlorella sorokiniana* (Chorophyta) by improvement of culture conditions and random mutagenesis. Mar Drugs 9:1607–1624

Das RK, Brar SK (2014) Enhanced fumaric acid production from brewery wastewater and insight into the morphology of *Rhizopus oryzae* 1526. Appl Biochem Biotechnol 172:2974–2988. https://doi.org/10.1007/s12010-014-0739-z

Das RK, Brar SK, Verma M (2015) A fermentative approach towards optimizing directed biosynthesis of fumaric acid by *Rhizopus oryzae* 1526 utilizing apple industry waste biomass. Fungal Biol 119:1279–1290. https://doi.org/10.1016/j.funbio.2015.10.001

Deng Y, Li S, Xu Q, Gao M, Huang H (2012) Production of fumaric acid by simultaneous saccharification and fermentation of starchy materials with 2-deoxyglucose-resistant mutant strains of *Rhizopus oryzae*. Bioresour Technol 107:363–367. https://doi.org/10.1016/j.biortech.2011.11.117

Dhillon GS, Brar SK, Kaur S, Verma M (2013a) Bioproduction and extraction optimization of citric acid from Aspergillus niger by rotating drum type solid-state bioreactor. Ind Crop Prod 41:78–84. https://doi.org/10.1016/j.indcrop.2012.04.001

Dhillon GS, Kaur S, Sarma SJ, Brar SK (2013b) Integrated process for fungal citric acid fermentation using apple processing wastes and sequential extraction of chitosan from waste stream. Ind Crop Prod 50:346–351. https://doi.org/10.1016/j.indcrop.2013.08.010

Díaz AB, de Ory I, Caro I, Blandino A (2012) Enhance hydrolytic enzymes production by *Aspergillus awamori* on supplemented grape pomace. Food Bioprod Process 90:72–78. https://doi.org/10.1016/j.fbp.2010.12.003

Díaz AB, Alvarado O, de Ory I, Caro I, Blandino A (2013) Valorization of grape pomace and orange peels: Improved production of hydrolytic enzymes for the clarification of orange juice. Food Bioprod Process 91:580–586. https://doi.org/10.1016/j.fbp.2013.01.007

Directive (EU) (2018) 2018/851 of the European Parliament and of the Council of 30 May 2018 amending Directive 2008/98/EC on waste

Do D, Theron C, Fickers P (2019) Organic wastes as feedstocks for non-conventional yeast-based bioprocesses. Microorganisms 7:229. https://doi.org/10.3390/microorganisms7080229

Doi T, Matsumoto H, Abe J, Morita S (2009) Feasibility study on the application of rhizosphere microflora of rice for the biohydrogen production from wasted bread. Int J Hydrogen Energy 34:1735–1743. https://doi.org/10.1016/j.ijhydene.2008.12.060

Dornelas K, Schneider R, Garcia do Amaral A (2017) Biogas from poultry waste—production and energy potential. Environ Monit Assess 189:407. https://doi.org/10.1007/s10661-017-6054-8

Durand A (2003) Bioreactor designs for solid state fermentation. Biochem Eng J 13:113–125. https://doi.org/10.1016/s1369-703x(02)00124-9

EFSA (2007) Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA J 587(587):1–6

El Achkar JH, Lendormi T, Hobaika Z, Salameh D, Louka N, Maroun RG, Lanoisellé J-L (2016) Anaerobic digestion of grape pomace: biochemical characterization of the fractions and methane production in batch and continuous digesters. Waste Manag 50:275–282. https://doi.org/10.1016/j.wasman.2016.02.028

El-Bakry M, Abraham J, Cerda A, Barrena R, Ponsá S, Gea T, Sánchez A (2015) From wastes to high value added products: novel aspects of SSF in the production of enzymes. Crit Rev Environ Sci Technol 45:1999–2042. https://doi.org/10.1080/10643389.2015.1010423

El-Bakry M, Gea T, Sanchez A (2016) Inoculation effect of thermophilic microorganisms on protease production through solid-state fermentation under non-sterile conditions at lab and bench scale (SSF). Bioprocess Biosyst Eng 39:585–592

Eleutheria N, Maria I, Vasiliki T, Alexandros E, Alexandros A, Vasileios D (2016) Energy recovery and treatment of winery wastes by a compact anaerobic digester. Waste Biomass Valor 7:799–805. https://doi.org/10.1007/s12649-016-9541-1

European Former Foodstuff Processor Association (EFFPA) (2018) Reducing food waste. https://www.effpa.eu/reducing-food-waste/. Accessed 3 Oct 2018

EUROSTAT (2019). https://ec.europa.eu/eurostat/tgm/table.do?tab=table&plugin=1&language=en&pcode=tag00043. Accessed 13 Jul 2019

FAOSTAT (2018). http://www.fao.org/faostat/en/#data/FBS. Accessed 9 Oct 2018

FEFAC (2012) Environment report, 2nd edn. European Feed Manufacturer's Federation, Brussels

Freitas A, Escaramboni B, Carvalho A, Lima V, Oliva-Neto P (2014) Production and application of amylases of *Rhizopus oryzae* and *Rhizopus microsporus* var. *oligosporus* from industrial waste in acquisition of glucose. Chem Pap 68:442–450. https://doi.org/10.2478/s11696-013-0466-x

García-Lomillo J, González-SanJosé ML (2017) Applications of wine pomace in the food industry: approaches and functions. Comprehens Rev Food Sci Food Saf 16:3–22. https://doi.org/10.1111/1541-4337.12238

Gervais P, Molin P (2003) The role of water in solid-state fermentation. Biochem Eng J 13:85–101

Ghorbel bellaaj O, Younes I, Maalej H, Hajji S, Nasri M (2012) Chitin extraction from shrimp shell waste using *Bacillus* bacteria. Int J Biol Macromol 51:1196–1201. https://doi.org/10.1016/j.ijbiomac.2012.08.034

Ghorbel-Bellaaj O, Hmidet N, Jellouli K, Younes I, Maâlej H, Hachicha R, Nasri M (2011) Shrimp waste fermentation with *Pseudomonas aeruginosa* A2: Optimization of chitin extraction conditions through Plackett–Burman and response surface methodology approaches. Int J Biol Macromol 48:596–602. https://doi.org/10.1016/j.ijbiomac.2011.01.024

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

Gibbs PA, Seviour RJ, Schmid F (2000) Growth of filamentous fungi in submerged culture: problems and possible solutions. Crit Rev Biotechnol 20:17–48. https://doi.org/10.1080/07388550091144177

Goksungur Y (2004) Optimization of the production of chitosan from beet molasses by response surface methodology. J Chem Technol Biotechnol 79:974–981. https://doi.org/10.1002/jctb.1065

Gupta S, Jaiswal AK, Abu-Ghannam N (2013) Optimization of fermentation conditions for the utilization of brewing waste to develop a nutraceutical rich liquid product. Ind Crop Prod 44:272–282. https://doi.org/10.1016/j.indcrop.2012.11.015

Gustavsson J, Cederberg C, Sonesson U, Van Otterdijk R, Meybeck A (2011) Global food losses and food waste—extent, causes and prevention. FAO, Düsseldorf

Halder SK, Mondal KC (2018) Microbial valorization of chitinous bioresources for chitin extraction and production of chito-oligomers and N-acetylglucosamine: trends, perspectives and prospects. In: Patra JK, Das G, Shin H-S (eds) Microbial biotechnology. Application in food and pharmacology, vol 2. Springer, Singapore, pp 69–107. https://doi.org/10.1007/978-981-10-7140-9_4

Haque MA, Kachrimanidou V, Koutinas A, Lin CSK (2016) Valorization of bakery waste for biocolorant and enzyme production by *Monascus purpureus*. J Biotechnol 231:55–64. https://doi.org/10.1016/j.jbiotec.2016.05.003

Hernández-García A, Velásquez-Orta SB, Novelo E, Yáñez-Noguez I, Monje-Ramírez I, Orta Ledesma MT (2019) Wastewater-leachate treatment by microalgae: biomass, carbohydrate

and lipid production. Ecotoxicol Environ Saf 174:435–444. https://doi.org/10.1016/j.ecoenv.2019.02.052

Hixson J, Wilkes E, Smith P, Forsyth K (2014) Understanding the composition of grape marc and its potential as livestock feed supplement. Energy Fuel 24:2479–2488

Hosseini SS, Khodaiyan F, Kazemi M, Najari Z (2018) Optimization and characterization of pectin extracted from sour orange peel by ultrasound assisted method. Int J Biol Macromol 125:621–629. https://doi.org/10.1016/j.ijbiomac.2018.12.096

Ibarruri J, Hernández I (2018) *Rhizopus oryzae* as fermentation agent in food derived sub-products. Waste Biomass Valor 9:2107–2115. https://doi.org/10.1007/s12649-017-0017-8

Ibarruri J, Hernández I (2019) Valorization of cheese whey and orange molasses for fungal biomass production by submerged fermentation with *Rhizopus* sp. Bioprocess Biosyst Eng 42:1285–1300. https://doi.org/10.1007/s00449-019-02127-4

Ibarruri J, Cebrián M, Hernández I (2019) Solid state fermentation of brewer's spent grain using *Rhizopus* sp. to enhance nutritional value. Waste Biomass Valor 10(12):3687–3700. https://doi.org/10.1007/s12649-019-00654-5

Iijima Y, Gang DR, Fridman E, Lewinsohn E, Pichersky E (2004) Characterization of geraniol synthase from the peltate glands of sweet basil. Plant Physiol 134:370–379. https://doi.org/10.1104/pp.103.032946

Ikram S, Huang LY, Zhang HJ, Wang J, Yin M (2017) Composition and nutrient value proposition of brewers spent grain. J Food Sci 82:2232–2242. https://doi.org/10.1111/1750-3841.13794

Izawa N, Kudo M, Nakamura Y, Mizukoshi H, Kitada T, Sone T (2015) Production of aroma compounds from whey using *Wickerhamomyces pijperi*. AMB Expr 5:015–0108

Jin G, Zhu Y, Rinzema A, Wijffels RH, Ge X, Xu Y (2019) Water dynamics during solid-state fermentation by *Aspergillus oryzae* YH6. Bioresour Technol 277:68–76

Jo GH, Jung WJ, Kuk JH, Oh KT, Kim YJ, Park RD (2008) Screening of protease-producing *Serratia marcescens* FS-3 and its application to deproteinization of crab shell waste for chitin extraction. Carbohydr Polym 74:504–508. https://doi.org/10.1016/j.carbpol.2008.04.019

Jung WJ, Jo GH, Kuk JH, Kim KY, Park RD (2005) Extraction of chitin from red crab shell waste by cofermentation with *Lactobacillus paracasei* subsp. *tolerans* KCTC-3074 and *Serratia marcescens* FS-3. Appl Microbiol Biotechnol 71:234. https://doi.org/10.1007/s00253-005-0126-3

Kawa-Rygielska J, Pietrzak W, Czubaszek A (2012) Characterization of fermentation of waste wheat-rye bread mashes with the addition of complex enzymatic preparations. Biomass Bioenergy 44:17–22. https://doi.org/10.1016/j.biombioe.2012.04.016

Kaza S, Yao LC, Bhada-Tata P, Van Woerden F (2018) What a Waste 2.0: a global snapshot of solid waste management to 2050. Washington

Khan M, Karmakar R, Das B, Diba F, Razu M (2016) Heterotrophic growth of micro algae, pp 1–19

Kim JH, Lee JC, Pak D (2011) Feasibility of producing ethanol from food waste. Waste Manag 31:2121–2125. https://doi.org/10.1016/j.wasman.2011.04.011

Konstantas A, Stamford L, Azapagic A (2019) Economic sustainability of food supply chains: Life cycle costs and value added in the confectionary and frozen desserts sectors. Sci Total Environ 670:902–914

Kosseva MR (2013) Chapter 5—recovery of commodities from food wastes using solid-state fermentation. In: Food industry wastes. Academic Press, San Diego, pp 77–102. https://doi.org/10.1016/B978-0-12-391921-2.00005-6

Kotlar CE, Ponce AG, Roura SI (2013) Improvement of functional and antimicrobial properties of brewery byproduct hydrolysed enzymatically. LWT- Food Sci Technol 50:378–385. https://doi.org/10.1016/j.lwt.2012.09.005

Kumar R, Shivakumar S (2014) Production of L-lactic acid from starch and food waste by amylolytic *Rhizopus oryzae* MTCC 8784. Int J ChemTech Res 6:527–537

Kumar D, Yadav KK, Muthukumar M, Garg N (2013) Production and characterization of alpha-amylase from mango kernel by *Fusarium solani* NAIMCC-F-02956 using submerged fermentation. J Environ Biol 34:1053–1058

Kupski L, de Carvalho Silvello MA, Fontes MRV, Lima TS, Treichel H, Badiale Furlong E (2015) *R. oryzae* cellulases: a new approach to degrading lignocellulosic material. J Food Biochem 39:129–138. https://doi.org/10.1111/jfbc.12097

Kusumaningtyas E, Widiastuti R, Maryam R (2006) Reduction of aflatoxin B1 in chicken feed by using *Saccharomyces cerevisiae*, *Rhizopus oligosporus* and their combination. Mycopathologia 162:307–311. https://doi.org/10.1007/s11046-006-0047-4

Kwan TH, Pleissner D, Lau KY, Venus J, Pommeret A, Lin CSK (2015) Techno-economic analysis of a food waste valorization process via microalgae cultivation and co-production of plasticizer, lactic acid and animal feed from algal biomass and food waste. Bioresour Technol 198:292–299. https://doi.org/10.1016/j.biortech.2015.09.003

Łaba W, Żarowska B, Chorążyk D, Pudło A, Piegza M, Kancelista A, Kopeć W (2018) New keratinolytic bacteria in valorization of chicken feather waste. AMB Expr 8:9. https://doi.org/10.1186/s13568-018-0538-y

Lam KF, Leung CCJ, Lei HM, Lin CSK (2014) Economic feasibility of a pilot-scale fermentative succinic acid production from bakery wastes. Food Bioprod Process 92:282–290. https://doi.org/10.1016/j.fbp.2013.09.001

Laufenberg G, Kunz B, Nystroem M (2003) Transformation of vegetable waste into value added products: (A) the upgrading concept; (B) practical implementation. Bioresour Technol 87:167–198. https://doi.org/10.1016/s0960-8524(02)00167-0

Lei H, Wang HL, Ning TT, Hao W, Xu CC (2012) Protein enrichment of potato starch residue by solid state fermentation with mixed strains. J Anim Vet Adv 11:2700–2705

Leung CCJ, Cheung ASY, Zhang AY-Z, Lam KF, Lin CSK (2012) Utilisation of waste bread for fermentative succinic acid production. Biochem Eng J 65:10–15. https://doi.org/10.1016/j.bej.2012.03.010

Liao W, Liu Y, Frear C, Chen S (2008) Co-production of fumaric acid and chitin from a nitrogen-rich lignocellulosic material—dairy manure—using a pelletized filamentous fungus *Rhizopus oryzae* ATCC 20344. Bioresour Technol 99:5859–5866. https://doi.org/10.1016/j.biortech.2007.10.006

Libonatti C, Agüeria D, García C, Basualdo M (2019) *Weissella paramesenteroides* encapsulation and its application in the use of fish waste. Rev Argent Microbiol 51:81–83. https://doi.org/10.1016/j.ram.2018.03.001

Lin C et al (2013) Food waste as a valuable resource for the production of chemicals, materials and fuels. Curr Situat Glob Perspect 6:426–464. https://doi.org/10.1039/c2ee23440h

Lincoln L, More SS (2018) Comparative evaluation of extracellular -d-fructofuranosidase in submerged and solid-state fermentation produced by newly identified *Bacillus subtilis* strain. J Appl Microbiol 125:441–456. https://doi.org/10.1111/jam.13881

Liu Z, Liao W, Liu Y (2016) A sustainable biorefinery to convert agricultural residues into value-added chemicals. Biotechnol Biofuels 9:197. https://doi.org/10.1186/s13068-016-0609-8

Lizardi-Jimenez MA, Hernandez-Martinez R (2017) Solid state fermentation (SSF): diversity of applications to valorize waste and biomass. 3 Biotech 7:44. https://doi.org/10.1007/s13205-017-0692-y

Magnuson JK, Lasure LL (2004) Organic acid production by filamentous fungi. In: Tkacz JS, Lange L (eds) Advances in fungal biotechnology for industry, agriculture, and medicine. Springer US, Boston, MA, pp 307–340. https://doi.org/10.1007/978-1-4419-8859-1_12

Mahboubi A, Ferreira J, Taherzadeh M, Lennartsson P (2017a) Production of fungal biomass for feed, fatty acids, and glycerol by *Aspergillus oryzae* from fat-rich dairy substrates. Fermentation 3:48. https://doi.org/10.3390/fermentation3040048

Mahboubi A, Ferreira JA, Taherzadeh MJ, Lennartsson PR (2017b) Value-added products from dairy waste using edible fungi. Waste Manag 59:518–525. https://doi.org/10.1016/j.wasman.2016.11.017

Mahmoud NS, Ghaly AE, Arab F (2007) Unconventional approach for demineralization of deproteinized crustacean shells for chitin production. Am J Biochem Biotechnol 3:1–9. https://doi.org/10.3844/ajbbsp.2007.1.9

Malilas W, Kang SW, Kim SB, Yoo HY, Chulalaksananukul W, Kim SW (2013) Lipase from *Penicillium camemberti* KCCM 11268: optimization of solid state fermentation and application to biodiesel production. Kor J Chem Eng 30:405–412. https://doi.org/10.1007/s11814-012-0132-y

Mamlouk D, Gullo M (2013) Acetic acid bacteria: physiology and carbon sources oxidation. Indian J Microbiol 53:377–384. https://doi.org/10.1007/s12088-013-0414-z

Mamma D, Topakas E, Vafiadi C, Christakopoulos P (2009) Biotechnological potential of fruit processing industry residues. In: Nigam PS, Pandey A (eds) Biotechnology for agro-industrial residues utilisation. Springer, Berlin, pp 273–291. https://doi.org/10.1007/978-1-4020-9942-7_14

Mantzouridou FT, Paraskevopoulou A, Lalou S (2015) Yeast flavour production by solid state fermentation of orange peel waste. Biochem Eng J 101:1–8. https://doi.org/10.1016/j.bej.2015.04.013

Marcelino Oliveira Cavalheiro J, Oliveira de Souza E, Singh Bora P (2007) Utilization of shrimp industry waste in the formulation of tilapia (*Oreochromis niloticus* Linnaeus) feed. Bioresour Technol 98:602–606. https://doi.org/10.1016/j.biortech.2006.02.018

Markou G, Wang L, Ye J, Unc A (2018) Using agro-industrial wastes for the cultivation of microalgae and duckweeds: contamination risks and biomass safety concerns. Biotechnol Adv 36:1238–1254. https://doi.org/10.1016/j.biotechadv.2018.04.003

Martinez G, Rebecchi S, Decorti D, Domingos J, Natolino A, Del Rio D, Bertin L, Da Porto C, Fava F (2016) Towards multi-purpose biorefinery platforms for the valorisation of red grape pomace: production of polyphenols, volatile fatty acids, polyhydroxyalkanoates and biogas. Green Chem 18:261–270. https://doi.org/10.1039/c5gc01558h

Martins S, Mussatto SI, Martinez-Avila G, Montanez-Saenz J, Aguilar CN, Teixeira JA (2011) Bioactive phenolic compounds: production and extraction by solid-state fermentation. A review. Biotechnol Adv 29:365–373. https://doi.org/10.1016/j.biotechadv.2011.01.008

Maruthiah T, Somanath B, Jasmin JV, Immanuel G, Palavesam A (2016) Production, purification and characterization of halophilic organic solvent tolerant protease from marine crustacean shell wastes and its efficacy on deproteinization. 3 Biotech 6:157–157. https://doi.org/10.1007/s13205-016-0474-y

Massarolo KC, Denardi de Souza T, Collazzo CC, Badiale Furlong E, Souza Soares LA (2017) The impact of *Rhizopus oryzae* cultivation on rice bran: gamma-oryzanol recovery and its antioxidant properties. Food Chem 228:43–49. https://doi.org/10.1016/j.foodchem.2017.01.127

Mazotto AM, Couri S, Damaso MCT, Vermelho AB (2013) Degradation of feather waste by *Aspergillus niger* keratinases: Comparison of submerged and solid-state fermentation. Int Biodeter Biodegr 85:189–195. https://doi.org/10.1016/j.ibiod.2013.07.003

McCarthy AL, O'Callaghan YC, Connolly A, Piggott CO, FitzGerald RJ, O'Brien NM (2013) *In vitro* antioxidant and anti-inflammatory effects of brewers' spent grain protein rich isolate and its associated hydrolysates. Food Res Int 50:205–212. https://doi.org/10.1016/j.foodres.2012.10.022

McKinney K, Combs J, Becker P, Humphries A, Filer K, Vriesekoop F (2015) Optimization of phytase production from *Escherichia coli* by altering solid-state fermentation conditions. Fermentation 1(1):13–23. https://doi.org/10.3390/fermentation1010013

Mena C, Adenso-Diaz B, Yurt O (2011) The causes of food waste in the supplier–retailer interface: evidences from the UK and Spain. Resour Conserv Recycl 55:648–658. https://doi.org/10.1016/j.resconrec.2010.09.006

Mohapatra S, Sarkar B, Samantaray DD, Daware A, Maity S, Pattnaik S, Bhattacharjee S (2017) Bioconversion of fish solid waste into PHB using *Bacillus subtilis* based submerged fermentation process. Environ Technol 38:3201–3208. https://doi.org/10.1080/09593330.2017.1291759

Mollea C, Marmo L, Bosco F (2013) Valorisation of cheese whey, a by-product from the dairy industry. In: Mazzalupo I (ed) Food industry. InTech, London. https://doi.org/10.5772/53159

Muhlack RA, Potumarthi R, Jeffery DW (2018) Sustainable wineries through waste valorisation: a review of grape marc utilisation for value-added products. Waste Manag 72:99–118. https://doi.org/10.1016/j.wasman.2017.11.011

Nair RB, Kabir MM, Lennartsson PR, Taherzadeh MJ, Horvath IS (2018) Integrated process for ethanol, biogas, and edible filamentous fungi-based animal feed production from dilute phosphoric acid-pretreated wheat straw. Appl Biochem Biotechnol 184:48–62. https://doi.org/10.1007/s12010-017-2525-1

Nigam PS, Pandey A (2009) Biotechnology for agro-industrial residues utilization. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-9942-7

Nout MJR, Aidoo KE (2011) Asian fungal fermented food. In: Hofrichter M (ed) Industrial applications. Springer, Berlin, Heidelberg, pp 29–58. https://doi.org/10.1007/978-3-642-11458-8_2

Novelli PK, Barros MM, Fleuri LF (2016) Novel inexpensive fungi proteases: production by solid state fermentation and characterization. Food Chem 198:119–124

Oh K-T, Kim Y-J, Nguyen VN, Jung W-J, Park R-D (2007) Demineralization of crab shell waste by *Pseudomonas aeruginosa* F722. Process Biochem 42:1069–1074. https://doi.org/10.1016/j.procbio.2007.04.007

Oliveira AC, Barata A, Batista AP, Gouveia L (2018) *Scenedesmus obliquus* in poultry wastewater bioremediation. Environ Technol 40:3735–3744. https://doi.org/10.1080/09593330.2018.1488003

Ortiz GE, Guitart ME, Cavalitto SF, Alberto EO, Fernandez-Lahore M, Blasco M (2015) Characterization, optimization, and scale-up of cellulases production by trichoderma reesei cbs 836.91 in solid-state fermentation using agro-industrial products. Bioprocess Biosyst Eng 38:2117–2128

Osman WHW, Mikami B, Saka N, Kondo K, Lin MI, Nagata T, Katahira M (2019) Identification of key residues for activities of atypical glutathione S-transferase of *Ceriporiopsis subvermispora*, a selective degrader of lignin in woody biomass, by crystallography and functional mutagenesis. Int J Biol Macromol 132:222–229

Östergren K et al. (2014) FUSIONS definitional framework for food waste. https://www.eu-fusions.org/phocadownload/Publications/FUSIONS%20Definitional%20Framework%20for%20Food%20Waste%202014.pdf. Accessede 24 Aug 2020

Pandey A (2003) Solid-state fermentation. Biochem Eng J 13:81–84. https://doi.org/10.1016/S1369-703X(02)00121-3

Pandey AK, Edgard G, Negi S (2016) Optimization of concomitant production of cellulase and xylanase from *Rhizopus oryzae* SN5 through EVOP-factorial design technique and application in Sorghum Stover based bioethanol production. Renew Energy 98:51–56. https://doi.org/10.1016/j.renene.2016.05.071

Paradelo R, Moldes AB, Dominguez JM, Barral MT (2009) Reduction of water repellence of hydrophobic plant substrates using biosurfactant produced from hydrolyzed grape marc. J Agric Food Chem 57:4895–4899. https://doi.org/10.1021/jf900353m

Parajuli R, Thoma G, Matlock MD (2019) Environmental sustainability of fruit and vegetable production supply chains in the face of climate change: a review. Sci Total Environ 650:2863–2879

Parveen J, Zahangir A, Nurul U (2007) Potential strain to produce bioprotein from cheaper carbon source: hope for millions. Am J Biotechnol Biochem 3:42–46. https://doi.org/10.3844/ajbbsp.2007.42.46

Peixoto CM, Dias MI, Alves MJ, Calhelha RC, Barros L, Pinho SP, Ferreira ICFR (2018) Grape pomace as a source of phenolic compounds and diverse bioactive properties. Food Chem 253:132–138. https://doi.org/10.1016/j.foodchem.2018.01.163

Pleissner D, Rumpold BA (2018) Utilization of organic residues using heterotrophic microalgae and insects. Waste Manag 72:227–239. https://doi.org/10.1016/j.wasman.2017.11.020

Pleissner D, Lau KY, Schneider R, Venus J, Lin CSK (2015) Fatty acid feedstock preparation and lactic acid production as integrated processes in mixed restaurant food and bakery wastes treatment. Food Res Int 73:52–61. https://doi.org/10.1016/j.foodres.2014.11.048

Portilla-Rivera O, Torrado A, Domínguez JM, Moldes AB (2008) Stability and emulsifying capacity of biosurfactants obtained from lignocellulosic sources using *Lactobacillus pentosus*. J Agric Food Chem 56:8074–8080. https://doi.org/10.1021/jf801428x

Potnis SS, Mashal BS, Bhartal SR, Patil DS, Gajbhar SS, Chakre GD (2016) Single cell protein production by probiotics grown on fruit waste. Int J Adv Biotechnol Res 7:112–117

Raa J, Gildberg A (1982) Fish silage: a review. Crit Rev Food Sci Nutr 16:383–419

Rai AK, Bhaskar N, Baskaran V (2015) Effect of feeding lipids recovered from fish processing waste by lactic acid fermentation and enzymatic hydrolysis on antioxidant and membrane bound enzymes in rats. J Food Sci Technol 52:3701–3710. https://doi.org/10.1007/s13197-014-1442-3

Ramos S et al (2016) SENSE tool: easy-to-use web-based tool to calculate food product environmental impact. Int J Life Cycle Assess 21:710–721. https://doi.org/10.1007/s11367-015-0980-x

Reddy GV, Ravindra Babu P, Komaraiah P, Roy KRRM, Kothari IL (2003) Utilization of banana waste for the production of lignolytic and cellulolytic enzymes by solid substrate fermentation using two *Pleurotus* species (*P. ostreatus* and *P. sajor-caju*). Process Biochem 38:1457–1462. https://doi.org/10.1016/S0032-9592(03)00025-6

Reyes-Becerril M, Ascencio F, Esther Macias M, Maldonado M, Rojas M, Esteban M (2012) Effects of marine silages enriched with *Lactobacillus sakei* 5-4 on haemato-immunological and growth response in Pacific red snapper (*Lutjanus peru*) exposed to *Aeromonas veronii*. Fish Shellfish Immunol 33:984–992. https://doi.org/10.1016/j.fsi.2012.08.014

Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Girones R, Herman L, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Cocconcelli P, Klein G, Prieto Maradona M, Querol A, Peixe L, Suarez J, Sundh I, Vlak J, Aguilera-Gómez M, Barizzone F, Brozzi R, Correia S, Heng L, Istace F, Lythgo C, Fernández Escámez P (2017) Scientific opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. EFSA J 15(3):178. https://doi.org/10.2903/j.efsa.2017.4664

Robinson T, Nigam P (2003) Bioreactor design for protein enrichment of agricultural residues by solid state fermentation. Biochem Eng J 13:197–203. https://doi.org/10.1016/s1369-703x(02)00132-8

Rodríguez Madrera R, Pando Bedriñana R, Suárez Valles B (2015) Production and characterization of aroma compounds from apple pomace by solid-state fermentation with selected yeasts. LWT-Food Sci Technol 64:1342–1353. https://doi.org/10.1016/j.lwt.2015.07.056

Rogers PL, Jeon YJ, Lee KJ, Lawford HG (2007) *Zymomonas mobilis* for fuel ethanol and higher value products. In: Olsson L (ed) Biofuels. Springer, Berlin, Heidelberg, pp 263–288. https://doi.org/10.1007/10_2007_060

Rombaut N, Savoire R, Thomasset B, Castello J, Van Hecke E, Lanoisellé J-L (2015) Optimization of oil yield and oil total phenolic content during grape seed cold screw pressing. Ind Crop Prod 63:26–33. https://doi.org/10.1016/j.indcrop.2014.10.001

Ruthu MPS, Rai AK, Bhaskar N (2014) Fermentative recovery of lipids and proteins from freshwater fish head waste with reference to antimicrobial and antioxidant properties of protein hydrolysate. J Food Sci Technol 51:1884–1892. https://doi.org/10.1007/s13197-012-0730-z

Saba I, Mukhtar H, Haq I (2013) Production of alkaline protease by *Bacillus subtilis* using solid state fermentation. Afr J Microbiol Res 7:1558–1568. https://doi.org/10.5897/ajmr12.1845

Sachindra NM, Bhaskar N, Siddegowda GS, Sathisha AD, Suresh PV (2007) Recovery of carotenoids from ensilaged shrimp waste. Bioresour Technol 98:1642–1646. https://doi.org/10.1016/j.biortech.2006.05.041

Sadh PK, Chawla P, Bhandari L, Kaushik R, Duhan JS (2017a) *In vitro* assessment of bio-augmented minerals from peanut oil cakes fermented by *Aspergillus oryzae* through Caco-2 cells. J Food Sci Technol 54:3640–3649. https://doi.org/10.1007/s13197-017-2825-z

Sadh PK, Saharan P, Duhan JS (2017b) Bio-augmentation of antioxidants and phenolic content of *Lablab purpureus* by solid state fermentation with GRAS filamentous fungi. Resour Eff Technol 3:285–292. https://doi.org/10.1016/j.reffit.2016.12.007

Sadh PK, Saharan P, Duhan S, Duhan JS (2017c) Bio-enrichment of phenolics and antioxidant activity of combination of *Oryza sativa* and *Lablab purpureus* fermented with GRAS filamentous fungi. Resour Eff Technol 3:347–352. https://doi.org/10.1016/j.reffit.2017.02.008

Sadh PK, Duhan S, Duhan JS (2018) Agro-industrial wastes and their utilization using solid state fermentation: a review. Bioresour Bioprocess 5:1. https://doi.org/10.1186/s40643-017-0187-z

Saito K, Hasa Y, Abe H (2012) Production of lactic acid from xylose and wheat straw by *Rhizopus oryzae*. J Biosci Bioeng 114:166–169. https://doi.org/10.1016/j.jbiosc.2012.03.007

San Martin D, Ramos S, Zufía J (2016) Valorisation of food waste to produce new raw materials for animal feed. Food Chem 198:68–74. https://doi.org/10.1016/j.foodchem.2015.11.035

Sánchez A, Ysunza F, Beltrán-García MJ, Esqueda M (2002) Biodegradation of viticulture wastes by *Pleurotus*: a source of microbial and human food and its potential use in animal feeding. J Agric Food Chem 50:2537–2542. https://doi.org/10.1021/jf011308s

Satari B, Karimi K, Taherzadeh MJ, Zamani A (2016) Co-production of fungal biomass derived constituents and ethanol from citrus wastes free sugars without auxiliary nutrients in airlift bioreactor. Int J Mol Sci 17:302. https://doi.org/10.3390/ijms17030302

Shin H-Y, Kim S-M, Lee JH, Lim S-T (2019) Solid-state fermentation of black rice bran with *Aspergillus awamori* and *Aspergillus oryzae*: effects on phenolic acid composition and antioxidant activity of bran extracts. Food Chem 272:235–241. https://doi.org/10.1016/j.foodchem.2018.07.174

Singh Ningthoujam D, Tamreihao K, Mukherjee S, Khunjamayum R, Jaya L, Singh Asem R (2018) Keratinaceous wastes and their valorization through keratinolytic microorganisms. IntechOpen, London. https://doi.org/10.5772/intechopen.80051

Sini TK, Santhosh S, Mathew PT (2007) Study on the production of chitin and chitosan from shrimp shell by using *Bacillus subtilis* fermentation. Carbohydr Res 342:2423–2429. https://doi.org/10.1016/j.carres.2007.06.028

Sloth JK, Jensen HC, Pleissner D, Eriksen NT (2017) Growth and phycocyanin synthesis in the heterotrophic microalga *Galdieria sulphuraria* on substrates made of food waste from restaurants and bakeries. Bioresour Technol 238:296–305. https://doi.org/10.1016/j.biortech.2017.04.043

Smetana S, Sandmann M, Rohn S, Pleissner D, Heinz V (2017) Autotrophic and heterotrophic microalgae and cyanobacteria cultivation for food and feed: life cycle assessment. Bioresour Technol 245:162–170. https://doi.org/10.1016/j.biortech.2017.08.113

Soccol CR, da Costa ESF, Letti LAJ, Karp SG, Woiciechowski AL, Vandenberghe LPS (2017) Recent developments and innovations in solid state fermentation. Biotechnol Res Innov 1:52–71. https://doi.org/10.1016/j.biori.2017.01.002

Souza Filho P, Zamani A, Taherzadeh M (2017) Production of edible fungi from potato protein liquor (PPL) in Airlift Bioreactor. Fermentation 3:12. https://doi.org/10.3390/fermentation3010012

Souza Filho PF, Nair RB, Andersson D, Lennartsson PR, Taherzadeh MJ (2018a) Vegan-mycoprotein concentrate from pea-processing industry byproduct using edible filamentous fungi. Fungal Biol Biotechnol 5:5. https://doi.org/10.1186/s40694-018-0050-9

Souza Filho P, Zamani A, Taherzadeh M (2018b) Edible protein production by filamentous fungi using starch plant wastewater. Waste Biomass Valor 10:2487–2496. https://doi.org/10.1007/s12649-018-0265-2

Srujana S, Varma Suresh S, Muralidharan A (2015) Production of bioethanol by solid state fermentation using paddy straw as a substrate. Int J Adv Res 3(1):212–215

Steiner J, Procopio S, Becker T (2015) Brewer's spent grain: source of value-added polysaccharides for the food industry in reference to the health claims. Eur Food Res Technol 241:303–315. https://doi.org/10.1007/s00217-015-2461-7

Stenmarck A, Jensen C, Quested T, Moates G (2016) FUSIONS Estimates of European food waste levels. IVL Swedish Environmental Research Institute, Stockholm. https://www.eu-fusions.org/phocadownload/Publications/Estimates%20of%20European%20food%20waste%20levels.pdf. Accessed 24 Aug 2020

Suresh S, Radha KV (2015) Effect of a mixed substrate on phytase production by *Rhizopus oligosporus* MTCC 556 using solid state fermentation and determination of dephytinization activities in food grains. Food Sci Biotechnol 24:551–559. https://doi.org/10.1007/s10068-015-0072-5

Suresh S, Radha KV (2016) Statistical optimization and mutagenesis for high level of phytase production by *Rhizopus oligosporus* MTCC 556 under solid state fermentation. J Environ Biol 37:253–259

Tai C, Li S, Xu Q, Ying H, Huang H, Ouyang P (2010) Chitosan production from hemicellulose hydrolysate of corn straw: impact of degradation products on *Rhizopus oryzae* growth and chitosan fermentation. Lett Appl Microbiol 51:278–284. https://doi.org/10.1111/j.1472-765X.2010.02893.x

Tengerdy RP, Szakacs G (2003) Bioconversion of lignocellulose in solid substrate fermentation. Biochem Eng J 13:169–179. https://doi.org/10.1016/S1369-703X(02)00129-8

Thomas L, Larroche C, Pandey A (2013) Current developments in solid-state fermentation. Biochem Eng J 81:146–161. https://doi.org/10.1016/j.bej.2013.10.013

Tominaga T, Kawaguchi K, Kanesaka M, Kawauchi H, Jirillo E, Kumazawa Y (2010) Suppression of type-I allergic responses by oral administration of grape marc fermented with *Lactobacillus plantarum*. Immunopharmacol Immunotoxicol 32:593–599. https://doi.org/10.3109/08923971003604786

Turner TL, Kim E, Hwang C, Zhang GC, Liu JJ, Jin YS (2017) Short communication: conversion of lactose and whey into lactic acid by engineered yeast. J Dairy Sci 100:124–128. https://doi.org/10.3168/jds.2016-11784

Ugwuanyi JO, McNeil B, Harvey LM (2009) Production of protein-enriched feed using agro-industrial residues as substrates. In: Nigam PS, Pandey A (eds) Biotechnology for agro-industrial residues utilisation, pp 77–103. https://doi.org/10.1007/978-1-4020-9942-7_5

Van Alfen NK (2014) Encyclopedia of agriculture and food systems, 2nd edn. Academic Press, New York

Vázquez JA, Docasal SF, Prieto MA, González MP, Murado MA (2008) Growth and metabolic features of lactic acid bacteria in media with hydrolysed fish viscera. An approach to bio-silage of fishing by-products. Bioresour Technol 99:6246–6257. https://doi.org/10.1016/j.biortech.2007.12.006

Vázquez JA, Meduíña A, Durán AI, Nogueira M, Fernández-Compás A, Pérez-Martín RI, Rodríguez-Amado I (2019) Production of valuable compounds and bioactive metabolites from by-products of fish discards using chemical processing, enzymatic hydrolysis, and bacterial fermentation. Mar Drugs 17:139

Venkatesagowda B, Ponugupaty E, Barbosa AM, Dekker RFH (2015) Solid-state fermentation of coconut kernel-cake as substrate for the production of lipases by the coconut kernel-associated fungus *Lasiodiplodia theobromae* VBE-1. Ann Microbiol 65:129–142. https://doi.org/10.1007/s13213-014-0844-9

Venkatramanan V, Shah S, Prasad R (eds) (2021a) Exploring synergies and trade-offs between climate change and the sustainable development goals. Springer, Singapore. https://doi.org/10.1007/978-981-15-7301-9

Venkatramanan V, Shah S, Prasad R (eds) (2021b) Sustainable bioeconomy: pathways to sustainable development goals. Springer Nature, Singapore. https://doi.org/10.1007/978-981-15-7321-7

Verma A, Singh H, Anwar S, Chattopadhyay A, Tiwari KK, Kaur S, Dhilon GS (2017) Microbial keratinases: industrial enzymes with waste management potential. Crit Rev Biotechnol 37:476–491

Vieira E, Rocha MAM, Coelho E, Pinho O, Saraiva JA, Ferreira IMPLVO, Coimbra MA (2014) Valuation of brewer's spent grain using a fully recyclable integrated process for extraction of proteins and arabinoxylans. Ind Crop Prod 52:136–143. https://doi.org/10.1016/j.indcrop.2013.10.012

Vieira E, Teixeira J, Ferreira I (2016) Valorization of brewers' spent grain and spent yeast through protein hydrolysates with antioxidant properties. Eur Food Res Technol 242:1975–1984. https://doi.org/10.1007/s00217-016-2696-y

Vieira EF, da Silva DD, Carmo H, Ferreira IMPLVO (2017) Protective ability against oxidative stress of brewers' spent grain protein hydrolysates. Food Chem 228:602–609. https://doi.org/10.1016/j.foodchem.2017.02.050

Wadhwa M, Bakshi MPS (2013) Utilization of fruit and vegetable wastes as livestock feed and as substrates for generation of other value-added products. FAO, Bangkok

Waldeck J, Daum G, Bisping B, Meinhardt F (2006) Isolation and molecular characterization of chitinase-deficient Bacillus licheniformis strains capable of deproteinization of shrimp shell waste to obtain highly viscous chitin. Appl Environ Microbiol 72:7879–7885. https://doi.org/10.1128/aem.00938-06

Waste and Resources Action Program (WRAP) (2019) Food and drink material hierarchy. http://www.wrap.org.uk/content/why-take-action-legalpolicy-case. Accessed 9 Jun 2019

Xiros C, Christakopoulos P (2012) Biotechnological potential of brewers spent grain and its recent applications. Waste Biomass Valor 3:213–232. https://doi.org/10.1007/s12649-012-9108-8

Yadav JS, Bezawada J, Ajila CM, Yan S, Tyagi RD, Surampalli RY (2014) Mixed culture of *Kluyveromyces marxianus* and *Candida krusei* for single-cell protein production and organic load removal from whey. Bioresour Technol 164:119–127. https://doi.org/10.1016/j.biortech.2014.04.069

Yadav JSS, Yan S, Pilli S, Kumar L, Tyagi RD, Surampalli RY (2015) Cheese whey: a potential resource to transform into bioprotein, functional/nutritional proteins and bioactive peptides. Biotechnol Adv 33:756–774. https://doi.org/10.1016/j.biotechadv.2015.07.002

Yadav JSS, Yan S, Ajila CM, Bezawada J, Tyagi RD, Surampalli RY (2016) Food-grade single-cell protein production, characterization and ultrafiltration recovery of residual fermented whey proteins from whey. Food Bioprod Process 99:156–165. https://doi.org/10.1016/j.fbp.2016.04.012

Yan S, Li J, Chen X, Wu J, Wang P, Ye J, Yao J (2011) Enzymatical hydrolysis of food waste and ethanol production from the hydrolysate. Renew Energy 36:1259–1265. https://doi.org/10.1016/j.renene.2010.08.020

Yang J-K, Shih I-L, Tzeng Y-M, Wang S-L (2000) Production and purification of protease from a Bacillus subtilis that can deproteinize crustacean wastes. Enzyme Microb Technol 26:406–413. https://doi.org/10.1016/S0141-0229(99)00164-7

Yang X, Zhu M, Huang X, Lin CSK, Wang J, Li S (2015) Valorisation of mixed bakery waste in non-sterilized fermentation for l-lactic acid production by an evolved *Thermoanaerobacterium* sp. strain. Bioresour Technol 198:47–54. https://doi.org/10.1016/j.biortech.2015.08.108

Younes I, Rinaudo M (2015) Chitin and chitosan preparation from marine sources. Structure, properties and applications. Mar Drugs 13:1133–1174

Zhang Y, Sun Z, Leung C, Han W, Lau K, Li M, Lin S (2013) Valorisation of bakery waste for succinic acid production. Green Chem 15:690–695. https://doi.org/10.1039/c2gc36518a

Valorization of Agri-Food Wastes

5

Germán Ayala Valencia, Cristiano José de Andrade, Jaciane Lutz Ienczak, Alcilene Rodrigues Monteiro, and Tomy J. Gutiérrez

Abstract

Agri-food wastes are inherently generated along the food production chain. In this sense, a significant reduction or better management of them could improve food security and also reduce hunger. It should be noted that several global organizations have estimated that food wastes could reach 126 million tons by 2020. Normally, agricultural wastes have been used as animal feeds and fertilizers. Nonetheless, most agricultural wastes have macro- and micronutrients, as well as bioactive compounds that could have a high added value after agricultural waste valorization processes, e.g., the latter can be extracted and incorporated as food additives for the manufacture of active and intelligent packaging, while macromolecules such as carbohydrates, proteins and lipids can lead to obtaining biosurfactants and single cell oils of high added value to be used in food sector. This chapter aims to provide some perspectives and advances in the valuation of agri-food wastes.

Keywords

Agri-food wastes · Valorization · Sustainable bioeconomy · Food practices · Industrial applications · Sustainable consumption

G. A. Valencia (✉) · C. J. de Andrade · J. L. Ienczak · A. R. Monteiro
Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, SC, Brazil
e-mail: g.ayala.valencia@ufsc.br

T. J. Gutiérrez
Grupo de Materiales Compuestos Termoplásticos (CoMP), Instituto de Investigaciones en Ciencia y Tecnología de Materiales (INTEMA), Facultad de Ingeniería, Universidad Nacional de Mar del Plata (UNMdP) y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Colón, Mar del Plata, Argentina

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_5

5.1 Introduction

"To achieve a sustainable and inclusive world with due recognition and respect to the planet, people, prosperity, peace and partnership, 'Sustainable Development Goals' were adopted by all the countries" (Venkatramanan et al. 2021a). One of the objectives of the Sustainable Development Goals (SDGs) is to reduce the loss of food along the supply chain and to valorize agri-food wastes. These two factors are the central themes of agro-industry sector (Corrado et al. 2019; Garcia-Garcia et al. 2019). In this context, the agri-food waste valorization has great potential in sustainable bioeconomy (Venkatramanan et al. 2021b) and several countries are already promoting strategies for the valorization of these materials under the concept of green economies or eco-economies taking into consideration the food manufacturing industry (Garcia-Garcia et al. 2019). Normally, the valorization of agri-food waste has focused on using the by-products obtained from some agricultural practices, such as landfills, animal feed, fertilizer supply, composting, incineration, and anaerobic digestion. In this sense, the recovery capacity and the effectiveness to create value from agri-food wastes remain quite limited (Otles and Kartal 2018).

Agri-food wastes are materials rich in macromolecules, such as carbohydrates, proteins, and lipids, as well as active compounds and pigments, which could be used within the food packaging industry as a strategy to obtain higher value-added materials from agri-food wastes and by-products. This chapter reviews the state of the art regarding the recovery of macromolecules, active compounds and pigments from agri-food wastes to be used as food packaging material. The use of agri-food wastes to produce biosurfactants and single cell oils (SCOs), as well as the perspectives and limitations of agri-food wastes will also be reviewed and analyzed.

5.2 Valorization of Agro-Industrial Wastes Within the Food Packaging Industry

Food packaging can be defined as any material used to contain and conserve the food quality, as well as to transmit specific information during the distribution and commercialization chain (Ghaani et al. 2016). Most food packaging has been made from petroleum-derived macromolecules because these materials have good mechanical, optical, and gas barrier properties. However, petroleum is a nonrenewable resource and food packaging manufactured from this source are nonbiodegradable (Valencia and Sobral 2018).

In recent years, the development of novel food packaging based on macromolecules derived from natural sources have increased, particularly, packaging based on macromolecules isolated from agri-food wastes, such as carbohydrates and proteins (Valencia and Sobral 2018), as well natural compounds with active and smart properties. In this sense, several reviews can be found highlighting the use of food packaging made from macromolecules isolated from agri-food waste.

Fruit and vegetable wastes are the main sources studied due to the high content of macromolecules. In line with this Nouraddini et al. (2018), recently manufactured

edible films from the mixture of eggplant flour (EF) containing the skin plus corn starch (CS), using the casting methodology. The resulting films were homogeneous, and the determination of the color parameters indicated that when increasing the proportion of EF, the values of the color parameters a^*, b^*, and L^* were increased. In addition, the incorporation of EF accelerated the biodegradability of the films compared to the control films based on CS. However, the mechanical properties of the EF/CS films decreased with the presence of EF. This work demonstrates the added value related to the post-harvest loss of eggplant within the food packaging industry.

Beverage wastes (BW) such as selecta orange (*Citrus sinensis*), passion fruit (*Passiflora edulis*), watermelon (*Citrullus lanatus*), lettuce (*Lactuca sativa*), courgette (*Cucurbita pepo*), carrot (*Daucus carota*), spinach (*Spinacea oleracea*), mint (*Mentha* sp.), taro (*Colocasia esculenta*), cucumber (*Cucumis sativus*), and rocket (*Eruca sativa*) have also mixed with potato skin flour (PSF) to produce edible films (Andrade and Ferreira 2016). Andrade and Ferreira (2016) found that films made from the BW/PSF mixture using the casting methodology did not require the addition of a plasticizer, since the sugars contained in the BW acted as one, thus improving the mechanical properties of the material. This is of great value for cost reduction for the development of sustainable food packaging materials. Brito et al. (2019) using the same BW indicated above for the development of edible films, they observed that a reduction in the particle size of the BW altered the mechanical properties of the films. These authors concluded that the most resistant films were those containing the coarsest BW fraction: 425–500 μm (Brito et al. 2019). The previously described materials were also tested by Fai et al. (2016) and Ferreira et al. (2016) as coatings of acerola (*Malpighia punicifolia* L.) and fresh-cut carrot (*Daucus carota* L.), finding a reduction in the weight loss of these fruits as well as no alterations in the pH values and content of soluble solids during storage, thus increasing the shelf life of these plant products.

Another agri-food waste is mango kernel (*Mangifera indica*), which is rich in starch. Edible coatings made from the isolated starch of this fruit have extended the shelf life of red chili (*Capsicum annuum* L.) and tomato (*Solanum lycopersicum*) fruit (Nawab et al. 2018, 2017).

Other studies have focused on adding value to wastes from the agri-food industry. For example, castor bean (*Ricinus communis* L.) is an oil seed of high economic value; however, the waste after oil extraction called castor bean cake (CBC) has no industrial application. Chambi et al. (2014) determined that CBC has a high protein content, which could be isolated at pH 12 and 50 °C without altering its amino acid profile. They used these isolated proteins for the manufacture of edible films, demonstrating that these materials had better or similar mechanical properties than those reported for other protein-based films (collagen and gelatin) or starch films (Chambi et al. 2014; Oliveira et al. 2015; Valencia and Sobral 2018).

Gelidium sesquipedale red algae are also widely used in the industry for agar extraction. The *G. sesquipedale* algae waste is rich in cellulose. In this regard, de Oliveira et al. (2019) used the solvent extraction method to recover highly crystalline cellulose (~70%) and even nanocellulose from the above-mentioned waste. The

same authors concluded that these biopolymers can be used to make food packaging. This study reports for the first time a complete valorization of *G. sesquipedale* red algae (de Oliveira et al. 2019).

5.2.1 Active Food Packaging

New strategies have been carried out with the objective of extracting active compounds and pigments from agri-food wastes and then applied as additives in food packaging materials, thus obtaining new materials with active and/or smart properties (Gutiérrez et al. 2016a; Gutiérrez 2018a). Active food packaging can have various functions, which are not provided by conventional packaging. Active food packaging has been shown to maintain food quality or safety, as well as extend the shelf life of food products such as fruits and vegetables, meat, dairy, and bakery products, among others (Domínguez et al. 2018; Janjarasskul and Suppakul 2018; Schumann and Schmid 2018). These packages prevent the gain or loss of moisture through a controlled barrier or gas permeability by using carbon dioxide (CO_2) or ethylene scavengers. This allows, e.g., to slow down the ripening processes in vegetal products, and the antimicrobial and/or antioxidant compounds contained in the active packages can reduce the growth of spoilage and pathogen microorganisms, thus demonstrating their active properties (Saberi and Golding 2018). The nanotechnology contained in active food packaging can also increase the performance of polymers by minimizing waste material in landfills and the level of pollution in the environment (Quintavalla and Vicini 2002; Janjarasskul and Suppakul 2018; Prasad et al. 2014, 2017).

According to Dainelli et al. (2008), active food packaging is defined as those materials containing chemical compounds "generally recognized as safe" (GRAS), which allow to improve the organoleptic or functional functions of food. These GRAS compounds could be released from the packaging and be absorbed by food. In line with this, Regulation 1935/2004 of the European Union (EU) promotes the general principles of safety and inertia for all materials in contact with food (Karamfilova 2016). The U.S. Food and Drug Administration (FDA 2017) also provides regulatory information on ingredients, additives, and packaging guidance documents for food contact or interactions.

In recent years, a new approach in the development of food packages began by adding agricultural wastes and derivatives as functional additives within food packaging materials (Majid et al. 2018). Figure 5.1 presents agricultural wastes and by-products that can be used to develop active packaging, while Table 5.1 shows the materials studied to manufacture active biodegradable packaging for the food industry.

The interactions between food and active packaging have allowed the development of these materials on a large scale. A desired function of the food package in general is to provide an inert external barrier against the environment (Rooney 2005). An active packaging must also provide desirable interactions in the surrounding atmosphere: CO_2, H_2O, and O_2. Vilela et al. (2018) proposed a guide

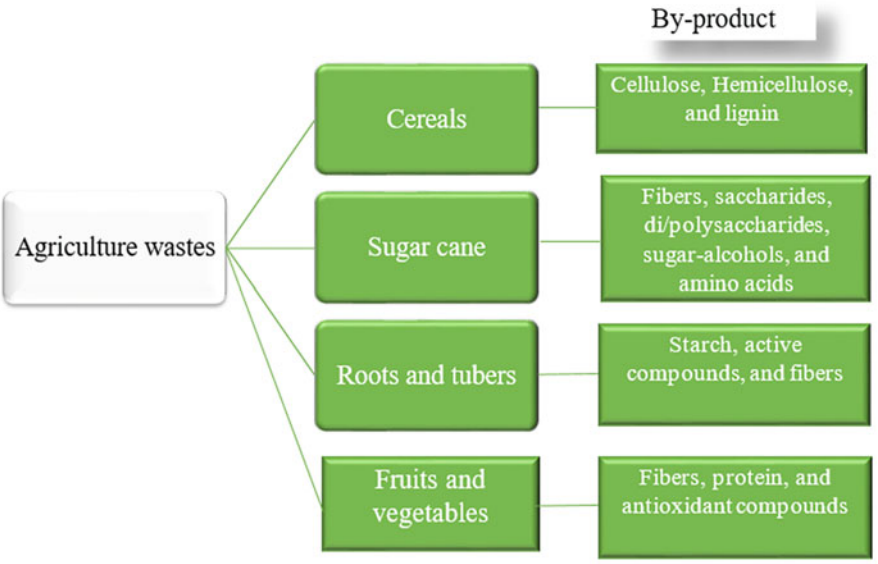


Fig. 5.1 Renewable sources from agricultural wastes for packaging development

containing the latest agents studied to be used as additives in terms of antioxidant and antimicrobial agents, as well as O_2 and ethylene scavengers in active food packaging to prevent oxidation and increase the shelf life of food. Table 5.1 also presents some researches on active packaging for food applications.

In this sense, Tosati et al. (2017) developed films from the mixture of starch-rich turmeric wastes with bovine gelatin to replace the artificial coating and avoid cross contamination in the sausage. Crizel et al. (2016) proposed blueberry fiber wastes as an additive of gelatin films to be used in the stabilization of fatty foods. Kanatt and Chawla (2018) developed a polymeric film from PLA and gelatin containing mango peel wastes. Due to the large mango production in tropical countries, wastes from these fruits can be a viable option for primary food packaging and to reduce the use of synthetic additives and polymers. Crizel et al. (2018) produced films from gelatin incorporating nutraceutical wastes obtained from the skin of papaya.

The introduction of antimicrobial and antifungal compounds such as bacteriophages, essential oils, or extracts obtained from plants, animal or plant enzymes, organic acids, bacteriocins among others into polymeric materials is based on the controlled release of these active agents from the food packaging material. The use of active compounds can be added to polymeric materials in order to obtain active plastic bags, sachets, coatings, and non-flexible packages (Otoni et al. 2016).

The migration of these active compounds is necessary to achieve desirable effectiveness against the development of microorganisms, molds, fungi, and oxidation. Scartazzini et al. (2019) developed edible gelatin films containing mint (*Mentha piperita*) essential oil and observed antimicrobial activity against the growth of

Table 5.1 Package materials used for food applications

Material	Active compounds	Targets	References
Polyethylene terephthalate/silicon oxide (PET-SiOx/LDPE)	Ethanol emitter sachet and O_2 absorber	Remove O_2 in the head space of the fresh bread packaging	Latou et al. (2010)
Polymeric resin and nonwoven (sachet)	Oregano, cinnamon, and lemongrass essential oils/antimicrobials	Inhibit microbial development in papaya fruits	Espitia et al. (2012)
Ethylene vinyl acetate (EVA) and low-density polyethylene (LDPE)	Polyphenolic compounds from the beer industry and rosemary extract	Provide antioxidant and antimicrobial activity	Barbosa-Pereira et al. (2014)
LDPE	Garlic acid, potassium chloride, and O_2 scavengers	Remove O_2 in the head space of packaging	Ahn et al. (2016)
Biomass from olive oil	Fibers from olive oil wastes	Increase the degradability of fossil polymers	Crizel et al. (2016)
Polyvinylidene chloride (PVDC)/nylon	BestKept® (O_2 scavenger)	Remove the chemical preservatives from sponge cakes. Maintenance of low oxygen (O_2) rate during storage	Janjarasskul et al. (2016)
Polyvinyl alcohol (PLA)/Cs	TiO_2 nanocomposite	Improve the functionality of PLA/Cs composites	Lian et al. (2016)
Potato starch	TiO_2 nanocomposite	Improves the properties of starch films	Oleyaei et al. (2016)
Turmeric starch waste/gelatin	Curcumin	Replace synthetic coatings and extend the shelf life of sausage	Tosati et al. (2017)
Flexible cast polyamide/polyethylene (PA/PE) + chitosan (Cs)	Flour from olive oil wastes	Antioxidant capacity of olive flour	Crizel et al. (2018)
Cs	Clove essential oil/halloysite nanoclays	Antioxidant capacity of clove essential oil	Lee et al. (2018)
Cs	TiO_2	Remove ethylene and antimicrobial activity on fruits and vegetables	Siripatrawan and Kaewklin (2018)
LDPE	O_2 scavengers and modified atmospheres	Whole bread	Upasen and Wattanachai (2018)
Fish gelatin	Anthocyanins	Potential active films for antioxidant packaging	Uranga et al. (2018)
Cs/gum Arabic	Cinnamon essential oil/antimicrobial	Use as an antimicrobial for food packaging	Xu et al. (2018)

(continued)

Table 5.1 (continued)

Material	Active compounds	Targets	References
Extruded CS/nano montmorillonites	Jamaica flower extract	Improve H-bonding interactions/compostability/ ecotoxicity	Gutiérrez et al. (2019)

Botrytis cinereal and *Rhizopus stolonifera*, thus opening the possibility for application in fruits and vegetables. Cerisuelo et al. (2012) developed nanoclay/EVOH-29 and bentonite/EVOH-29 composites by adding carvacrol as an antimicrobial. The authors observed that the diffusion of carvacrol in active films was influenced by the water content, temperature, and chemical interactions between polymers and active compounds. Xu et al. (2018) developed edible antimicrobial films based on chitosan (Cs) and Arabic gum incorporating cinnamon (*Cinnamomum zeylanicum*) essential oil, the resulting films were shown to retain the oil inside the film structure during storage when the Arabic gum content was higher. In addition, the antibacterial activities of the films were influenced by the storage environment and the concentration of cinnamon essential oil into the film. Salari et al. (2018) incorporated cellulose nanocrystals and silver nanoparticles into the chitosan (Cs) nanocomposites for the development of an active antimicrobial food packaging with improved mechanical and barrier properties. Siripatrawan and Kaewklin (2018) also added the TiO_2 nanocomposites into chitosan (Cs) to scavenge ethylene, thus developing antimicrobial active food packaging. Other research using active compounds, nanomaterials, and nanocomposites show the new trends in the development of active food packaging (Table 5.1).

On the other hand, synthetic polymers and their mixtures with renewable polymers with or without active compounds have been the focus of many research studies, since these have allowed to obtain more biodegradable food packaging materials. However, these mixtures have had as limitation the inherent polymeric immiscibility caused by the physicochemical properties of these polymers. This has limited its industrial applications yet (Huang et al. 2019). Sanches-Silva et al. (2010) incorporated chitosan (Cs) and astaxathin from shrimp wastes into synthetic plastic to obtain active packaging with antioxidant and antimicrobial properties.

5.2.2 Smart Food Packaging

According to Valencia et al. (2019), "smart or intelligent" food packaging is composed of self-adhesive labels attached to individual packages, which communicates the status of the product to the end users based on stimuli-responses. Mostly smart packaging is used as indicators of freshness in seafood, fish, meat, and fruits by monitoring the growth of microbials, chemical changes associated with food, or gases involved in the ripening process (Ghaani et al. 2016). Most of the information about smart films can be found in the specialized literature (Balbinot-Alfaro et al. 2019; Ghaani et al. 2016; Valencia et al. 2019). It is worth noting that

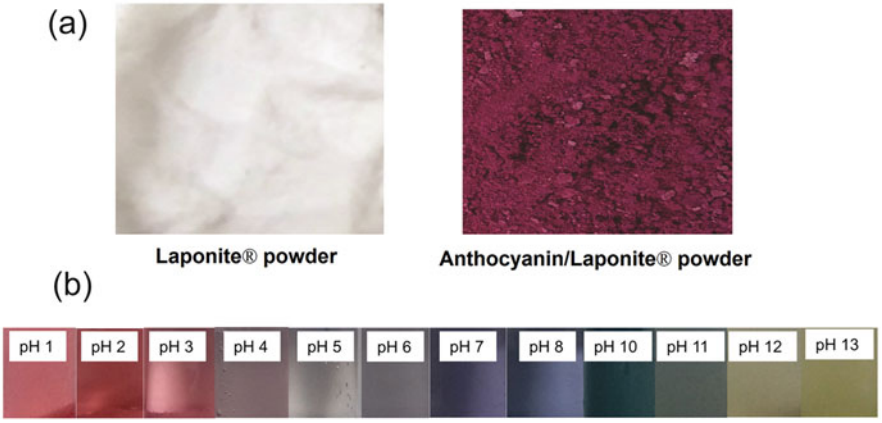


Fig. 5.2 (**a**) Laponite® powder and anthocyanin/laponite® powder and (**b**) color changes of anthocyanin/laponite® powder as a function of pH. (Source: Adapted with permission from Capello et al. (2019))

other intelligent materials such as those having shape memory are also considered as one of them (Herniou--Julien et al. 2019).

Regarding valorization of agri-food wastes, some papers have been aimed at recovering natural pigments such as anthocyanins due to their bathochromic effect when they are exposed to different pH values (Gutiérrez 2018b). Capello et al. (2019) recovered approximately 90% anthocyanins from eggplant peel at 25 °C and pH = 1, using laponite® nanoparticles. These authors suggested that anthocyanin/laponite® could be used to manufacture smart food packaging (Fig. 5.2).

Blueberry (*Vaccinium corymbosum* L.) and blackberry (*Rubus fruticosus*) wastes are rich in anthocyanins and have been used to manufacture pH-sensitive (smart) films by casting and thermocompression methodologies. These films have shown a color change at different pHs and can be used to control the food freshness (Andretta et al. 2019; Luchese et al. 2017, 2018; Nogueira et al. 2019). Further research should explore the potential of other anthocyanin-rich wastes such as peels from eggplant (Capello et al. 2019), jambolão (Brito et al. 2017), sweet potato (Yong et al. 2019), among others, for the manufacture of smart films. It should be noted that betalain-rich wastes obtained from beets have also been used as additives for the development of pH-sensitive films (Gutiérrez et al. 2016b).

5.3 Production of Biosurfactants from Agri-Food Wastes

Biosurfactants are amphiphilic molecules synthesized and produced under the concept of green chemistry, essentially by microbial cells (Inamuddin and Prasad 2021a). Biosurfactants have been applied as agricultural biostimulants, alternative pesticides, antimicrobials, and skin care oils (Inamuddin and Prasad 2021a, b). Some means for obtaining biosurfactants from agri-food wastes will be discussed below.

5.3.1 Lipopeptides

Lipopeptides are one of the best-known biosurfactant classes, among which surfactin, iturin, bacillomycin, and mycosubtilin can be highlighted. Its chemical structure consists of lipids covalently linked to peptides.

5.3.1.1 Surfactin

One of the best-known antimicrobial biosurfactants is surfactin. Due to the high cost of production, various alternatives have been suggested in the literature to obtain this compound from olive wastes, sewage from breweries and cassava, sugar cane and beet molasses, buttermilk among others (de Andrade et al. 2016, 2017). The chemical structure of surfactin is composed of seven amino acids forming a lactone ring linked to a β-OH fatty acid chain (Fig. 5.3). The amino acid sequence of the lactone ring is given as follows: glutamate (Glu), leucine (Leu), Leu, valine (Val), aspartate (Asp), Leu, Leu, or Val (de Andrade et al. 2016).

The production of surfactin by means of *Bacillus subtilis* using cassava wastewater as an alternative culture medium has been reported by de Andrade et al. (2016). The authors concluded that the use of agro-industrial wastes to produce surfactin is an interesting strategy, due to its low cost and sustainability. However, despite these advantages, the high protein content hinders the ultrafiltration of surfactin.

The cashew apple juice has also been used by Felix et al. (2019) as an alternative culture medium for obtaining surfactin using 20 different strains of *Bacillus*, including *B. subtilis*, *B. thuringiensis*, *B. cereus*, and *B. anthracis*, and the purification of the compound was given by acid precipitation followed by solvent extraction. These authors evaluated the phytotoxicity and cytotoxicity of surfactin obtained, as well as germination index as a biomarker of agricultural stimulation in lettuce, finding that a higher germination index (~80%) associated with the application of surfactin (from 12 to 350 mg/L).

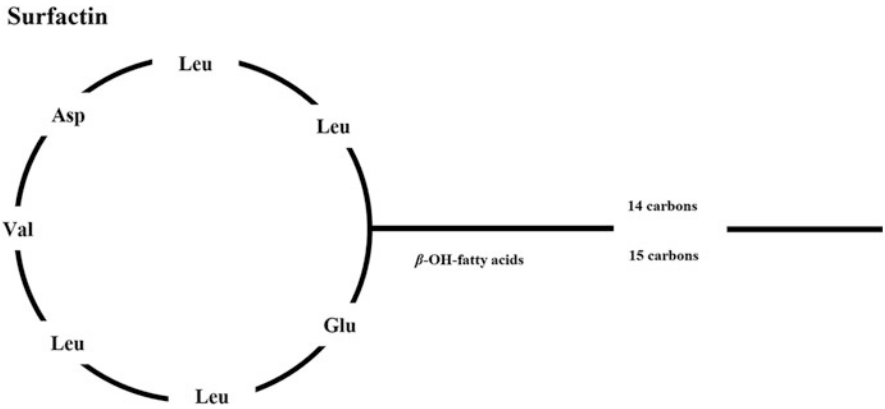


Fig. 5.3 Chemical structure of surfactin. (Source: Reprinted with permission from de Andrade et al. (2016))

Other potential wastes for surfactin production are distiller grains, the main waste of the liquor industry. Zhi et al. (2017) also described the production of surfactin by *B. amyloliquefaciens* MT45 and *B. amyloliquefaciens* X82 using distiller grains, thus achieving a high yield: ~3.5 g of surfactin/L. This demonstrates the potential for the valorization of agri-food wastes.

5.3.2 Glycolipids

Glycolipids are chemically composed of one or more monosaccharide moieties chemically linked to lipids (hydrophobic moiety). Glycolipids have a wide range of structures, and they show significantly higher production yields (g/L) compared to the yields of lipopeptide production (~mg/L).

5.3.2.1 Rhamnolipids

Rhamnolipids are glycolipidic biosurfactants which are produced from different bacterial species, in particular by *Pseudomonas aeruginosa*. The chemical structure of the rhamnolipids includes four congeners: α-L-rhamnopyranosyl-α-L-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate (Rha-Rha-C10-C10), α-L-rhamnopyranosyl-α-L-rhamnopyranosyl-β-hydroxydecanoate (Rha-Rha-C10), and their mono-rhamnolipid congeners: Rha-C10-C10 and Rha-C10 (Fig. 5.4) (Radzuan et al. 2018).

Regarding the production of rhamnolipid using alternative culture media from agri-food wastes, Radzuan et al. (2017) used agricultural wastes obtained from palm oil refining to produce rhamnolipids by *P. aeruginosa* PAO1 (0.43 g of rhamnolipid/L). Radzuan et al. (2018) also investigated the production of rhamnolipid by *P. aeruginosa* PA01 using as a single carbon source, the palm fatty acid distillate and the fatty acid methyl ester. These authors concluded that both the substrates have potential as a low-cost carbon source for large-scale rhamnolipid production. Gudiña et al. (2016) achieved the production of rhamnolipids (~5 g of rhamnolipids/L) from oily wastes from olive oil extraction using *P. aeruginosa* #112.

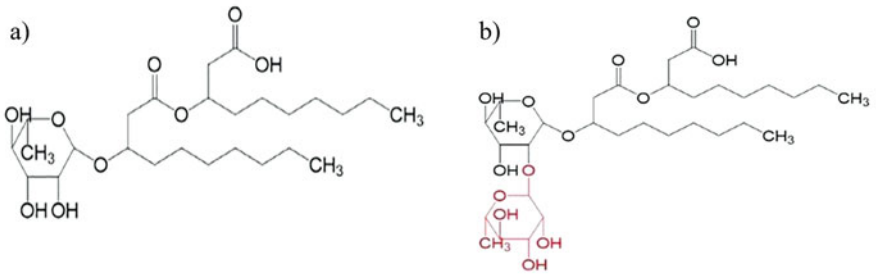


Fig. 5.4 Chemical structure of rhamnolipid: (**a**) mono-rhamnolipid, and (**b**) di-rhamnolipid. (Source: Adapted with permission from Wu et al. (2019))

5.3.2.2 Mannosylerythritol Lipids

Mannosylerythritol lipid is a mixture of partially acylated derivatives of 4-O-β-D-manopyranosyl-D-erythritol. There are four homologs of mannosylerythritol lipids-A, -B, -C, and-D, which are classified exclusively based on the acetylation of C-4′ and C-6′ (mannose) (Fig. 5.5).

de Andrade et al. (2017) described the successful production and purification (ultrafiltration) of mannosylerythritol lipid-B obtained from cassava wastewater by means of *Pseudozyma tsukubaensis*. A similar study was conducted by Niu et al. (2019) for mannosylerythritol lipids manufacturing from soybean oil. These authors obtained 61 g of mannosylerythritol/L lipids for the optimized bioprocess, while the non-optimized medium had a yield of ~10 g of mannosylerythritol/L lipids. Santos et al. (2019) used lignocellulose hydrolysates to produce mannosylerythritol lipids through *Moesziomyces antarcticus*, finding that the furfural of lignocellulose hydrolysates has a greater inhibitory effect on the production of mannosylerythritol lipids.

5.3.2.3 Sophorolipids

The sophorolipids are composed of a sophorose disaccharide as a hydrophilic part linked to a long-chain hydroxylated fatty acid as a hydrophobic part (Fig. 5.6). Sophorolipids have the highest yields among the glycolipids. Kaur et al. (2019) used agri-food wastes mainly rice to produce sophorolipids in a bioreactor using an

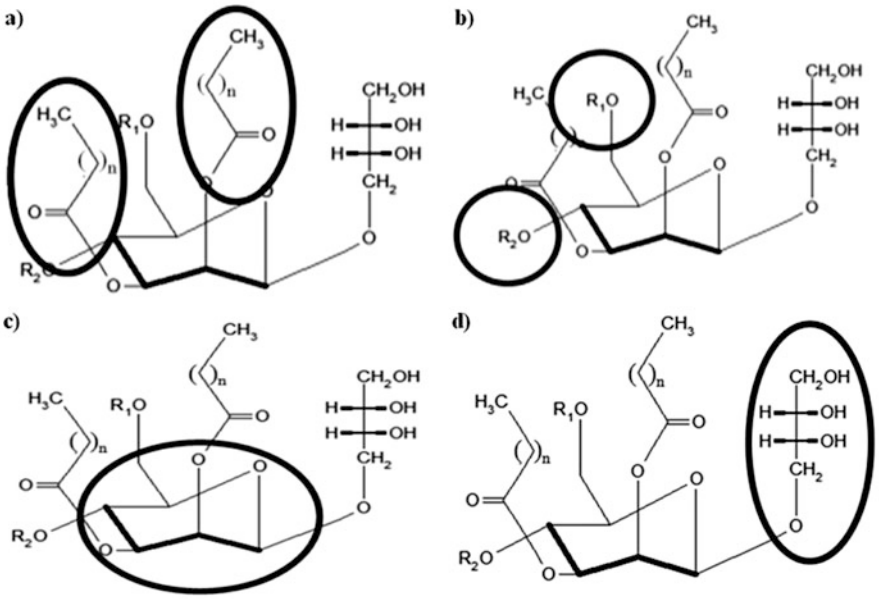


Fig. 5.5 Chemical structure of mannosylerythritol lipids: (**a**) fatty acids, (**b**) acetylation of C-4′ and/or C-6′ in mannose, (**c**) mannose, (**d**) erythritol. (Source: Reprinted with permission from de Andrade et al. (2017))

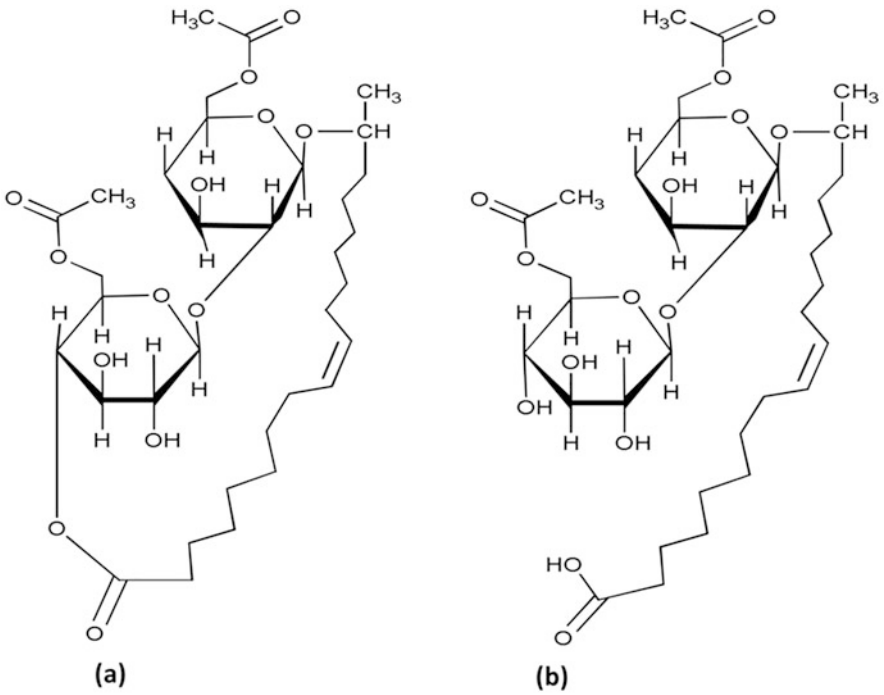


Fig. 5.6 Chemical structure of (**a**) lactonic sophorolipid and (**b**) acidic sophorolipid. (Source: Adapted with permission from Jadhav et al. (2019))

enzymatic bioprocess. The authors managed to produce ~115 g of sophorolipids/L after 92 h (~1.25 g/L h). However, the enzymatic pretreatment makes this bioprocess unfeasible due to the high cost. Jadhav et al. (2019) also produced (submerged fermentation) sophorolipids from sunflower acid oil (a waste of sunflower oil refining) by using *Starmerella bombicola* (fungi). The highest production reached ~50 g of sophorolipids/L at a 5 L-bioreactor scale (~0.25 g/L h).

5.4 Production of SCOs Using Agri-Food Wastes

The use of agri-food wastes as a low-cost alternative carbon source is essential to achieve the production of single cell oil (SCOs) at competitive prices (Ageitos et al. 2011). SCOs have been presented as edible oils, produced from unicellular microorganisms (oleaginous microorganisms) through a cultivation process (Ratledge and Cohen 2008). All microorganisms are capable of synthesizing lipids, although some microbial cells such as yeasts, molds, and microalgae can accumulate significant amounts of lipids in their cells relative to dry weight (>20%), and for this reason, they are defined as oleaginous microorganisms (OM) (Papanikolaou and Aggelis 2011).

Table 5.2 Lipid content and PUFA profile for the most studied yeasts, molds, and microalgae compared to soybean and corn oil

Oil source	Maximum lipid content (% w/w)	PUFA (% w/w)						References
		16:0	18:1	18:2	18:3	20:3	20:4	
Soybean	18	11	24	54	7	ND	ND	Ratledge (1997)
Corn	13	11	13	54	1	ND	ND	
R. glutinis (yeast)	72	37	3	47	8	ND	ND	Ratledge and Cohen (2008)
R. toruloides (yeast)	66	18	3	66	ND	ND	ND	
M. alpine (mold)	70	15	20	10	8	5	40	Dyal and Narine (2005)
S. platensis (microalgae)	25	44.60	8.55	12.73	20.92	ND	ND	Bhakar et al. (2013) and Colla et al. (2004)

ND not detected

All edible oils produced today are rich in polyunsaturated fatty acids (PUFAs) and are intended for human consumption as nutraceuticals, although some are used for animal feed (Ratledge and Cohen 2008), food additives, biopolymers, drugs, and cosmetics (Vasconcelos et al. 2019). PUFAs have shown great importance not only for human consumption but also to produce biofuels such as biodiesel and jet fuel (Ratledge 2013). The SCOs produced by OM have had a high PUFA content compared to the most important genetically modified plants used to produce these oils on a large scale (Table 5.2).

Table 5.2 shows the content of PUFAs in oleaginous microorganisms such as *Rhodotorula glutinis*, *Rhodosporidium toruloides*, *Mortierella alpine*, and *Spirulina platensis*, these being higher compared to oilseeds such as soybeans and corn. The profile of PUFAs from the SCOs is interesting, since they have the high long chain content and the number of unsaturation in the PUFAs. This characteristic is important because they have therapeutic properties, in terms of reducing the level of cholesterol in the blood (Koutinas et al. 2014). It is worth noting that some species of molds can produce high levels of arachidonic acid (ARA, 20:4) (Table 5.2), which is essential for the brain, muscles, and liver, and this is more important in infants and the elderly (Kikukawa et al. 2018).

5.4.1 Carbon Sources for SCO Production

As indicated above, the prices of agri-food wastes for obtaining high value-added products such as SCOs are crucial for the sustainability of this process based on green chemistry and economics. The price (US$/ton) of some agri-food wastes

leading to obtain SCOs can be given below: wheat straw (90–100), sugarcane bagasse (100–300), sugarcane molasses (200–400), CS (299–319), whey powder (300–500), corn syrup (390–450), and glycerol (650–700). The use of these agri-food wastes and wastewaters produced from the food industry not only has been of interest for their recovery but also reduces pollution damage to soils and water bodies, since they have high oxygen demand values for degradation. For this reason, these agri-food wastes have also drawn attention to the production of SCOs by aerobic microorganisms (Huang et al. 2013).

Lignocellulosic hydrolysates have been studied extensively as a low-cost carbon source for SCO production bioprocesses using *Lipomyces starkeyi*. In accordance with this, Anschau et al. (2014) obtained a yield and productivity of SCO of 0.236 g/g and 0.111 g/L h, respectively, using sugarcane bagasse as an agri-food waste and *L. starkeyi* as an oleaginous microorganism (Table 5.3). Ykema et al. (1988) obtained an SCO productivity of 0.995 g/L h using whey permeate as a carbon source for *Cryptococcus curvatus* cultivation (Table 5.3).

The cultivation of *R. toruloides* was carried out by Fei et al. (2016) using corn stove hydrolysate for SCO production. These authors achieved a productivity of SCO 0.4 g/L h, and a yield of 0.29 g/g. Vieira et al. (2014) investigated the cultivation of *R. glutinis* CCT 2182, *R. toruloides* CCT 0783, *R. minuta* CCT

Table 5.3 SCO productivity obtained from oleaginous microorganisms (OMs) using different carbon sources and different cultivation methods

Microorganisms	Carbon source	Fermentation process	Productivity (g/L h)	References
C. curvatus	Whey permeates	Partial recycling	0.995	Ykema et al. (1988)
M. circinelloides, f. lusitanicus CBS 277.49	Thin stillage (corn ethanol plant)	Batch (air lift)	0.095	Mitra et al. (2012)
Mortierella isabellina (DSM 1414)	Whey powder 6.0% lactose (treated with lactase)	Batch (STR)	0.200	Demir et al. (2013)
L. starkeyi DSM 70296	Hemicellulose hydrolysate (sugarcane bagasse)	Continuous (CSTR)	0.111	Anschau et al. (2014)
R. toruloides CCT 0783	Sugarcane molasses	Pulsed fed-batch (STR)	0.410	Vieira et al. (2014)
R. toruloides DSMZ 4444	Corn hydrolysate	Fed-batch	0.400	Fei et al. (2016)
R. glutinis TISTR5159	Hydrolyzed pineapple pulp waste	Batch (Erlenmeyer flasks)	0.100	Tinoi and Rakariyatham (2016)
C. curvatus DSM 70022	Rich-in-starch wheat wastes	Batch (Erlenmeyer flasks)	2.000	Chaturvedi et al. (2019)

STR stirred tank reactor, *CSTR* continuous stirred tank reactor

1751 and *L. starkeyi* DSM 70296 as oleaginous microorganisms, obtaining a yield in descending order as follows: *R. toruloides* (0.41 g/L h) < *R. glutinis* (0.27 g/L h) < *R. minuta* (0.135 g/L h) < *L. starkeyi* (0.13 g/L h), using sugarcane molasses as a carbon source.

Several carbon sources were also tested by Chaturvedi et al. (2019) for SCO production such as banana, cassava, potato and yam peels, corn and rice wastes, wheat bran, and starchy barley wastes. The authors concluded that wheat bran was the best substrate for *C. curvatus* cultivation, showing a productivity and yield of 2.00 g/L h and 16.0 g_{lipid}/g_{starch}, respectively. Demir et al. (2013) used deproteinized whey powder for SCO production by *M. isabellina*. The results obtained by Demir et al. (2013) at the laboratory scale showed that the whey solution previously treated with lactase had better results: productivity 0.2 g/L h and yield 17.13 g/L of SCO.

Another agri-food waste used for SCO production has been pineapple pulp. With this in mind, Tinoi and Rakariyatham (2016) obtained an SCO productivity of 0.1 g/L h using *R. glutinis* as an oleaginous microorganism (Table 5.3). Another microorganism cultured by Mitra et al. (2012) for SCO production from thin stillage was *Mucor circinelloides*. These authors found an SCO productivity of 0.095 g/L h.

5.5 Prospects and Limitations of Agri-Food Waste Valorization

Agri-food wastes have potential compounds to be incorporated into food products, which reduce the carbon footprint. In this sense, the correct use of agri-food wastes could help the circular bioeconomy, thus reducing the gap between waste valorization and product recovery (Dahiya et al. 2018; Venkatramanan et al. 2021a, b). There are significant opportunities to value a significant number of identified agri-food wastes. However, future research should consider the quantities and types of agri-food wastes available, patterns of generation of these materials, the qualitative and quantitative characteristics of agri-food wastes, as well as the variability of the agri-food waste generation and quality (Garcia-Garcia et al. 2019). It is also necessary to optimize and expand the recovery/separation process of biopolymers, active compounds, and pigments from agri-food wastes, as well as the biological processes used to produce biosurfactants and SCOs (Dahiya et al. 2018). The safety and inertness of biopolymers, active compounds, pigments, biosurfactants, and SCOs from agri-food wastes should also be studied.

5.6 Conclusion

The agri-food industry inherently generates wastes. In this sense, there is a wide range of opportunities to directly recover value-added products from these agri-food wastes. In this chapter, the valuation of agri-food waste was reviewed in terms of obtaining bioactive compounds and pigments, as well as the manufacture of biosurfactants and SCOs by bioprocesses using oleaginous microorganisms. This

demonstrates the potential of agri-food wastes to obtain value-added products, generating the possibility of circular economies and green chemistry.

Acknowledgments G. A. Valencia, C. J. Andrade, J. L. Ienczak, and A. R. Monteiro would like to thank the Federal University of Santa Catarina (UFSC) and CAPES (Coordination for the Improvement of Higher Education Personnel) for their support. G.A. Valencia gratefully acknowledge to CNPq (National Council for Scientific and Technological Development) for the research grant (405432/2018-6).

T. J. Gutiérrez would like to thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Mar del Plata (UNMdP), and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) (grant PICT-2017-1362) for financial support.

Conflict of Interest: The author declares no conflict of interest.

References

Ageitos JM, Vallejo JA, Veiga-crespo P, Villa TG (2011) Oily yeasts as oleaginous cell factories. Appl Microbiol Biotechnol 90(4):1219–1227. https://doi.org/10.1007/s00253-011-3200-z

Ahn BJ, Gaikwad KK, Lee YS (2016) Characterization and properties of LDPE film with gallic-acid-based oxygen scavenging system useful as a functional packaging material. J Appl Polym Sci 133(44):1–8. https://doi.org/10.1002/app.44138

Andrade RMS, Ferreira MSL (2016) Development and characterization of edible films based on fruit and vegetable residues. J Food Sci 81(2):E412–E418. https://doi.org/10.1111/1750-3841.13192

Andretta R, Luchese CL, Tessaro IC, Spada JC (2019) Development and characterization of pH-indicator films based on cassava starch and blueberry residue by thermocompression. Food Hydrocoll 93:317–324. https://doi.org/10.1016/j.foodhyd.2019.02.019

Anschau A, Xavier MCA, Hernalsteens S, Franco TT (2014) Effect of feeding strategies on lipid production by *Lipomyces starkeyi*. Bioresour Technol 157:214–222. https://doi.org/10.1016/j.biortech.2014.01.104

Balbinot-Alfaro E, Craveiro DV, Lima KO, Leão H, Costa G, Lopes DR, Prentice C (2019) Intelligent packaging with ph indicator potential. Food Eng Rev 11:1–10. https://doi.org/10.1007/s12393-019-09198-9

Barbosa-Pereira L, Angulo I, Lagarón JM, Paseiro-Losada P, Cruz JM (2014) Development of new active packaging films containing bioactive nanocomposites. Innov Food Sci Emerg Technol 26:310–318. https://doi.org/10.1016/j.ifset.2014.06.002

Bhakar RN, Kumar R, Pabbi S (2013) Total lipids and fatty acid profile of different *Spirulina* strains as affected by salinity and incubation time. Vegetos 26(2s):148–154. https://doi.org/10.5958/j.2229-4473.26.2s.133

Brito B d N d C, Pena R d S, Lopes AS, Chisté RC (2017) Anthocyanins of jambolão (*Syzygium cumini*): extraction and pH-dependent color changes. J Food Sci 82(10):2286–2290. https://doi.org/10.1111/1750-3841.13847

Brito T, Carrajola J, Gonçalves ECB, Martelli-tosi M, Ferreira MS (2019) Fruit and vegetable residues flours with different granulometry range as raw material for pectin-enriched biodegradable film preparation. Food Res Int 121:412–421. https://doi.org/10.1016/j.foodres.2019.03.058

Capello C, Leandro GC, Campos CEM, Hotza D, Carciofi BAM, Valencia GA (2019) Adsorption and desorption of eggplant peel anthocyanins on a synthetic layered silicate. J Food Eng 262:162–169. https://doi.org/10.1016/j.jfoodeng.2019.06.010

Cerisuelo JP, Alonso J, Aucejo S, Gavara R, Hernández-Muñoz P (2012) Modifications induced by the addition of a nanoclay in the functional and active properties of an EVOH film containing carvacrol for food packaging. J Membr Sci 423–424:247–256. https://doi.org/10.1016/j.memsci.2012.08.021

Chambi HNM, Lacerda RS, Makishi GLA, Monica A, Bittante QB, Gomide CA, Sobral PJA (2014) Protein extracted from castor bean (*Ricinus communis* L.) cake in high pH results in films with improved physical properties. Ind Crop Prod 61:217–224. https://doi.org/10.1016/j.indcrop.2014.07.009

Chaturvedi S, Bhattacharya A, Nain L, Prasanna R, Khare SK (2019) Valorization of agro-starchy wastes as substrates for oleaginous microbes. Biomass Bioenergy 127:105294. https://doi.org/10.1016/j.biombioe.2019.105294

Colla LM, Bertolin TE, Costa JAV (2004) Fatty acids profile of spirulina platensis grown under different temperatures and nitrogen concentrations. Z Naturforsch C 59(1–2):55–59. https://doi.org/10.1515/znc-2004-1-212

Corrado S, Caldeira C, Eriksson M, Jørgen O, Hauser H, Van Holsteijn F, Liu G, Östergren K, Parry A, Secondi L (2019) Food waste accounting methodologies: challenges, opportunities, and further advancements. Glob Food Sec 20:93–100. https://doi.org/10.1016/j.gfs.2019.01.002

Crizel T d M, Costa TMH, Rios A d O, Flôres SH (2016) Valorization of food-grade industrial waste in the obtaining active biodegradable films for packaging. Ind Crop Prod 87:218–228. https://doi.org/10.1016/j.indcrop.2016.04.039

Crizel T d M, Rios A d O, Alves VD, Bandarra N, Moldão-Martins M, Flôres SH (2018) Active food packaging prepared with chitosan and olive pomace. Food Hydrocoll 74:139–150. https://doi.org/10.1016/j.foodhyd.2017.08.007

Dahiya S, Kumar AN, Sravan JS, Chatterjee S, Sarkar O, Mohan SV (2018) Food waste biorefinery: sustainable strategy for circular bioeconomy. Bioresour Technol 248(Part A):2–12. https://doi.org/10.1016/j.biortech.2017.07.176

Dainelli D, Gontard N, Spyropoulos D, Zondervan-van den Beuken E, Tobback P (2008) Active and intelligent food packaging: legal aspects and safety concerns. Trends Food Sci Technol 19 (Supplement 1):S103–S112. https://doi.org/10.1016/j.tifs.2008.09.011

de Andrade CJ, Barros FC, De Andrade LM, Rocco SA, Sforça L, Pastore M, Jauregi P (2016) Ultrafiltration based purification strategies for surfactin produced by *Bacillus subtilis* LB5A using cassava wastewater as substrate. J Chem Technol Biotechnol 91(12):3018–3027. https://doi.org/10.1002/jctb.4928

de Andrade CJ, de Andrade LM, Rocco SA, Sforça ML, Pastore GM, Jauregi P (2017) A novel approach for the production and purification of mannosylerythritol lipids (MEL) by *Pseudozyma tsukubaensis* using cassava wastewater as substrate. Sep Purif Technol 180:157–167. https://doi.org/10.1016/j.seppur.2017.02.045

de Oliveira JP, Bruni GP, Fabra MJ, Zavareze E d R, López-Rubio A, Martínez-Sanz M (2019) Development of food packaging bioactive aerogels through the valorization of *Gelidium sesquipedale* seaweed. Food Hydrocoll 89:337–350. https://doi.org/10.1016/j.foodhyd.2018.10.047

Demir M, Turhan I, Kucukcetin A, Alpkent Z (2013) Bioresource technology oil production by *Mortierella isabellina* from whey treated with lactase. Bioresour Technol 128:365–369. https://doi.org/10.1016/j.biortech.2012.10.078

Domínguez R, Barba FJ, Gómez B, Putnik P, Bursa D, Pateiro M, Santos EM, Lorenzo JM (2018) Active packaging films with natural antioxidants to be used in meat industry: a review. Food Res Int 113:93–101. https://doi.org/10.1016/j.foodres.2018.06.073

Dyal SD, Narine SS (2005) Implications for the use of Mortierella fungi in the industrial production of essential fatty acids. Food Res Int 38(4):445–467. https://doi.org/10.1016/j.foodres.2004.11.002

Espitia PJP, Soares N d FF, Botti LCM, de Melo NR, Pereira OL, da Silva WA (2012) Assessment of the efficiency of essential oils in the preservation of postharvest papaya in an antimicrobial packaging system. Braz J Food Technol 15(4):307–316. https://doi.org/10.1590/s1981-67232012005000027

Fai AEC, de Souza MRA, de Barros ST, Bruno NV, Ferreira MSL, Gonçalves ÉCB d A (2016) Postharvest biology and technology development and evaluation of biodegradable fi lms and

coatings obtained from fruit and vegetable residues applied to fresh-cut carrot (*Daucus carota* L.). Postharv Biol Technol 112:194–204. https://doi.org/10.1016/j.postharvbio.2015.09.021

FDA (2017) Code of Federal Regulations (CFR) citations for color additives, food ingredients and packaging. https://www.fda.gov/food/ingredients-additives-gras-packaging-guidance-documents-regulatory-information/code-federal-regulations-cfr-citations-color-additives-food-ingredients-and-packaging. Accessed 24 Aug 2020

Fei Q, Brien MO, Nelson R, Chen X, Lowell A, Dowe N (2016) Biotechnology for biofuels enhanced lipid production by *Rhodosporidium toruloides* using different fed-batch feeding strategies with lignocellulosic hydrolysate as the sole carbon source. Biotechnol Biofuels 9:130. https://doi.org/10.1186/s13068-016-0542-x

Felix AKN, Martins JJL, Almeida JGL, Giro MEA, Cavalcante KF, Melo VMM, Pessoa ODL, Rocha MVP, Gonçalves LRB, Aguiar RS d S (2019) Purification and characterization of a biosurfactant produced by *Bacillus subtilis* in cashew apple juice and its application in the remediation of oil-contaminated soil. Colloids Surf B Biointerfaces 175:256–263. https://doi.org/10.1016/j.colsurfb.2018.11.062

Ferreira MSL, Fai EC, Andrade CT, Paulo H, Azero G, Cba É (2016) Edible films and coatings based on biodegradable residues applied to acerolas (*Malpighia punicifolia* L.). J Sci Food Agric 96(5):1634–1642. https://doi.org/10.1002/jsfa.7265

Garcia-Garcia G, Stone J, Rahimifard S (2019) Opportunities for waste valorisation in the food industry—a case study with four UK food manufacturers. J Clean Prod 211:1339–1356. https://doi.org/10.1016/j.jclepro.2018.11.269

Ghaani M, Cozzolino CA, Castelli G, Farris S (2016) An overview of the intelligent packaging technologies in the food sector. Trends Food Sci Technol 51:1–11. https://doi.org/10.1016/j.tifs.2016.02.008

Gudiña EJ, Rodrigues AI, De Freitas V, Azevedo Z, Teixeira JA, Rodrigues LR (2016) Valorization of agro-industrial wastes towards the production of rhamnolipids. Bioresour Technol 212:144–150. https://doi.org/10.1016/j.biortech.2016.04.027

Gutiérrez TJ (2018a) Active and intelligent films made from starchy sources/blackberry pulp. J Polym Environ 26(6):2374–2391. https://doi.org/10.1007/s10924-017-1134-y

Gutiérrez TJ (2018b) Are modified pumpkin flour/plum flour nanocomposite films biodegradable and compostable? Food Hydrocoll 83:397–410. https://doi.org/10.1016/j.foodhyd.2018.05.035

Gutiérrez TJ, Suniaga J, Monsalve A, García NL (2016a) Influence of beet flour on the relationship surface-properties of edible and intelligent films made from native and modified plantain flour. Food Hydrocoll 54:234–244. https://doi.org/10.1016/j.foodhyd.2015.10.012

Gutiérrez TJ, Guzmán R, Jaramillo CM, Famá L (2016b) Effect of beet flour on films made from biological macromolecules: native and modified plantain flour. Int J Biol Macromol 82:395–403. https://doi.org/10.1016/j.ijbiomac.2015.10.020

Gutiérrez TJ, Toro-Márquez LA, Merino D, Mendieta JR (2019) Hydrogen-bonding interactions and compostability of bionanocomposite films prepared from corn starch and nano-fillers with and without added Jamaica flower extract. Food Hydrocoll 89:283–293. https://doi.org/10.1016/j.foodhyd.2018.10.058

Herniou--Julien C, Mendieta JR, Gutiérrez TJ (2019) Characterization of biodegradable/non-compostable films made from cellulose acetate/corn starch blends processed under reactive extrusion conditions. Food Hydrocoll 89:67–79. https://doi.org/10.1016/j.foodhyd.2018.10.024

Huang C, Chen XF, Xiong L, Chen XD, Ma L, Chen Y (2013) Single cell oil production from low-cost substrates: the possibility and potential of its industrialization. Biotechnol Adv 31 (2):129–139. https://doi.org/10.1016/j.biotechadv.2012.08.010

Huang T, Qian Y, Wei J, Zhou C (2019) Polymeric antimicrobial food packaging and its applications. Polymers (Basel) 11(3):560. https://doi.org/10.3390/polym11030560

Inamuddin AMI, Prasad R (eds) (2021a) Microbial biosurfactants: preparation, properties and applications. Springer, Singapore. (ISBN: 978-981-15-6606-6) https://www.springer.com/gp/book/9789811566066

Inamuddin AMI, Prasad R (2021b) Advanced antimicrobial materials and applications. Springer, Singapore. (ISBN: 978-981-15-7098-8) https://www.springer.com/gp/book/9789811570971

Jadhav JV, Pratap AP, Kale SB (2019) Evaluation of sun flower oil refinery waste as feedstock for production of sophorolipid. Process Biochem 78:15–24. https://doi.org/10.1016/j.procbio.2019.01.015

Janjarasskul T, Suppakul P (2018) Active and intelligent packaging: the indication of quality and safety. Crit Rev Food Sci Nutr 58(5):808–831. https://doi.org/10.1080/10408398.2016.1225278

Janjarasskul T, Tananuwong K, Kongpensook V (2016) Shelf life extension of sponge cake by active packaging as an alternative to direct addition of chemical preservatives. LWT- Food Sci Technol 72:166–174. https://doi.org/10.1016/j.lwt.2016.04.049

Kanatt SR, Chawla SP (2018) Shelf life extension of chicken packed in active film developed with mango peel extract. J Food Saf 38(1):e12385. https://doi.org/10.1111/jfs.12385

Karamfilova E (2016) Food contact materials—Regulation (EC) 1935/2004. https://www.europarl.europa.eu/RegData/etudes/STUD/2016/581411/EPRS_STU(2016)581411_EN.pdf. Accessed 24 Aug 2020

Kaur G, Wang H, Ho M, Roelants SLKW, Soetaert W, Sze C, Lin K (2019) Efficient sophorolipids production using food waste. J Clean Prod 232:1–11. https://doi.org/10.1016/j.jclepro.2019.05.326

Kikukawa H, Sakuradani E, Ando A, Shimizu S, Ogawa J (2018) Arachidonic acid production by the oleaginous fungus *Mortierella alpina* 1S-4: a review. J Adv Res 11:15–22. https://doi.org/10.1016/j.jare.2018.02.003

Koutinas AA, Chatzifragkou A, Kopsahelis N, Papanikolaou S, Kookos IK (2014) Design and techno-economic evaluation of microbial oil production as a renewable resource for biodiesel and oleochemical production. Fuel 116:566–577. https://doi.org/10.1016/j.fuel.2013.08.045

Latou E, Mexis SF, Badeka AV, Kontominas MG (2010) Shelf life extension of sliced wheat bread using either an ethanol emitter or an ethanol emitter combined with an oxygen absorber as alternatives to chemical preservatives. J Cereal Sci 52(3):457–465. https://doi.org/10.1016/j.jcs.2010.07.011

Lee MH, Kim SY, Park HJ (2018) Effect of halloysite nanoclay on the physical, mechanical, and antioxidant properties of chitosan films incorporated with clove essential oil. Food Hydrocoll 84:58–67. https://doi.org/10.1016/j.foodhyd.2018.05.048

Lian Z, Zhang Y, Zhao Y (2016) Nano-TiO_2 particles and high hydrostatic pressure treatment for improving functionality of polyvinyl alcohol and chitosan composite films and nano-TiO_2 migration from film matrix in food simulants. Innov Food Sci Emerg Technol 33:145–153. https://doi.org/10.1016/j.ifset.2015.10.008

Luchese CL, Sperotto N, Spada JC, Tessaro IC (2017) Effect of blueberry agro-industrial waste addition to corn starch-based films for the production of a pH-indicator film. Int J Biol Macromol 104(Part A):11–18. https://doi.org/10.1016/j.ijbiomac.2017.05.149

Luchese CL, Uranga J, Spada JC, Tessaro IC, de la Caba K (2018) Valorisation of blueberry waste and use of compression to manufacture sustainable starch films with enhanced properties. Int J Biol Macromol 115:955–960. https://doi.org/10.1016/j.ijbiomac.2018.04.162

Majid I, Nayik GA, Dar SM, Nanda V (2018) Novel food packaging technologies: innovations and future prospective. J Saudi Soc Agric Sci 17(4):454–462. https://doi.org/10.1016/j.jssas.2016.11.003

Mitra D, Rasmussen ML, Chand P, Reddy V, Yao L, Grewell D, Verkade JG, Wang T, Van Leeuwen JH (2012) Bioresource technology value-added oil and animal feed production from corn-ethanol stillage using the oleaginous fungus *Mucor circinelloides*. Bioresour Technol 107:368–375. https://doi.org/10.1016/j.biortech.2011.12.031

Nawab A, Alam F, Hasnain A (2017) Mango kernel starch as a novel edible coating for enhancing shelf- life of tomato (*Solanum lycopersicum*) fruit. Int J Biol Macromol 103:581–586. https://doi.org/10.1016/j.ijbiomac.2017.05.057

Nawab A, Alam F, Haq MA, Haider MS, Lutfi Z, Kamaluddin S, Hasnain A (2018) Innovative edible packaging from mango kernel starch for the shelf life extension of red chili powder. Int J Biol Macromol 114:626–631. https://doi.org/10.1016/j.ijbiomac.2018.03.148

Niu Y, Wu J, Wang W, Chen Q (2019) Production and characterization of a new glycolipid , mannosylerythritol lipid, from waste cooking oil biotransformation by *Pseudozyma aphidis* ZJUDM34. Food Sci Nutr 7(3):937–948. https://doi.org/10.1002/fsn3.880

Nogueira GF, Soares CT, Cavasini R, Fakhouri FM, de Oliveira RA (2019) Bioactive films of arrowroot starch and blackberry pulp: physical , mechanical and barrier properties and stability to pH and sterilization. Food Chem 275:417–425. https://doi.org/10.1016/j.foodchem.2018.09.054

Nouraddini M, Esmaiili M, Mohtarami F (2018) Development and characterization of edible films based on eggplant flour and corn starch. Int J Biol Macromol 120(Part B):1639–1645. https://doi.org/10.1016/j.ijbiomac.2018.09.126

Oleyaei SA, Zahedi Y, Ghanbarzadeh B, Moayedi AA (2016) Modification of physicochemical and thermal properties of starch films by incorporation of TiO_2 nanoparticles. Int J Biol Macromol 89:256–264. https://doi.org/10.1016/j.ijbiomac.2016.04.078

Oliveira T, Makishi GL, Chambi HN, Bittante AMQ, Lourenço R, Sobral PJ (2015) Cellulose fiber reinforced biodegradable films based on proteins extracted from castor bean (*Ricinus communis* L.) cake. Ind Crop Prod J 67:355–363. https://doi.org/10.1016/j.indcrop.2015.01.036

Otles S, Kartal C (2018) Food waste valorization. In: Galanakis CM (ed) Sustainable food systems from agriculture to industry, 1st edn. Academic Press, Cambridge, MA. https://doi.org/10.1016/b978-0-12-811935-8.00011-1

Otoni CG, Espitia PJP, Avena-bustillos RJ, Mchugh TH (2016) Trends in antimicrobial food packaging systems: emitting sachets and absorbent pads. Food Res Int 83:60–73. https://doi.org/10.1016/j.foodres.2016.02.018

Papanikolaou S, Aggelis G (2011) Lipids of oleaginous yeasts. Part I: biochemistry of single cell oil production. Eur J Lipid Sci Technol 113(8):1031–1051. https://doi.org/10.1002/ejlt.201100014

Prasad R, Kumar V, Prasad KS (2014) Nanotechnology in sustainable agriculture: present concerns and future aspects. Afr J Biotechnol 13(6):705–713

Prasad R, Bhattacharyya A, Nguyen QD (2017) Nanotechnology in sustainable agriculture: recent developments, challenges and perspectives. Front Microbiol 8:1014. https://doi.org/10.3389/fmicb.2017.01014

Quintavalla S, Vicini L (2002) Antimicrobial food packaging in meat industry. Meat Sci 62 (3):373–380. https://doi.org/10.1016/s0309-1740(02)00121-3

Radzuan MN, Banat IM, Winterburn J (2017) Production and characterization of rhamnolipid using palm oil agricultural refinery waste. Bioresour Technol 225:99–105. https://doi.org/10.1016/j.biortech.2016.11.052

Radzuan MN, Banat IM, Winterburn J (2018) Biorefining palm oil agricultural re fi nery waste for added value rhamnolipid production via fermentation. Ind Crop Prod 116:64–72. https://doi.org/10.1016/j.indcrop.2018.02.045

Ratledge C (1997) Microbial lipids. In: Rehm H-J, Reed G (eds) Biotechnology: products of secondary metabolism. Wiley, New York, pp 133–197. https://doi.org/10.1002/9783527620890.ch4

Ratledge C (2013) Microbial oils: an introductory overview of current status. Res. Artic Dossier Micro 20(6):D602. https://doi.org/10.1051/ocl/2013029

Ratledge C, Cohen Z (2008) Microbial and algal oils: do they have a future for biodiesel or as commodity oils? Lipid Technol 20(7):155–160. https://doi.org/10.1002/lite.200800044

Rooney ML (2005) Introduction to active food packaging technologies. In: Han JH (ed) Innovations in food packaging. Elsevier, Amsterdam, pp 63–79. https://doi.org/10.1016/b978-012311632-1/50037-1

Saberi B, Golding JB (2018) Postharvest application of biopolymer-based edible coatings to improve the quality of fresh horticultural produce. In: Gutiérrez TJ (ed) Polymers for food applications. Springer, Cham, pp 211–250. https://doi.org/10.1007/978-3-319-94625-2_9

Salari M, Sowti M, Rezaei R, Ghanbarzadeh B (2018) Development and evaluation of chitosan based active nanocomposite films containing bacterial cellulose nanocrystals and silver nanoparticles. Food Hydrocoll 84:414–423. https://doi.org/10.1016/j.foodhyd.2018.05.037

Sanches-Silva A, Costa HS, Losada PP, Sendón R, Sánchez-Machado DI, Valdez HS, Varona IA, López-Cervantes J (2010) News from EU research: preparation of active packaging with antioxidant and antimicrobial activity based on astaxanthin and chitosan. Nutr Bull 35 (3):268–271. https://doi.org/10.1111/j.1467-3010.2010.01834.x

Santos MV, Faria NT, Castelo F (2019) Production of mannosylerythritol lipids from lignocellulose hydrolysates : tolerance thresholds of *Moesziomyces antarcticus* to inhibitors. J Chem Technol Biotechnol 94(4):1064–1072. https://doi.org/10.1002/jctb.5853

Scartazzini L, Tosati JV, Cortez DHC, Rossi MJ, Flo SH (2019) Gelatin edible coatings with mint essential oil (*Mentha arvensis*): film characterization and antifungal properties. J Food Sci Technol 56:1–12. https://doi.org/10.1007/s13197-019-03873-9

Schumann B, Schmid M (2018) Packaging concepts for fresh and processed meat—recent progresses. Innov Food Sci Emerg Technol 47:88–100. https://doi.org/10.1016/j.ifset.2018.02.005

Siripatrawan U, Kaewklin P (2018) Fabrication and characterization of chitosan-titanium dioxide nanocomposite film as ethylene scavenging and antimicrobial active food packaging. Food Hydrocoll 84:125–134. https://doi.org/10.1016/j.foodhyd.2018.04.049

Tinoi J, Rakariyatham N (2016) Optimization of pineapple pulp residue hydrolysis for lipid production by *Rhodotorula glutinis* TISTR5159 using as biodiesel feedstock. Biosci Biotechnol Biochem 80(8):1641–1649. https://doi.org/10.1080/09168451.2016.1177444

Tosati JV, Messias VC, Carvalho PIN, Pollonio MAR, Meireles MAA, Monteiro AR (2017) Antimicrobial effect of edible coating blend based on turmeric starch residue and gelatin applied onto fresh frankfurter sausage. Food Bioproc Tech 10(12):2165–2175. https://doi.org/10.1007/s11947-017-1985-1

Upasen S, Wattanachai P (2018) Packaging to prolong shelf life of preservative-free white bread. Heliyon 4(9):e00802. https://doi.org/10.1016/j.heliyon.2018.e00802

Uranga J, Etxabide A, Guerrero P, de Caba K (2018) Development of active fish gelatin films with anthocyanins by compression molding. Food Hydrocoll 84:313–320. https://doi.org/10.1016/j.foodhyd.2018.06.018

Valencia GA, Sobral PJ d A (2018) Recent trends on nano-biocomposite polymers for food packaging. In: Gutiérrez TJ (ed) Polymers for food applications. Springer, Gewerbestrasse, pp 101–130. https://doi.org/10.1007/978-3-319-94625-2_5

Valencia GA, Luciano CG, Monteiro AR (2019) Smart and active edible coatings based on biopolymers. In: Gutiérrez TJ (ed) Polymers for Agri-food applications. Springer, Gewerbestrasse, pp 391–416. https://doi.org/10.1007/978-3-030-19416-1_20

Vasconcelos B, Teixeira JC, Dragone G, Teixeira JA (2019) Oleaginous yeasts for sustainable lipid production-from biodiesel to surf boards, a wide range of "green" applications. Appl Microbiol Technol 103(9):3651–3667. https://doi.org/10.1007/s00253-019-09742-x

Venkatramanan V, Shah S, Prasad R (eds) (2021a) Exploring synergies and trade-offs between climate change and the sustainable development goals. Springer, Singapore. https://doi.org/10.1007/978-981-15-7301-9

Venkatramanan V, Shah S, Prasad R (eds) (2021b) Sustainable bioeconomy: pathways to sustainable development goals. Springer Nature, Singapore. https://doi.org/10.1007/978-981-15-7321-7

Vieira JPF, Ienczak JL, Rossell CEV, Pradella JGC, Franco TT (2014) Microbial lipid production: screening with yeasts grown on Brazilian molasses. Biotechnol Lett 36(12):2433–2442. https://doi.org/10.1007/s10529-014-1624-0

Vilela C, Kurek M, Hayouka Z, Röcker B, Yildirim S, Dulce M, Antunes C, Nilsen-nygaard J, Kvalvåg M, Freire CSR (2018) A concise guide to active agents for active food packaging. Trends Food Sci Technol 80:212–222. https://doi.org/10.1016/j.tifs.2018.08.006

Wu L, Lai L, Lu Q, Mei P, Wang Y, Cheng L, Liu Y (2019) Comparative studies on the surface/interface properties and aggregation behavior of mono-rhamnolipid and di-rhamnolipid. Colloids Surf B Biointerfaces 181:593–601. https://doi.org/10.1016/j.colsurfb.2019.06.012

Xu T, Gao C, Yang Y, Shen X, Huang M, Liu S (2018) Retention and release properties of cinnamon essential oil in antimicrobial films based on chitosan and gum arabic. Food Hydrocoll 84:84–92. https://doi.org/10.1016/j.foodhyd.2018.06.003

Ykema A, Verbree EC, Kater MM, Smit H (1988) Optimization of lipid production in the oleaginous yeast *Apiotrichum curvatum* in wheypermeate. Appl Microbiol Biotechnol 29 (2–3):211–218. https://doi.org/10.1007/bf00939309

Yong H, Wang X, Bai R, Miao Z, Zhang X, Liu J (2019) Development of antioxidant and intelligent pH-sensing packaging films by incorporating purple-fleshed sweet potato extract into chitosan matrix. Food Hydrocoll 90:216–224. https://doi.org/10.1016/j.foodhyd.2018.12.015

Zhi Y, Wu Q, Xu Y (2017) Production of surfactin from waste distillers' grains by co-culture fermentation of two *Bacillus amyloliquefaciens* strains. Bioresour Technol 235:96–103. https://doi.org/10.1016/j.biortech.2017.03.090

Turning Wastes into Resources: Exploiting Microbial Potential for the Conversion of Food Wastes into Polyhydroxyalkanoates

6

Iolanda Corrado, Marco Vastano, Nicoletta Cascelli, Giovanni Sannia, and Cinzia Pezzella

Abstract

Polyhydroxyalkanoates (PHA) are microbial polyesters produced by a wide range of microorganisms as storage materials. Besides displaying material properties similar to those of petroleum-based plastics, their intrinsic biodegradability makes them "green" candidates for solving plastic pollution issues. The PHA diversity, determined by monomer size as well as by polymer structure, translates into a wide range of material properties finding applications in different sectors. This tunability is due to the complex metabolic network that drives PHA biosynthesis in vivo, which makes every microorganism unique in its producing abilities. Despite such potentialities, the production of PHAs at large scale is hindered by the high cost of carbon substrate necessary to feed PHA producing microbes. In this regard, the use of food wastes as starting feedstock for microbial fermentation would represent a cost-effective way to boost PHA exploitation. This chapter examines the state of the art of food wastes conversion into PHAs, focusing on the strategies applied to develop microbial strains for producing PHAs with tailored properties and high yield. Examples of PHA production based on natural or engineered strains will be examined, and prospects and challenges for the effective exploitation of the processes will be presented.

Keywords

Polyhydroxyalkanoates (PHA) · Biorefinery · Strain engineering · Waste valorization

I. Corrado · M. Vastano · N. Cascelli · G. Sannia
Department of Chemical Sciences, University Federico II, Naples, Italy

C. Pezzella (✉)
Department of Agricultural Sciences, University Federico II, Naples, Italy
e-mail: cpezzella@unina.it

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_6

6.1 Introduction

The exploitation of fossil resources to satisfy the current demand for plastic materials is a serious threat for the environment, with consequences in terms of global warming, human health risks, and ecosystem toxicity (Harding et al. 2007). Since their introduction in human everyday life, petro-plastics, due to their intrinsic resistance to microbial degradation, have accumulated in the environment, generating a real pollution emergency. European policies in relation to waste management, emission reduction, and sustainable development strongly encourage the search for new green solutions to the plastic issue (Directive 2008/98/EC on waste).

Polyhydroxyalkanoates (PHAs) are biodegradable and naturally synthesized polyesters, accumulated by various microorganisms as carbon, energy and redox storage material, in response to stressful/unbalanced growth conditions. In the last decade, PHAs have been emerging as "green" candidates for solving plastic pollution issues due to their spectrum of properties, very close to those of petroleum-based plastics.

Since the first identification of PHA accumulation in *Bacillus megaterium* by Lemoigne in 1926, over 90 PHA producing species and about 150 (R)-hydroxyalkanoic acids, as monomer constituents of natural PHAs, have been discovered (Ojumu et al. 2004). According to monomer chain length, PHAs have been classified in two main categories: short chain length (scl)-PHA (C4 and C5) and medium chain length (mcl)-PHA ($C \geq 6$). Polyhydroxybutyrate (PHB), a scl-PHA, is by far the most well-studied PHA polymer, accumulated to up to 80% of cell dry weight by native as well as recombinant microorganisms (Aldor and Keasling 2003). Due to its thermoplastic properties, similar to those of polypropylene, PHB has found application in food packaging as well as in agricultural purposes (Reddy et al. 2003). On the other hand, mcl-PHAs characterized by high elasticity and low crystallinity emerged as suitable materials for novel applications in cosmetics, paint formulations, medical devices, and tissue engineering (Vastano et al. 2017).

Three main pathways regulate PHA biosynthesis in vivo. The biosynthetic routes to PHA monomers, which are strictly interconnected with the central metabolic pathways, compete with and/or rely on tricarboxylic acid (TCA) cycle, fatty acid biosynthesis and degradation, and are based on central metabolites and cofactors, i.e. acetyl-CoA and NADPH (Fig. 6.1).

The complex metabolic network that drives PHA biosynthesis in vivo makes every microorganism unique in its producing abilities. PHA composition and properties are strictly influenced by the supplied carbon source, the activated pathway, the properties of the enzymes involved, as well as by growth and operating conditions. The term "PHAome" was coined to describe the diverse and dynamic modifications the PHA spectrum undergoes within the cell (Chen and Hajnal 2015). Beside monomer composition, these modifications also reflect in the diversity of polymer structure (homopolymers, random or block-copolymers) and molecular weights, resulting from controlling operative culture conditions and/or external substrate feeding (Aldor and Keasling 2003; Chen and Hajnal 2015).

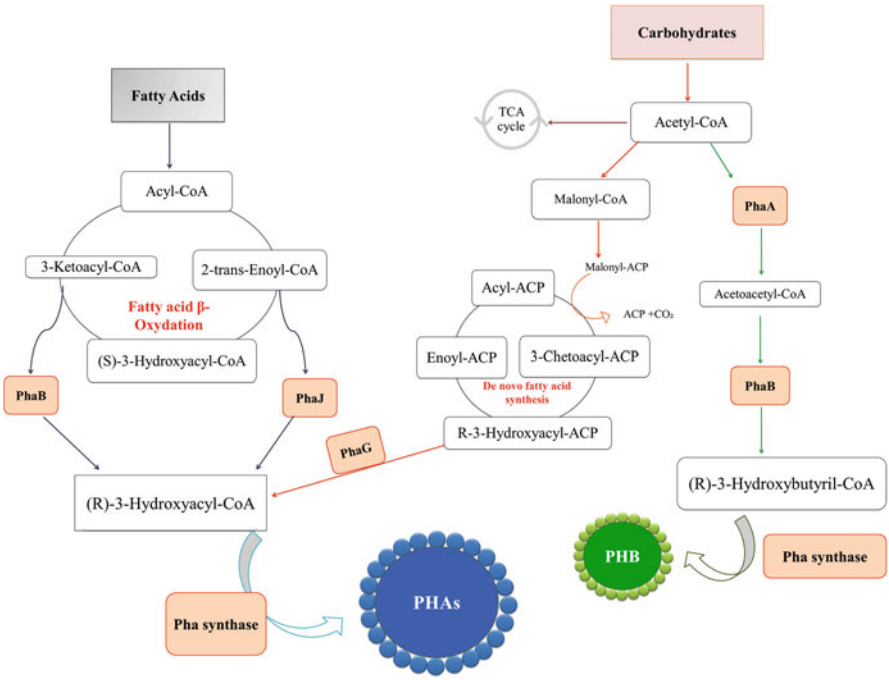
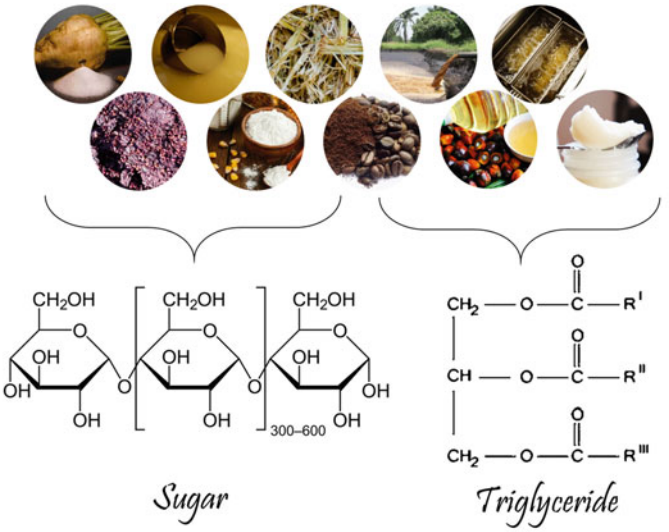


Fig. 6.1 Schematic representation of the main metabolic pathways involved in PHA synthesis. (Note: *PhaA* β-ketothiolase, *PhaB* ketoacyl-CoA reductase, *PhaG* transacylase, *PhaJ* enoyl-CoA hydratase, *TCA cycle* tricarboxylic acid cycle)

This scenario left room to metabolic engineering to channel precursors into preferred routes in order to control PHA composition (Chen and Jiang 2017; Lee et al. 2019). At the same time, since the incorporation of specific precursors is linked to the substrate specificity of the PHA synthetic enzymes, protein engineering was applied to PHA biosynthetic enzymes, especially to the PHA synthase (PhaC), to modulate the abundance of a specific monomer into the synthesized polymers (Lee et al. 2019).

PHA heterogeneity gives rise to a wide range of materials, from thermoplastic polymers, to elastomers or even sticky resins. This feature, coupled to the intrinsic biodegradability and the renewable origin, confers an enormous potential to PHAs exploitation. It has been estimated that PHA production can save on average 2 kg CO_2 emitted (about 30 MJ of fossil resources) for 1 kg PHA produced, compared to fossil-derived plastics (Essel and Carus 2012). However, PHA production reached industrial scale only in few examples (Pakalapati et al. 2018), with production capacities from 1000 tonnes/year (Biomer, Germany; Kaneka Corporation, Japan) to 10,000 tonnes/year (Tianjin GreenBio Materials, PR China). The main obstacle to effective PHA application is the cost associated with the substrates used to feed the microbial process that was estimated to account for more than 50% of the production



Food Wastes	Main examples	Composition
Fruits and vegetables	Pulp fruit, vinasse,	Sugars, carbohydrates
Waste oils	Palm oil, rapeseed oil, waste frying oil	High content of lipis, free fatty acids and triacyilgrylcerol
Animal by products	Animal Fats	
Organic crops	Lignocellulosic wastes, wheat bran	sugars, lipids, carbohydrates, and mineral acids

Fig. 6.2 Main examples of food wastes and their principal components

costs (Koller et al. 2017). This makes the whole process not cost-efficient when compared with petroleum-based plastics and has encouraged the use of inexpensive waste biomasses to reduce the economic impact of the raw material (Sabapathy et al. 2017). In addition, the use of edible carbohydrates like glucose or lipids of nutritional value would provoke the "plate-versus-plastics" controversy, adding ethics concerns about bioplastic exploitation.

To be sustainable, PHA production processes should encompass aspects of economic, environmental protection, optimized engineering, and ethics (Koller 2019). A possible solution is to directly link the food production sector with biopolymer production, by using carbon-rich waste streams of food and feed production as raw materials for biopolymer manufacturing (Nielsen et al. 2017). The valorization of these wastes through a microbial process would also represent a solution to their disposal. About 10^{12} kg of food is discarded per year (Kwan et al. 2018), and many carbon-rich food waste streams are produced from different productive sectors: from the cheese industry, to food companies producing waste

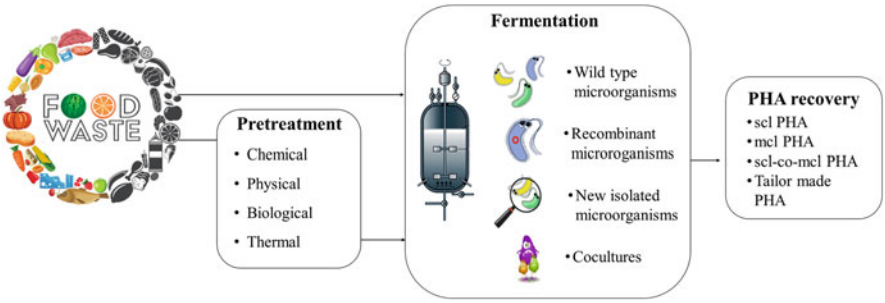


Fig. 6.3 Main steps of PHA production from food wastes

frying oils; from waste animal fats from slaughterhouse to lignocellulosic wastes from different agro-sectors (Fig. 6.2).

Several studies explored the use of these waste materials as substrate for microbial PHA production. A survey of the scientific literature (Scopus search) published over the past 35 years reveals an increasing trend in the number of articles containing the keyword "PHA", with a positive correlation with those resulting from the search for "PHA and waste". In the published papers, different aspects have been addressed: the effect of waste composition on microbial metabolism and, consequently, the choice of proper PHA producer for the selected waste; the presence of readily metabolizable C-sources or the need for a pretreatment step to hydrolyse complex substrates into small convertible compounds; the necessity to detoxify the hydrolysates to remove inhibiting compounds.

This chapter covers the research carried out in the last decade on the exploitation of food waste materials for PHA production, focusing on the different strategies applied to optimize and customize biopolymer production processes (Fig. 6.3).

6.2 Exploring PHA Production from Pure Cultures and New Microbial Isolates

Bacterial strains able to produce PHAs are generally classified into two groups: (1) microorganisms that accumulate PHAs under limitation of some nutrients like oxygen, nitrogen or phosphorous in presence of C-sources excess; (2) microorganisms which do not require nutrient limitation for PHA biosynthesis, because polymer production is associated with growth (Kourmentza et al. 2017).

Microbial PHA production from food wastes was thoroughly investigated in order to reduce the production costs. In this section, the state of the art of food waste conversion into PHAs is discussed, focusing on the most abundant wastes worldwide and on the most relevant microbial processes in terms of PHA production yield, costs and innovation (Tables 6.1 and 6.2).

Table 6.1 Examples of PHA production from lipid-rich waste by pure cultures

Waste source	Microorganism	Fermentation strategy	% PHA (w/w)	Monomeric composition	References
Waste frying oil	*Pseudomonas fluorescens* S48	Batch flask	12	PHB	Gamal et al. (2011)
Waste frying oil	*Pseudomonas fluorescens* S48	One-stage bioreactor	30	PHB	Gamal et al. (2012)
		Two-stage bioreactor	47		
		Fed-batch bioreactor	50		
Waste frying oil	*Pseudomonas fluorescens* S48	High cell density fed-batch culture in bioreactor	55	PHB	Gamal et al. (2013)
Waste frying oil	*Pseudomonas resinovorans*	Batch flask	28	mcl-PHA	Cruz et al. (2016)
Olive oil distillate	*Pseudomonas resinovorans*	Batch flask	31	mcl-PHA	
Olive oil distillate	*Cupriavidus necator*	Batch flask	62	PHB	
Two-phase olive mill waste	*Azotobacter chroococcum* H23 *Azotobacter vinelandii* UWD	Batch flask	44 33	PHB	Cerrone et al. (2010)
Two-phase olive mill waste	*Haloferax mediterranei*	Batch flask	43	P(3HB-co-3HV)	Alsafadi and Al-Mashaqbeh (2017)
Waste palm oil	*Pseudomonas* sp. Gl01	Batch bioreactor	43	mcl-PHA	Możejko and Ciesielski (2013)
Waste rapeseed oil	*Pseudomonas* sp. Gl01	Batch bioreactor	20	mcl-PHA	Mozejko et al. (2011)
Waste rapeseed oil	*Pseudomonas* sp. Gl01	Fed-batch bioreactor	44	mcl-PHA	Możejko and Ciesielski (2014)
Waste animal fats	*Ralstonia eutropha*	Batch bioreactor	70	PHB	Riedel et al. (2015)

Note: *3HB* 3-hydroxybutyrate, *3HV* 3-hydroxyvalerate

Table 6.2 Examples of PHA production from food and agro-industrial wastes by pure microbial cultures

Waste source	Microorganism	Pretreatment	Fermentation strategy	Monomeric composition	%PHA (w/w)	References
Spent coffee grounds	*Cupriavidus necator* H16	Solvent oil extraction	Fed-batch bioreactor	PHB	89	Obruca et al. (2014a)
	Burkholderia cepacia	Solvent oil extraction and detoxification	Batch flask	P(3HB-co-3HV)	55	Obruca et al. (2014a)
	Cupriavidus necator DSM 428	$scCO_2$ oil extraction	Fed-batch bioreactor	PHB	78	Cruz et al. (2014)
Sugarcane molasses	*Bacillus megaterium* BA-019	–	Fed-batch bioreactor	PHB	42	Kulpreecha et al. (2009)
Molasses	*Pseudomonas* sp.	–	Batch flask	PHB	21	Chaudhry et al. (2011)
Desugarized sugar molasses	*Bacillus megaterium uyuni* S29	–	Batch bioreactor	PHB	55	Schmid et al. (2019)
Vinasse	*Haloferax mediterranei*	Adsorption of phenolic compounds with activated carbon	Batch flask	P(3HB-co-3HV)	70	Bhattacharyya et al. (2014)
	Haloarcula marismortui			PHB	30	Pramanik et al. (2012)
Cassava starch wastewater	*Cupriavidus* sp. KKU38	Enzymatic saccharification	Batch flask	PHB	62	Poomipuk et al. (2014)
Starch	*Haloferax mediterranei*	Enzymatic saccharification	Fed-batch bioreactor	P(3HB-co-3HV)	51	Chen et al. (2006)
	Bacillus cereus CFR06	–	Batch flask	PHB	40	Halami (2008)
	Bacillus thuringiensis	–	Batch flask	PHB	73	Gowda and Shivakumar (2014)
	Azotobacter chroococcum	–	Batch flask	PHB	46	Kim (2000)

(continued)

Table 6.2 (continued)

Waste source	Microorganism	Pretreatment	Fermentation strategy	Monomeric composition	%PHA (w/w)	References
	Bacillus megaterium PHB29	–	Batch flask	PHB	73	Aneesh et al. (2016)
	Halogeometricum borinquense E3	–	Batch flask	P(3HB-co-3HV)	45	Salgaonkar et al. (2019)
White grapes pomace	*Pseudomonas resinovorans*	Enzymatic saccharification	Two-stage batch bioreactor	mcl-PHA	23	Follonier et al. (2014)
Apple pomace	*Pseudomonas putida* KT2440	–	Batch flask	mcl-PHA	25	Urbina et al. (2018)
Apple pulp	*Pseudomonas citronellolis* NRLL B2504	–	Batch bioreactor	mcl-PHA	30	Rebocho et al. (2019)
Pineapple peel	*Ralstonia eutropha* ATCC 17697	Acidic hydrolysis	Batch flask	P(3HB-co-3HV)	45	Vega-Castro et al. (2016)
Orange and passion fruit wastes	*Cupriavidus necator*	Polygalacturonases (pectinases)	Batch flask	N.D.	N.D.	Locatelli et al. (2019)
Lignocellulosic material	*Pandoraea* sp. B-6	–	Batch flask	PHB	25	Liu et al. (2019)
Wheat straw (lignin)	*Burkholderia sacchari* DSM 17165	AFEX-pre-treatment and enzymatic saccharification	Fed-batch bioreactor	PHB	72	Cesário et al. (2014)
	Ralstonia eutropha NCIMB 11599	Enzymatic saccharification	Batch flask	PHB	62	Annamalai and Sivakumar (2016)
Bagasse (lignin)	*Ralstonia eutropha*	Acidic hydrolysis	Batch flask	PHB	60	Yu and Stahl (2008)
Industrial oil products	*Pseudomonas aeruginosa* 42A2	–	Batch flask	ND	25	Rodríguez-Carmona et al. (2012)

Dairy waste, rice bran and brackish water	*Bacillus megaterium* sp.	–	Two step fed-batch bioreactor	PHB	ND	RamKumar Pandian et al. (2010)
Soybean effluent	*Halomonas* sp. SF 2003	–	Batch bioreactor	P(3HB-co-3HV)	23	Lemechko et al. (2019)
Rice mill effluent	*Acinetobacter junii* BP25	–	Batch flask	PHB	92	Sabapathy et al. (2019)
Rice-based ethanol stillage	*Haloferax mediterranei*		Batch flask	P(3HB-co-3HV)	71	Bhattacharyya et al. (2014)
MSW (municipal solid wastes)	*Rhodospirillum rubrum*	MIP	Batch flask	PHB	16	Revelles et al. (2017)
Whey	*Pseudomonas hydrogenovora*	–	Fed-batch bioreactor	P(3HB-co-3HV)	12	Koller et al. (2008)
	Bacillus megaterium	–	Batch flask	PHB	37	Obruca et al. (2011)
	Caulobacter segnis DSM 29236	–	Batch bioreactor	PHB	37	Bustamante et al. (2019)
	Haloferax mediterranei	–	Batch bioreactor	PHB	50	Koller et al. (2007)
Digestate liquor	*Cupriavidus necator*	–	Fed-batch bioreactor	PHB	90	Passanha et al. (2013)
Fish solid extract	*Bacillus subtilis* KP17 2548	–	Batch flask	PHB	70	Mohapatra et al. (2017)

Note: *PHB-HV* poly 3-hydroxybutyrate-co-3-hydroxyvalerate, *AFEX* ammonia fibre expansion, *MIP* microwave induced pyrolysis, *SsCO*$_2$ supercritical fluid extraction

6.2.1 Fatty Wastes

Vegetable oils were studied as possible candidates for PHA production. Due to their composition in medium and long chain fatty acids, oil-containing substrates can act as precursor for PHA with medium/long-monomer length (Table 6.1).

Waste frying oils (WFO) from food industry are mainly composed of triglycerides, containing long fatty acids (FFAs) with saturated and/or unsaturated bonds. Despite their common reuse in biodiesel industry, WFO were also tested as substrates for PHA production. Compared to pure vegetable oil, WFO provide components that improve growth and PHA accumulation such as food residues, readily available nitrogen compounds, peroxides, and short chain compound formed during heating (Verlinden et al. 2011). An example of WFO exploitation as carbon source at industrial scale was developed using *P. fluorescens* S48. Different strategies were investigated. With a continuous WFO feeding at 0.55 mL/L/h, the authors obtained a PHA content of 55.34% (w/w) and 0.64 g/L cell dry weight (cdw) after 54 h (Gamal et al. 2013). When operated in fed batch, with WFO supplying, a higher cell dry weight (3.46 g/L) and 49.71% polymer content (w/w) were achieved in a shorter time (48 h) (Gamal et al. 2012). In both cases, the feeding of carbon source improved its solubility and availability for bacteria.

Olive oil distillate (OOD) is a by-product from olive refining industry representing 0.05–0.1% of total processed oil. It is mainly composed of free fatty acids (>50 wt.%) with a lower concentration in triglycerides, diglycerides, and monoglycerides than WFO (less than 10 wt.%). As for WFO, the concentration of saturated fatty acids is lower than that of unsaturated FFA (Cruz et al. 2016). Using OOD as substrate for PHA production, up to 62% of PHB (w/w) was obtained using *C. necator*, and 31% (w/w) with *P. resinovorans* (Cruz et al. 2016). In the latter case, the polymer was mainly composed of 3-hydroxyoctaonate (3HO) and 3-hydroxydecanoate (3HD) with smaller amount of 3hydroxyhexanoate (3HHx).

An interesting bioprocess, combining anaerobic and aerobic steps, was reported by Cerrone and co-authors using the Two-Phase Olive Mill Waste (TPOMW), a semisolid waste generated in the olive mill industry by the two-phase extraction system (Cerrone et al. 2010). This waste is characterized by a high concentration of organic matter and elevated hydro-soluble carbohydrate content (Dionisi et al. 2005). The first biological anaerobic treatment transformed TPOMW, during the hydrolytic and acidogenic phases, into propionic, butyric or valeric acids; these were then used as precursors for the synthesis of 3-hydroxybutyric (3HB) and 3-hydroxyvaleric (3HV) in the subsequent aerobic step. Nevertheless, despite the capability of *A. chroococcum* H23 and *A. vinelandii* UWD to grow on this pretreated waste, they produced only homopolymers of PHB (*A. chroococcum* 44% (w/w), *A. vinelandii* 33% (w/w)); according to the authors this is due to the high concentration of carbohydrates (10 g/L) with respect to that of volatile fatty acids (30 mg/L) within the medium.

Alsafadi and Al-Mashaqbeh (2017) proposed a one-stage cultivation step on WFO, by exploiting extremophilic organisms, which exhibits tolerance to a range of environmental stressors. As a fact, *H. mediterranei* is able to incorporate

3HV units in the synthesized polymer, using Olive Mill Waste (OMW) as carbon source, without the need of a fermentation step or any additional feeding with costly 3HV-related precursors. *H. mediterranei* cultivation conditions were optimized in medium containing 15% OMW by investigating several parameters affecting PHA production. High salt concentration inhibits PHA biosynthetic pathway activating an osmotic balance response (Vega-Castro et al. 2016). The highest PHA content (43% (w/w)) was achieved at 37 °C, 170 rpm and 22% salt concentration.

Song, Haba and co-workers reported the synthesis of mcl-PHA by *Pseudomonas* species using waste palm oil (WPO) as carbon source (Song et al. 2008; Haba et al. 2007). *Pseudomonas* sp. Gl01 strain was not able to grow on WPO in its original form, and a preliminary saponification step to break down the triglycerides into free fatty acids was required. After this pretreatment, up to 43% of mcl-PHA (w/w) after 17 h was produced (Możejko and Ciesielski 2013). Interestingly, nitrogen limitation is unnecessary for stimulating biopolymer synthesis. *Pseudomonas* sp. Gl01 was also reported to produce mcl-PHA from waste rapeseed oil as carbon source (Mozejko et al. 2011). In this case, oxygen concentration plays a crucial role, with lower oxygen supply being responsible for higher PHAs accumulation. In addition, the authors proposed a pulsed feeding strategy as more favourable approach for mcl-PHA production, reaching 44% (w/w) at 41 h of fermentation (Możejko and Ciesielski 2014).

In addition to vegetable oils, waste animal lipids from the food processing and slaughtering industries have a huge potential as carbon feedstock for PHA production. There are many examples of PHA production from tallow; however, its low availability to microorganisms renders the addition of an emulsifying agent mandatory. Riedel et al. (2015) achieved a total production of 70% of PHB (w/w) by *R. eutropha* using a pre-emulsified tallow (mechanical mixing of tallow with arabic gum before inoculation). To increase the yield, they investigated the possibility to feed liquefied (by heat treatment) tallow during the fermentation in a fed-batch process. However, in this condition a lower polymer accumulation (63% (w/w)) with respect to the batch process was achieved (Riedel et al. 2015).

6.2.2 Spent Coffee Grounds (SCG)

SCG is a solid residue derived from coffee processing and consumption. Coffee is one of the world's most common beverages and its consumption has grown in the last 150 years: about 6.0 million ton of SCG are estimated to be produced worldwide and disposed as solid wastes (Tokimoto et al. 2005). Apart from coffee oils (about 15% weight), SCG contains carbohydrates, especially hemicelluloses (37% weight) and cellulose (9% weight), proteins (13% weight), and lignin (29% weight). Many studies showed SCG potentiality for PHA production as a low-cost oil-containing waste. When compared to other waste oils (rapeseed, palm, sunflower), oils extracted from SCG (by using *n*-hexane) assured the highest PHB accumulation and biomass recovery (70.3% PHA (w/w) and 14.2 g/L cell dry weight, cdw), in shake-flasks experiments using *C. necator* H16 (Obruca et al. 2014b). The authors

attributed the superior properties of coffee oil to its high content of free fatty acids, easily utilized by the bacterial culture. When scaled up in fed-batch mode, a productivity of 0.82 g PHA per g of oil was achieved. Furthermore, the addition of pure plant oils or recycled ones was also found effective to solve the difficulty related to the natural foaming effect of SCG, because they act as antifoaming agent. Since oil extraction reduces the calorific value of SCG by only 9%, the residual SCG can be used as fuel to partially cover heat and energy demands of the fermentation process, thus improving the economic feasibility of the PHA producing process.

Valorization of residual SCG after oil extraction was also pursued by Obruca et al. (2014b). A detoxification step, aimed at extracting SCG polyphenols, was applied before SCG hydrolysis to improve the fermentability of the hydrolysate (SCGH). *B. cepacia* was able to utilize the SCGH and produced a PHB-co-HV copolymer up to 51.6% (w/w). Levulinic acid present in the SCGH acted as precursor for 3HV monomer. The introduction of a polyphenol extraction step before the acidic hydrolysis enhanced PHA yields of about 25% (w/w) and allowed also to recover these important side products with potential high market value.

In order to avoid the use of hazardous organic solvents like *n*-hexane, Cruz et al. (2014) extracted SCG oil by supercritical fluid extraction with CO_2 obtaining a yield higher than 90% (w/w). The oils were directly fed to *C. necator* DSM 428 in fed-batch mode. The culture reached a 16.7 g/L cdw, with a PHB content of 78.4% (w/w) (13.1 g/L). In contrast, batch mode operation produced lower amounts of PHB (55% (w/w), 6 g/L) (Cruz et al. 2014).

6.2.3 Sugar Industry Waste

Several processes have been studied using molasses for PHA production. Sugarcane molasses is a by-product of sugar industry, rich in nutrients, growth factors and minerals, sucrose and glucose residues, not suitable for food. The absence of the proper enzymes (i.e. α-galactosidases and β-furanosidases) required to metabolize the major carbohydrates components of molasses (sucrose, stachyose and raffinose) has limited the exploitation of this waste to few classes of microorganisms. *Pseudomonas* species have been considered for a long time the best microorganisms to produce PHAs by molasses, accumulating up to 20.6% of PHB (w/w) (Chaudhry et al. 2011). Recently, in a promising study by Kulpreecha et al. (2009), sugarcane molasses was used as C-source by *B. megaterium* BA-019 achieving a cdw of 72.7 g/L and a PHB content of 42% (w/w) (Kulpreecha et al. 2009). Schmid et al. (2019) on the other hand, recently investigated the potential of the halophilic bacterium *B. megaterium uyuni* S29 in accumulating PHB by desugarized sugar beet molasses, a saline by-product of fractionation of beet molasses obtained from the separation of the sugar, betaine, and the refined fraction, which has a lower economic value than regular sugar beet molasses and is currently used as fertilizer and as nutrient additive for animal feed (Schmid et al. 2019). Fermentation of sugarcane molasses and other linked by-products led to a prevalent synthesis of the homopolymer PHB (Table 6.2).

Another significant sugar industry waste is vinasse, an acidic compost with a pH of 3.5–5.0, rich of organic and water-soluble components. It is the major by-product of ethanol production by molasses, left after alcohol distillation from the fermentation broth. Recent research on using it as a C-source for PHA production focused on extremely halophilic species like *H. marismortui* and *H. mediterranei*, notable for their ability to produce P(3HB-co-3HV) without the addition of organic acids (Pramanik et al. 2012; Bhattacharyya et al. 2014). Halophilic organisms have also the advantage of reducing the risk of microbial contamination even in a not rigidly sterile environment, due to the high salinity of the fermentation broth. The inhibitory effects of polyphenols into vinasse and the accumulation of salts after fermentations are two of the possible side effects in using them. While the second issue is affordable using two-stage desalination of spent medium to reuse salts, pretreatments like adsorption on activated carbon, at certain pH ranges, can reduce phenols allowing the usage of vinasse as C-source above the concentration of 10% wt. After this pretreatment, *H. mediterranei* was able to produce up to 19.7 g/L of P(3HB-co-3HV) (70% of polymer (w/w)), from a vinasse concentrations of up to 50% weight, while *H. marismortui* accumulated 4.5 g/L PHB, corresponding to 30% (w/w) of production, starting from 100% of vinasse.

6.2.4 Starch

Starch is a polysaccharide found in many wastes, like food (cereals, fruits, tubers, legume) and agricultural ones (roots). Worldwide, scientists tried to use this renewable carbon source for the production of different value-added products, i.e. bioethanol and maltose syrup (Keshavarz and Roy 2010; Lareo et al. 2013). There are only few reports about the use of starch directly by bacteria: most of microorganisms do not have the metabolic apparatus for its metabolization, thus a preliminary hydrolysis (chemical or enzymatic) step is required. Poomipuk et al. (2014) isolated a new *Cupriavidus* sp. KKU38 from cassava starch wastewater and tested it for PHAs production using hydrolysed cassava starch as C-source, under selected conditions and nitrogen starvation (Poomipuk et al. 2014). A PHB content of 5.9 g/L corresponding to 61.6% (w/w) was reported. Interestingly, *H. mediterranei* (Chen et al. 2006) produced P(3HB-co-3HV) copolymer from salt medium supplemented with previously hydrolysed starch.

In order to skip substrates pretreatment, and consequently reduce the overall process cost, more attention has been paid to microorganisms with the ability to metabolize starch and simultaneously produce PHA. Halami (2008) found that the isolated *B. cereus* CFR06 secretes the enzyme amylase for substrate hydrolysis and produces 0.48 g/L of PHB (48.0% (w/w) and 1.0 g/L cdw) (Halami 2008). *B. thuringiensis* is also able to produce approximately 60–72% of PHB (w/w) with a cdw of 3.6 g/L (Gowda and Shivakumar 2014). A production of 25 g/L of PHB (46% (w/w)) was reported when soluble starch was utilized as a carbon source by *A. chroococcum* in batch mode with oxygen limitation (Kim 2000). Aneesh et al. (2016) focused on PHB production by a new environmental isolate *B. megaterium*

PHB29 with 73.46% of PHB (w/w) accumulation (Aneesh et al. 2016). Salgaonkar et al. (2019) explored the interesting potential of the Archaea *H. boriquense* E3 strain to produce copolymers P(3HB-co-3HV) from cassava starch wastes with a 44.7% (w/w) of accumulation (Salgaonkar et al. 2019).

6.2.5 Pomaces

Pomaces are fruit residues obtained by the pressing of grapes and distillation of fruits like apricots and cherries. They are constituted by fruit skins, pulp and seeds, and are considered as no-value wastes, usually subjected to landfill disposal, incineration or composting. Due to their high polysaccharide content (cellulose, hemicellulose, starch and pectin), pomaces have been used for the production of enzymes, organic acids, fuels and also PHA. In their interesting work, Follonier et al. (2014) investigated the production of mcl-PHA by the native PHA producer *P. resinovorans* carrying out a two-step process (Follonier et al. 2014). Nine types of fruit pomaces including apricots, cherries and red/white grapes were used as C-source for biomass growth in the first step. All these wastes underwent a preliminary enzymatic hydrolysis to produce fermentable sugars, otherwise inaccessible to *P. resinovorans*. WFO were added in the second step of a batch fermentation to sustain PHA synthesis. All fruit pomaces hydrolysates were assayed for the presence of possible growth inhibitors like phenols and tannins. The best PHA production was achieved by White Solaris grapes reaching 6.1 g/L of cdw, 21.3 g/(L_{pomace}) of PHA and 23.3% of mcl-PHA accumulation (w/w) (Fig. 6.4).

Urbina et al. (2018) used apple pomace (cider and apple juice by-products), rich of a heterogeneous mixture of sugars like fructose, glucose, xylose and sucrose, directly as substrate for mcl-PHA production by *P. putida* KT2440 (Urbina et al. 2018). A PHA concentration of 1.1 g/L with a 25.5% PHA (w/w) and 4.3 g/L cdw was achieved employing the fed-batch strategy. Compared to the Follonier's two-step process, despite the comparable production yields, the process reported by Urbina is more economically sustainable because no saccharification step of fruit residues are needed and no fatty acids were supplemented during the fermentation process.

Untreated apple pulp wastes gained the attention of Rebocho research group who produced about 30% mcl-PHA (w/w) using *P. citronellolis* NRLL B-2504 (Rebocho et al. 2019). The authors succeeded in obtaining mcl-films by solvent casting and characterized them. The films display enhanced hydrophobicity, presenting a lower permeability to CO_2 and O_2 than silicone rubber, are less rigid and even less resistant to deformations than other natural polyesters as PHB, P(3HB-co-3HV), P(3-HB-co-3HHx) and polylactic acid (PLA), thus they turned out interesting for applications in food packaging and biomedical fields.

Among fruit pomaces attracting recent attention, pineapple peel wastes, particularly abundant in Colombia agro-industries, were tested as cheap C-source for scl-PHA (containing 3HV, prevalently) production by *R. eutropha* ATCC 17697 with a maximum of 44.8% (w/w) accumulation (Vega-Castro et al. 2016).

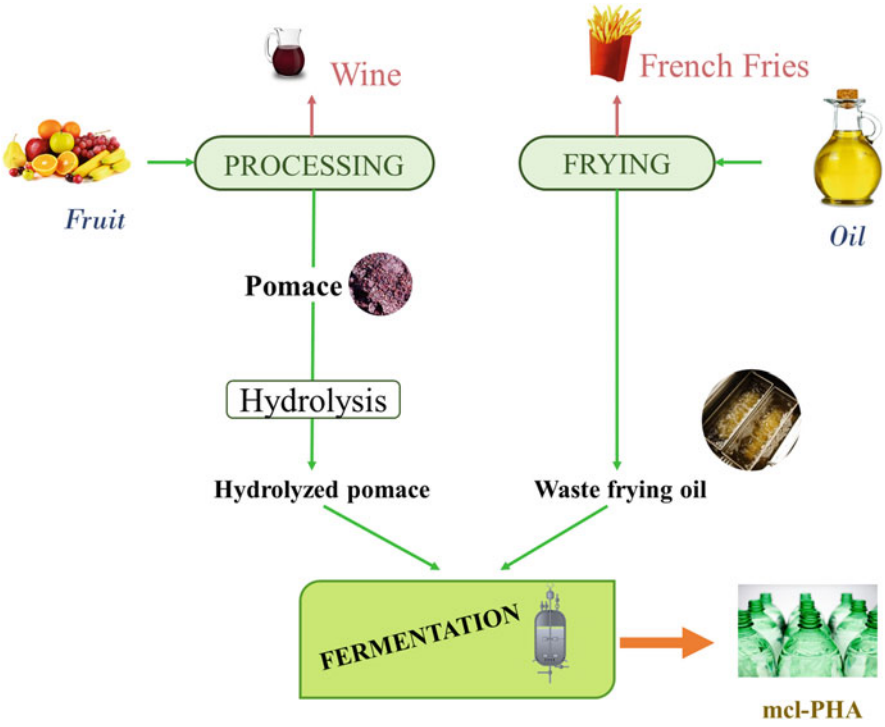


Fig. 6.4 Overview of the production process of mcl-PHA using *P. resinovorans* grown on pomaces as C-source and WFO as mcl-PHA precursor (Follonier et al. 2014)

6.2.6 Lignocellulosic Wastes

Lignocellulosic biomasses are plant residues obtained from different manufacturing processes, made of hemicelluloses, cellulose, pectin and lignin. To be used as C-sources for microbial processes, these materials need to be hydrolysed and often detoxified to remove growth-inhibiting compounds. Lignocellulosic residues from wheat straw (Cesário et al. 2014), bagasse (Yu and Stahl 2008), and wheat bran (Annamalai and Sivakumar 2016) were investigated for PHA production, testing different pretreatment methods, in which lignin depolymerization is always the limiting step. Laccases, lignin peroxidases and other extracellular oxidative enzymes, from fungi and bacteria, were applied as enzymatic pretreatment, reaching similar production yields (as shown in Table 6.2).

Liu et al. (2019) very recently reported an interesting direct bioconversion system of lignin into PHA by *Pandoraea* sp. B-6 (Liu et al. 2019) without any pretreatment. From the genome analysis of the isolated microorganisms, putative genes coding for lignin depolymerases like laccases, peroxidases and Fenton-reaction enzymes were individuated. Even if they are far from setting an ideal production process in terms of

PHA content in both cases, the authors built the bases for the lignin bioconversion into PHAs in one step, reducing the overall costs of the process and opening up new perspectives in this field.

6.2.7 Dairy Industry Wastes

The dairy industry embraces several production sectors, milk, cheese, butter, milk powder or condensate, each of them producing different kind of wastes. Dairy industry wastes are characterized by high BOD and COD content, representing polluting materials with high disposal costs. Ramkumar Pandian et al. (2010) analysed a new potential PHB producer, *B. megaterium* sp. isolated from brackish water. They optimized the production medium made of a mixture of dairy wastes, rice bran and sea water as sources using RSM methodology, thus obtaining a maximum of 11.3 g/L of PHB (RamKumar Pandian et al. 2010).

Whey is the main by-product from cheese manufacture. It is rich in fermentable nutrients such as lactose, soluble proteins vitamins and mineral salts. About 50% of whey is recycled as animal feed, the remaining part usually destined for disposal. Whey can be considered a cheap source for microbial fermentation aimed at PHA production. Generally, whey does not require any extensive pretreatment before fermentation: the only limit is the bacterial strain ability of using lactose directly for their growth. Many microorganisms are reported to produce PHB directly from whey, some of the most interesting in terms of production yields being *B. megaterium* (Obruca et al. 2011), *H. mediterranei* and *P. hydrogenovora* (Koller et al. 2008). In their recent work, Bustamante et al. (2019) found a new PHA producer by cheese way, *C. segnis* DSM 29236, using an in silico screening approach (Bustamante et al. 2019). They achieved 37% of PHB (w/w) reaching up to 9.2 g/L, the highest concentration reported to date for a wild-type microorganism capable of converting lactose from whey into PHA.

6.2.8 Other Wastes

The treatment of wastewaters, comprising liquid wastes discharged by domestic or commercial residences and agricultural activities, was also coupled to the conversion into PHAs. Lemechko et al. (2019) recently reported the production of a P(3HB-co-3HV) copolymer using soybean industry effluents with the addition of valeric acid for tuning the proportion of 3HV into the chains (Lemechko et al. 2019). In a recent study, PHA production was investigated using different microbial strains pre-isolated from sludge collected by different processes and fed with raw wastewaters mainly containing milk, soybean and fruit juice residues (Lam et al. 2017). Despite the low PHA yields achieved by both single and mixed strains cultures, due to the inhibitory effects of the high organic loading, this approach succeeded in reducing drastically COD and BOD of the wastewater. Higher yields of PHB production were, on the other hand, achieved by *A. junii* BP 25 using rice mill

effluent supplemented with some nutrients after a statistical medium optimization using PB and RSM technique (Table 6.2) (Sabapathy et al. 2019).

Waste rice-based ethanol stillage, a distillery spent wash, was proved to be a good substrate for PHA production. It does not contain antibacterial substances like phenolic residues, thus no pretreatment is required, making the process costly competitive. Bhattacharyya et al. (2014) produced and characterized the copolymer P(3HB-co-3HV) obtained by the halophilic strain *H. mediterranei* grown on this waste, obtaining a polymer accumulation of 71% (w/w) and optimizing the recovery and the reuse of the spent saline production medium, which is a possible solution for solving the major bottleneck in the industrial production of PHA by halophilic strains (Bhattacharyya et al. 2014).

Syngas obtained from pyrolysis and gasification of solid municipal wastes was efficiently introduced in chemical platforms for the production of PHA by using microorganisms able to metabolize this gas as C-source and/or energy for growth (Sahoo et al. 2021). Revelles et al. (2017) verified the PHA production from syngas by the photosynthetic microorganism *R. rubrum*, using the microwave-induced pyrolysis (MIP) as an eco-friendly and productive alternative to the standard thermal conversion of solid wastes. The principal by-product of MIP is the carbonaceous solid fraction (char) that can be used as solid fuel or amendment for carbon sequestration according to the biorefinery concept (Revelles et al. 2017).

The anaerobic digestion of solid organic wastes is a pretreatment process which degrades and stabilizes these wastes producing, at the same time, renewable energy in the form of methane and hydrogen. The digestate resulting as by-product of the process, rich of residual nutrients, is usually used as plant fertilizer. An interesting valorization of the digested liquors is their use as feedstock for PHA production. Passanha et al. (2013) set up different growth experiments of *C. necator* on different microfiltered digestates (Passanha et al. 2013). They achieved a major PHA accumulation yield than that obtained in rich media (12 g/L, 90% (w/w)) and they also demonstrated the reusability of the residual media after appropriate removal of biomass and complementation of the consumed nutrients. Another significant example of solid organic wastes revaluation is provided by Mohapatra et al. (2017). They tested for the first time the bioconversion of fish solid waste extract into PHB by using *B. subtilis* (KP172548), in one stage of batch cultivation at industrial scale (Table 6.2).

6.3 PHA Production from Mixed Microbial Cultures (MMCs)

In recent years, PHA production by MMCs gained increasing attention due to its inherent advantages. It is a cost-effective strategy, since sterilization of culture media is not required, and control and process operation are facilitated. Moreover, the presence of multiple species broadens the choice of possible feedstocks, as the consortium tends gradually to adapt itself to the carbon source provided each time. Preliminary studies, based on life cycle analysis, revealed that MMCs PHA

production may be more favourable than pure culture in economic as well as environmental terms (Gurieff and Lant 2007).

The MMCs based process for PHA production requires three essential stages: (1) selection of suitable feedstocks; (2) enrichment of MMCs with biopolymer producing microorganisms; (3) PHA production. The most significative examples of MMCs applied to PHA production are reported in Table 6.3.

Unlike most pure cultures, MMCs tend to accumulate glycogen as storing reserve instead of PHA. Therefore, PHA production from food wastes requires a previous transformation of complex organic compounds into short chain volatile fatty acids (VFA), which can be effectively stored as PHA by mixed microbial cultures (Albuquerque et al. 2010a, b). In most cases, VFA production is obtained by an anaerobic acidogenic fermentation and, according to the specific raw materials, different parameters should be taken into account to maximize VFA yields such as pH, temperature, hydraulic retention time, sludge retention time, and organic loading rate (Strazzera et al. 2018). Recently, Luo and co-workers reviewed the possible strategies for enhancing VFA production from waste activated sludge derived from wastewater treatment, including the co-digestion with different substrates and the mechanical, chemical-physical and biological pretreatments. They concluded that the VFA production performances vary considerably with sludge characteristics, thus process parameters should be optimized for each specific co-substrate (Luo et al. 2019). Lee et al. (2014) studied the influence of temperature (30, 40 and 55 °C) on acidogenic fermentation of palm oil mill effluent (POME). Mesophilic conditions (30 and 40 °C) considerably outperformed thermophilic condition (55 °C) revealing acidification degrees of 48% and 7%, respectively (Lee et al. 2014).

Besides influencing the production yield, the anaerobic fermentation conditions also affect the composition of the synthesized polymer. Gouveia et al. (2017) focused on the possibility of tailoring PHA by controlling the acidogenic reactor operating conditions, namely pH, using cheese whey as model feedstock (Gouveia et al. 2017). They operated the acidogenic reactor under dynamic pH change conditions in the range 4.5–7. The variation imposed on pH led to different monomer precursor profiles, which resulted in PHA copolymer with different compositions. Using the same feedstock, through the manipulation of the acidogenic reactor conditions, it was possible to produce PHA with composition in terms of 3HB and 3HV percentage of 70/30, 83/17 and 95/5 at pH values 6, 5 and 4.5, respectively (Gouveia et al. 2017). Similarly, Huang et al. studied the effect of pH and the concentrations of β-cyclodextrin and glycerol on the anaerobic digestion of wasted activated sludge in order to increase the abundance of odd-carbon VFA (Huang et al. 2018).

Although less explored, there are also alternative strategies to acidogenic fermentation for monomer precursor fuelling. Moita Fidalgo et al. (2014) compared the PHAs production performances of MMC from bio-oil, the liquid fraction resulting from pyrolysis processes (Moita Fidalgo et al. 2014). Two strategies for bio-oil upgrade were applied, anaerobic fermentation and vacuum distillation, and the resulting liquid streams were tested for PHA production. The first one was rich in VFA, the second mainly in phenolic and long chain fatty acids. The vacuum distilled

Table 6.3 PHA production using MMCs

Sludge	Type of fermentation	Waste material	Type of polymer	References
Anaerobic wastewater treatment plant	CSTR subjected to a dynamic pH change	Cheese whey	P (3HB-co-3HV)	Gouveia et al. (2017)
Wasted activated sludge	Batch and semi-continuous reactors	Wasted activated sludge	P (3HB-co-3HV)	Huang et al. (2018)
Activated sludge from wastewater treatment plant	Sequencing batch reactors	Bio-oil (liquid fraction from pyrolysis processes)	P (3HB-co-3HV)	Moita Fidalgo et al. (2014)
Biological nutrient removal (Bardenpho process) sludge	Sequencing batch reactors	Hydrothermal liquors	P (3HB-co-3HV)	Wijeyekoon et al. (2018)
Activated sludge from the municipal wastewater treatment plant	Sequencing batch reactors with pulse and batch feed	Brewery wastewater	P (3HB-co-3HV)	Tamang et al. (2019)
Mixed consortia of *Pseudomonas* sp.	Sequencing batch reactors	Oil mill wastewater	P (3HB-co-3HO)	Ntaikou et al. (2014)
PHA-accumulating mixed culture acclimatized to the fermented molasses feedstock	CSTR system (acidogenic fermentation) and sequencing batch reactors	Sugar molasses	P (3HB-co-3HV)	Albuquerque et al. (2010b)
Sludge from a full-scale anaerobic digester operated in continuous mode under anaerobic conditions for a period of over 2 years	2-stage CSTR system (acidogenic fermentation and culture enrichment); batch reactor (PHA production)	Sugar molasses	P (3HB-co-3HV)	Albuquerque et al. (2010a)
Activated sludge from the "Roma Nord" (Italy) full-scale municipal treatment plant	*Sequencing batch reactors*	Olive oil mill wastewater	P (3HB-co-3HV)	Campanari et al. (2014)
Activated sludge taken from a long-term operating parent SBR	Sequencing batch reactors	Food waste fermentation leachate	P (3HB-co-3HV)	Wen et al. (2018)
Heterotrophic aerobic bacterium from a facultative anaerobic pond of POME and sludge from a waste stabilization pond	Bio-PORec®	Palm oil mill effluent	P3HB	Md Din et al. (2012)

(continued)

Table 6.3 (continued)

Sludge	Type of fermentation	Waste material	Type of polymer	References
Aerobic consortia acquired from an operating activated sludge process (ASP) treating ten MLD of composite wastewater from domestic and industrial processes	Sequencing batch reactors under aerobic and anoxic microenvironments	Food waste and effluent from acidogenic biohydrogen production process	P (3HB-co-3HV)	Venkateswar Reddy and Venkata Mohan (2012)
Phototrophic mixed cultures	CSTR system (acidogenic fermentation), phototrophic selector reactor (MMC selection), batch reactor (PHA production)	Fermented cheese whey	P (3HB-co-3HV)	Fradinho et al. (2019)
Activate sludge	CSTR system (acidogenic fermentation), sequencing batch reactors (MMC selection), fed batch (PHA production)	Cheese whey and sugarcane molasses	P (3HB-co-3HV)	Duque et al. (2014)

bio-oil was not as effective as the digested one since it favoured growth instead of PHA production (Moita Fidalgo et al. 2014).

Wijeyekoon et al. (2018) tested, for the first time, the oxidative hydrothermal liquors from wet oxidation of organic residues for PHA production. They selected two sources of organic biomass, municipal wastewater sludge and food waste to subject to sub-critical wet oxidation to convert organic material into VFA, particularly acetic acid (Wijeyekoon et al. 2018). The enriched culture produced up to 41% of a PHA copolymer (77% Polyhydroxybutyrate (PHB) and 23% Polyhydroxyvalerate (PHV)) (w/w). Similarly, Tamang et al. (2019) investigated the potentiality of acidified brewery wastewater, compared to the anaerobically treated one, as carbon source for PHA production by an enriched MMC. They reported a similar maximum of PHA production in optimized conditions for both wastewaters (45% (w/w)), in spite of the higher VFA concentration in acidified brewery wastewaters (Tamang et al. 2019). Ntaikou et al. (2014) applied a clarification step, based on aluminium sulphate to induce flocculation and precipitation of solids, to the acidified oil mill wastewater. The treated waste was then tested for PHA production from a *Pseudomonas* sp. enriched culture. Although

clarification had no direct effect on the profile of the produced VFA, it positively affected PHA production, altering the values of total suspended solids and total chemical oxygen demand (Ntaikou et al. 2014).

The most common approach for enriching a mixed culture with the PHA-storing phenotype was through an aerobic dynamic feeding (ADF), called "Feast and Famine": this process configuration originates periods of excess (Feast) and lack (Famine) of external carbon substrate, resulting in the selection of microbial populations with an enhanced capacity to store PHA.

In the MMC-based process, culture selection/enrichment and PHA production occur in different Sequencing Batch Reactors (SBR), operated independently (different optimum conditions—namely different nutrient concentrations—were shown to favour each step). Through optimization of the culture enrichment stage, Albuquerque et al. (2010b) were able to achieve 74% PHA content (w/w) in batch production stage using fermented molasses as feedstock fermentation (Albuquerque et al. 2010a, b). The same research group also investigated the possibility to operate the culture enrichment in a 2-stage continuous stirred tank reactor (CSTR) under feast and famine conditions. The effect of inlet VFA concentration and hydraulic retention times (HRT) of the first and second reactors on system's selection efficiency was tested. It was shown that the feast reactor residual substrate concentration affected the selective pressure for PHA storage (Albuquerque et al. 2010a).

Once the starting inoculum was enriched with a consortium with high PHA-storing capacity, according to the selected feedstock, process parameters have been optimized for biopolymer production. Campanari et al. (2014) studied the effect of organic loading rates (OLR) ranging from 2.40 to 8.40 gCOD/(Ld) in MMC PHA production using dephenolized OMW (Campanari et al. 2014). Wen et al. (2018) reported the effect of sodium chloride concentration on PHA production from food waste fermentation leachate under different (OLR) (1.35–8.43 gCOD/(Ld) (Wen et al. 2018). Also, the micro-environment aeration is crucial for PHA yield as reported by Md Din et al. (2012) for production of PHA from POME and Venkateswar Reddy and Venkata Mohan (2012) for production of PHA from food waste and effluent from acidogenic biohydrogen production process (Md Din et al. 2012; Venkateswar Reddy and Venkata Mohan 2012).

PHA production with MMCs enriched by ADF limits the exploitability to only aerobic organisms, while the diversity of bacterial species that can produce and accumulate PHA is much wider. Fradinho et al. (2019) developed a different enrichment strategy based on an anaerobic permanent feast. In anaerobic conditions, the organisms must activate internal mechanisms to oxidize reduced molecules produced during cell metabolism (like NADH, NADPH). One of these mechanisms is based on the accumulation of PHA that requires the reduction of monomer precursors during its synthesis (Fig. 6.1). Therefore, the permanent feast strategy allowed the selection of microorganisms able to regulate internal reducing power via PHA production. The authors tested the selected Phototrophic Mixed Culture (PMC) for PHA production from fermented cheese whey and obtained a biopolymer with a 3HV content of 12%. Interestingly, the used light intensities (20 W/L) opened up the

possibility for direct sunlight illumination for processes carried out in sunny regions (Fradinho et al. 2019).

A common problem to all MMC process from wastes or by-product is the seasonal availability of the feedstock. Duque et al. (2014) developed a system operating with two feedstocks with different annual availability (cheese whey and sugarcane molasses). The two raw materials induce different VFA profile during biological conversion. By mixing the two feedstocks in defined volume proportions, polymers with target compositions were achieved (Duque et al. 2014).

6.4 Engineered Strains for PHA Production

Genetic approaches have been widely applied to improve the performance of microorganisms potentially exploitable for PHA production process (Favaro et al. 2018). A huge of examples have been described in the last decade, focusing on different aspects: (1) optimizing PHA yield in native PHA producers, through host cell genome manipulation and/or recombinant gene expression; (2) introducing catabolic operons to allow metabolization of new C-sources in native PHA producers; (3) conferring PHA producing abilities to non-native producers endowed with advantageous metabolic/physiological features, i.e. halophilic bacteria, microorganisms naturally able to metabolize complex C-sources; (4) modulating PHA composition acting on precursors supplying pathways (Favaro et al. 2018). Engineering strategies were also adopted to design microbial factories able to convert different food wastes into PHAs (Table 6.4).

Cheese whey is a potential carbon source for PHA production, being rich in lactose, lipids and soluble proteins. However, only a limited number of wild-type microorganisms is able to metabolize lactose: among them, *Escherichia coli* has been the main object of genetic manipulation. This host is also attractive due to the absence of enzymes for PHA degradation and the easiness of polymer downstream processing (Reddy et al. 2003). *E.coli* was engineered to efficiently produce PHB from cheese whey by recombinant expression of the PHA biosynthetic operon from *Alcaligenes latus* (Lee et al. 1997; Woo Suk Ahn et al. 2000) and *C. necator* (Pais et al. 2014). In the latter example, implementation of PHB production was achieved by generating *E. coli* mutants with reduced organic acid production capacity with the aim to direct the strain's metabolism towards biopolymer synthesis. The selected mutant displayed the highest reduction in organic acid synthesis coupled to an almost threefold increase of PHB yield with respect to the original *E. coli* strain (Pais et al. 2014).

Among natural PHA producers, *C. necator* was engineered with the *E. coli lac* operon to allow lactose utilization using cheese whey as carbon feedstock. Interestingly, the *lac* genes were introduced within an intracellular depolymerase coding gene (*phaZ1*) achieving its inactivation. Disruption of *phaZ1* ensured lower PHB degradation and higher polymer yield compared to the wild-type strain (Povolo et al. 2010).

Table 6.4 Engineering strategies for waste valorization into PHA

Strain	Engineering/gene targets	Waste material	Type of polymer	References
C. necator DSM 545	*phaZ* depolymerase inactivation Insertion of *E. coli lacZ*, *lacI*, *lacO* genes for lactose utilization	Cheese whey	PHB	Povolo et al. (2010)
C. necator H16	Replacement of endogenous *phaC* with *Aeromonas caviae phaC*	Waste cooking oils	P (3HB-co-3HHx)	Kamilah et al. (2013)
C. necator H16	Improved activities of enzymes involved in oxidative stress response	Waste frying oils	P (3HB-co-3HV)	Obruca et al. (2013)
E. coli CML3-1	Recombinant expression of *C. necator phaABC* operon Reduced synthesis of organic acids	Cheese whey	PHB	Pais et al. (2014)
Delftia acidovorans DSM39	Recombinant expression of *Pseudomonas stutzeri* BT3 lipase genes *lipC* and *lipH*	Agricultural fatty by-products	P (3HB-co-4HB)	Romanelli et al. (2014)
E. coli SKB99	Recombinant expression of: *Panibacillus* sp. amylase coding gene and *R. eutropha phaABC* operon	Starch	PHB	Bhatia et al. (2015)
R. eutropha H16	Recombinant expression of: scl-mcl-PHA synthase from *Rhodococcus aetherivorans* and *P. aeruginosa phaJ*	Waste animal fats and waste frying oils	P (3HB-co-3HHx)	Riedel et al. (2015)
R. eutropha NCIMB11599	Recombinant expression of *E. coli xylA* (xylose isomerase) and *xylB* (xylulokinase)	Hydrolysate solution of sunflower stalk	PHB	Kim et al. (2016)
E. coli	Recombinant expression of: mutated *Pseudomonas* sp.61-3 *phaC1Ps*(ST/QK) (lactic acid polymerizing enzyme); *Megasphaera elsdenii* propionyl-CoA-transferase; *R. eutropha phaA* and *phaB*	Lignocellulosic waste (hydrolysate from woody extract)	P(LA-co-3HB)	Takisawa et al. (2017)
Burkholderia sacchari	Recombinant expression of endogenous *xylA* (xylose isomerase) and *xylB* (xylulokinase)	Xylose	PHB	Guamán et al. (2018)
R. eutropha Re2133/pCB81	Deletion of endogenous *phaB* Replacement of endogenous *phaC* with *Rhodococcus aetherivorans phaC2*	Food waste ferment	P (3HB-co-3HHx)	Bhatia et al. (2019)
Pseudomonas putida KT2440	Deletion of *tctA* (tricarboxylate transporter coding gene)	Waste vegetable oil	mcl-PHA	Borrero-de Acuña et al. (2019)

Similar approaches were applied to design microbial strains able to utilize carbon sources potentially derivable from food wastes (Bhatia et al. 2015; Takisawa et al. 2017; Kim et al. 2016; Guamán et al. 2018). Starch, for example, is a renewable carbon source, available in large quantities in waste bread or mixed domestic wastes (Tsang et al. 2019). An *E. coli* strain expressing the PHB synthetic genes from *R. eutropha* was transformed with a functional amylase coding gene from *Panibacillus* sp. to achieve PHB accumulation utilizing starch as the sole carbon source (Bhatia et al. 2015). Lignocellulosic wastes are the most abundant resources on earth, representing a promising substrate for PHA producing platforms. Xylose is the major components of the hydrolysed lignocellulose, thus engineering approaches were applied to transfer the *E. coli* xylose catabolic genes *xylAB* (coding for xylose isomerase and xylulokinase) to a native PHA producer, such as *R. eutropha* (Kim et al. 2016). The recombinant strain accumulated PHB from both xylose and glucose/xylose containing media and achieved high biopolymer yields when tested on the hydrolysate solution of sunflower stalks, as a model lignocellulosic biomass. Similarly, overexpression of the endogenous xylose assimilation operon in *B. sacchari*, a non-model bacterium with high capacity for PHB accumulation, proved to be an effective strategy to improve both xylose utilization and PHB yield (Guamán et al. 2018).

An important target of strain engineering is the modulation of polymer composition, aimed at the incorporation of mcl monomers in the synthesized PHA. In fact, the incorporation of mcl units within scl-mcl copolymer was shown to positively affect material properties, reducing stiffness and brittleness which characterize PHB polymers. Two main approaches were pursued: (1) altering the specificity of PHA synthetic enzymes towards mcl-precursors; (2) engineering metabolic routes promoting the activation of the pathways fuelling mcl-precursors (β-oxidation and synthesis of fatty acids). These strategies, either individually or in combination, were applied to bioprocesses fed with different wastes. In particular, WFOs, a source of fatty acids, cheap and widely available, were investigated by many authors as a starting feedstock for PHA producing bioprocesses. In one of the early examples, Kamilah and co-workers engineered *C. necator* by replacing the endogenous scl-specific PhaC synthase with a PHA synthase gene of *A. caviae* endowed with different specificity. When the recombinant strain was fed with WFOs and a properly selected nitrogen source, it produced a P(3HB-co-3HHx) copolymer, while the wild type accumulated only PHB (Kamilah et al. 2013). A similar approach was applied to construct a *R. eutropha* mutant expressing a scl/mcl-PHA, synthase from *R. aetherivorans*, favouring the synthesis of P(HB-co-3HHx), together with an enoyl-CoA hydratase gene (*phaJ*) from *P. aeruginosa*. The combination of both genetic modifications allowed to boost the incorporation of HHx moieties in the copolymer synthesized using waste animal fats as inexpensive raw material (Riedel et al. 2015). On the other hand, a random chemical mutagenesis was applied to *C. necator* to select for the variants with the best producing performances from waste frying oils. The selected mutant displayed not only the ability to produce polymer with improved yields, but also an increased incorporation of 3HV in the final copolymer (Obruca et al. 2013). The authors characterized some phenotypic traits

of the mutant, coming to the hypotheses that the increased NADPH/NADP levels observed in the mutant may support polymer accumulation, the highest activity of malic enzyme may reduce the availability of oxaloacetate for the utilization of propionyl-CoA in 2-methylcitrate cycle, thus resulting in more propionyl-CoA available for incorporation into the copolymer (Obruca et al. 2013).

More recently, implementation of PHA production yield from waste vegetable oils was achieved in a natural mcl-PHA producer, *P. putida* KT2440, by knocking out the *tctA* (tricarboxylate transporter) gene, coding for the key transport enzyme of carboxylic acids. The inactivation of the transport systems for these preferred carbon sources resulted in a nearly twofold increment in the mcl-PHA volumetric productivity with respect to the wild-type strain (Borrero-de Acuña et al. 2019). In order to combine the native ability of *D. acidovorans* DSM39 to incorporate 4-hydroxybutyrate (4HB) monomer with the valorization of agricultural fatty by-products (udder, lard and tallow), this strain, unable to grow on fatty substrates, was engineered with *lip* genes from *P. stutzeri* BT3. The recombinant strain proved to be able to accumulate P3HB-P4HB copolymer directly from slaughterhouse residues without the supplementation of any precursor (Romanelli et al. 2014).

Furthermore, examples of copolymers synthesized from non-fatty wastes were reported. Taking advantage of the *E. coli* abilities to metabolize both xylose and galactose derived from woody-extract hemicellulosic hydrolysate, Takisawa et al. designed a microbial cell factory for the production of P(LA-co-3HB) copolymer from lignocellulosic feedstock (Takisawa et al. 2017). To this aim, the authors carried out a fine *E. coli* engineering, developing a recombinant strain that expressed, besides PhaA and PhaB from *R. eutropha*, a propionyl-CoA transferase (PCT) from *Megasphaera elsdenii* able to catalyse Coenzyme A addition to lactic acid, and a mutated PHA synthase from *Pseudomonas* sp. 61-3 endowed with the ability to polymerize lactoyl-CoA precursor (Yang et al. 2011). In another example, Bhatia et al. (2019) applied a tailor-made designed *R. eutropha* strain to the production of P(3HB-co-3HHx) copolymer from anaerobically digested food waste derived volatile fatty acids (Bhatia et al. 2019). The strain was engineered with a deletion of the acetoacetyl-CoA reductase (PhaB) and a replacement of the native PHA synthase with phaC2 from *R. aetherivorans*, characterized by a high specificity towards mcl-PHA precursors (Jeon et al. 2014). A response surface design study showed that in mixtures, butyrate is the main organic acid involved in PHA production, acting as precursor for 3HHx monomer (Bhatia et al. 2019), without the addition of any additional precursor for mcl-monomer units.

6.5 Biorefinery-Inspired Approaches for PHA Production

Despite the huge attempts made over the past decades to design sustainable and cost-competitive PHA-based bioprocess, large-scale production of PHA is still limited due to the cost of substrates and of recovery processes (Rodriguez-Perez et al. 2018). The use of agro-food wastes was estimated to reduce PHA production cost up to 50%, and several attempts in this direction have been discussed in this chapter. In

Table 6.5 Examples of waste valorization processes for co-production of PHA with other compounds

Strain	Co-product	Waste material	References
P. aeruginosa IFO3924	Rhamnolipid	Palm oil	Marsudi et al. (2008)
Rhodobacter sphaeroides O.U.001	Biohydrogen	Olive mill wastewater (OMW)	Eroğlu et al. (2008)
P. aeruginosa L2-1	Rhamnolipid	Cassava wastewater/ waste cooking oil	Costa et al. (2009)
P. aeruginosa 7a	Rhamnolipid	Waste cooking oil	Costa et al. (2009)
Sinorhizobium meliloti MTCC100	EPS	Rice bran hydrolysate	Saranya Devi et al. (2012)
Bacillus sp. CFR-67	α-amylase	Wheat and rice bran hydrolysates	Shamala et al. (2012)
Bacillus sp. CFR-67	α-amylase	Wheat and rice bran hydrolysates	Sreekanth et al. (2013)
Bacillus thuringiensis IAM 12077	α-amylase	Nine agriculture/food wastes	Gowda and Shivakumar (2014)
Bacillus cereus EGU43	Biohydrogen	Pea shell slurry	Patel et al. (2015)
Rhodopseudomonas palustris sp.	Biohydrogen	Olive mill wastewater (OMW)	Padovani et al. (2016)
Rhodobacter capsulatus ATCC17015	Biohydrogen	Fruit/vegetable wastes	Montiel Corona et al. (2017)
Burkholderia thailandensis	Rhamnolipid	Used cooked oil	Kourmentza et al. (2018)
Paracoccus sp. LL1	Astaxanthin	Waste cooking oil	Kumar and Kim (2019)
MMC	Biohydrogen	Dairy waste streams	Colombo et al. (2019)
Recombinant *E. coli Pseudomonas resinovorans*	Biodiesel	Waste frying oils	Vastano et al. (2019)

view of a zero-waste policy, the efficient exploitation of resources is crucial to support the sustainability of the process. In this regard, designing systems for the production of multiple products would boost process competitiveness by further lowering manufacturing costs and assuring a more efficient utilization of raw materials (Li et al. 2017). Several examples of co-production of PHA with other value-added products (amino acids, proteins, alcohols, hydrogen, biosurfactants, exopolysaccharides) have been described in the recent literature (Kumar et al. 2018). It is worth to note that PHA synthesis is strictly related to energy and carbon metabolism, thus it was shown to positively affect the production of other cellular metabolites, i.e. amino acids, or more generally, all the products connected with cellular oxidation/reduction balance (Li et al. 2017). From an economical and technical point of view, the most advantageous processes are those which couple an optimized accumulation of intracellular PHA together with the recovery of extracellular products.

Few examples of multi-product processes starting from agro-food wastes were reported (Table 6.5). The simultaneous production of PHA and biosurfactants, amphiphilic compounds able to decrease surface tension, was achieved in several bioprocess fed with waste oils. Among biosurfactants, rhamnolipids have attracted interests since they are produced in large quantities by microbial fermentation, especially in the presence of hydrophobic substrates, as a strategy to enhance bioavailability of the C-source (Kourmentza et al. 2018). In addition, their recovery as extracellular products are relatively easy, thus increasing process feasibility. Different *P. aeruginosa* strains displayed a wide range of PHA and rhamnolipids yields when grown in the presence of various raw materials (waste oils, cassava wastewater and palm oil). However, the use of these opportunistic pathogens represents a limiting factor for the process. More recently, Kourmentza et al. (2018) isolated a non-pathogenic strain of *Burkholderia thailandensis*, able to co-produce high yields of PHB and rhamnolipids using waste cooking oil as low-cost carbon source (Kourmentza et al. 2018).

Exopolysaccharides (EPS) are polymers secreted by several bacteria under stress conditions, with the aim to protect the cells and provide energy source under adverse conditions. The concurrent synthesis of both PHA and EPS polymers was observed in many microorganisms, since their accumulation is triggered by quite similar environmental stimuli (Kumar and Kim 2018). Supplementation of growth medium with rice bran hydrolysate was found to enhance the co-production of both PHA and EPS in *S. meliloti* MTCC100 (Saranya Devi et al. 2012).

In a PHA production process coupled to the synthesis of a co-product, the higher market value the co-product has, the more economically attractive will be the process. This is the case of compounds belonging to carotenoids such as adonixanthin, astaxanthin, β-carotene, etc., characterized by very high market value (about US$2000/kg). Few photosynthetic and dark-fermentative bacteria were reported to produce pigments together with PHA and/or H_2 (Kumar et al. 2018). *R. sphaeroides*, grown on oil mill wastewater (OMW) produced H_2, coupled to both PHA and carotenoids (Eroğlu et al. 2008). When fed with waste cooking oil as substrate, the halophilic strain of *Paracoccus* sp. LL1 co-generated the copolymer P(3HB-co-HV) together with high yields of astaxanthin-rich carotenoids. Additionally, these co-products were secreted in the form of vesicles, with a further advantage to the recovery process (Kumar and Kim 2019; Kumar et al. 2018).

Process sustainability is also encouraged when inexpensive waste feedstocks are used to support the co-generation of PHA with bioproducts characterized by high large-scale production costs, i.e. microbial enzymes. Besides accumulating a P (3HB-co-HV) copolymer, *Bacillus* sp. CFR-67 was found to produce high titres of α-amylase, an industrially relevant enzyme, when grown on a mixture of wheat bran and/or rice bran hydrolysates supplemented with corn starch and ammonium acetate (Shamala et al. 2012; Sreekanth et al. 2013). On the other hand, the intrinsic α-amylase production by *B. thuringiensis* IAM 12077 was explored to support the hydrolysis of nine different agriculture and food wastes (rice husk, wheat bran, ragi husk, jowar husk, jackfruit seed powder, mango peel, potato peel, bagasse and straw). The enzymatically treated substrates promoted PHB accumulation at levels

comparable to that of the acid-hydrolysed ones, thus supporting the one-step biomass pretreatment and PHB production process (Gowda and Shivakumar 2014).

Several native isolates, mainly photosynthetic and dark-fermentative bacteria, were reported to co-produce biohydrogen and PHA in response to different culture conditions (Padovani et al. 2016; Montiel Corona et al. 2015, 2017; Patel et al. 2015). A dark-fermentation effluent from fruit and vegetable wastes was tested as substrate for the simultaneous production of H_2 and PHA (Montiel Corona et al. 2017). Among the tested microorganisms, *R. capsulatus* achieved an increase in PHB production and H_2, when light-dark cycles were applied in alternative to continuous illumination, with also a benefit in terms of saved energy (Montiel Corona et al. 2017). A more advantageous process to couple PHA with H_2 production was achieved through a two-step system employing mixed microbial cultures (MMC) and two diary waste streams coming from cheese whey deproteinization. During the first step, dark fermentation of the sugar content of the wastes resulted in high daily H_2 volume, together with production of organic acids. The latter were used in the second step, as substrates for aerobic PHA production, reaching high conversion yields and PHA accumulation for both the fermented diary streams (Colombo et al. 2019).

Finally, an original example of waste frying oil conversion into two added-value products, i.e. PHA and biodiesel, was recently reported by Vastano et al. (2019). The authors used a WFO with a high content of free fatty acids (FFAs), unsuitable for direct transesterification into biodiesel, to design a process aimed at (1) reducing the FFAs content allowing its conversion into biodiesel and (2) simultaneously producing PHA. The bioprocess was verified using both recombinant (*E. coli*) and native (*P. resinovorans*) PHAs producing cell factories. Proper strain designing and process optimization allowed to address the FFAs into PHA metabolism, achieving up to 1.5 g L^{-1} of mcl-PHAs, together with an efficient conversion (80% (w/w) yield) of the treated WFO into biodiesel (Vastano et al. 2019).

6.6 Conclusion and Perspectives

The current state of the art about "food waste conversion to PHA" depicted in this chapter highlights the main strengths and weaknesses of the process. The principal hurdles to waste exploitation are related to their availability and/or to the necessity to store them properly, anticipating their seasonal shortage. Furthermore, the variability in waste composition may influence process productivity as well as polymer recovery. In this regard, monitoring the quality of the wastes and how it impacts on process performances would be useful to define the crucial process variables and to develop strategies to cope with them.

Although representing a green alternative to petroleum-derived plastics, PHA exploitation is still limited by the high costs related to its large-scale production. The use of waste materials allows to reduce the overall cost, but the production process is still not economically competitive. A winning strategy seems to be to design processes, which combine the production of PHA with that of other added-value

compounds. PHA synthetic pathway is centred on acetyl-CoA, a connecting link of the majority of the biomolecule synthetic pathways, thus co-production of any metabolite with PHA must balance the overall cellular metabolic flux. This implies a strict optimization of process conditions and subtle strain designing, in order to avoid competition for the main substrate. Protein engineering together with mathematical model analyses will represent powerful tools to get insight into the metabolic fluxes underlying multi-products processes, and to identify the main targets for performance improvement.

In conclusion, the conversion of food wastes into PHA represents a very promising possibility to face both waste disposal and biopolymer production from renewable sources. The future in this field will rely on the integration of polymer production within a "Waste Biorefinery" aimed at valorizing wastes as renewable feedstocks to recover biobased products and energy, in line with the concept of circular economy-based process.

References

Ahn WS, Park SJ, Lee SY (2000) Production of poly(3-hydroxybutyrate) by fed-batch culture of recombinant Escherichia Coli with a highly concentrated whey solution. Appl Environ Microbiol 66(8):3624–3627. https://doi.org/10.1128/AEM.66.8.3624-3627.2000

Albuquerque MGE, Concas S, Bengtsson S, Reis MAM (2010a) Mixed culture Polyhydroxyalkanoates production from sugar molasses: the use of a 2-stage CSTR system for culture selection. Bioresour Technol 101(18):7112–7122. https://doi.org/10.1016/j.biortech.2010.04.019

Albuquerque MGE, Torres CAV, Reis MAM (2010b) Polyhydroxyalkanoate (PHA) production by a mixed microbial culture using sugar molasses: effect of the influent substrate concentration on culture selection. Water Res 44(11):3419–3433. https://doi.org/10.1016/j.watres.2010.03.021

Aldor IS, Keasling JD (2003) Process design for microbial plastic factories: metabolic engineering of Polyhydroxyalkanoates. Curr Opin Biotechnol 14(5):475–483. https://doi.org/10.1016/j.copbio.2003.09.002

Alsafadi D, Al-Mashaqbeh O (2017) A one-stage cultivation process for the production of poly-3-(hydroxybutyrate-co-hydroxyvalerate) from olive mill wastewater by Haloferax Mediterranei. New Biotechnol 34:47–53. https://doi.org/10.1016/j.nbt.2016.05.003

Aneesh BP, Arjun JK, Kavitha T, Harikrishnan K (2016) Production of short chain length polyhydroxyalkanoates by Bacillus megaterium PHB29 from starch feed stock. Int J Curr Microbiol App Sci 5(7):816–823. https://doi.org/10.20546/ijcmas.2016.507.094

Annamalai N, Sivakumar N (2016) Production of polyhydroxybutyrate from wheat bran hydrolysate using Ralstonia eutropha through microbial fermentation. J Biotechnol 237:13–17. https://doi.org/10.1016/j.jbiotec.2016.09.001

Bhatia SK, Shim YH, Jeon JM, Brigham CJ, Kim YH, Kim HJ, Seo HM et al (2015) Starch based polyhydroxybutyrate production in engineered Escherichia coli. Bioprocess Biosyst Eng 38(8):1479–1484. https://doi.org/10.1007/s00449-015-1390-y

Bhatia SK, Gurav R, Choi TR, Jung HR, Yang SY, Song HS, Jeon JM, Kim JS, Lee YK, Yang YH (2019) Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) production from engineered Ralstonia eutropha using synthetic and anaerobically digested food waste derived volatile fatty acids. Int J Biol Macromol 133:1–10. https://doi.org/10.1016/j.ijbiomac.2019.04.083

Bhattacharyya A, Saha J, Haldar S, Bhowmic A, Mukhopadhyay UK, Mukherjee J (2014) Production of poly-3-(hydroxybutyrate-co-hydroxyvalerate) by Haloferax mediterranei using

rice-based ethanol stillage with simultaneous recovery and re-use of medium salts. Extremophiles 18(2):463–470. https://doi.org/10.1007/s00792-013-0622-9

Borrero-de Acuña J, Aravena-Carrasco C, Gutierrez-Urrutia I, Duchens D, Poblete-Castro I (2019) Enhanced synthesis of medium-chain-length poly(3-hydroxyalkanoates) by inactivating the tricarboxylate transport system of Pseudomonas putida KT2440 and process development using waste vegetable oil. Process Biochem 77:23–30. https://doi.org/10.1016/j.procbio.2018.10.012

Bustamante D, Segarra S, Tortajada M, Ramón D, del Cerro C, Auxiliadora Prieto M, Iglesias JR, Rojas A (2019) In silico prospection of microorganisms to produce polyhydroxyalkanoate from whey: Caulobacter segnis DSM 29236 as a suitable industrial strain. Microb Biotechnol 12 (3):487–501. https://doi.org/10.1111/1751-7915.13371

Campanari S, Silva FAE, Bertin L, Villano M, Majone M (2014) Effect of the organic loading rate on the production of Polyhydroxyalkanoates in a multi-stage process aimed at the valorization of olive oil mill wastewater. Int J Biol Macromol 71:34–41. https://doi.org/10.1016/j.ijbiomac.2014.06.006

Cerrone F, Sánchez-Peinado M d M, Juárez-Jimenez B, González-López J, Pozo C (2010) Biological treatment of two-phase olive mill wastewater (TPOMW, Alpeorujo): polyhydroxyalkanoates (PHAs) production by Azotobacter strains. J Microbiol Biotechnol 20 (3):594–601. https://doi.org/10.4014/jmb.0906.06037

Cesário MT, Raposo RS, de Almeida MCMD, van Keulen F, Ferreira BS, da Fonseca MMR (2014) Enhanced bioproduction of poly-3-hydroxybutyrate from wheat straw lignocellulosic hydrolysates. New Biotechnol 31(1):104–113. https://doi.org/10.1016/j.nbt.2013.10.004

Chaudhry WN, Jamil N, Ali I, Ayaz MH, Hasnain S (2011) Screening for polyhydroxyalkanoate (PHA)-producing bacterial strains and comparison of PHA production from various inexpensive carbon sources. Ann Microbiol 61(3):623–629. https://doi.org/10.1007/s13213-010-0181-6

Chen G, Hajnal I (2015) The 'PHAome'. Trends Biotechnol 33(10):559–564. https://doi.org/10.1016/j.tibtech.2015.07.006

Chen GQ, Jiang XR (2017) Engineering bacteria for enhanced polyhydroxyalkanoates (PHA) biosynthesis. Synth Syst Biotechnol 2(3):192–197. https://doi.org/10.1016/j.synbio.2017.09.001

Chen CW, Don TM, Yen HF (2006) Enzymatic extruded starch as a carbon source for the production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by Haloferax mediterranei. Process Biochem 41(11):2289–2296. https://doi.org/10.1016/j.procbio.2006.05.026

Colombo B, Calvo MV, Sciarria TP, Scaglia B, Kizito SS, D'Imporzano G, Adani F (2019) Biohydrogen and polyhydroxyalkanoates (PHA) as products of a two-steps bioprocess from deproteinized dairy wastes. Waste Manag 95:22–31. https://doi.org/10.1016/j.wasman.2019.05.052

Costa SGVAO, Lépine F, Milot S, Déziel E, Nitschke M, Contiero J (2009) Cassava wastewater as a substrate for the simultaneous production of rhamnolipids and polyhydroxyalkanoates by Pseudomonas aeruginosa. J Ind Microbiol Biotechnol 36(8):1063–1072. https://doi.org/10.1007/s10295-009-0590-3

Cruz MV, Paiva A, Lisboa P, Freitas F, Alves VD, Simões P, Barreiros S, Reis MAM (2014) Production of polyhydroxyalkanoates from spent coffee grounds oil obtained by supercritical fluid extraction technology. Bioresour Technol 157:360–363. https://doi.org/10.1016/j.biortech.2014.02.013

Cruz MV, Freitas F, Paiva A, Mano F, Dionísio M, Ramos AM, Reis MAM (2016) Valorization of fatty acids-containing wastes and byproducts into short- and medium-chain length polyhydroxyalkanoates. New Biotechnol 33(1):206–215. https://doi.org/10.1016/j.nbt.2015.05.005

Dionisi D, Carucci G, Papini MP, Riccardi C, Majone M, Carrasco F (2005) Olive oil mill effluents as a feedstock for production of biodegradable polymers. Water Res 39(10):2076–2084. https://doi.org/10.1016/j.watres.2005.03.011

Duque AF, Oliveira CSS, Carmo ITD, Gouveia AR, Pardelha F, Ramos AM, Reis MAM (2014) Response of a three-stage process for PHA production by mixed microbial cultures to feedstock

shift: impact on polymer composition. New Biotechnol 31(4):276–288. https://doi.org/10.1016/j.nbt.2013.10.010

Eroğlu I, Tabanoğlu A, Gündüz U, Eroğlu E, Yücel M (2008) Hydrogen production by Rhodobacter sphaeroides O.U.001 in a flat plate solar bioreactor. Int J Hydrog Energy 33 (2):531–541. https://doi.org/10.1016/j.ijhydene.2007.09.025

Essel R, Carus M (2012) Meta-analysis of 30 LCAs. Bioplastics Magazine 7(2):46–49. https://doi.org/10.1186/1742-6405-6-12

Favaro L, Basaglia M, Casella S (2018) Improving polyhydroxyalkanoate production from inexpensive carbon sources by genetic approaches: a review. Biofuels Bioprod Biorefin 13:208–227. https://doi.org/10.1002/bbb.1944

Follonier S, Goyder MS, Silvestri AC, Crelier S, Kalman F, Riesen R, Zinn M (2014) Fruit pomace and waste frying oil as sustainable resources for the bioproduction of medium-chain-length polyhydroxyalkanoates. Int J Biol Macromol 71:42–52. https://doi.org/10.1016/j.ijbiomac.2014.05.061

Fradinho JC, Oehmen A, Reis MAM (2019) Improving polyhydroxyalkanoates production in phototrophic mixed cultures by optimizing accumulator reactor operating conditions. Int J Biol Macromol 126:1085–1092. https://doi.org/10.1016/j.ijbiomac.2018.12.270

Gamal RF, Hassan E, El-tayeb T, Abou-taleb K (2011) Production of polyhydroxyalkanoate (PHAs) and copolymer [P(HB-co-HV)] by soil bacterial isolates in batch and two-stage batch cultures. Egypt J Microbiol 46(1):109–123. https://doi.org/10.21608/ejm.2011.266

Gamal RF, Hassan EA, El-tayeb TS, Abou-taleb KA (2012) Production of PHAs from waste frying oil by Pseudomonas fluorescens S48 using different bioreactor feeding strategies. Egypt J Microbiol 47(1):1–15. https://doi.org/10.21608/ejm.2012.249

Gamal RF, Abdelhady HM, Khodair TA, El-Tayeb TS, Hassan EA, Aboutaleb KA (2013) Semi-scale production of PHAs from waste frying oil by Pseudomonas fluorescens S48. Braz J Microbiol 44(2):539–549. https://doi.org/10.1590/S1517-83822013000200034

Gouveia AR, Freitas EB, Galinha CF, Carvalho G, Duque AF, Reis MAM (2017) Dynamic change of PH in acidogenic fermentation of cheese whey towards polyhydroxyalkanoates production: impact on performance and microbial population. New Biotechnol 37:108–116. https://doi.org/10.1016/j.nbt.2016.07.001

Gowda V, Shivakumar S (2014) Agrowaste-based polyhydroxyalkanoate (PHA) production using hydrolytic potential of Bacillus Thuringiensis IAM 12077. Braz Arch Biol Technol 57 (1):55–61. https://doi.org/10.1590/S1516-89132014000100009

Guamán LP, Oliveira-Filho ER, Barba-Ostria C, Gomez JGC, Taciro MK, da Silva LF (2018) XylA and XylB overexpression as a successful strategy for improving xylose utilization and poly-3-hydroxybutyrate production in Burkholderia Sacchari. J Ind Microbiol Biotechnol 45 (3):165–173. https://doi.org/10.1007/s10295-018-2007-7

Gurieff N, Lant P (2007) Comparative life cycle assessment and financial analysis of mixed culture polyhydroxyalkanoate production. Bioresour Technol 98(17):3393–3403. https://doi.org/10.1016/j.biortech.2006.10.046

Haba E, Vidal-Mas J, Bassas M, Espuny MJ, Llorens J, Manresa A (2007) Poly 3-(hydroxyalkanoates) produced from oily substrates by Pseudomonas Aeruginosa 47T2 (NCBIM 40044): effect of nutrients and incubation temperature on polymer composition. Biochem Eng J 35(2):99–106. https://doi.org/10.1016/j.bej.2006.11.021

Halami P (2008) Production of polyhydroxyalkanoate from starch by the native isolate Bacillus cereus CFR06. World J Microbiol Biotechnol 24:805–812. https://doi.org/10.1007/s11274-007-9543-z

Harding KG, Dennis JS, von Blottnitz H, Harrison STL (2007) Environmental analysis of plastic production processes: comparing petroleum-based polypropylene and polyethylene with biologically-based poly-β-hydroxybutyric acid using life cycle analysis. J Biotechnol 130 (1):57–66. https://doi.org/10.1016/j.jbiotec.2007.02.012

Huang L, Chen Z, Xiong D, Wen Q, Ji Y (2018) Oriented acidification of wasted activated sludge (WAS) focused on odd-carbon volatile fatty acid (VFA): regulation strategy and microbial community dynamics. Water Res 142:256–266. https://doi.org/10.1016/j.watres.2018.05.062

Jeon JM, Brigham CJ, Kim YH, Kim HJ, Yi DH, Kim H, Rha CK, Sinskey AJ, Yang YH (2014) Biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (P(HB-co-HHx)) from butyrate using engineered Ralstonia eutropha. Appl Microbiol Biotechnol 98(12):5461–5469. https://doi.org/10.1007/s00253-014-5617-7

Kamilah H, Tsuge T, Yang TA, Sudesh K (2013) Waste cooking oil as substrate for biosynthesis of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate): turning waste into a value-added product. Malays J Microbiol 9(1):51–59. https://doi.org/10.21161/mjm.45012

Keshavarz T, Roy I (2010) Polyhydroxyalkanoates: bioplastics with a green agenda. Curr Opin Microbiol 13(3):321–326. https://doi.org/10.1016/j.mib.2010.02.006

Kim B (2000) Production of poly(3-hydroxybutyrate) from inexpensive substrates. Enzym Microb Technol 27:774–777. https://doi.org/10.1016/s0141-0229(00)00299-4

Kim HS, Oh YH, Jang YA, Kang KH, David Y, Ju Hyun Y, Song BK et al (2016) Recombinant Ralstonia eutropha engineered to utilize xylose and its use for the production of poly (3-hydroxybutyrate) from sunflower stalk hydrolysate solution. Microb Cell Factories 15 (1):1–13. https://doi.org/10.1186/s12934-016-0495-6

Koller M (2019) Biopolyesters from agro-industrial by-products for securing food safety. Appl Food Biotechnol 6:1–6

Koller M, Hesse P, Bona R, Kutschera C, Atlić A, Braunegg G (2007) Biosynthesis of high quality polyhydroxyalkanoate co- and terpolyesters for potential medical application by the archaeon Haloferax mediterranei. Macromol Symp 253:33–39. https://doi.org/10.1002/masy.200750704

Koller M, Bona R, Chiellini E, Fernandes EG, Horvat P, Kutschera C, Hesse P, Braunegg G (2008) Polyhydroxyalkanoate production from whey by Pseudomonas hydrogenovora. Bioresour Technol 99(11):4854–4863. https://doi.org/10.1016/j.biortech.2007.09.049

Koller M, Maršálek L, de Sousa Dias MM, Braunegg G (2017) Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner. New Biotechnol 37:24–38. https://doi.org/10.1016/j.nbt.2016.05.001

Kourmentza C, Plácido J, Venetsaneas N, Burniol-Figols A, Varrone C, Gavala H, Reis M (2017) Recent advances and challenges towards sustainable polyhydroxyalkanoate (PHA) production. Bioengineering 4(2):1–43. https://doi.org/10.3390/bioengineering4020055

Kourmentza C, Costa J, Azevedo Z, Servin C, Grandfils C, De Freitas V, Reis MAM (2018) Burkholderia thailandensis as a microbial cell factory for the bioconversion of used cooking oil to polyhydroxyalkanoates and rhamnolipids. Bioresour Technol 247:829–837. https://doi.org/10.1016/j.biortech.2017.09.138

Kulpreecha S, Boonruangthavorn A, Meksiriporn B, Thongchul N (2009) Inexpensive fed-batch cultivation for high poly(3-hydroxybutyrate) production by a new isolate of Bacillus megaterium. J Biosci Bioeng 107(3):240–245. https://doi.org/10.1016/j.jbiosc.2008.10.006

Kumar P, Kim B (2018) Valorization of polyhydroxyalkanoates production process by co-synthesis of value-added products. Bioresour Technol 269:544–556. https://doi.org/10.1016/j.biortech.2018.08.120

Kumar P, Kim B (2019) Paracoccus sp. strain LL1 as a single cell factory for the conversion of waste cooking oil to polyhydroxyalkanoates and carotenoids. Appl Food Biotechnol 6 (1):53–60. https://doi.org/10.22037/afb.v6i1.21628

Kumar P, Jun HB, Kim BS (2018) Co-production of polyhydroxyalkanoates and carotenoids through bioconversion of glycerol by Paracoccus sp. strain LL1. Int J Biol Macromol 107:2552–2558. https://doi.org/10.1016/j.ijbiomac.2017.10.147

Kwan TH, Hu Y, Lin CSK (2018) Techno-economic analysis of a food waste valorisation process for lactic acid, lactide and poly(lactic acid) production. J Clean Prod 181:72–87. https://doi.org/10.1016/j.jclepro.2018.01.179

Lam W, Wang Y, Chan PL, Chan SW, Tsang YF, Chua H, Yu PHF (2017) Production of polyhydroxyalkanoates (PHA) using sludge from different wastewater treatment processes and the potential for medical and pharmaceutical applications. Environ Technol 38 (13–14):1779–1791. https://doi.org/10.1080/09593330.2017.1316316

Lareo C, Ferrari MD, Guigou M, Fajardo L, Larnaudie V, Ramírez MB, Martínez-Garreiro J (2013) Evaluation of sweet potato for fuel bioethanol production: hydrolysis and fermentation. Springerplus 2(1):1–11. https://doi.org/10.1186/2193-1801-2-493

Lee SY, Middelberg APJ, Lee YK (1997) Poly(3-hydroxybutyrate) production from whey using recombinant Escherichia coli. Biotechnol Lett 19(10):1033–1035. https://doi.org/10.1023/A:1018411820580

Lee WS, Chua ASM, Yeoh HK, Ngoh GC (2014) Influence of temperature on the bioconversion of palm oil mill effluent into volatile fatty acids as precursor to the production of polyhydroxyalkanoates. J Chem Technol Biotechnol 89(7):1038–1043. https://doi.org/10.1002/jctb.4197

Lee Y, Cho IJ, Choi SY, Lee SY (2019) Systems metabolic engineering strategies for non-natural microbial polyester production. Biotechnol J 14(9):1800426. https://doi.org/10.1002/biot.201800426

Lemechko P, Le Fellic M, Bruzaud S (2019) Production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) using agro-industrial effluents with tunable proportion of 3-hydroxyvalerate monomer units. Int J Biol Macromol 128:429–434. https://doi.org/10.1016/j.ijbiomac.2019.01.170

Li T, Elhadi D, Chen GQ (2017) Co-production of microbial polyhydroxyalkanoates with other chemicals. Metab Eng 43:29–36. https://doi.org/10.1016/j.ymben.2017.07.007

Liu D, Yan X, Si M, Deng X, Min X, Shi Y, Chai L (2019) Bioconversion of lignin into bioplastics by Pandoraea sp. B-6: molecular mechanism. Environ Sci Pollut Res 26(3):2761–2770. https://doi.org/10.1007/s11356-018-3785-1

Locatelli GO, Finkler L, Finkler CLL (2019) Orange and passion fruit wastes characterization, substrate hydrolysis and cell growth of Cupriavidus necator, as proposal to converting of residues in high value added product. Anais Da Academia Brasileira de Ciencias 91(1):1–9. https://doi.org/10.1590/0001-3765201920180058

Luo K, Pang Y, Yang Q, Wang D, Li X, Lei M, Huang Q (2019) A critical review of volatile fatty acids produced from waste activated sludge: enhanced strategies and its applications. Environ Sci Pollut Res 26:13984–13998. https://doi.org/10.1007/s11356-019-04798-8

Marsudi S, Unno H, Hori K (2008) Palm oil utilization for the simultaneous production of polyhydroxyalkanoates and rhamnolipids by Pseudomonas aeruginosa. Appl Microbiol Biotechnol 78:955–961. https://doi.org/10.1007/s00253-008-1388-3

Md Din M, Mohanadoss P, Ujang Z, van Loosdrecht M, Yunus S, Chelliapan S, Zambare V, Olsson G (2012) Development of Bio-PORec® system for polyhydroxyalkanoates (PHA) production and its storage in mixed cultures of palm oil mill effluent (POME). Bioresour Technol 124:208–216. https://doi.org/10.1016/j.biortech.2012.08.036

Mohapatra S, Sarkar B, Samantaray DP, Daware A, Maity S, Pattnaik S, Bhattacharjee S (2017) Bioconversion of fish solid waste into PHB using Bacillus subtilis based submerged fermentation process. Environ Technol 38(24):3201–3208. https://doi.org/10.1080/09593330.2017.1291759

Moita Fidalgo R, Ortigueira J, Freches A, Pelica J, Gonçalves M, Mendes B, Lemos P (2014) Bio-oil upgrading strategies to improve PHA production from selected aerobic mixed cultures. New Biotechnol 31:297–307. https://doi.org/10.1016/j.nbt.2013.10.009

Montiel Corona V, Revah S, Morales M (2015) Hydrogen production by an enriched photoheterotrophic culture using dark fermentation effluent as substrate: effect of flushing method, bicarbonate addition, and outdoor–indoor conditions. Int J Hydrog Energy 40:9096–9105. https://doi.org/10.1016/j.ijhydene.2015.05.067

Montiel Corona V, Le Borgne S, Revah S, Morales M (2017) Effect of light-dark cycles on hydrogen and poly-β-hydroxybutyrate production by a photoheterotrophic culture and Rhodobacter capsulatus using a dark fermentation effluent as substrate. Bioresour Technol 226:238–246. https://doi.org/10.1016/j.biortech.2016.12.021

Możejko J, Ciesielski S (2013) Saponified waste palm oil as an attractive renewable resource for mcl-polyhydroxyalkanoate synthesis. J Biosci Bioeng 116:485–492. https://doi.org/10.1016/j.jbiosc.2013.04.014

Możejko J, Ciesielski S (2014) Pulsed feeding strategy is more favorable to medium-chain-length polyhydroxyalkanoates production from waste rapeseed oil. Biotechnol Prog 30(5):1243–1246. https://doi.org/10.1002/btpr.1914

Mozejko J, Przybyłek G, Ciesielski S (2011) Waste rapeseed oil as a substrate for medium-chain-length polyhydroxyalkanoates production. Eur J Lipid Sci Technol 113(12):1550–1557. https://doi.org/10.1002/ejlt.201100148

Nielsen C, Rahman A, Rehman AU, Walsh MK, Miller CD (2017) Food waste conversion to microbial polyhydroxyalkanoates. Microb Biotechnol 10(6):1338–1352. https://doi.org/10.1111/1751-7915.12776

Ntaikou I, Valencia Peroni C, Kourmentza C, Ilieva VI, Morelli A, Chiellini E, Lyberatos G (2014) Microbial bio-based plastics from olive-mill wastewater: generation and properties of polyhydroxyalkanoates from mixed cultures in a two-stage pilot scale system. J Biotechnol 188:138–147. https://doi.org/10.1016/j.jbiotec.2014.08.015

Obruca S, Marova I, Melusova S, Mravcova L (2011) Production of polyhydroxyalkanoates from cheese whey employing Bacillus megaterium CCM 2037. Ann Microbiol 61(4):947–953. https://doi.org/10.1007/s13213-011-0218-5

Obruca S, Snajdar O, Svoboda Z, Marova I (2013) Application of random mutagenesis to enhance the production of polyhydroxyalkanoates by Cupriavidus necator H16 on waste frying oil. World J Microbiol Biotechnol 29(12):2417–2428. https://doi.org/10.1007/s11274-013-1410-5

Obruca S, Benesova P, Petrik S, Oborna J, Prikryl R, Marova I (2014a) Production of polyhydroxyalkanoates using hydrolysate of spent coffee grounds. Process Biochem 49(9):1409–1414. https://doi.org/10.1016/j.procbio.2014.05.013

Obruca S, Petrik S, Benesova P, Svoboda Z, Eremka L, Marova I (2014b) Utilization of oil extracted from spent coffee grounds for sustainable production of polyhydroxyalkanoates. Appl Microbiol Biotechnol 98(13):5883–5890. https://doi.org/10.1007/s00253-014-5653-3

Ojumu TV, Yu J, Solomon BO (2004) Production of polyhydroxyalkanoates, a bacterial biodegradable polymer. Afr J Biotechnol 3(1):18–24. https://doi.org/10.5897/AJB2004.000-2004

Padovani G, Carlozzi P, Seggiani M, Cinelli P, Vitolo S, Lazzeri A (2016) PHB-rich biomass and BioH2 production by means of photosynthetic microorganisms. Chem Eng Trans 49:55–60. https://doi.org/10.3303/CET1649010

Pais J, Farinha I, Freitas F, Serafim LS, Martínez V, Martínez JC, Arévalo-Rodríguez M, Auxiliadora Prieto M, Reis MAM (2014) Improvement on the yield of polyhydroxyalkanotes production from cheese whey by a recombinant Escherichia coli strain using the proton suicide methodology. Enzym Microb Technol 55:151–158. https://doi.org/10.1016/j.enzmictec.2013.11.004

Pakalapati H, Chang CK, Show PL, Arumugasamy SK, Lan JCW (2018) Development of polyhydroxyalkanoates production from waste feedstocks and applications. J Biosci Bioeng 126(3):282–292. https://doi.org/10.1016/j.jbiosc.2018.03.016

Passanha P, Esteves SR, Kedia G, Dinsdale RM, Guwy AJ (2013) Increasing polyhydroxyalkanoate (PHA) yields from Cupriavidus necator by using filtered digestate liquors. Bioresour Technol 147:345–352. https://doi.org/10.1016/j.biortech.2013.08.050

Patel SKS, Kumar P, Singh M, Lee JK, Kalia VC (2015) Integrative approach to produce hydrogen and polyhydroxybutyrate from biowaste using defined bacterial cultures. Bioresour Technol 176:136–141. https://doi.org/10.1016/j.biortech.2014.11.029

Poomipuk N, Reungsang A, Plangklang P (2014) Poly-β-hydroxyalkanoates production from cassava starch hydrolysate by Cupriavidus sp. KKU38. Int J Biol Macromol 65:51–64. https://doi.org/10.1016/j.ijbiomac.2014.01.002

Povolo S, Toffano P, Basaglia M, Casella S (2010) Polyhydroxyalkanoates production by engineered Cupriavidus necator from waste material containing lactose. Bioresour Technol 101(20):7902–7907. https://doi.org/10.1016/j.biortech.2010.05.029

Pramanik A, Mitra A, Arumugam M, Bhattacharyya A, Sadhukhan S, Ray A, Haldar S, Mukhopadhyay UK, Mukherjee J (2012) Utilization of vinasse for the production of

polyhydroxybutyrate by Haloarcula marismortui. Folia Microbiol 57(1):71–79. https://doi.org/10.1007/s12223-011-0092-3

RamKumar Pandian S, Deepak V, Kalishwaralal K, Rameshkumar N, Jeyaraj M, Gurunathan S (2010) Optimization and fed-batch production of PHB utilizing dairy waste and sea water as nutrient sources by Bacillus megaterium SRKP-3. Bioresour Technol 101(2):705–711. https://doi.org/10.1016/j.biortech.2009.08.040

Rebocho AT, Pereira JR, Freitas F, Neves LA, Alves VD, Sevrin C, Grandfils C, Reis MAM (2019) Production of medium-chain length polyhydroxyalkanoates by Pseudomonas citronellolis grown in apple pulp waste. Appl Food Biotechnol 6(1):71–82. https://doi.org/10.22037/afb.v6i1.21793

Reddy CSK, Ghai R, Rashmi, Kalia VC (2003) Polyhydroxyalkanoates: an overview. Bioresour Technol 87(2):137–146. https://doi.org/10.1016/S0960-8524(02)00212-2

Revelles O, Beneroso D, Menéndez JA, Arenillas A, García JL, Prieto MA (2017) Syngas obtained by microwave pyrolysis of household wastes as feedstock for polyhydroxyalkanoate production in Rhodospirillum rubrum. Microb Biotechnol 10(6):1412–1417. https://doi.org/10.1111/1751-7915.12411

Riedel SL, Jahns S, Koenig S, Bock MCE, Brigham CJ, Bader J, Stahl U (2015) Polyhydroxyalkanoates production with Ralstonia eutropha from low quality waste animal fats. J Biotechnol 214:119–127. https://doi.org/10.1016/j.jbiotec.2015.09.002

Rodríguez-Carmona E, Bastida J, Manresa A (2012) Utilization of agro-industrial residues for poly (3-hydroxyalkanoate) production by Pseudomonas aeruginosa 42A2 (NCIMB 40045): optimization of culture medium. J Am Oil Chem Soc 89(1):111–122. https://doi.org/10.1007/s11746-011-1897-6

Rodriguez-Perez S, Serrano A, Pantión AA, Alonso-Fariñas B (2018) Challenges of scaling-up PHA production from waste streams. A review. J Environ Manag 205:215–230. https://doi.org/10.1016/j.jenvman.2017.09.083

Romanelli MG, Povolo S, Favaro L, Fontana F, Basaglia M, Casella S (2014) Engineering Delftia acidovorans DSM39 to produce polyhydroxyalkanoates from slaughterhouse waste. Int J Biol Macromol 71:21–27. https://doi.org/10.1016/j.ijbiomac.2014.03.049

Sabapathy PC, Devaraj S, Kathirvel P (2017) Parthenium hysterophorus: low cost substrate for the production of polyhydroxyalkanoates. Curr Sci 112(10):2106–2111. https://doi.org/10.18520/cs/v112/i10/2106-2111

Sabapathy PC, Devaraj S, Parthipan A, Kathirvel P (2019) Polyhydroxyalkanoate production from statistically optimized media using rice mill effluent as sustainable substrate with an analysis on the biopolymer's degradation potential. Int J Biol Macromol 126:977–986. https://doi.org/10.1016/j.ijbiomac.2019.01.003

Sahoo J, Patil P, Verma A, Lodh A, Khanna N, Prasad R, Pandit S, Fosso-Kankeu E (2021) Biochemical aspects of syngas fermentation. In: Prasad R, Singh J, Kumar V, Upadhyay CP (eds) Recent developments in microbial technologies. Springer Nature, Singapore, pp 395–424

Salgaonkar BB, Mani K, Bragança JM (2019) Sustainable bioconversion of cassava waste to poly (3-hydroxybutyrate-co-3-hydroxyvalerate) by Halogeometricum borinquense strain E3. J Polym Environ 27(2):299–308. https://doi.org/10.1007/s10924-018-1346-9

Saranya Devi E, Vijayendra SVN, Shamala TR (2012) Exploration of rice bran, an agro-industry residue, for the production of intra- and extra-cellular polymers by Sinorhizobium meliloti MTCC 100. Biocatal Agric Biotechnol 1(1):80–84. https://doi.org/10.1016/j.bcab.2011.08.014

Schmid MT, Song H, Raschbauer M, Emerstorfer F, Omann M, Stelzer F, Neureiter M (2019) Utilization of desugarized sugar beet molasses for the production of poly(3-hydroxybutyrate) by halophilic Bacillus megaterium uyuni S29. Process Biochem 86:9–15. https://doi.org/10.1016/j.procbio.2019.08.001

Shamala TR, Vijayendra SVN, Joshi GJ (2012) Agro-industrial residues and starch for growth and co-production of polyhydroxyalkanoate copolymer and α-amylase by Bacillus sp. CFR-67. Braz J Microbiol 43(3):1094–1102. https://doi.org/10.1590/S1517-83822012000300036

Song JH, Jeon CO, Choi MH, Yoon SC, Park W (2008) Polyhydroxyalkanoate (PHA) production using waste vegetable oil by Pseudomonas sp. strain DR2. J Microbiol Biotechnol 18 (8):1408–1415

Sreekanth MS, Vijayendra SVN, Joshi GJ, Shamala TR (2013) Effect of carbon and nitrogen sources on simultaneous production of α-amylase and green food packaging polymer by Bacillus sp. CFR 67. J Food Sci Technol 50(2):404–408. https://doi.org/10.1007/s13197-012-0639-6

Strazzera G, Battista F, Garcia NH, Frison N, Bolzonella D (2018) Volatile fatty acids production from food wastes for biorefinery platforms: a review. J Environ Manag 226:278–288. https://doi.org/10.1016/j.jenvman.2018.08.039

Takisawa K, Ooi T, Matsumoto K'i, Kadoya R, Taguchi S (2017) Xylose-based hydrolysate from eucalyptus extract as feedstock for poly(lactate-co-3-hydroxybutyrate) production in engineered Escherichia coli. Process Biochem 54:102–105. https://doi.org/10.1016/j.procbio.2016.12.019

Tamang P, Banerjee R, Köster S, Nogueira R (2019) Comparative study of polyhydroxyalkanoates production from acidified and anaerobically treated brewery wastewater using enriched mixed microbial culture. J Environ Sci (China) 78:137–146. https://doi.org/10.1016/j.jes.2018.09.001

Tokimoto T, Kawasaki N, Nakamura T, Akutagawa J, Tanada S (2005) Removal of lead ions in drinking water by coffee grounds as vegetable biomass. J Colloid Interface Sci 281(1):56–61. https://doi.org/10.1016/j.jcis.2004.08.083

Tsang YF, Kumar V, Samadar P, Yang Y, Lee J, Ok YS, Song H, Kim KH, Kwon EE, Jeon YJ (2019) Production of bioplastic through food waste valorization. Environ Int 127:625–644. https://doi.org/10.1016/j.envint.2019.03.076

Urbina L, Wongsirichot P, Corcuera MÁ, Gabilondo N, Eceiza A, Winterburn J, Retegi A (2018) Application of cider by-products for medium chain length polyhydroxyalkanoate production by Pseudomonas putida KT2440. Eur Polym J 108:1–9. https://doi.org/10.1016/j.eurpolymj.2018.08.020

Vastano M, Pellis A, Immirzi B, Dal Poggetto G, Malinconico M, Sannia G, Guebitz GM, Pezzella C (2017) Enzymatic production of clickable and PEGylated recombinant polyhydroxyalkanoates. Green Chem 19(22):5494–5504. https://doi.org/10.1039/c7gc01872j

Vastano M, Corrado I, Sannia G, Solaiman DKY, Pezzella C (2019) Conversion of no/low value waste frying oils into biodiesel and polyhydroxyalkanoates. Sci Rep 9(1):1–8. https://doi.org/10.1038/s41598-019-50278-x

Vega-Castro O, Contreras-Calderon J, León E, Segura A, Arias M, Pérez L, Sobral PJA (2016) Characterization of a polyhydroxyalkanoate obtained from pineapple peel waste using Ralsthonia eutropha. J Biotechnol 231:232–238. https://doi.org/10.1016/j.jbiotec.2016.06.018

Venkateswar Reddy M, Venkata Mohan S (2012) Influence of aerobic and anoxic microenvironments on polyhydroxyalkanoates (PHA) production from food waste and acidogenic effluents using aerobic consortia. Bioresour Technol 103(1):313–321. https://doi.org/10.1016/j.biortech.2011.09.040

Verlinden RAJ, Hill DJ, Kenward MA, Williams CD, Piotrowska-Seget Z, Radecka IK (2011) Production of polyhydroxyalkanoates from waste frying oil by Cupriavidus necator. AMB Express 1(1):1–8. https://doi.org/10.1186/2191-0855-1-11

Wen Q, Ji Y, Hao Y, Huang L, Chen Z, Sposob M (2018) Effect of sodium chloride on polyhydroxyalkanoate production from food waste fermentation leachate under different organic loading rate. Bioresour Technol 267:133–140. https://doi.org/10.1016/j.biortech.2018.07.036

Wijeyekoon S, Carere CR, West M, Nath S, Gapes D (2018) Mixed culture polyhydroxyalkanoate (PHA) synthesis from nutrient rich wet oxidation liquors. Water Res 140:1–11. https://doi.org/10.1016/j.watres.2018.04.017

Yang TH, Jung YK, Kang HO, Kim TW, Park SJ, Lee SY (2011) Tailor-made type II Pseudomonas PHA synthases and their use for the biosynthesis of polylactic acid and its copolymer in recombinant Escherichia coli. Appl Microbiol Biotechnol 90(2):603–614. https://doi.org/10.1007/s00253-010-3077-2

Yu J, Stahl H (2008) Microbial utilization and biopolyester synthesis of bagasse hydrolysates. Bioresour Technol 99(17):8042–8048. https://doi.org/10.1016/j.biortech.2008.03.071

Bacterial Cellulose Production from Agro-Industrial and Food Wastes

7

G. K. Chua, N. I. F. Mahadi, and F. H. Y. Tan

Abstract

Bacterial cellulose (BC) is a popular substitution of plant cellulose due to its higher purity and better properties. It has vast application in various industries, e.g. in paper production, in wound healing, in food packaging and many more. In commercial scale, *Gluconacetobacter xylinus* is the common species used. Large-scale production of bacterial cellulose, however, is costly with defined chemical medium, i.e. Hestrin and Schramm (HS) medium. Thus, most researchers are seeking alternative from the available wastes in order to reduce the cost. Numerous agro-industrial wastes were utilized as the feedstock for BC production, e.g. pineapple waste, citrus peel waste and extracted date syrup. Most of these agro-wastes are considered as defined medium as the changes of the composition are rather small. The other potential waste that can be used as a feedstock is the household food wastes. Since food waste generation and disposal are major problem in most of the countries, valorization of this waste for BC production may be a win-win situation. Nevertheless, food waste if used as a medium may impose the problem of inconsistent quality of BC as food waste collected typically has inconsistent composition and thus a complex undefined medium. This chapter is centred on comparing the feasibility of using food waste as a low-cost medium to produce BC. Moreover, the effect of food waste medium on the quality of BC is compared with the BC produced from pineapple peel juice medium. In addition, the pre-treatment of food waste and its effect on the properties of the BC are briefly discussed.

G. K. Chua (✉) · N. I. F. Mahadi · F. H. Y. Tan
Department of Chemical Engineering, College of Engineering, University Malaysia Pahang, Lebuhraya Tun Razak, Gambang, Kuantan, Malaysia
e-mail: chua@ump.edu.my

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_7

Keywords

Bacterial cellulose · Biovalorization · Agro-industrial wastes · Food wastes

7.1 Introduction

Inappropriate waste management leads to accumulation of large amount of kitchen and food waste. One-third of the food produced for human consumption in the world is lost or waste every year (FAO 2019). This amounted to approximately 1.3 billion tonnes of food (FAO 2019). The lost or wastage of food occurs from the agricultural production throughout the food value chain up to the final household consumption. The food that losses through these processes are partly still safe for consumption and full with nutrition. In the developed countries, most of the food lost at the consumption level, while for developing countries like Malaysia, Bangladesh, and India, food loss happens at the production to processing stage (FAO 2018). As reported by Yong et al. (2019), municipal solid waste (MSW) generation by Malaysia is estimated to be 49,670 tonnes per day by year 2030, and around 45% of the MSW consists of organic fraction, which is the food wastes. Therefore, an estimation of 8.16 million tonnes of food waste is wasted yearly in Malaysia in specific.

Common practise of handling food wastes is incineration or landfill. In Malaysia, 89% of the MSW will end up in the landfill (Yong et al. 2019). Along the decomposition process after landfill, greenhouse gases are released which contribute to the global warming (FAO 2018). Furthermore, the resources and energy input to the production of food have been wasted. Therefore, recycling of food waste to enhance its value should be done. Currently, portion of organic food waste in the MSW is only anaerobically digested to produce methane as biogas. A better option would be to use it as a substrate for producing other useful higher value products, such as bioactive compounds, enzymes, organic acids, pigments, and xanthan gum through fermentation process (Sindhu et al. 2019). This would ascertain the sustainability of the environment and economy. In this chapter, the focus will be on using the food waste to produce bacterial cellulose.

7.2 Food Wastes

Food wastes produced in the agricultural production stage up to the industrial processing stage are generally called as agro-industrial wastes. The examples are wheat straw (Chen et al. 2013), waste fibre sludge (Cavka et al. 2013), extracted date syrup (Lotfiman et al. 2016), citrus peel waste (Güzel and Akpinar 2019), and sugarcane molasses (Machado et al. 2018). This type of waste though complex is rather consistent in composition, which can be characterized easily. It contains mostly the carbon source in the form of simple sugar or complex fibre. As an example, pineapple peel juice from pineapple wastes are high in sugar content (73.76 g/L of total sugar), which consists of mainly glucose, fructose, and sucrose

(Abdullah and Mat 2008). Pineapple peel solids, on the other hand, are cellulose fibres that can be further treated to obtain simple sugar (Abdullah and Mat 2008). Similarly, after enzymatic hydrolysis of waste fibre sludge obtained from sulphate-based (SAFS) process in kraft pulping and that from sulphite (SIFS) process in lignocellulosic biorefinery, the composition was determined by Cavka et al. (2013) to be dominant by glucan (69.1% w/w in SAFS and 89.7% w/w in SIFS), followed by xylan in SAFS (15.4% w/w) and mannan in SIFS (2.7% w/w). The rest of the components were found to be arabinan, galactan, lignin, and ash. The fluctuation of the composition of agro-industrial wastes typically is within the acceptable limit.

Food wastes generated in the stage of household consumption (including those from the restaurants), however, are inconsistent in the composition, and the fluctuation is rather big. The composition is dependent on many factors, which include the culture and behaviour of the consumers, waste collection and management systems, etc. (Wan Mohamed Radzi et al. 2010; Sindhu et al. 2019).

Table 7.1 shows the physical composition of food wastes collected from six sources around Kuantan town, Pahang state, Malaysia (Chua et al. 2019). The source of wastes can be generally divided into two categories: pre-processing wastes (morning and night markets) and post-processing wastes (restaurants and household). The waste types are being grouped into three groups physically, one contributed to carbon source, the others contributed to nitrogen source, and the last group gives minerals and vitamins. It can be seen from Table 7.1 that all food wastes consist mainly a high amount of carbon source. This certainly is an advantage as microorganisms relies on carbon source to grow and reproduce. In other words, this type of waste is a suitable source of carbon for microorganisms, thus a potential replacer of pure glucose. Besides carbon, most of the food wastes have nitrogen source and other nutrients in a significant amount. These inevitably would assist microorganisms for growth and maintenance, especially the presence of micronutrients.

The chemical composition of the food wastes can be quantified by either using elemental analyser or proximate analysis. Before quantification, the wastes need to be homogenized with a heavy duty lab blender. Proximate analysis was performed on the wastes, and the results are depicted in Table 7.2. The moisture content of the solid waste, total solid content, volatile solid content and the ash content were determined using Standard Methods (APHA 2012). On the other hand, the amounts of proteins, carbohydrate and lipids in the waste hydrolysate were determined using Bradford assay (Bradford 1976), phenol-sulphuric acid method (Dubois et al. 1956) and Bligh & Dyer method (Bligh and Dyer 1959), respectively.

Table 7.2 shows that most of the wastes have high content of moisture, especially the waste collected from morning market. This is because this waste contains mainly rotten fruits and vegetables, which is high in moisture content. In addition to that, fruits and vegetables are high in fibres. As a result, it gives low calorie values, reflecting in its low total and volatile solid content. To use food wastes in a suspension culture, food waste hydrolysate will be used instead of its semi-solid form. Highest soluble protein was identified in the Chinese restaurant's waste, in line with the physical composition presented in Table 7.1. Nonetheless, amount of

Table 7.1 Physical composition of the food waste collected around Kuantan area in Pahang, Malaysia

Wastes composition		Post-processing								Pre-processing			
		University's café (%w/w)		Indian restaurant (%w/w)		Chinese restaurant (%w/w)		Household (%w/w)		Morning market (%w/w)		Night market (%w/w)	
Carbon source	Rice/mee/roti canai	52	85	40	49	12	33	21	79	0	100	0	66
	Vegetables	11		7		17		20		24		66	
	Fruits	22		2		4		38		76		0	
Nitrogen source	Bone/meat/seafood	15		35		52		0		0		0	
Others	Egg shells/prawn shells	0		16		15		21		0		34	

Source: Chua et al. (2019)

Table 7.2 Chemical composition of food wastes collected around Kuantan area in Pahang, Malaysia

Items	University's café	Indian restaurant	Chinese restaurant	Household	Morning market	Night market
Moisture content (%)	66.67 ± 2.82	66.13 ± 0.00	70.93 ± 0.00	78.60 ± 0.00	89.19 ± 0.29	67.80 ± 0.00
Total solids (%)	33.33 ± 2.82	33.87 ± 0.00	29.07 ± 0.00	21.40 ± 0.00	10.81 ± 0.14	32.20 ± 0.00
Volatile solids (%)	27.50 ± 0.25	17.05 ± 0.52	4.62 ± 0.14	7.72 ± 0.13	0.52 ± 0.08	20.51 ± 0.46
Ash content (%)	5.83 ± 2.68	16.82 ± 0.52	24.45 ± 0.14	13.68 ± 0.13	10.29 ± 0.08	11.69 ± 0.46
Carbohydrate (mg/g)	17.98 ± 1.79	3.48 ± 0.08	2.42 ± 0.21	4.88 ± 0.60	15.51 ± 0.69	8.10 ± 0.10
Protein (mg/g)	0.14 ± 0.01	0.94 ± 0.02	1.70 ± 0.06	1.03 ± 0.03	0.96 ± 0.12	0.99 ± 0.01
Lipid (mg/g)	28.68 ± 0.90	8.96 ± 0.00	13.19 ± 0.00	3.03 ± 0.00	0.91 ± 0.00	0.21 ± 0.03

Source: Chua et al. (2019)

soluble carbohydrate determined in the hydrolysate is lower than that determined in the physical composition. Existing carbohydrate in the complex fibre or solid form may be one of the reasons. Consequently, food wastes must be further treated to break down all the complex nutrients in the solid forms into the dissolved components for easy uptake of the microorganism. Depending upon the source, composition of the food wastes varies greatly. As a consequence, a common strategy is difficult to apply to all food wastes. Certain kind of pre-treatment may be required to be carried out before it is fit for use as a fermentation medium.

7.2.1 Pre-treatment of Food Wastes

The main challenge in utilizing food wastes directly as a fermentation medium is their heterogeneous nature as some microbes cannot consume it in this form. Therefore, some way of physical, chemical, enzymatic or combined pre-treatment of waste is required to make it more accessible to the microorganisms. This is applied to both agro-industrial wastes and food wastes as most of these wastes are not in pure liquid form. There will be solids like bone, meat pieces, fibres, fruit skins, etc.

Common thermal conversion methods include incineration, gasification and pyrolysis. Nonetheless, these methods are not suitable for wastes with high moisture content. Besides, it is high in energy demand. Other thermal pre-treatment methods include liquid hot water, steam, autohydrolysis and aquasolv pre-treatment (Triantafyllidis et al. 2013). These methods are widely used at industrial scale where the heating process is applied to destroy the structure of material, and disintegration of cell membranes leads to solubilisation of organic compounds (Ariunbaatar et al. 2014). Different heating temperature and treatment times will result in different outcomes. The limitation of thermal pre-treatment is that some inhibitors such as the furfural and soluble phenolic compounds will form (Hendriks and Zeeman 2009). Mechanical pre-treatment, on the other hand, is a physical process like chipping, milling or grinding that used to reduce the size of the waste, so that to increase the surface area of the waste (Ariunbaatar et al. 2014). Other examples of mechanical pre-treatment are sonication, maceration and microwave. These methods can be easily implemented, will not generate odour and consume moderate energy.

A simple and commonly used method is chemical pre-treatment. It is used for the destruction of organic compounds by means of acids, alkaline or oxidants. Acid pre-treatment can be classified into dilute or concentrated acid under low or high temperature. The examples of acid under this pre-treatment are nitric acid, sulphuric acid, phosphoric acid and hydrochloric acid. This pre-treatment is mainly used to remove hemicelluloses and solubilize lignin effectively. On the other hand, the reagents used in alkaline pre-treatment are sodium hydroxide, calcium hydroxide, potassium hydroxide, aqueous ammonia and ammonium hydroxide to increase cellulose accessibility by solubilizing lignin and hemicellulose (Taherzadeh and Karimi 2008). The effect of chemical pre-treatment is highly dependent on the

Table 7.3 Advantages and disadvantages of technologies involved

Pre-treatment methods	Advantages	Disadvantages
Chemical—Acid	Simple and direct process	Expensive special corrosive-resistant reactors required
Chemical—Alkali	Efficient in solubilizing proteins and lignin	Longer reaction time than acid hydrolysis
Thermal	Widely use in the industry	Generation of inhibitors; high energy demand
Mechanical	Easily implemented, no odour generation and consume moderate energy	Only reduce the size of substrate
Enzymatic	Low capital cost and little dependence of chemicals	Requires longer retention time than other processes

types of method used and the characteristics of substrate. It is more suitable to apply on the substrate that is rich in lignin (Li and Noike 1992).

Microorganisms, viz. brown, white and soft rot-fungi are used in biological pre-treatment to degrade lignin and hemicellulose so that the biodegradability of organic matter can be enhanced. The lignin is more resistant than cellulose and hemicellulose, thus the efficiency of microorganism to degrade lignin is slower (Taherzadeh and Karimi 2008). This technique required longer time due to low biological hydrolysing rate but is economic and low in energy demand (Hendriks and Zeeman 2009). Besides, it uses less chemicals and mild reaction conditions (Saritha and Arora 2012). Alternatively, enzyme obtained from these microorganisms can be directly applied to achieve similar results but in shorter time frame. An example of the enzyme is Viscozyme® L (Guan and Yao 2007), a mixture of carbohydrases produced by *Aspergillus aculeatus*. This mixture of enzymes is able to disintegrate cell wall tissue and help in protein release besides degrading complex carbohydrate into simple sugars. Other commercial enzymes that were used to hydrolyse carbohydrates into simpler form of sugars are α-amylase and glucoamylase (Kiran et al. 2015). In short, enzymes secreted by the microorganism or the commercial enzymes aim to enhance the availability of sugars by degrading the non-starch polysaccharides in the plant materials that exist in the food wastes, for example vegetables and fruits. Table 7.3 summarizes the advantages and disadvantages of different technologies on pre-treating the food wastes on large scale. An efficient pre-treatment may be the combination of these methods. For example, mechanical pre-treatment which breakdown the size of wastes will increase the surface area of the particle, which in turn will increase the rate of reaction for the processes, namely enzymatic pre-treatment or chemical pre-treatment.

7.3 Bacterial Cellulose

Cellulose is an important material that can be synthesized by plants, algae and microorganisms. The homogenous D-glucose sugar units are linked linearly by a β-linkage and form the cellulose. The degree of polymerization ranges from 2000 to 10,000 units (Jonas and Farah 1998; Gallegos et al. 2016; Hussain et al. 2019). Conventional source of cellulose is normally obtained from the plant. However, plant cellulose has impurities such as hemicellulose and lignin which require harsh treatment for removal. The wastes from the treatment, if not handled properly, may lead to environmental pollution. Furthermore, producing cellulose from the plant had created great problems to biosphere. To overcome these problems, the use of bacterial cellulose (BC) has been suggested.

Bacterial cellulose is also termed as microbial cellulose (Gallegos et al. 2016). It is described as a strong white gelatinous pellicle on the surface of a liquid medium. The bacterial cellulose produced by bacteria is much better than the cellulose from plants. It is because of its high purity, ultra-fine network structure, high biodegradability and unique mechanical strength (Ullah et al. 2016; Hussain et al. 2019). Other than that, it has a higher crystalline structure, tensile strength, water holding capacity, biocompatibility and free from other structural components, such as lignin and hemicellulose (Hussain et al. 2019). The other useful properties that it possesses are good light transmittance, in situ moldability, high porosity, good stability, low immunogenic potential, and the capability for cell adhesion and proliferation (Ullah et al. 2016). As a result, BC has been widely recognized as a multifunctional biomaterial and performs better in areas such as food, cosmetics, biomedicine, paper production, and textile industry (Moniri et al. 2017; Velásquez-Riaño and Bojacá 2017). BC may also be used in human food and animal feed industries, especially as a thickening and gelling substance, stabilizer, water binding material, and packaging material (Ullah et al. 2016). The BC has also been shown to be a good low-calorie food additive (Shi et al. 2014).

7.3.1 Synthesis of Bacterial Cellulose

Bacterial cellulose is typically produced by acetic acid bacteria from genera *Aerobacter, Acetobacter, Achromobacter, Agrobacterium, Alacaligenes, Azotobacter, Pseudomonas, Rhizobium* and *Sarcina*. Other microorganisms with such capability are algae and fungi. *Acetobacter* species (Hussain et al. 2019; Gallegos et al. 2016) can produce huge quantities of good quality cellulose that meet commercial requirements. *Acetobacter xylinum* is the most efficient producer of BC. It has the capability to utilize different sugars or compounds as the carbon source.

Figure 7.1 shows the synthesis pathway from glucose to cellulose in *G. xylinus* (adapted from Jonas and Farah (1998) and Ullah et al. (2016)). The synthesizing process involves four major enzymatic catalysed steps. Step 1 is the phosphorylation of glucose to glucose-6-phosphate by enzyme glucokinase. Step 2 is an isomerization process that is driven by enzyme phosphoglucomutase. In this step,

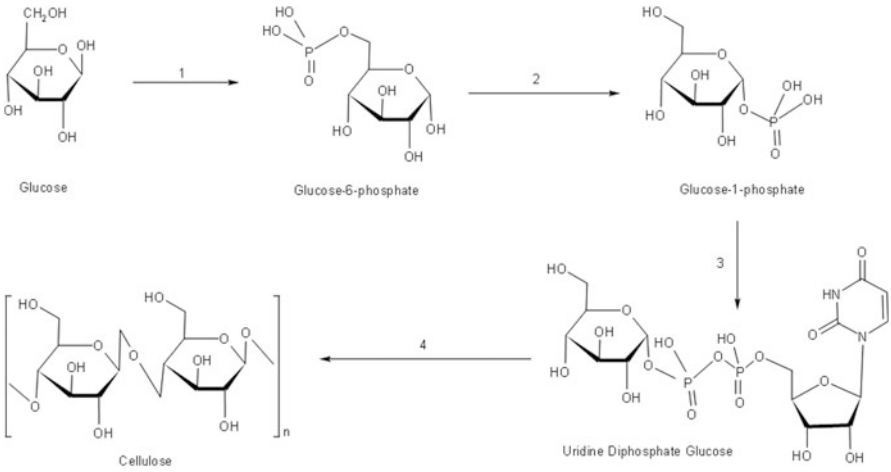


Fig. 7.1 Synthesis pathway from glucose to cellulose in *G. xylinus*. *Note*: The enzymes involved in four steps are: (1) glucokinase, (2) phosphoglucomutase, (3) UDP-glucose pyrophosphorylase and (4) cellulose synthase. (*Source*: Adapted from Jonas and Farah (1998) and Ullah et al. (2016))

glucose-6-phosphate is isomerized into glucose-1-phosphate. Enzyme UDP--glucose pyrophosphorylase is involved in step 3 to convert glucose-1-phosphate into uridine diphosphate glucose (UDP). Final step is the formation of glucan chains from UDP-glucose to form cellulose with the assistance of enzyme cellulose synthase. This synthesizing process was reported to occur between the space of cytoplasmic membrane and outer membrane of the microorganism. The cellulose produced will be aggregated and crystallized to form microfibrils, which leave the pores at the surface of the bacteria and aggregated with other synthesize microfibrils. Finally, these aggregated microfibrils polymerize to become a ribbon of crystalline cellulose. The final bacterial cellulose harvested is typically in the form of colourless and odourless gel after proper washing process (Ullah et al. 2016). Nonetheless, bacteria from different genus may produce cellulose in different forms, e.g. pellicle or cellulose ribbons (*Acetobacter*), cellulose fibrils (*Achromobacter, Aerobacter, Alcaligenes*), short fibrils (*Agrobacterium, Rhizobium*), amorphous cellulose (*Sarcina*), etc. (Jonas and Farah 1998). Cellulose formation was reported to occur at the air/cellulose pellicle interface as oxygen is an important requirement for the bacteria to grow.

7.3.2 Media for Bacterial Cellulose Production

To obtain high yield of BC, nutrient composition of the growing media is a key factor. Carbon, nitrogen, oxygen, hydrogen and micronutrients such as iron, zinc and vitamins are typical components required for the synthesis of various metabolites and healthy growth of microorganisms. Microbes degrade or polymerize these

components to synthesize specific metabolites. Thus, availability of these nutrients to microbes will affect the production rate of the specific metabolites. For BC production, main nutrients required in the culture media are carbon and nitrogen sources, together with salts for pH buffering.

7.3.2.1 Influence of Carbon Source

A carbon source is the major requirements for cells, which provide energy for growth and synthesis of primary and secondary metabolites. According to Keshk and Sameshima (2005) and Molina-Ramírez et al. (2017), glucose is the major preferred carbon source by *Komagataeibacter* sp. Nevertheless, it is able to synthesize glucose from various substrate, e.g. fructose, sucrose, glycerol (Keshk and Sameshima 2005). Besides growth, glucose is polymerized into cellulose by this microbe. Therefore, a large amount of hydrolysable carbon source will ensure a high yield of BC.

Besides glucose, a wide range of other sugars have been tested for their ability to become the main carbon source for *Gluconacetobacter xylinus* in producing bacterial cellulose, for example galactose, xylose, mannose, sucrose, glycerol (Jonas and Farah 1998; Ramana et al. 2000). Jonas and Farah (1998) reported that besides arabitol and mannitol, all other sugars give a lower yield of cellulose as compared to glucose. They stated that the reason behind is not clear, but the researcher postulated that it might be due to the stability of medium pH as gluconic acid was not produced by the bacteria fed with these sugars. Ramana et al. (2000), on the other hand, reported that sucrose, glucose and mannitol were suitable for optimum levels of cellulose production. When other sugars are present together with glucose in the media, bacteria will normally consume glucose first, and other sugars will only enter into the metabolism after depletion of glucose.

7.3.2.2 Influence of Nitrogen Source

Besides growth, nitrogen is required for the synthesis of proteins and nucleic acids, or more generally, for cell maintenance. Provided that there is plenty of carbon available and all nutrients required are present in the initial medium, nitrogen amount in a medium will determine the amount of biomass produced by a particular cell line. Typical nitrogen sources are ammonia, nitrogen-based salts such as ammonium sulphate and ammonium chloride, and complex nitrogen source such as yeast extract and soya bean meal (Harvey and McNeil 2008). Sometimes amino acids such as methionine and glutamate were also used.

Yodsuwan et al. (2012) reported that yeast extract when used alone as a nitrogen source in the medium supplemented with sucrose will produce higher yield of bacterial cellulose in relative to the medium that added ammonium sulphate. They found that combination of two nitrogen sources (5 g/L each) will increase the bacterial cellulose yield significantly. The best combination is determined to be yeast extract and Casein hydrolysate, with 1.15-fold increment in yield in relation to control (yeast extract + ammonium sulphate).

7.3.2.3 Commercial Media for BC Production

Hestrin and Schramm (HS) medium is the most common medium used for BC production. It contains (w/v): 2% glucose, together with peptone (0.5%), yeast extract (0.5%), disodium phosphate (0.27%) and citric acid (0.115%) (Molina-Ramírez et al. 2017). It is a chemically defined medium as the components and concentrations of the chemicals are known. Undefined media, as opposed to chemically defined media, consist of components in unknown concentration from natural sources. Other types of media modified from HS medium are HS-ascorbic acid medium, Hassid-Barker medium, modified HS media, Yamanaka medium, yeast extract-peptone-dextrose medium, acetate buffered medium, etc. (Hussain et al. 2019).

The cost of medium in a fermentation process accounts for 50–65% of the total production cost. The chemically defined media discussed above is expensive and impractical to be used for commercial scale BC production. Consequently, most of the researchers sorted for alternative cheap medium like agro-industrial wastes, wastes from pulp mills, textile mills, biorefineries, etc. (Hussain et al. 2019). Food wastes, which are reported to contain high amount of nutrients, are another potential resources.

7.4 Agro-industrial Waste

Various agro-industrial wastes had been used as a feedstock for the production of bacterial cellulose. Some examples are citrus peels, pineapple and watermelon peels, extracted date syrup, sago by-product, etc. Citrus peels which include lemon, mandarin, orange and grapefruit had been acid hydrolysed with dilute sulphuric acid to obtain the hydrolysate and used as the medium for bacterial cellulose production without any supplementation (Güzel and Akpinar 2019). *Komagataeibacter hansenii* GA2016 was used in their study, where 3.92% (w/w) yield could be obtained from the mandarin peel hydrolysate. They determined the composition of mandarin peel hydrolysate to be consisted of protein (39.9 mg/L), glucose (7.21 g/L), galactose (6.11 g/L), arabinose (2.33 g/L), citric acid (0.65 g/L), acetic acid (0.25 g/L) and total phenolic (5.42 gGAE/L). Even though mandarin peel hydrolysate gave the highest yield, all bacterial cellulose produced from the citrus peel wastes had similar properties with those produced using commercial media.

Pineapple peel and watermelon peel were tested by Kumbhar et al. (2015) to culture *Komagataeibacter hansenii* MCM B-967 in producing bacterial cellulose. The peel juices were supplemented with 5% table sugar, 0.7% ammonium sulphate and 0.02% cycloheximide. Comparing with HS medium (53.3 g/100 g reducing sugar, 3.69 g/100 g total nitrogen), pineapple peel juice (44.42 g/100 g) and watermelon peel juice (35.55 g/100 g) had a lower reducing sugar content and lower total nitrogen contents (1.82 g/100 g and 2.04 g/100 g, respectively). Different from HS medium which contains mainly glucose, both pineapple and watermelon peel juice contains glucose, fructose and sucrose (Kumbhar et al. 2015). The cellulose yield was found to be highest in pineapple peel juice medium (12.5 g/

100 ml, wet weight), followed by watermelon peel juice medium (10 g/100 ml) and HS medium (3 g/100 ml). Kumbhar et al. (2015) observed that the microfibril ribbon from HS medium is long, uniform and arranged in an ordered manner, but the microfibril ribbon produced from the fruit peels juice medium is shorter and in irregular arrangement. This difference is postulated to be due to the presence of other sugars that hinder the aggregation of cellulose chains into microfibrils.

Ultrasonic extracted date syrup was used by Lotfiman et al. (2016) as a carbon source to substitute glucose in the HS medium for culturing of *Acetobacter xylinum* 0416. The sugar contents in the date syrup were determined to be 47.1% glucose, 28% fructose and 4.7% sucrose. The study was performed using 20–50 g/L of extracted date syrup, and the highest yield of bacterial cellulose (5.8 g/L) was obtained in the culture supplemented with 30 g/L of extracted date syrup. Similar observation with that of Kumbhar et al. (2015) was reported by Lotfiman et al. (2016), where the microfibrils formed in the medium supplemented with extracted date syrup were in irregular arrangement.

Another study performed by Voon et al. (2019) used enzymatic hydrolysed sago by-product as carbon source and two types of bacteria, i.e. *Beijerinckia fluminensis* WAUPM53 and *Gluconacetobacter xylinus* 0416, to produce bacterial cellulose. The sago by-product hydrolysate in the medium has a glucose concentration of 2% and is supplemented with 0.5% peptone, 0.5% yeast extract and 0.27% disodium phosphate. The yield of cellulose is lower than HS medium (0.52 g/L) when sago by-product hydrolysate (0.47 g/L) was used as the carbon source and fermented by *B. fluminensis* WAUPM53. Nonetheless, the cellulose yield produced by *G. xylinus* 0416 was comparable in both the media (1.57 g/L in HS medium and 1.55 g/L in sago by-product medium) and significantly higher than that of *B. fluminensis* WAUPM53.

Concluding from the studies presented above, agro-industrial wastes with high lignocellulosic content or high sugar contents are potential cheap media in replacing commercial chemically defined medium like HS medium. Agro-industrial wastes typically contain various type of sugars initially or after hydrolysis, these sugars are not simple glucose that can be directly utilized by the bacteria. Metabolism of these complex sugars requires longer time and following different pathway, thus reducing gluconic acid production which in turn increases the yield of cellulose produced. High amount of glucose in the medium may not be good as along the metabolism of glucose, gluconic acid will be produced. When the glucose concentration is high, gluconic acid produced will also be great in amount. Presence of large amount of gluconic acid resulting in dropping of pH medium, which affects the cell viability and thus drops in the cellulose yield. Moreover, concentration of carbon source is also an influential factor on the arrangement of the BC fibre network. Too high a concentration of carbon source results in a denser fibre network and reduces water holding capacity. Therefore, it can be concluded that the properties of BC are affected by nutrient sources and their concentration.

7.4.1 Cultivation Conditions

Bacteria cellulose can be cultured in either static or stirring modes. A more common mode that has been practised for long is static mode. In static culture, the medium is placed in shallow trays and inoculated with bacteria. To produce thin film of cellulose as depicted in Fig. 7.2a, a 7 days' culture is sufficient. If Nata de Coco (a thicker form of BC, which is a popular dessert massively produced in Philippines) is the target, the culture period can take up to 14–21 days. Figure 7.2b depicts Nata de Pina produced using pineapple peel juice. Producing BC in static mode is expensive as the productivity is low. The efficiency of BC production in stationary cultures is strongly connected with the area of air–liquid interface. The bigger the surface, the higher is the efficiency. Therefore, to increase the cellulose yield, a large area of culture vessel with shallow depth will be required. This is impractical in the commercial set-up as it will take up a large space and increase the risk of contamination with other microorganisms, which in turn will reduce the yield as competition for nutrients among the bacteria and the invaded species will occur.

In agitated culture, aeration is improved. Increased level of oxygen dissolved in the medium resulted in high cell concentration and productivity. Intense mixing in the agitated culture not only influenced the bacteria cell growth, the high shear stress generated also affects the physical and structural properties of the BC. The shape of BC produced become either irregular in wet form and spherical after freeze-dried (Fig. 7.3). The properties of the BC produced through agitated culture have a lower crystallinity, crystal size, and degree of polymerization as compared to those produced through static culture (Reiniati et al. 2017; Czaja et al. 2004). However, Chen et al. (2018) reported that scaling up agitated culture to 75 l was able to improve these properties of BC.

Besides batch static or agitated cultures as described above, bacterial cellulose is sometimes cultivated in semi-continuous or fed-batch and also in continuous mode. Mode of operation chosen depends on the requirement of cellulose thickness. Further, the mode of operation greatly affects the yield and productivity.

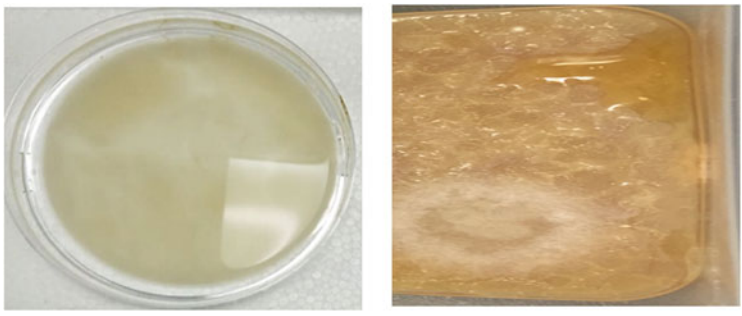


Fig. 7.2 (**a**) Thin film of BC from 7 days culture, (**b**) thick layer of BC from 14 days culture

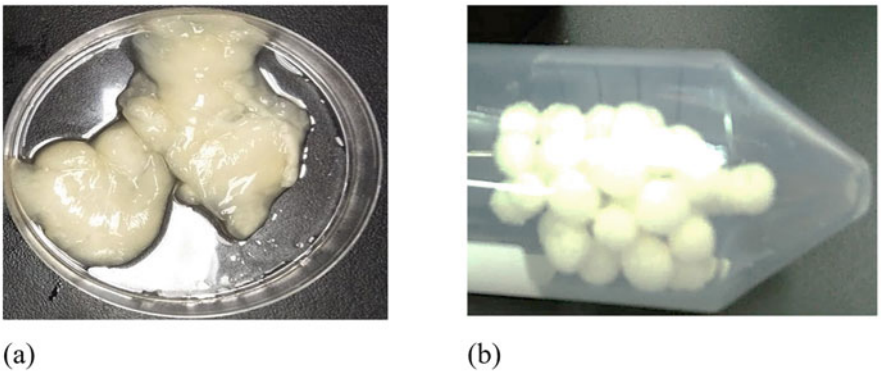


Fig. 7.3 BC produced using hydrothermal pre-treated food waste hydrolysate. Culture conditions: pH 5.5, 30°C, 10% inoculum, 250 rpm. (**a**) Wet condition, (**b**) freeze-dried condition

Other culture conditions such as medium pH, culture temperature, and inoculum density only affected cell density and viability, which will directly influence the cellulose yield obtained. They have minimal impact on the cellulose properties obtained. Typical culture conditions employed by most of the researchers is 28–30 °C, medium pH 5–6 and 10% v/v inoculum density.

Studies have shown that food waste hydrolysate is a better media for BC production as it contains both carbon and nitrogen sources that are required for cell growth, maintenance, and product formation. Pineapple peel juice, on the other hand, contains solely carbon source. Without extra supplementation, pineapple peel juice alone may not be sufficient in substituting commercial media. Pineapple peel juice is a defined waste medium, while food waste hydrolysate is a complex undefined waste medium. This is because pineapple peel juice has been reported to contain mainly 2.04% fructose, 2.18% glucose and 3.04% sucrose by Siti Roha et al. (2013) for pineapple species N36. Even though the composition of sugars is highly dependent on species and maturity, the composition is within a range that could be determined. Conversely, food wastes that are collected every day from the café will be varied in unpredictable range. Thus, to use food waste hydrolysate for commercial production will be a great challenge as the product quality and product yield will be dependent on the composition of the food wastes hydrolysate obtained daily.

7.5 Bacterial Cellulose Production Using Food Wastes Hydrolysate and Pineapple Peel Juice

Figure 7.4 shows the bacterial cellulose produced using hydrothermal pre-treated food waste hydrolysate (a and b) and pineapple peel juice (c and d). Cellulose produced using hydrothermal treated food waste hydrolysate has a higher yield, and thus, thicker gel is formed. It looks slightly opaque as compared to the cellulose

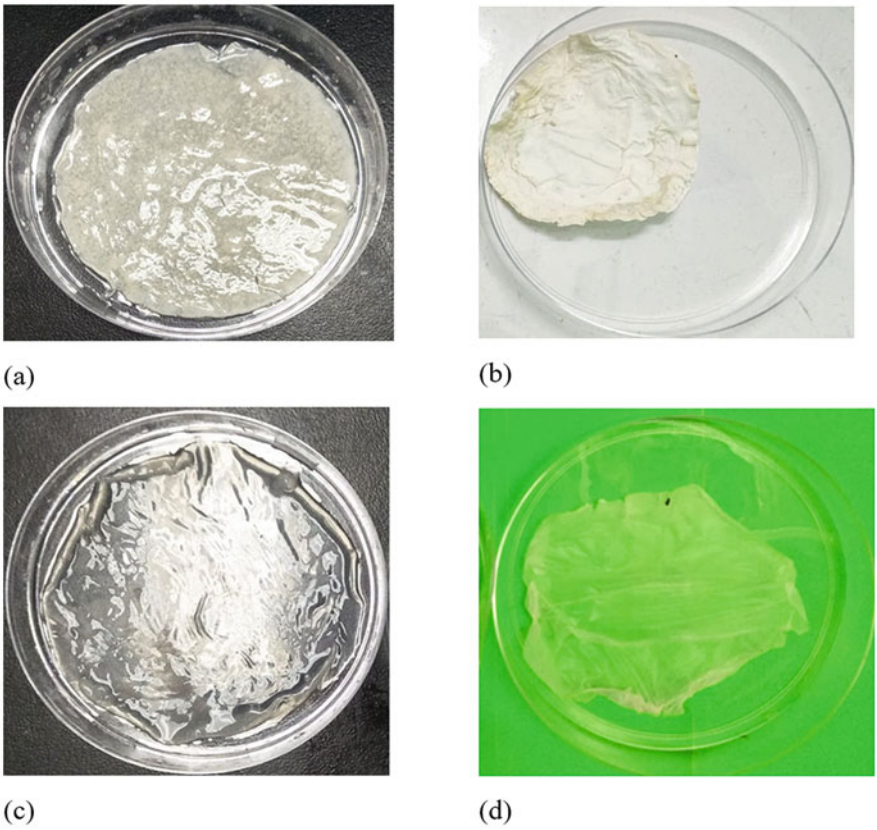


Fig. 7.4 BC produced using hydrothermal pre-treated food waste hydrolysate (**a**, **b**) and pineapple peel juice (**c** and **d**). (Note: Culture conditions: pH 5.5, 30°C, 10% inoculum, static condition; (**a**) and (**c**) are wet condition and (**b**) and (**d**) oven-dried condition)

from pineapple peel juice. Figure 7.4b is the resultant BC after oven-dried, which looks like a filter paper. Figure 7.4c and d, on the other hand, are cellulose produced using the pineapple peel juice as a medium without any supplementation. It can be seen that the cellulose obtained is a transparent thin gel in the wet condition and resembles a membrane at the oven-dried condition.

Figure 7.5 shows the scanning electron microscopy (SEM) image of the BC produced using different media under static conditions. It can be seen that the morphology of BC produced is quite similar, except that the cellulose fibre produced with pineapple peel juice is rather pure and clean if compared to that using food waste hydrolysate. The red circle in Fig. 7.5b illustrates foreign particles that were entrapped in the cellulose fibre structure. In addition to this, BC produced from food waste hydrolysate is more compact and less porous as depicted in Fig. 7.5b by the arrows. This is supported by the results of water holding capacity, where the water holding capacity of the BC from food waste hydrolysate was 6.5% lower than that of

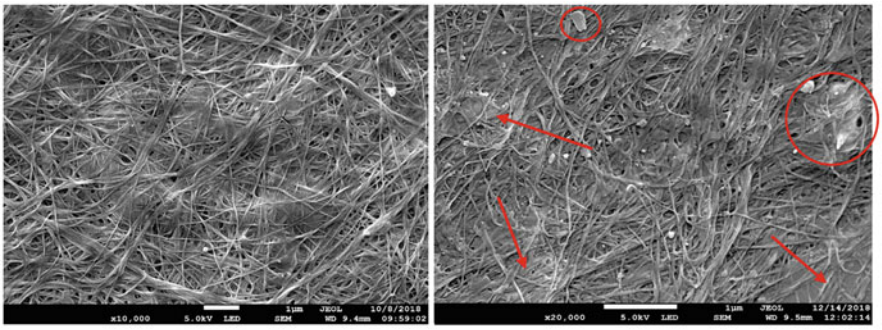


Fig. 7.5 SEM image of BC produced by using (**a**) pineapple peel juice (×10,000 magnification) and (**b**) food waste hydrolysate (×20,000 magnification)

BC from pineapple peel juice. Compact arrangement of cellulose fibre means lower number of pores, thus lowering the ability of BC to absorb water (UI-Islam et al. 2012). The foreign particles entrapped would also occupy the pores and further reduced the pore numbers. Besides, the compact area was believed to be oil that trapped between the fibres, which further repelled the water molecules. It can be noted that hydrothermal pre-treated food waste hydrolysate is a potential cheap media for commercial scale BC production. Nonetheless, further study is required to identify a feasible system of waste collection and pre-treatment in order to ensure more consistent composition and better removal of oil for cheap and efficient production of BC.

7.6 Conclusion

Bacterial cellulose (BC) is gaining importance due to its higher purity and better properties. Further, bacterial cellulose has numerous industrial applications. Nevertheless, large-scale production of bacterial cellulose demands feedstock which are cheap and available in plenty. In this regard, agricultural wastes such as pineapple waste, citrus peel waste and extracted date syrup are found to be potential candidate. Besides the agricultural wastes, food wastes are equally important from the point of view of bacterial cellulose production. Most of these agro-wastes are considered as defined medium as the changes of the composition is rather small. On the other hand, food wastes are known for its inconsistent composition and thus a complex undefined medium. This chapter discussed the bacterial cellulose production for agricultural and food wastes. It can be concluded that hydrothermal pre-treated food waste hydrolysate is a potential cheap media for commercial scale BC production. However, intensive study is required to identify a feasible system of waste collection and pre-treatment in order to ensure more consistent composition and better removal of oil for cheap and efficient production of BC.

Acknowledgement The authors gratefully acknowledge the financial support received from Universiti Malaysia Pahang (RDU 170329).

References

Abdullah B, Mat H (2008) Characterization of solid and liquid pineapple waste. Reaktor 12:48–52
American Public Health Association (APHA) (2012) Standard methods for the examination of water and wastewater. Standard methods, p 541
Ariunbaatar J, Panico A, Esposito G, Pirozzi F, Lens PNL (2014) Pretreatment methods to enhance anaerobic digestion of organic solid waste. Appl Energy 123:143–156
Bligh E, Dyer W (1959) A rapid method of total lipid extraction and purification. Can J Biochem Phys 37:911–917
Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
Cavka A, Guo X, Tang S-J, Winestrand S, Jönsson LJ, Hong F (2013) Production of bacterial cellulose and enzyme from waste fiber sludge. Biotechnol Biofuels 6:25–34
Chen L, Hong F, Yang X et al (2013) Biotransformation of wheat straw to bacterial cellulose and its mechanism. Bioresour Technol 135:464–468
Chen G, Wu G, Alriksson B et al (2018) Scale-up of production of bacterial nanocellulose using submerged cultivation. J Chem Technol Biol 93:3418–3427
Chua GK, Tan FHY, Chew FN, Mohd-Hairul AR (2019) Nutrients content of food wastes from different sources and its pre-treatment. AIP Conf Proc 2124:020031. https://doi.org/10.1063/1.5117091
Czaja W, Romanovicz D, Brown RM (2004) Structural investigations of microbial cellulose produced in stationary and agitated culture. Cellulose 11:403–411
Dubois M, Gilles K, Hamilton J et al (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356
FAO (2018) Gender and food loss in sustainable food value chains – A guiding note. Food and Agriculture Organization of the United Nations, Rome. http://www.fao.org/3/I8620EN/i8620en.pdf. Accessed 13 July 2019
FAO (2019) Food and Agriculture Organization of the United Nations. http://www.fao.org/food-loss-and-food-waste/en/. Accessed 13 July 2019
Gallegos AMA, Carrera SH, Parra R et al (2016) Bacterial cellulose: a sustainable source to develop value-added products – a review. Bioresources 11(2):5641–5655
Guan X, Yao H (2007) Optimization of Viscozyme L-assisted extraction of oat bran protein using response surface methodology. Food Chem 106:345–351
Güzel M, Akpinar Ö (2019) Production and characterization of bacterial cellulose from citrus peels. Waste Biomass Valorization 10:2165–2175
Harvey LM, McNeil B (2008) The design and preparation of media for bioprocesses. In: McNeil B, Harvey LM (eds) Practical fermentation technology. Wiley, West Sussex
Hendriks ATWM, Zeeman G (2009) Pre-treatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 100:10–18
Hussain Z, Sajjad W, Khan T et al (2019) Production of bacterial cellulose from industrial wastes: a review. Cellulose 26:2895–2911
Jonas R, Farah LF (1998) Production and application of microbial cellulose. Polym Degrad Stab 59:101–106
Keshk SMAS, Sameshima K (2005) Evaluation of different carbon sources for bacterial cellulose production. Afr J Biotechnol 4:478–482
Kiran EU, Trzcinski AP, Liu Y (2015) Enhancing the hydrolysis and methane production potential of mixed food waste by an effective enzymatic pretreatment. Bioresour Technol 183:47–52
Kumbhar JV, Rajwade JM, Paknikar KM (2015) Fruit peels support higher yield and superior quality bacterial cellulose production. Appl Microbiol Biotechnol 99:6677–6691

Li YY, Noike T (1992) Upgrading of anaerobic digestion of waste activated sludge by thermal pretreatment. Water Sci Technol 26:857–866

Lotfiman S, Awang Biak D, Ti T, Kamarudin S, Nikbin S (2016) Influence of date syrup as a carbon source on bacterial cellulose production by Acetobacter xylinum 0416. Adv Polym Technol 37:1085–1091. https://doi.org/10.1002/adv.21759

Machado RTA, Meneguin AB, Sábio RM et al (2018) *Komagataeibacter rhaeticus* grown in sugarcane molasses-supplemented culture medium as a strategy for enhancing bacterial cellulose production. Ind Crop Prod 122:637–646

Molina-Ramírez C, Castro M, Osorio M et al (2017) Effect of Different Carbon Sources on Bacterial Nanocellulose Production and Structure Using the Low pH Resistant Strain *Komagataeibacter Medellinensis*. Materials 10:639. https://doi.org/10.3390/ma10060639

Moniri M, Moghaddam AB, Azizi S et al (2017) Production and status of bacterial cellulose in biomedical engineering. Nanomaterials 7:257. https://doi.org/10.3390/nano7090257

Ramana KV, Tomar A, Singh L (2000) Effect of various carbon and nitrogen sources on cellulose synthesis by *Acetobacter xylinum*. World J Microbiol Biotechnol 16:245–248

Reiniati I, Hrymak AN, Margaritis A (2017) Kinetics of cell growth and crystalline nanocellulose production by *Komagataeibacter xylinus*. Biochem Eng J 127:21–31

Saritha M, Arora A (2012) Biological pretreatment of lignocellulosic substrates for enhanced delignification and enzymatic digestibility. Indian J Microbiol 52:122–130

Shi Z, Zhang Y, Phillips GO et al (2014) Utilization of bacterial cellulose in food. Food Hydrocolloid 35:539–545

Sindhu R, Gnansounou E, Rebello S, Binod P, Varjani S, Thakur IS, Nair RB, Pandey A (2019) Conversion of food and kitchen waste to value-added products. J Environ Manage 241:619–630

Siti Roha AM, Zainal S, Noriham A, Nadzirah KZ (2013) Determination of sugar content in pineapple waste variety N36. Int Food Res J 20:1941–1943

Taherzadeh MJ, Karimi K (2008) Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. Int J Mol Sci 9:1621–1651

Triantafyllidis KS, Lappas AA, Stöcker M (2013) The role of catalysis for the sustainable production of bio-fuels and bio-chemical. Elsevier, Amsterdam

UI-Islam M, Khana T, Parka JK (2012) Water holding and release properties of bacterial cellulose obtained by in situ and ex situ modification. Carbohyd Polym. 88:596–203

Ullah H, Santos HA, Khan T (2016) Applications of bacterial cellulose in food, cosmetics and drug delivery. Cellulose 23:2291–2314

Velásquez-Riaño M, Bojacá V (2017) Production of bacterial cellulose from alternative low-cost substrates. Cellulose 24:2677–2698

Voon WWY, Muhialdin BJ, Yusof NL, Rukayadi Y, Meor Hussin AS (2019) Bio-cellulose production by *Beijerinckia fluminensis* WAUPM53 and *Gluconacetobacter xylinus* 0416 in sago by-product medium. Appl Biochem Biotechnol 187:211–220

Wan Mohamed Radzi CWJ, Abdul Murad MHS, Bakar O (2010) Food intake in Malaysian culture and society: focus on the younger generation. http://repository.um.edu.my/id/eprint/86384

Yodsuwan N, Owatworakit A, Ngaokla A, Tawichai N, Soykeabkaew N (2012) Effect of carbon and nitrogen sources on a bacterial cellulose production for bionanocomposite materials. In: First Mae Fah Luang University International Conference 2012, Chiang Rai, Thailand. http://mfuic2012.mfu.ac.th/electronic_proceeding/Documents/00_PDF/P-SC-B/P-SC-B-20%20Nutthawut%20Yodsuwan%20(Should%20change%20font%20in%20figure).pdf

Yong ZJ, Bashir MJK, Ng CA, Sethupathi S, Lim JW, Show PL (2019) Sustainable waste-to-energy development in Malaysia: appraisal of environmental, financial, and public issues related with energy recovery from municipal solid waste. Processes 7:676–705

Transformation Process of Agricultural Waste to Chemical Production via Solid-State Fermentation

8

Farhan M. Said, Nor Farhana Hamid, Mohamad Al-Aamin Razali, Nur Fathin Shamirah Daud, and Siti Mahira Ahmad

Abstract

Agricultural waste is generated significantly worldwide. In tropical country like Malaysia, the largest biomass contributing sector is from palm oil plantation. In every palm oil production, the biomass produced was fourfold greater than the palm oil produced. In 2020, the wastes produced reached up to 39 million tons. The wastes comprise of the empty fruit bunches (EFB), palm kernel shell (PKS), mesocarp fiber (MF), palm oil mill effluent (POME), oil palm trunks (OPT), oil palm leaves (OPL), and oil palm fronds (OPF). Most of the wastes are being thrown to landfill, decomposed anaerobically, and led to severe environmental problems. Generally, the agricultural wastes are rich in carbohydrate content which are crucial for fermentation process. Thus, utilization of the waste to useful product, such as chemical production, via fermentation process especially, solid-state fermentation, is vast possibility.

Keywords

Fungal pigments · Oil palm · Oil palm frond · *Monascus* · Solid-state fermentation · Pigments

8.1 Introduction

Oil palm (*Elaeis guineensis*) trees originated from West Africa and have been grown widely in Malaysia (Sumathi et al. 2008; Wahid et al. 2005). Malaysia is known as the major producer and exporter of oil palm for decades, after Indonesia, which

F. M. Said (✉) · N. F. Hamid · M. A.-A. Razali · N. F. S. Daud · S. M. Ahmad
Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, Gambang, Pahang, Malaysia
e-mail: farhan@ump.edu.my

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_8

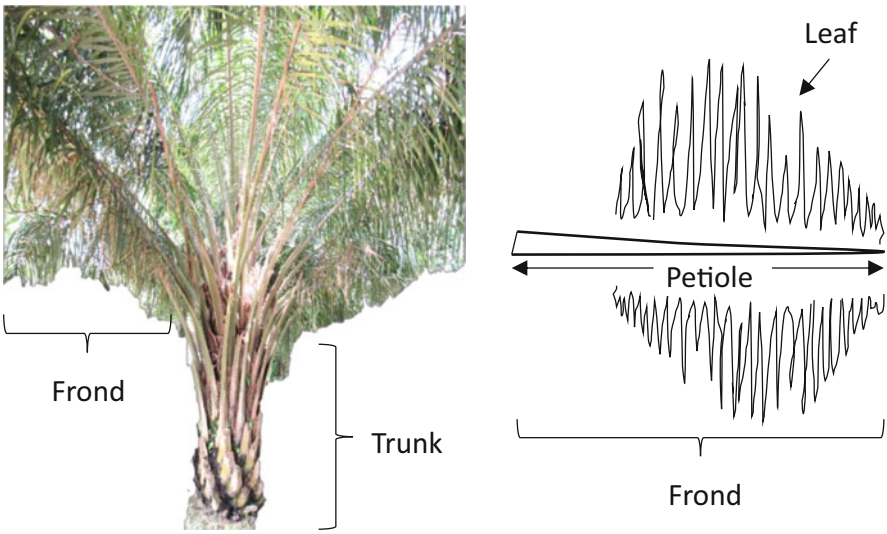


Fig. 8.1 Structure of an oil palm tree and oil palm frond

contributes nearly 60% of world's production. In Malaysia, the oil palm industry plantation covers about 5.81 million hectares, which allows to produce about 19.92 million and approximately 80–90 million tons of oil and biomass, respectively (MPOB 2018).

The progression of the oil palm industry in Malaysia has resulted in collective output of agricultural wastes. The wastes such as oil palm trunks (OPTs), mesocarp fiber (MF), palm kernel shell (PKS), empty fruit bunches (EFBs), palm oil mill effluent (POME), and oil palm fronds (OPFs) are generated gradually, during harvesting, processing, or replantation (Awalludin et al. 2015). Nearly all of the parts of oil palm trees, especially the waste, are commercially utilized, mainly in energy and manufacturing sectors (Sulaiman et al. 2015). Yet, OPFs have very limited usage. Each year, massive OPFs are produced, which is about 46.71% of the total waste from palm oil plantation (Inayat et al. 2019). These large quantities make it a very favorable source to be a substrate for fermentation process. In addition, the pre-eminent carbohydrates content in OPF make it useful for microbial development. Figure 8.1 shows the structure of tree and frond of oil palm.

8.2 Oil Palm Frond

Oil palm fronds (OPFs) refer to the leaves part of the palm tree and consist of three main components, namely leaflets, rachis, and petiole (Zahari et al. 2003). The top part of the frond, which are the rachis and leaflets, comprises of the major nutrient composition. While the petiole has a significant amount of cellulosic materials and sugars, which is fit for fermentation purposes.

Table 8.1 Organic composition of oil palm frond

Cellulose (%)	Hemicellulose (%)	Lignin (%)	Location	References
41.88	33.61	20.65	Malaysia	Long Wee Lai et al. (2016)
45.0	32.0	16.9	Malaysia	Megashah et al. (2018)
38.61	22.81	9.72	China	Wu et al. (2019)
32.7	22.5	15.2	Malaysia	Zakaria et al. (2014)

Table 8.2 Chemical compound in fractions of oil palm frond

Fractions	Petiole (%)	Leaflet (%)	Rachis (%)	Stem (%)
Hemicellulose	7.15	12.10	13.85	7.42
Cellulose	8.53	3.90	19.57	11.41
Lignin	2.86	5.91	1.79	2.53
Starch	1.87	1.26	0.94	1.55
Crude protein	0.90	2.55	1.76	0.80
Pectin	0.07	0.84	0.24	0.06

Source: Roslan et al. (2014)

Table 8.3 Free sugar composition in oil palm frond

Free sugar compositions (%)			Location	References
Glucose	Sucrose	Fructose		
73.00	16.00	11.00	Johor, Malaysia	Che Maail et al. (2014)
77.96	16.22	5.82	Penang, Malaysia	Lee and Halim (2014)
44.16	11.25	1.46	Pahang, Malaysia	Zani et al. (2019)
72.58	20.27	7.15	Penang, Malaysia	Saad et al. (2016)

The organic composition of oil palm frond (OPF) is presented in Table 8.1. Hemicellulose, cellulose, and lignin form the key constituent of OPF. The variation of the compositions was affected by several factors which include age of plant, soil condition, climate, and testing approaches. In addition, the variation also affected by the asymmetrical sectional zone which differs along the length of OPF (Nordin et al. 2016). The degradation of lignocellulosic material into cellulose which is then converted into simple sugar is ideal for the efficient growth of fungal cultures.

Table 8.2 further shows the chemical compositions of different sections of OPF. The cellulose and total carbohydrate of the petiole show higher yield over the midrib and leaflet (Roslan et al. 2014). The high cellulose content in OPF can be converted into valuable fermentable sugars/free sugar, which later can be used as the fermentation feedstock. Pretreatment of OPF could enhance conversion of cellulose to fermentable sugar (Hamid and Said 2016; Razali and Said 2017). The pretreatment could break the lignocellulosic material of OPF. However, an efficient pretreatment process should be considered based on the characteristics, for instance, (1) depolymerized hemicellulose, (2) preserved and decrystallized cellulose, (3) low energy input, and (4) cost effective (Agbor et al. 2011). Table 8.3 shows the free sugar contained in OPF, and Table 8.4 displays the macro minerals

Table 8.4 Elemental content (%) of different fractions of oil palm frond

Fractions	Si	P	S	Cl	K	Ca	Mn	Others
Leaflet	0.39	0.01	0.03	0.08	0.15	0.66	0.11	0.01
Petiole	0.15	0.02	0.03	0.11	0.5	0.77	nd	0.04
Rachis	0.26	0.02	0.04	0.12	0.43	0.87	0.1	0.01

Source: Roslan et al. (2014)

composition of OPF. OPF also contains crude fiber (41.00–43.51%) and crude protein (5.00–8.74%) (Atiqah and Sudin 2015; Harahap et al. 2018). Whereas, the total amino acids in OPF were 174.1 l g/g, mainly consist of serine (111.0 l g/g), glutamic acid (22.7 l g/g), and proline (27.1 l g/g) (Zahari et al. 2012).

8.3 Pretreatment of Oil Palm Frond (OPF)

Oil palm frond (OPF) is a lignocellulose material which is composed of lignin, cellulose, and hemicelluloses. Cellulose is the source that can be converted into glucose. While, the hemicelluloses and lignin impart the strength of the plant cell walls, besides protecting cellulose from enzymatic degradation (Lai and Idris 2013). Hence, the pretreatment of OPF must be applied to breakdown the lignocellulosic material into fermentable sugar.

A variety of physical (i.e., hydro-thermolysis), chemical (i.e., acid, alkali), physico-chemical (i.e., steam explosion, ammonia fiber explosion), and biological pretreatment systems have been designed to improve the accessibility of the enzymes to the cellulosic fibers (Mosier et al. 2005). Each pretreatment method has its own pros and cons. For instance, biological pretreatment such as degradation of lignin was conducted using white-rot fungi tends to offer a low chemical and energy consumptions at longer degradation time (Hatakka 1983). While, chemical pretreatments, which include ammonia fiber explosion (Lau et al. 2010), dilute acid hydrolysis (Amirkhani et al. 2015), and organosolv process (Teramura et al. 2016), have been observed to involve high processing cost and regarded as non-environmental friendly due to the use of toxic chemicals.

According to Sukri et al. (2013), autohydrolysis pretreatment is the best technique for the degradation of hemicellulose from lignocellulosic biomass. It is because autohydrolysis pretreatment can be conducted in a short period of time and environmentally friendly. In addition, by this technique, the corrosion issue can be eliminated, besides the sugar degradation can also be reduced. During the process, the morphology of OPF is distorted, where many pores are created and the lignocellulosic fibers are cracked (Lim et al. 2010), consequently exposing the cellulosic materials to be easily converted to the fermentable sugar. Figure 8.2 shows the scanning electron microscope (SEM) micrograph of the OPF. Table 8.5 shows the yield of the fermentable sugars produced by OPF after being treated using different technique.

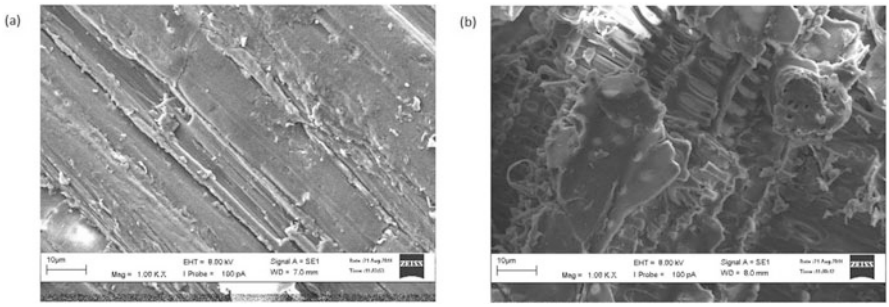


Fig. 8.2 SEM micrograph of the OPF under ×1000 magnification, (**a**) before autohydrolysis pretreatment, (**b**) after autohydrolysis pretreatment. (Source: Said and Hamid 2019)

Table 8.5 Yields of fermentable sugars after pretreatment process

Pretreatment technique	Fermentable sugars produced mg/g biomass
Alkali pretreatment	97.72 ± 9.5
Acid pretreatment	43.61 ± 11.5
Autohydrolysis pretreatment	127.00 ± 3.8

Source: Lim et al. (2010)

8.4 Role of Microorganisms to Transform the Waste into Valuable Products

Fermentable sugar or free sugar which is in OPF makes the component easily accessible to the microorganisms. Besides, it provides a viable solution to detrimental environmental effects. Microbes (i.e., *Monascus* sp.) are potential candidate for reprocessing and eventual utilization of OPFs to be valuable products (i.e., pigments, statins, antioxidants). These OPF can be used as raw material/substrate for the growth of various microbes which in turn used for various fermentation purposes, for instance, production of bioethanol (Srianta et al. 2014; Tan et al. 2016), pigment (Hamid and Said 2016; Razali and Said 2017), and biohydrogen (Hossain et al. 2016; Inayat et al. 2017).

8.4.1 *Monascus* sp.

Monascus sp. is a species of fungi in the family of *Monascaceae* (Jůzlová et al. 1996). Traditionally, *Monascus* species have been widely used in food and medicines for hundreds of years, particularly in the Orient country. *Monascus* can be classified into six species, which are *M. purpureus*, *M. pilosus, M. ruber, M. pallens, M. floridanus*, and *M. sanguineus* (Pattanagul et al. 2007). *Monascus* spp. are known as the best producers of primary metabolites (i.e., protein, acid, and

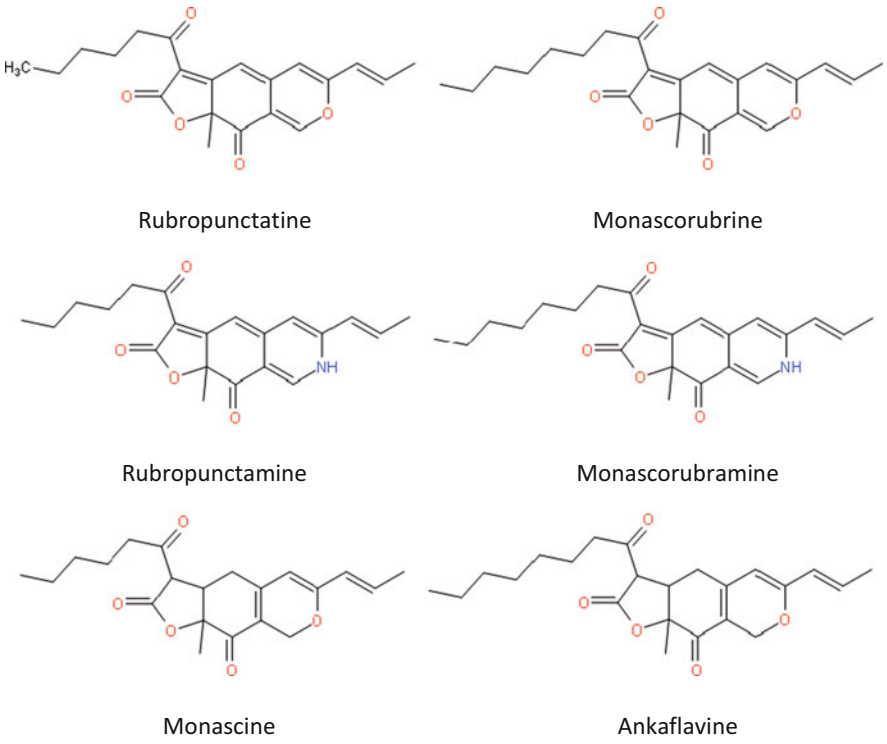


Fig. 8.3 Pigments of *Monascus* sp. (Source: Dufossé et al. 2005)

.

esters) and secondary metabolites products (i.e., pigments, antioxidant, monakolin K, antibacterial agent, and cholesterol lowering drugs) (Li et al. 2016; Pengnoi et al. 2017). For pigment production, *Monascus purpureus* is one of the most reported genus used (Torres et al. 2016). Several studies have revealed that colorants produced from *M. purpureus* do not only function as food colorants but is also able to exhibit biological activities such as antioxidant activities (Hsu and Pan 2012), anti-inflammatory (Cheng et al. 2012), anti-microbial, anti-obesity, and anti-cancer activities (Feng et al. 2012), as well as a supplement to fight hypercholesterolemia and hypertension (Lin et al. 2008).

Monascus pigments mainly have at least six molecular structures, which are categorized into three clusters based on their color and the azaphilone skeleton as in Fig. 8.3. They are orange pigments; monascorubrin ($C_{23}H_{26}O_5$) and rubropunctatin ($C_{21}H_{22}O_5$), red pigments; monascorubramine ($C_{23}H_{27}NO_4$) and rubropuntamine ($C_{21}H_{23}NO_4$) and the yellow pigments; monascin ($C_{21}H_{26}O_5$) and ankaflavin ($C_{23}H_{30}O_5$) (Nimnoi and Lumyong 2011; Pattanagul et al. 2007; Zhou et al. 2008). The color description is depending on the association of amino acid in the molecule structure. While, the orange pigments possess oxo-lactone ring, the red

pigments are the nitrogen analogs of the orange pigments (Nimnoi and Lumyong 2011). Besides being an edible *Monascus* pigment, it also possessed other beneficial properties such as stable at high range of temperature and stable at wide range of pH (Zhou et al. 2008).

8.5 Fermentation Condition in Solid-State Fermentation

There are two major factors that affect the performance in solid-state fermentation (SSF): biological and physico-chemicals factors.

8.5.1 Biological Factors

8.5.1.1 Types of Microorganisms

The process of microorganism selection is the most important factor that influences the fermentation product. The selection should consider a number of necessary features such as non-toxic, non-pathogenic in nature, ease of separation, yield of reasonable color, temperature tolerant, and pH tolerant (Babitha 2009). In addition, this process is dependent on the desired final product, type of medium, and growth condition (Krishna 2005). Fungi, bacteria, and yeast are generally known to be capable of growing in solid substrate (Raimbault 1998).

Filamentous fungi (i.e., *Monascus* sp.) are considered as prominent microorganism in SSF due to its natural physiological capabilities (Mitchell et al. 2011). In addition, the mode of mycelia growth is known to favor a relatively low flowing-water environment, which is suitable to the SSF condition. The hyphal mode of fungal growth, good tolerance to high osmotic pressure, and low water activity (Aw) conditions have caused the fungi to continue dominating as the best microorganism in SSF. Besides, the growth of hyphae provides power and strength to allow the filamentous fungi to penetrate the substrates and utilize the available nutrient.

8.5.1.2 Substrates

Solid substrate is one of the crucial factors in SSF. The substrate acts as a physical support and nutrients provider. Generally, agriculture or low-cost by-products of agro-industry are mostly utilized as solid substrates in SSF (Couto and Sanromán 2006). There are many low-cost by-products that can be utilized as the medium for fermentation of *Monascus,* which is able to produce the potential outcome in metabolites production. The agro-industrial residues, for instance OPF, are rich in nutrient and fermentable sugar; hence, it is applicable to be used as raw materials for the fermentation process (Panesar et al. 2016).

The reuse of agro-industrial waste in SSF for pigment production is quite significant as it can be easily obtained and its disposal is regarded to be environmentally friendly, for instance, corn steep liquor (Hamano and Kilikian 2006), sugarcane baggase (Silveira et al. 2013), soy bean meal (Choi et al. 2016), jackfruit seed (Babitha et al. 2007), and oil palm frond (Said and Hamid 2019). In several cases,

it is necessary to add other nutrients to the medium such as glucose or/and nitrogen sources (i.e., amino acids, ammonium nitrate) as suggested by Carvalho et al. (2003) in order to top up for the insufficient nutritional composition of the substrate.

8.5.2 Physico-Chemical Factors

8.5.2.1 Nitrogen Source

Additional nitrogen source to the substrate is essential to enhance the metabolite production; especially if the substrate contained limited nitrogen content. Nitrogen source such as peptone and sodium nitrate effectively supports the secondary metabolite production (Babitha et al. 2006). The yields of pigment production are significantly influenced by the percentage of nitrogen source in the substrate. The type of nitrogen apparently affects the pH condition and subsequently affects the growth and the pigment produced (Danuri 2008).

However, it was reported that the use of yeast extract to the *Monascus* sp. is not suitable for pigment production because it is only stimulating the biomass growth, not the pigment (Jůzlová et al. 1996). Hence, the selection of nitrogen source is important to promote the growth and pigment production. With the OPF as a substrate for *M. purpureus* FTC 5356 via SSF, 2% of peptone stimulated the best pigment formation (Said and Hamid 2019). Lower or higher % of nitrogen than the optimum value may interrupt the product formation.

8.5.2.2 Carbon Source

Carbon source is a crucial source for the fungal growth. The most commonly used carbon sources for *Monascus* species include glucose, maltose, starch, sucrose, and galactose. Glucose is found as the best carbon source for pigment production and *Monascus* growth (Lin and Demain 1991). Recently, the agro-industrial wastes, such as wheat flour, jackfruit powder, or OPF, have been utilized in SSF to support the growth of *Monascus* species (Hamid and Said 2016; Razali and Said 2017). The agro-industrial wastes which are rich in starch content improve the growth of *Monascus* species.

8.5.2.3 Trace Metal

Trace metal has been reported to influence the secondary metabolite production of *Monascus* species, especially when the substrate contained limited or no trace metal. The most commonly used trace metals include Zn^{2+}, Mn^{2+}, and Fe^{2+}. Low concentration of mineral supplements is significant for fungal growth. However, a very high concentration of trace metals can result in a negative effect, especially on pigment formation (Said 2010). Zinc is very important for the growth of fungal at low concentrations, but it was found to be toxic at higher concentrations (Bau and Wong 1979). OPF is a good substrate for the growth of *M. purpureus.* When the *M. purpureus* FTC 5356 grown on OPF, there is no need to add trace metals. As mentioned in the previous section, the OPF contains sufficient amount of the chemical constituents to support the growth of *M. purpureus.*

8.5.2.4 Initial Moisture Content

The success of SSF process is dependent on the initial moisture content of the substrate (Rashid et al. 2011). Generally, SSF restricted with little or no free-flowing water. The range of substrate moisture content is between 30 and 80% w/w (Manan et al. 2017). The moisture of the substrate serves as the mediator for nutrients, enzymes, vitamins, and metabolites transfer, including as an aid for oxygen absorption (Oriol et al. 1988).

At high moisture level, the substrates tend to agglomerate and lead to the poor aeration and possible anaerobic condition (Farinas 2015), which is favorable for bacterial growth. High moisture content increases the chance of bacterial contamination. In addition, too high moisture level leads to the suboptimal product formation, as it reduced the mass transfer process, and it dropped the heat transfer in the substrate (Singhania et al. 2009).

However, at lower moisture level, it limits the contact of nutrients to the microorganism (Babitha et al. 2007) and increases the water tension (Batten et al. 2006). Thus, it inhibits the growth of fungi (Manan 2014). Therefore, it is crucial to ensure optimum moisture level of the substrate. For pigment production of *M. purpureus* FTC 5356 using OPF via SSF, initial moisture content of 50% improved the production of red pigment (Said and Hamid 2019).

8.5.2.5 Temperature

Temperature plays a vital role in SSF fungal development and metabolite production (Raimbault and Alazard 1980). In the SSF process, the temperature can rise as heat is produced during fungal respiration (Manan 2014). The heat that produced during fermentation needs to be eliminated in order to avoid overheating, which can affect the growth of microorganism, and subsequently disrupt the product formation (Pandey 2001). Yet, the temperature of the system should not be too low because the reaction rate of biochemical process can proportionally decrease with the decrease of the temperature (Pandey 2001). Temperature between 35 °C and 40 °C is considered optimum for fungal growth (Raimbault and Alazard 1980). For *M. purpureus* FTC 5357 using OPF, 30 °C is an appropriate temperature for the fungal growth via SSF.

8.5.2.6 Particle Size

Particle size of substrate is also significant factor in SSF (Niladevi et al. 2007). Smaller particle size increases the accessibility of fungi to the nutrient in the substrate. However, oxygen diffusion will be restricted at smaller particle size, due to the inter-particle space limitation (Pandey 2001). On the other hand, larger particle size allows good oxygen diffusion due to the smaller surface area to the volume ratio. Nevertheless, larger particle size might distract the accessibility of fungi to the nutrient. Therefore, appropriate particle size is required to fulfill the oxygen, nutrient, and mycelial growth demand. For the growth of *M. purpureus* FTC 5356 in OPF, 0.5 mm particle size is applicable for the fungal growth.

8.5.2.7 Initial pH

The initial pH of substrate is one of the important factors that can affect the yield of the final product because it can influence the growth and metabolic activity of microorganism during fermentation. However, it is very challenging to measure and control the pH during SSF, due to the substantial amount of water. For pigment production, pH 2–4 tends to produce yellow pigments, whereas pH 6–8 promoted red pigments production (Kang et al. 2014; Mukherjee and Singh 2011). For *M. purpureus* FTC 5356 grown in OPF via SSF, pH 7 was found to be better for both fungal and pigment productions (Said and Hamid 2019).

8.5.2.8 Aeration

In SSF, the major roles of aeration are to supply oxygen (O_2) for aerobic fermentation and removal of carbon dioxide (CO_2), water vapor, heat, and volatile components, which are produced during the process (Manan 2014). Microorganisms differ in their need for oxygen. Oxygen partial pressures above 1 atm will affect the aerobic microorganisms negatively, leading to growth inhibition and reduction in product formation and cell yield (Onken and Liefke 1989). For *Monascus* red pigment produced via SSF, higher aeration enhances the product formation (Chysirichote 2016; Razali and Said 2017; Said et al. 2010). With OPF as a substrate for *Monascus* pigment production, aeration rate of 1.0–1.2 vvm was reported to promote better pigment production (Razali and Said 2017; Said and Razali 2019).

However, too high oxygen supply may be toxic to the microorganisms (Onken and Liefke 1989). In heat and mass transfer mechanism, the aeration of saturated humidified air is a common strategy in SSF. Supply of humidified air aids to control the temperature and moisture gradients of the substrates (Umsza-Guez et al. 2011). Yet, the effect of oxygen on microbial growth and pigment production might vary not only due to the strain of microorganisms but also due to the culture conditions.

8.6 Conclusion

Agricultural waste especially oil palm frond (OPF) is a promising source for fermentation process, to be transformed into valuable products. Besides the utilization of abandoned waste, it offers the cost-effective strategy for the production of valuable product (i.e., pigment). As regards the utilization of OPF by *M. purpureus* via SSF for red pigment production, factors such as nitrogen source, initial moisture content, pH, and aeration play significant. Yet, pretreatment of OPF is needed prior to the SSF, to obtain the optimum yield of product formation. The utilization of low-cost substrate in red pigment production can be significantly improved by use of solid-state fermentation under conditions specified above.

References

Agbor VB, Cicek N, Sparling R, Berlin A, Levin DB (2011) Biomass pretreatment: fundamentals toward application. Biotechnol Adv 29(6):675–685. https://doi.org/10.1016/j.biotechadv.2011.05.005

Amirkhani H, Yunus R, Rashid U, Salleh FS, Radhiah ABD, Syam S (2015) Low temperatures dilute acid hydrolysis of oil palm frond. Chem Eng Commun 202:1235–1244

Atiqah N, Sudin Y (2015) A comparative study on the chemical composition of fermented oil palm fronds (opf) by using probiotics with fresh unfermented OPF. J Agrobiotechnol 6:71–78

Awalludin MF, Sulaiman O, Hashim R, Nadhari WNAW (2015) An overview of the oil palm industry in Malaysia and its waste utilization through thermochemical conversion, specifically via liquefaction. Renew Sust Energ Rev 50:1469–1484. https://doi.org/10.1016/j.rser.2015.05.085

Babitha S (2009) Microbial pigments. In: Singh nee' Nigam P, Pandey A (eds) Biotechnology for agro-industrial residues utilisation: utilisation of agro-residues. Springer, Netherlands, pp 147–162. https://doi.org/10.1007/978-1-4020-9942-7_8

Babitha S, Soccol CR, Pandey A (2006) Jackfruit seed - a novel substrate for the production of Monascus pigments through solid state fermentation. Food Technol Biotechnol 44(4):465–471

Babitha S, Soccol CR, Pandey A (2007) Solid-state fermentation for the production of Monascus pigments from jackfruit seed. Bioresour Technol 98(8):1554–1560. https://doi.org/10.1016/j.biortech.2006.06.005

Batten KM, Scow KM, Davies KF, Harrison SP (2006) Two invasive plants alter soil microbial community composition in serpentine grasslands. Biol Invasions 8(2):217–230. https://doi.org/10.1007/s10530-004-3856-8

Bau Y-S, Wong H-C (1979) Zinc effects on growth, pigmentation and antibacterial activity of Monascus purpureus. Physiol Plant 46(1):63–67. https://doi.org/10.1111/j.1399-3054.1979.tb03187.x

Carvalho JCD, Pandey A, Babitha S, Soccol CR (2003) Production of Monascus biopigments: an overview. Agro Food Ind Hi Tech 14:37–42

Che Maail CMH, Ariffin H, Hassan MA, Shah UKM, Shirai Y (2014) Oil palm frond juice as future fermentation substrate: a feasibility study. Biomed Res Int 2014:1–8. https://doi.org/10.1155/2014/465270

Cheng M-J, Wu M-D, Yuan G-F, Su Y-S, Yanai H (2012) Secondary metabolites produced by the fungus Monascus pilosus and their anti-inflammatory activity. Phytochem Lett 5(3):567–571. https://doi.org/10.1016/j.phytol.2012.05.015

Choi J, Rahman MM, Lee SY, Chang KH, Lee SM (2016) Effects of dietary inclusion of fermented soybean meal with Phaffia rhodozyma on growth, muscle pigmentation, and antioxidant activity of juvenile rainbow trout (Oncorhynchus mykiss). Turk J Fish Aquat Sci 16:91–101

Chysirichote T (2016) Effect of aeration mode on the red pigment production by Monascus ruber on rice during fermenting in the packed bed bioreactor. KKU Resour J 21(2):176–184

Couto SR, Sanromán MÁ (2006) Application of solid-state fermentation to food industry—a review. J Food Eng 76(3):291–302. https://doi.org/10.1016/j.jfoodeng.2005.05.022

Danuri H (2008) Optimizing Angkak pigments and lovastatin production by *Monascus purpureus*. HAYATI J Biosci 15(2):61–66. https://doi.org/10.4308/hjb.15.2.61

Dufossé L, Galaup P, Yaron A, Arad SM, Blanc P, Chidambara Murthy KN, Ravishankar GA (2005) Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? Trends Food Sci Technol 16(9):389–406. https://doi.org/10.1016/j.tifs.2005.02.006

Farinas CS (2015) Developments in solid-state fermentation for the production of biomass-degrading enzymes for the bioenergy sector. Renew Sust Energ Rev 52:179–188. https://doi.org/10.1016/j.rser.2015.07.092

Feng Y, Shao Y, Chen F (2012) Monascus pigments. Applied microbiology fermentation. Eur J Appl Microbiol Biotechnol 9:199–209

Hamano PS, Kilikian BV (2006) Production of red pigments by Monascus ruber in culture media containing corn steep liquor. Braz J Chem Eng 23:443–449. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0104-66322006000400002&nrm=iso

Hamid NF, Said FM (2016) Factorial design screening for the red pigment production by Monascus purpureus FTC 5356. Jurnal Teknologi 78(11–2):13–17. https://doi.org/10.11113/jt.v78.9937

Harahap RP, Jayanegara A, Nahrowi F, S. (2018) Evaluation of oil palm fronds using fiber cracking technology combined with Indigofera sp. in ruminant ration by Rusitec. AIP Conf Proc 2021 (2018):0500081–0500086. https://doi.org/10.1063/1.5062758

Hatakka AI (1983) Pretreatment of wheat straw by white-rot fungi for enzymatic saccharification of cellulose. Appl Microbiol Biotechnol 18:350–357

Hossain MA, Laxmi J, Ganesan P, Sahu J, Ramesh S, Poh SC (2016) Microwave pyrolysis of oil palm fiber (OPF) for hydrogen production: parametric investigation. Energy Convers Manag 115:232–243. https://doi.org/10.1016/j.enconman.2016.02.058

Hsu WH, Pan TM (2012) Monascus purpureus-fermented products and oral cancer: a review. Appl Microbiol Biotechnol 93(5):1831–1842. https://doi.org/10.1007/s00253-012-3891-9

Inayat A, Ahmad MM, Mutalib MIA, Yusup S, Khan Z (2017) Economic analysis and optimization for bio-hydrogen production from oil palm waste via steam gasification. Energy Sources B Econ Plann Policy 12(2):158–165. https://doi.org/10.1080/15567249.2014.937881

Inayat M, Sulaiman SA, Kurnia JC (2019) Catalytic co-gasification of coconut shells and oil palm fronds blends in the presence of cement, dolomite, and limestone: Parametric optimization via Box Behnken Design. J Energy Inst 92(4):871–882. https://doi.org/10.1016/j.joei.2018.08.002

Jůzlová P, Martínková L, Křen V (1996) Secondary metabolites of the fungus Monascus: a review. J Ind Microbiol 16(3):163–170. https://doi.org/10.1007/bf01569999

Kang B, Zhang X, Wu Z, Wang Z, Park S (2014) Production of citrinin-free Monascus pigments by submerged culture at low pH. Enzym Microb Technol 55:50–57. https://doi.org/10.1016/j.enzmictec.2013.12.007

Krishna C (2005) Solid-state fermentation systems-an overview. Crit Rev Biotechnol 25 (1–2):1–30. https://doi.org/10.1080/07388550590925383

Lai LW, Idris A (2013) Destruption of oil palm trunks and fronds by microwave- alkali pretreatment. Bioresour Technol 8(2):2792–2804

Lai LW, Ibrahim M, Md Rahim N, Hashim EF, Ya'cob MZ, Idris A, Akhtar J (2016) Study on composition, structural and property changes of oil palm frond biomass under different pretreatments. Cellul Chem Technol 50(9–10):951–959

Lau MJ, Lau MW, Gunawan C, Dale BE (2010) Ammonia Fiber Expansion (AFEX) pretreatment, enzymatic hydrolysis, and fermentation on empty palm fruit bunch fiber (EPFBF) for cellulosic ethanol production. Appl Biochem Biotechnol 162(7):1847–1857

Lee CK, Halim FAA (2014) Oil palm fronds juice: A potential feedstock for bioethanol production. Int J Sci Res Publ 4(12):1–7

Li X, Xu J, Tang X, Liu Y, Yu X, Wang Z, Liu W (2016) Anthocyanins inhibit trastuzumab-resistant breast cancer in vitro and in vivo. Mol Med Rep 13(5):4007–4013. https://doi.org/10.3892/mmr.2016.4990

Lim SH, Ibrahim D, Ibrahim CO (2010) Microscopic studies of oil palm frond during processing for saccharification. Int J Bioeng 4(2):1–13

Lin TF, Demain AL (1991) Effect of nutrition of Monascus sp. on formation of red pigments. Appl Microbiol Biotechnol 36(1):70–75. https://doi.org/10.1007/bf00164701

Lin YL, Wang TH, Lee MH, Su NW (2008) Biologically active components and nutraceuticals in the Monascus-fermented rice: a review. Appl Microbiol Biotechnol 77(5):965–973. https://doi.org/10.1007/s00253-007-1256-6

Manan MA (2014) Design aspects of solid state fermentation. PhD Thesis, The University of Manchester

Manan MA, Mohamad R, Ariff A (2017) Monascus spp.: a source of natural microbial color through fungal biofermentation. J Microbiol Exp 5(3). https://doi.org/10.15406/jmen.2017.05.00148

Megashah LN, Ariffin H, Zakaria MR, Hassan MA (2018) Properties of cellulose extract from different types of oil palm biomass. IOP Conf Ser Mater Sci Eng 368(1):1–11. https://doi.org/10.1088/1757-899X/368/1/012049

Mitchell DA, de Lima Luz LF, Krieger N, Berovič M (2011) 2.25 - Bioreactors for solid-state fermentation. In: Moo-Young M (ed) Comprehensive biotechnology, 2nd edn. Academic Press, London, pp 347–360. https://doi.org/10.1016/B978-0-08-088504-9.00107-0

Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96:673–686. https://doi.org/10.1016/j.biortech.2004.06.025

MPOB (2018) Overview of the Malaysian oil palm industry 2017 (Malaysian). http://www.mpob.gov.my/

Mukherjee G, Singh SK (2011) Purification and characterization of a new red pigment from Monascus purpureus in submerged fermentation. Process Biochem 46(1):188–192. https://doi.org/10.1016/j.procbio.2010.08.006

Niladevi KN, Sukumaran RK, Prema P (2007) Utilization of rice straw for laccase production by Streptomyces psammoticus in solid-state fermentation. J Ind Microbiol Biotechnol 34 (10):665–674. https://doi.org/10.1007/s10295-007-0239-z

Nimnoi P, Lumyong S (2011) Improving solid-state fermentation of Monascus purpureus on agricultural products for pigment production. Food Bioprocess Technol 4(8):1384–1390. https://doi.org/10.1007/s11947-009-0233-8

Nordin NA, Sulaiman O, Hashim R, Kassim MHM (2016) Characterization of different parts of oil palm fronds (Elaeis Guineensis) and its properties. Int J Adv Sci Eng Inf Technol 6(1):74–76

Onken U, Liefke E (1989) Effect of total and partial pressure (oxygen and carbon dioxide) on aerobic microbial processes. Adv Biochem Eng Biotechnol 40:137–169

Oriol E, Raimbault M, Roussos S, Viniegra-Gonzales G (1988) Water and water activity in the solid state fermentation of cassava starch by Aspergillus niger. Appl Microbiol Biotechnol 27 (5):498–503. https://doi.org/10.1007/bf00451620

Pandey A (2001) Solid-state fermentation in biotechnology: fundamentals and applications. Asiatech Publishers, New Delhi

Panesar PS, Kaur R, Singla G, Sangwan RS (2016) Bioprocessing of agro-industrial wastes for production of food-grade enzymes: progress and prospects. Appl Food Biotechnol 3 (4):208–227. https://doi.org/10.22037/afb.v3i4.13458

Pattanagul P, Pinthong R, Phianmongkhol A, Leksawasdi N (2007) Review of angkak production (Monascus purpureus). Chiang Mai J Sci 34:319–328

Pengnoi P, Mahavan R, Khanongnuch C, Lumyong S (2017) Antioxidant properties and production of monacolin K, citrinin, and red pigments during solid state fermentation of purple rice (Oryzae sativa) varieties by Monascus purpureus. Czech J Food Sci 35(1):32–39. https://doi.org/10.17221/154/2016-CJFS

Raimbault M (1998) General and microbiological aspects of solid substrate fermentation. Electron J Biotechnol 1(3):1–20

Raimbault M, Alazard D (1980) Culture method to study fungal growth in solid fermentation. Eur J Appl Microbiol Biotechnol 9(3):199–209. https://doi.org/10.1007/bf00504486

Rashid JIA, Samat N, Wan Yusoff WM (2011) Optimization of temperature, moisture content and inoculum size in solid state fermentation to enhance mannanase production by Aspergillus terreus SUK-1 using RSM. Pak J Biol Sci 14(9):533–539. https://doi.org/10.3923/pjbs.2011.533.539

Razali MA-A, Said FM (2017) Red pigment production by monascus purpureus in stirred-drum bioreactor. Sci Herit J 1(1):13–15. https://doi.org/10.26480/gws.01.2017.13.15

Roslan AM, Ahmad M, Mohd K (2014) Investigation of oil palm frond properties for use as biomaterials and biofuels. Trop Agric Dev 58(1):26–29. https://doi.org/10.11248/jsta.58.26

Saad MEM, Amani AHF, Keong LC (2016) Optimization of bioethanol production process using oil palm frond juice as substrate. Malay J Microbiol 12(4):308–314. https://doi.org/10.21161/mjm.84016

Said FM (2010) *Monascus Ruber* ICMP 15220 fermentation for the production of pigments. Dissertation, Massey University. https://mro.massey.ac.nz/bitstream/handle/10179/2654/02_whole.pdf?sequence=1&isAllowed=y

Said FM, Hamid NF (2019) Natural red colorant via solid-state fermentation of oil palm frond by Monascus purpureus FTC 5356: Effect of operating factors. J Eng Sci Technol 14 (5):2576–2589

Said FM, Razali MAA (2019) Effect of factors on the red pigment production in the stirred drum bioreactor: fractional factorial design approach. AIP Conf Proc 2155, 020008-1-020008–8. https://doi.org/10.1063/1.5125512

Said FM, Chisti Y, Brooks J (2010) The effects of forced aeration and initial moisture level on red pigment and biomass production by Monascus ruber in packed bed solid state fermentation. Int J Environ Sci Dev 1(1):1–4

Silveira ST, Daroit DJ, Sant'Anna V, Brandelli A (2013) Stability modeling of red pigments produced by Monascus purpureus in submerged cultivations with sugarcane bagasse. Food Bioprocess Technol 6(4):1007–1014. https://doi.org/10.1007/s11947-011-0710-8

Singhania RR, Patel AK, Soccol CR, Pandey A (2009) Recent advances in solid-state fermentation. Biochem Eng J 44(1):13–18. https://doi.org/10.1016/j.bej.2008.10.019

Srianta I, Ristiarini S, Nugerahani I, Sen SK, Zhang BB, Xu GR, Blanc PJ (2014) Recent research and development of Monascus fermentation products. Int Food Res J 21(1):1–12

Sukri SSM, Rahman RA, Norzita N (2013) Pretreatment of oil palm fronds for improving hemicellulose content for higher recovery of xylose. Jurnal Teknologi 62(2):39–42

Sulaiman SA, Balamohan S, Moni MNZ, Atnaw SM, Mohamed AO (2015) Feasibility study of gasification of oil palm fronds. J Mech Eng Sci 9:1744–1757. https://doi.org/10.15282/jmes.9.2015.20.0168

Sumathi S, Chai SP, Mohamed AR (2008) Utilization of oil palm as a source of renewable energy in Malaysia. Renew Sust Energ Rev 12(9):2404–2421

Tan JP, Jahim JM, Harun S, Wu TY, Mumtaz T (2016) Utilization of oil palm fronds as a sustainable carbon source in biorefineries. Int J Hydrog Energy 41(8):4896–4906. https://doi.org/10.1016/j.ijhydene.2015.08.034

Teramura H, Sasaki K, Oshima T, Matsuda F, Okamoto M, Shirai T, Kawaguchi H, Ogino C, Hirano K, Sazuka T, Kitano H, Kikuchi J, Kondo A (2016) Organosolv pretreatment of sorghum bagasse using a low concentration of hydrophobic solvents such as 1-butanol or 1-pentanol. Biotechnol Biofuels 9(27):1–11. https://doi.org/10.1186/s13068-016-0427-z

Torres FA, Zaccarim BR, de Lencastre Novaes LC, Jozala AF, Dos Santos CA, Teixeira MF, Santos-Ebinuma VC (2016) Natural colorants from filamentous fungi. Appl Microbiol Biotechnol 100(6):2511–2521. https://doi.org/10.1007/s00253-015-7274-x

Umsza-Guez MA, Díaz AB, de Ory I, Blandino A, Gomes E, Caro I (2011) Xylanase production by Aspergillus awamori under solid state fermentation conditions on tomato pomace. Braz J Microbiol 42:1585–1597. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1517-83822011000400046&nrm=iso

Wahid M, Abdullah S, Henson I (2005) Oil palm - achievements and potential. Plant Prod Sci 8:288–297. https://doi.org/10.1626/pps.8.288

Wu H, Zhou Z, Yang Y, Meng Q (2019) Effect of steam explosion of oil palm frond and empty fruit bunch on nutrient composition and ruminal fermentation characteristics. Trop Anim Health Prod 52:1223–1228. https://doi.org/10.1007/s11250-019-02117-4

Zahari MWO, Abu Hassan O, Wong HK, Liang JB (2003) Utilization of oil palm frond - based diets for beef and dairy production in Malaysia. Asian Aust J Anim Sci 16(4):625–634

Zahari MAKM, Zakaria MR, Ariffin H, Mokhtar MN, Salihon J, Shirai Y, Hassan MA (2012) Renewable sugars from oil palm frond juice as an alternative novel fermentation feedstock for value-added products. Bioresour Technol 110:566–571. https://doi.org/10.1016/j.biortech.2012.01.119

Zakaria MR, Fujimoto S, Hirata S, Hassan MA (2014) Ball milling pretreatment of oil palm biomass for enhancing enzymatic hydrolysis. Appl Biochem Biotechnol 173(7):1778–1789. https://doi.org/10.1007/s12010-014-0964-5

Zani SHM, Asri FM, Azmi NS, Yussof HW, Zahari MAKM (2019) Optimization of process parameters for bioethanol production from oil palm frond juice by Saccharomyces cerevisiae using response surface methodology as a tool. IOP Conf Ser Mater Sci Eng 702(1):012003. https://doi.org/10.1088/1757-899X/702/1/012003

Zhou B, Pu YW, Zhu MJ, Liang SZ (2008) Effects of nitrogen sources on Monascus yellow pigment production by Monascus mutant. Mod Food Sci Technol 2:123–127

Bioleaching from Coal Wastes and Tailings: A Sustainable Biomining Alternative

9

Alexsandra Valério, Danielle Maass, Cristiano José de Andrade, Débora de Oliveira, and Dachamir Hotza

Abstract

Mineral coal is one of the most employed natural resources that represent potential environmental issues. The mine tailing contains several valuable minerals such as zinc, molybdenum, vanadium, chromium, iron, and copper. Currently, the most part of mine tailings is disposed at large tailing ponds. Another important tailing from mineral coal is fly ash, the main residue from thermoelectric plants, which may also contain valuable minerals. Currently, the most part of coal fly ash produced is used as raw material for cement fabrication or disposed at ash ponds. In this sense, biomining and bioleaching is an economically and environmentally attractive technology that can be used for metal recovery from residues such as mine tailing and coal ash, in line up with the concept of green chemistry. There are sparse data available on bioleaching of coal ash using either autotrophic or heterotrophic microorganisms. Therefore, the aim of this chapter was to describe the key aspects related to biomining and bioleaching of mine tailing and coal ash, pointing out the state of the art and some future perspectives.

Keywords

Mining tailing · Coal ash · Bioleaching · Microorganism · Biomining

A. Valério · C. J. de Andrade · D. de Oliveira · D. Hotza (✉)
Department of Chemical Engineering and Food Engineering (EQA), Federal University of Santa Catarina (UFSC), Florianópolis, SC, Brazil
e-mail: d.hotza@ufsc.br

D. Maass
Institute of Science and Technology (ICT), Federal University of São Paulo (UNIFESP), São José dos Campos, SP, Brazil

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_9

9.1 Introduction

Mineral coal is a complex and varied mixture of organic components. Its quality as fuel is related to some parameters as calorific value, content of mineral matter and sulfur moisture, among others. According to the concentration of carbon, the rank of a coal can be classified (from lower to higher) in lignite, sub-bituminous, bituminous, and anthracite (Deska et al. 2018; Maass et al. 2019a, b). Coal mining plays a key role in economic development of several countries (Burchart-Korol et al. 2014). It is projected that the worldwide coal production increases ~3% from 2015 to 2040, reaching ~9.4 billion tons, and that the global coal consumption will remain stabilized at ~160 quadrillion Btu (from 2015 to 2040), since China and the United States (main producers) will decrease their productions, whereas India will increase its production (https://www.eia.gov/outlooks/ieo/pdf/0484(2017).pdf).

In terms of global energy matrix, coal-based energy represents currently ~8% of total world energy consumption. In this sense, about 30% of coal-based energy is used as primary energetic needs and 26% for other uses than electric power generation. One of the main issues in using coal as an energy source or fuel is that it has a significant impacts on environment in mining and transportation processes, as well as in the burning process when compared to petroleum and gas (Wang et al. 2014). Hence, it is necessary to understand the environmental influence of electricity coal from a life cycle perspective. Coal life cycle (CLC) refers to the various phases and whole process from the raw coal mining to the final waste disposal, as presented in Fig. 9.1. A rational division of the CLC is necessary to analyze the level, and the pollution form as well as the environmental behavior characteristics and ecological risk implicated in each phase (Wang et al. 2014). There are several forms to classify CLC, in which one of the simplest is composed by extraction stage, transportation stage, and power plant (Steinmann et al. 2014).

The power plant or energy production phase is environmentally harmful, since it generates greenhouse gases, fly ash, and suspended particulate matter (Table 9.1). The greenhouse gases aggravate the climate change effect, by increasing atmospheric pollution, and threatening the workers and surrounding residents' health. Surface water and underground water are also seriously polluted when the waste water from coal combustion is disposal improperly into the environment (Wang et al. 2014). Usually, coal-based power plants produce ~750 million tons of bottom ash and fly ash per year. Fly ash represents 77.5% of the total ash produced, which corresponds to the world's fifth largest raw material resource. Regarding the total ash produced, $\geq$50% of coal ashes are currently utilized, mainly as construction material component, embankments in roads, or fly ash-based ceramics (Ahmaruzzaman 2010; Dwivedi and Jain 2014; Gonçalves et al. 2018; Pangayao et al. 2015; Zhu et al. 2018). Coal fly ash may be classified by American (ASTM-C-618) and European (EN-450-1) standards, as presented in Table 9.2 (Temuujin et al. 2019).

Thus, in general, the sum of SiO_2 + Al_2O_3 + Fe_2O_3 (wt.%) is used to distinguish classes F and C, whereas LOI can be used to discern all classes. In addition, the coal fly ash is named as siliceous coal fly ash when the sum of SiO_2 + Al_2O_3 + Fe_2O_3 is $\geq$70 and the reactive CaO $<$ 10%; whereas calcareous coal fly ash are defined when

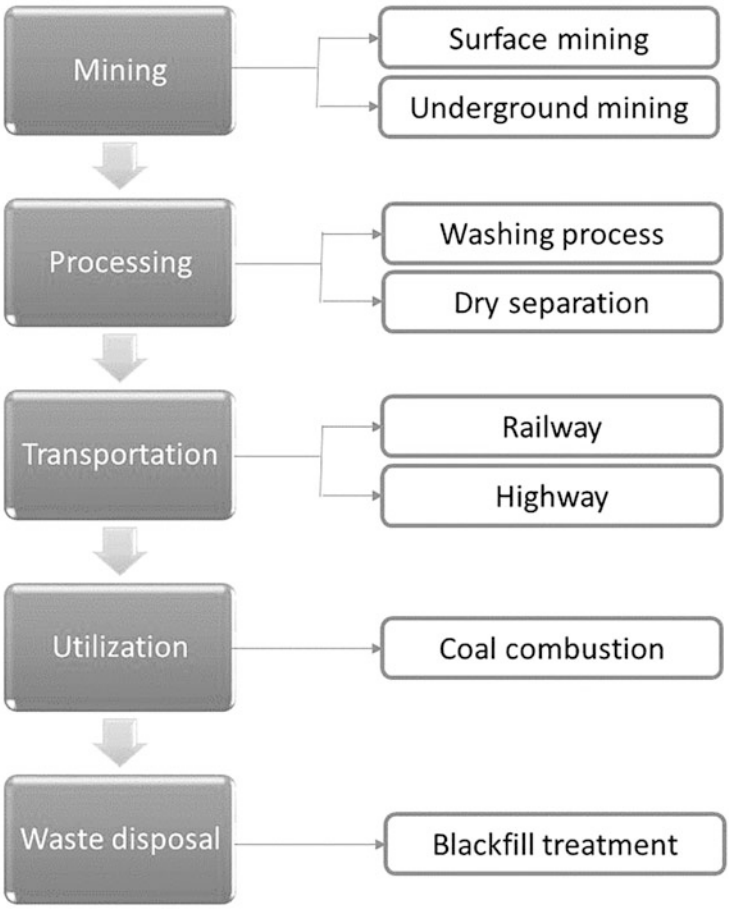


Fig. 9.1 Flowchart of the coal life cycle. (Source: Adapted from Steinmann et al. 2014)

the sum of SiO_2 + Al_2O_3 + $Fe_2O_3 \geq 50$ and reactive CaO $> 10\%$ (Temuujin et al. 2019).

Pyrometallurgy and hydrometallurgy are processes traditionally used in coal wastes treatment. Pyrometallurgy processes extract and refine metals that are inherently present in coal tailing at high temperatures ($\geq$400 °C), whereas hydrometallurgical processes employ acidic and alkaline solutions to dissolve the solid material with subsequent precipitation of the metallic ions. Both, pyrometallurgy and hydrometallurgy processes require high amounts of energy and have great potential to negatively impact the environment, mainly due to the formation of uncontrolled harmful products and the presence of heavy metals in liquid effluents (Guo et al. 2009; Xie et al. 2015).

The replacement of conventional processes, such as pyrometallurgy and hydrometallurgy, by large-scale environmentally friendly processes has been increasing

Table 9.1 Characteristics of wastes and outputs in each phase of the coal life cycle. (Source: Adapted from Wang et al. 2014)

Phase	Main wastes and outputs	Environmental impact
Coal mining	Coal production, solid waste (coal gangue, etc.), mine water, gas (CO_2, CO, NO_x, CH_4, etc.), land use change	Water resources depletion land resource damage; air pollution; biodiversity reduction
Coal processing	Wastewater, waste gas, solid waste (such as solid impurities in raw coal, etc.). Main pollution factors: CO_2, CO, NO_x, CH_4, S, Pb, Cd, Hg, As, etc.	Increasing in air particulate matter; and water and soil pollution
Coal transportation	Dust, tail gas, noise, etc. Main pollution factors: CO, NO_x, Pb, Cd, Hg, As, etc.	Air pollution along the traffic route, agriculture, fishery, and animal husbandry are damaged
Coal utilization	Power generation, wastewater, waste gas, solid waste (coal cinder, coal ash, etc.). Main pollution factors: CO_2, CO, SO_x, NO_x, Pb, Cd, Hg, As, etc.	Air quality declines; surface water is polluted; land is pressed
Waste disposal	Recycling: recycling the waste to converse available material Backfill treatment: waste gas, wastewater (such as filtrate), landfill, land pollution Main pollution factors: CO_2, CO, NO_x	Harmful elements pollute soil, underground water, and plants

Table 9.2 Coal fly ash classification

Components (wt.%)	Class F	Class C	Class A	Class B	Class C
$SiO_2 + Al_2O_3 + Fe_2O_3$	≥70	≥50	≥70	–	–
SO_3%	≤5	≤5	≤3	–	–
Moisture%	≤3	≤3	–	–	–
[a]LOI %	≤6	≤6	≤5	2–7	4–9

Source: Adapted from Temuujin et al. (2019)
[a]*LOI* loss on ignition

worldwide, which is related to the concept of green chemistry (Brierley and Brierley 2001; Ghosh et al. 2016; Mani and Kumar 2014). An alternative is a biological process known as biomining, which is a generic term used to describe the extraction and recovery of metals presents in ores or wastes by biological systems (mostly prokaryotic microorganisms) (Johnson 2014).

Therefore, this chapter describes the most recent key scientific findings on biomining and bioleaching of mine tailing and coal ash, including current researches and perspectives for this technology.

9.2 Microbiology in Biomining Operations

9.2.1 Biomining Techniques

Biohydrometallurgy, bioleaching or biooxidation designates specific biomining process (Mahmoud et al. 2017). Bioleaching is the conversion of insoluble metals present in ores into soluble forms, for instance the conversion of sulfide into sulfuric metals in mines with the presence of sulfur (Campodonico et al. 2016; Erüst et al. 2013), whereas biooxidation is the decomposition of the mineral matrix by microorganisms, which encapsulates the metal of interest, exposing it and thus increasing its accessibility for further extraction. The most common sulfide minerals are pyrite (FeS_2), chalcopyrite ($CuFeS_2$), sphererite (ZnS), and galena (PbS). They may trap precious metals (gold, silver and uranium) and other base metals (cobalt, molybdenum, nickel, copper and zinc) (Brierley and Brierley 2013; Clark et al. 2006; Mahmoud et al. 2017).

Industrial biohydrometallurgy operations are variable and depend on the type of ore, geographical location, metallic content and specific minerals present—oxides or sulfides. One of the most common configurations used for metal recovery involves an irrigation process in which leachate solutions percolate through the crushed ore. Three main strategies of biohydrometallurgy processes are used for the mining industry: stirred tanks, dump, and heap (Brierley 2008; Jerez 2017a, b).

Dump biohydrometallurgy is a highly economical method of recovering copper from very low-grade ores (<0.5 wt.%) due to the large quantities of cupric rocks and low production costs (Brierley 2008). These landfills contain tons of run-of-mine material. Acidified water is used at the top of the dumps to make the environment conducive for the growth of microorganisms that, together with the acidic liquid that percolates the ore, enhance the leaching of metals (Natarajan 2018).

Heap bioleaching is relatively similar to the dump biohydrometallurgy strategy, nevertheless finer particles of mining wastes and also at smaller scale (Bharadwaj and Ting 2011). Heap bioleaching metal oxidation process comes from a stacking of fragmented ores irrigated with an acidic solution, which allows a better suitability of the medium for the microbial development. This procedure results in the generation of an acid effluent containing solubilized metals that are recovered by solvent extraction and electroextraction. Finally, the solution with the leached metals is neutralized with CaO and treated with cyanide reagents in order to aid the metals solubilization. The process can be improved by the presence of aerators for oxygen insertion in the heaps (Brierley and Brierley 2013; Natarajan 2018).

Both heaps and dumps biohydrometallurgy processes have limited aeration and less control during the process. In this sense, biomining in agitated tanks appears as an interesting alternative. Biomining in agitated tanks is costly, thus high concentrated and metal-rich ores should be used for this process (Bharadwaj and Ting 2011). However, higher recovery rates occur in this type of process when compared to stacked and landfill biohydrometallurgical processes. The tanks are equipped with agitators that aerate the reactor and end up keeping the fine particles of concentrated ores in suspension, ensuring also the satisfactory transfer of oxygen

and carbon dioxide in the solution in which the microorganisms are included after previous inoculation (Brierley and Brierley 2013).

9.2.2 Bioleaching and Biooxidation Microorganisms

Bioleaching and biooxidation microorganisms, including their relations, have the greatest impact on the metal cycle in the mineralization environment mediating metal transformation, since the energy required for their metabolism is obtained from the metabolism of organic compounds or the oxidation of inorganic compounds—heterotrophs or autotrophs, respectively (Erüst et al. 2013).

Autotrophs use light (photoautotrophs) or chemical energy from metals or sulfur—electron donors—(chemolithoautotrophic) to turn CO_2 into organic carbon molecules for the production of biomass. Heterotrophs, on the other hand, oxidize organic carbon, in which it generates many by-products such as acetic acid and citric acid that solubilize metals from minerals (Anjum et al. 2012; Jain and Sharma 2004; Natarajan 2018).

The interaction of microorganisms with metals present in ores and residues depends on their thermotolerance and sensitivity to acidic pH values. Regarding microbial thermotolerance, bacteria and fungi used in biohydrometallurgy are essentially mesophilic (20–40 °C), moderately thermophilic (40–60 °C) or thermophilic (above 60 °C). In addition, bacteria and fungi used in biohydrometallurgy are also acidophilic (Brierley and Brierley 2013; Hoque and Philip 2011; Jerez 2017a, b).

Acidithiobacillus ssp. (bacteria) are commonly used in biomining processes, especially *A. ferroxidans*, *A. ferridurans*, *A. thiooxidans,* and *A. ferrorivans* (Johnson 2014). *Acidithiobacillus* genus belongs to the Gram-negative Proteobacteria phylum, which is rod-shaped and with flagellate structures for mobility.

Regarding biomining, as already mentioned *A. ferroxidans* shows some important advantages (when compared to others microorganisms) such as higher growth rate on acidic environments, metabolic versatility to oxidize both iron and sulfur, and higher thermoresistance. Nevertheless, *A. thiooxidans* oxidize only sulfide compounds, which is its main drawback (Bosecker 1997; Hoque and Philip 2011; Natarajan 2018).

Bacteria of the genus *Lepstopirum*, *Acidiphilum,* and *Sulfobacillus* also commonly applied into biohydrometallurgical processes, especially *Leptospirum* spp. The optimal conditions of *Leptospirum* ssp. (highest growth rate) are usually at pH values from 1.5 to 4.0, under moderate temperature. In addition, iron (II) and (III) ions should be available. However, this species does not oxidize sulfur and sulfuric compounds such as thiosulfate (Bosecker 1997; Erüst et al. 2013; Mahmoud et al. 2017).

Most microbial species that are involved in biomining processes are chemolithoautotrophic. However, there are heterotrophic organisms usually present in non-sulfuric mines. Among the bacteria, the genera *Bacillus* and *Pseudomonas* are the most common, while among the fungi the most important are *Aspergillus* and *Penicillium* (Anjum et al. 2012; Bosecker 1997; Jain and Sharma 2004).

Bacillus is a Gram-positive genus, which has optimum growth at acidic pH and are mostly mesophilic. This genus is capable of leaching silver, copper, lead, tin, and especially manganese (Das et al. 2011; Jain and Sharma 2004). The genus *Aspergillus*, in which stands out the species *A. niger*, includes filamentous fungi, which grow preferentially in oxygen-rich environments and in pH ranges between 5.0 and 7.0. However, unlike most biomining microorganisms, they tolerate a wide variation in the pH values of the culture medium.

9.2.3 Biomining Mechanisms

Generally, the action of microorganisms in biomining processes occurs by two different mechanisms: direct or indirect contact. In the direct contact, there is the adhesion of bacteria to the mineral matrix and electron transfer to chemical reactions through enzymatic action without the aid of iron ions. In the indirect contact, there is bacterial adhesion to the mineral surface, with the use of ferric intermediate in the layer of extracellular polymeric substances (EPS) produced for bioleaching reactions. The oxidation of ferrous ions in ferric occurs in solution, generally to leach the ore surface that contain the metal of interest (Natarajan 2018).

In the indirect mechanism of contact, the microorganism adheres (partially) to the surface of the mineral and, therefore, the electrochemical interactions are attributed to the solubilization of metals. Nevertheless, in the indirect mechanism of noncontact, organic, or inorganic acids generated at the end of the procedure, with the culture medium already practically depleted (extremely low pH and high temperature), can help themselves as a leaching agent (Anjum et al. 2012). Moreover, in the indirect cooperative mechanism, there is the dissolution of sulfur colloids, sulfuric intermediates and various minerals fragmented by planktonic bacterial cells. This suggests that bioleaching occurs either through EPS from microorganisms that have adhered to fragmented minerals or through iron ions in the colloid solution and sulfur intermediates (Mahmoud et al. 2017).

Alternatively, there is biomining mechanism related to the dissolution of sulfuric metals. In general, this process is achieved due to the proton attack on the acid medium and the oxidation process that release the occluded metals in ores. In this sense, there are two plausible pathways for dissolution reactions: thiosulfate and polysulfide pathways, as illustrated by Fig. 9.2. The difference between the mechanisms is determined according to the minerals present in the process, the solubility in acid medium (reactivity with H^+) and formation of the valence bands of minerals (Rohwerder 2003; Sand et al. 2001).

Minerals that do not have solubility in the acidic environment, i.e., their valence band is formed only by electrons from the metal atoms, are oxidized via ferric ion electroextraction, more specifically in the disulfide, forming sulfonic acid group to provide the possibility of sulfur-metal bond breakdown and formation of ferrous ions and thiosulfate ($S_2O_3^-$) (Rohwerder 2003). Thiosulfate, a soluble compound, is oxidized to tetrathionate and is subsequently degraded to other minor sulfur

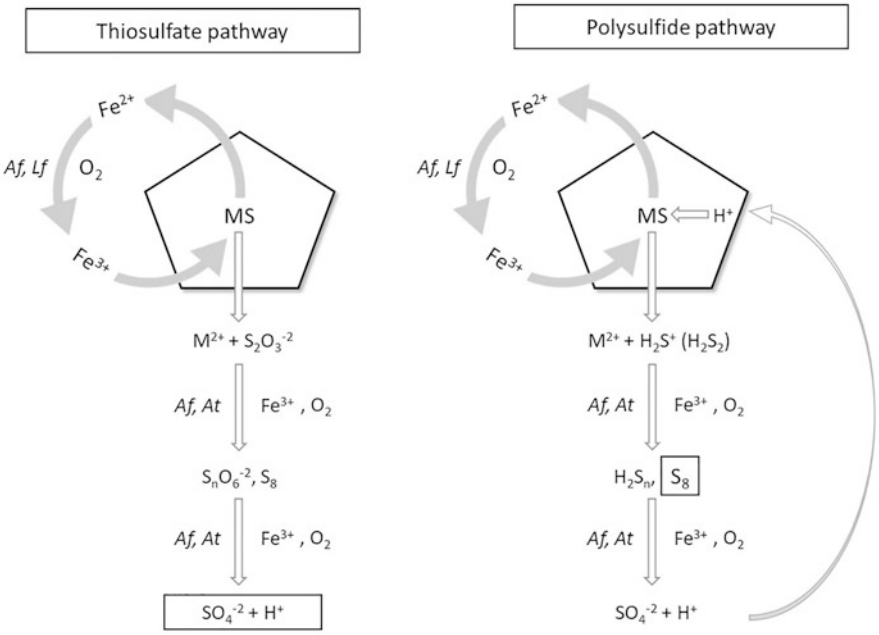


Fig. 9.2 Biomining mechanisms: thiosulfate and polysulfide pathways (Source: Adapted from Rohwerder (2003)). *Note*: The acronyms *Af*, At and *Lf* represent the bacterial species: *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans,* and *Leptospirillum ferrooxidans*, respectively. MS stands for metal sulfide

compounds until they are transformed into sulfate ion at the end of the metabolic pathway (Vera et al. 2013).

Minerals that have their valence bands shared between metals and sulfur are directly soluble in the acid medium and follow the polysulfate or polysulfide pathway, and this is the case for most sulfides (sphalerite, ZnS; galena, PbS; chalcopyrite, $CuFeS_2$; and hauerite, MnS_2) (Tributsch and Rojas-Chapana 2007). At low pH levels, the electro-oxidation of ferric iron combined with proton attack is able to solubilize the metal and lead to the release of elemental sulfur in the medium and be subjected to oxidation by bacteria that are able to accomplish of this reaction, forming sulfuric acid in the medium (decreasing pH values) (Mahmoud et al. 2017; Vera et al. 2013).

Non-sulfidic minerals are bioleached for organic molecules, particularly organic acids (gluconic, malic, succinic, citric, and oxalic acids) that were biosynthesized by heterotrophic microorganisms. These by-products interact with the mineral surface and transform metals into organometallic complexes by protonation, chelation and other mechanisms (Erüst et al. 2013).

Among the different mechanisms in non-sulfidic minerals, four stand out: bioreduction, acidification, complexolysis, and alkalinization. In bioreduction, the metal solubilization occurs due to its reduction in an acidic environment, such as iron

and manganese mainly under the influence of oxalic acid (Brombacher et al. 1997). Acidification is found in environments with a pH lower than 5.0, where protons and oxygen are formed and associated with water to remove the metal from the mineral surface (Johnson 2006). This acidification is the result of acid metabolites from microbiological oxidation or the use of alkaline substrates. Complexolysis is the formation through heterotrophic agents that excrete organic radicals and/or binders by fermentation or degradation of organic molecules, complexing and chelating substances that mobilize the metals constituting the minerals (Fe, Al, Cu, Mn, Mg, etc.). Alkalinization is more specific for silicates, since the bonding of silica with oxygen weakens at high pH values, as in the case of microorganisms that grow in urea media and generate ammonia (NH_3) (Jain and Sharma 2004).

9.3 Biomining: Metal Recovery from Ores

Extractive metallurgy or mining is the extraction of metals from raw materials by physical and chemical processes involving manipulation of their properties in bulk as well as at the atomic level (Anjum et al. 2012). Metals are extracted from ore by a wide range of techniques related to extraction metallurgy (Johnson and du Plessis 2015). The global trend toward urbanization and industrialization supports the increasing demand for industrial metals, low-grade, complex ores, old waste deposits related to past mining activities, and other secondary sources have received much more attention in recent years (Johnson 2014).

Disposal of mine tailings is one of the most important environmental issues during the lifetime of a mine (Brierley 2008). In the European Union, mining and quarrying are producing 727 million tons waste, which is 28.3% of the total waste amount. Some of these wastes are inert, thus they, very likely, do not represent a significant environmental risk. However, other fractions, especially tailings from processing of sulfide minerals like pyrite (FeS_2), pyrrhotite ($Fe_{1-x}S_2$), or chalcocite (Cu_2S) represent significant environmental risks due to their tendency to oxidize in the presence of water or air. Pyrite formation is commonly related to internal sources, i.e., to intrinsic sulfur to organic matter, or to external sources such as sulfuric gas present in bogs or associated with the intrusion of marine sediments (Yang et al. 2018).

For both environmental and economic exploitation of such ores and resources, efficient technologies for the recovery of metals need to be developed, such as bacteria-assisted leaching, which is a promising emerging technology (Anjum et al. 2012; Mahmoud et al. 2017; Schippers et al. 2013). Thus, biotechnological methods for recovery of metals are becoming increasingly important for current industrial applications (Banerjee et al. 2017). According to Mahmoud et al. (2017), an important concept suitable for all applications for any kind of resources is known as "resources efficiency." It aims to optimize metal recovery of base metals (Cu, Ni, Zn) and precious metals (gold, silver), and also other valuable associated minerals (Co, Platinum-Group Metals (PGM)) for both economic and environmental reasons.

Banerjee et al. (2017) reported that copper and gold biomining practices are well-developed and accepted. Globally, around 5% of gold, 15% of copper, and lesser percentages of the other base metals, are nowadays bioleached (Brierley and Brierley 2013). According to Schippers et al. (2013), the production of copper from low-grade ores is the most important industrial application and a significant part of world copper production. The most expressive countries that contribute to the copper biomining are Chile, Australia, the USA, and China. Regarding gold biomining, it is worth mentioning that the main producers are Australia, South Africa, Peru, Ghana, China, and Brazil (Banerjee et al. 2017). About 80% of the bioleached copper originates from projects with secondary copper ores. In regard of the remaining 20%, low-grade primary ore is increasingly bioleached via dump/stockpile leaching (Schippers et al. 2013).

Thus, the development of bioleaching processes is the key to recover the metals in increasingly abundant low-grade ores (Banerjee et al. 2017; Brierley and Brierley 2013; Schippers et al. 2013). Bioleaching methods are less energy intensive, environmentally considerate, and have the potential to generate by-products that can be of value to other processes within the mine (Banerjee et al. 2017).

9.3.1 Extracting Metals from Primary Ores

Currently, biomining perform about 15–25% of world copper production, 5% gold and lower percentages of cobalt, nickel, uranium, and zinc (Mahmoud et al. 2017). Another example of industrial biomining applications is in situ bioleaching. This method consists of leaching ore in its natural location, without excavation (Banerjee et al. 2017; Sinclair and Thompson 2015). This method was used extensively in Canada in the 1970s to recover uranium from worked out deep mines (Johnson 2014). In situ mineral bioprocessing may will be the next major development in the mining sector as the need to utilize deep-buried ore bodies while at the same time minimizing energy costs and carbon footprints could well involve extracting metals from fractured geological strata using microbiologically generated leach liquors (Johnson 2014, 2018; Schippers et al. 2013).

At this point, it is important to mention that the bioleaching processes by microorganisms also have the potential for soil remediation. The investigation of such processes with the aim of decontamination may have a positive impact on the environment, especially for abandoned or unused mines, which represent a significant contamination threat and pose safety risks.

9.3.2 Nanoparticles Production by Biomining

Nanotechnology is commonly considered to deal with particles <100 nm in at least one dimension (Jeevanandam et al. 2018; Lyddy 2009; Valério et al. 2015), with a range of chemical composition, and shape. Nanoparticles may improve the application field in areas such as electronics, optics, sensing, cosmetics, textiles, agriculture,

catalysis, drug delivery, bioseparation, among others (Lyddy 2009; Prasad 2014; Prasad et al. 2014, 2016, 2017). Due to the development of environmentally sustainable processes, the synthesis of nanoparticles has advanced from physical and chemical processes to biological processes (Prasad et al. 2018a).

When compared to the production of nanomaterials by physical and chemical routes, the biological route presents some advantages, mainly due to the alignment with the principles of green chemistry and lower size (Prasad 2017). In this sense, the production of metallic nanoparticles by microorganisms is a relatively novel approach (Temple and Le Roux 1964; Prasad et al. 2016, 2018a, b; Prasad 2019a, b; Srivastava et al. 2021).

Theoretically, any source of metallic ions could be used for the production of metallic nanoparticles by microorganisms (bottom-up approach), including mining tailing as coal ash and pyrite (Maass et al. 2019b; Yeheyis et al. 2009; Prasad 2019a, b). Nevertheless, only a few papers have described the production of nanoparticles by microorganisms from mining tailing. Some researchers showed that bacteria such as *Actinobacter* ssp., *Aquaspirillum magnetotacticum*, *Geobacter metallireducens* GS-15, *Magnetotactic* bacterium, *Magnetospirillum magnetotacticum* and *M. gryphiswaldense* are able to synthesize iron particles (Dickson 1999; Lovley et al. 1987; Narayanan and Sakthivel 2010).

Khan et al. (2014) reported the production of water-soluble silica nanoparticles (mostly from 22 to 24 nm) by *Fusarium oxysporum* (heterotrophic microorganism). *F. oxysporum* was grown in MGYP medium (Malt extract 0.3%, yeast extract 0.3%, peptone 0.5%, glucose 1%, and agar 2%). The mycelial biomass (60 g) was mixture with coal fly ash (10 g) obtained from a thermal power plant. The authors claim that ~72% of silica was synthesized into Si nanoparticles or bioleached.

As illustrated in Fig. 9.3, it would be possible to carry out the bioleaching of specific elements from coal fly ash such as Si, Al, or Fe by a microbial consortium (step I). Then, a heterotrophic microorganism *Aspergillus niger* would produce specific nanoparticles, for instance Si or Al nanoparticles (step II). Ideally, the microbial consortium should have synergism on bioleaching. In addition, the same heterotrophic microorganism should be used in step I and step II. However, it can be inoculated after step I. According to Demirbaş and Balat (2004), desulfurization is the most applied coal enrichment method, which employs alkaline solutions, organic or inorganic acids, in particular hydrochloric acid and phosphoric acid. Moreover, oxidizing agents such as ferric sulfate, hydrogen peroxide are efficient for the removal of ash and sulfate sulfur. Nevertheless, oxidizing agents are ineffective for reduction of organic sulfur.

Therefore, the bioleaching of coal fly ash by a microbial consortium is an environmentally friendly process, which uses thermal power plant residue as an alternative ore. In addition, it could produce specific nanoparticles, in particular Si, Al, and Fe nanoparticles, considering the respective high contents in coal ash.

Mine tailings are one of the most important environmental issues concerning the mine lifetime. The coal extraction, for example, generates huge volumes of sulfide wastes, which are rich in pyrite. Maass et al. (2019b) reported the extraction of residual iron from coal mining (pyrite) and the transformation into nanoparticles by

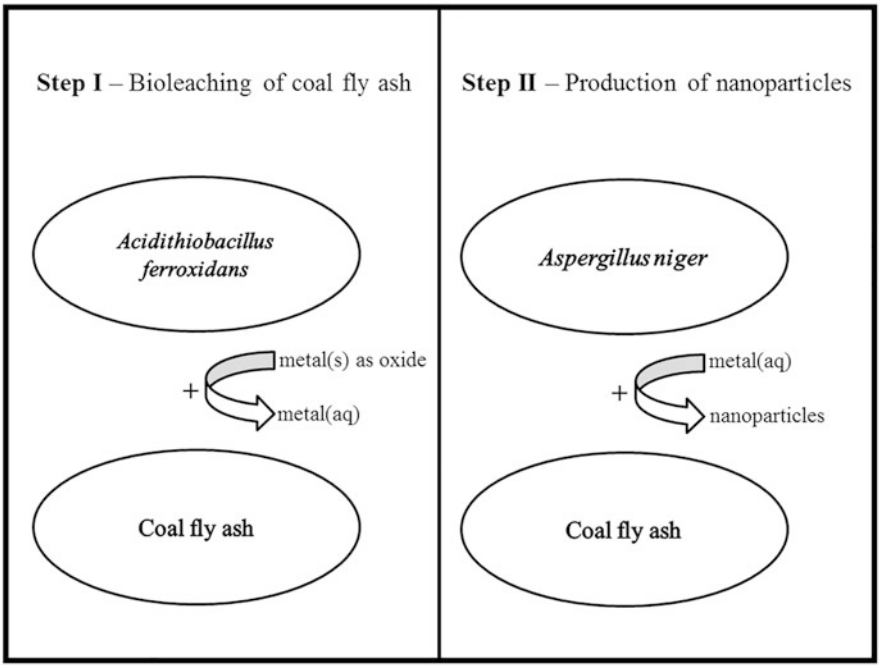


Fig. 9.3 Bioleaching of coal fly ash followed by the production of nanoparticles

Rhodococcus erythropolis ATCC 4277 free-cells in a stirred tank reactor. The iron-based nanoparticles (<50 nm) were identified as iron oxides (β-Fe_2O_3 and α-Fe_2O_3). Despite microbial processes for pyritic sulfur removal have advanced to the pilot scale, there are no commercial processes based on this approach, once there are faster physical and chemical routes to recovery pyrite from mining tailing. Later, Maass et al. (2019a) reported the influence of culture conditions (stirring rate, biomass concentration, and coal tailings ratio) on the particle size in the biotransformation of coal tailings into iron-containing nanoparticles using *Rhodococcus erythropolis* ATCC 4277. The authors found that a more intense stirring rate (150 rpm) of the culture medium and a higher coal tailings ratio led to the synthesis of smaller particles (<50 nm).

Figure 9.4 shows a flowchart with a possible integrate route to obtain iron nanoparticles from residual iron from coal tailing. However, physical and chemical properties of this nanoparticles, as well as their reactivity and microorganism metal removal capacity from contaminated soils and mining tailing, are not yet completely conceptualized, rising the opportunity to researchers to develop new and detailed studies.

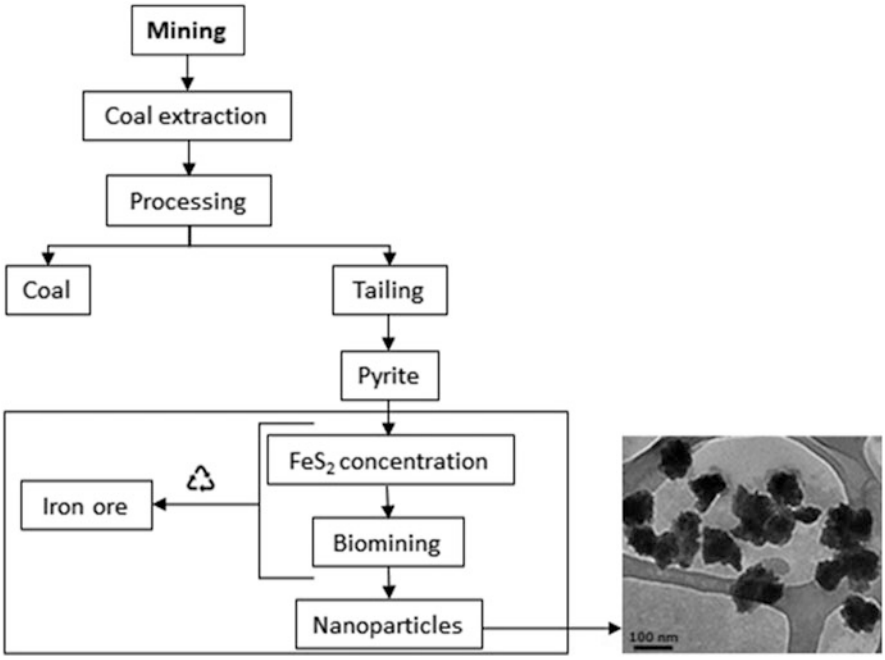


Fig. 9.4 Integrated system for the nanoparticles synthesis using residual iron from coal tailings

9.4 Coal Bioleaching by Microorganisms

The hydrometallurgical industry has been replacing traditional chemical approaches by simple and effective technologies such as bioremediation, biosorption, bioaccumulation, and bioleaching. Nevertheless, concerning bioleaching of inorganic minerals from coal ash by fungi, (micro) algae or yeasts, there is very little research in the literature.

Coal plays a fundamental role in powering modern economies. However, ~50% of the world's total coal deposits are low quality. Sulfur compounds restrict the direct industrial application of coal, which lead to the need of enrichment. The coal enrichment methods can be divided into physical (gravity and magnetic separation), chemical (leaching), physicochemical (flotation and oil agglomeration) or biotechnological processes (Demirbaş and Balat 2004). Coal can be classified into lignite, sub-bituminous, bituminous, and anthracite. Lignite, which is geologically the most recent, is considered the most feasible for desulfurization by microorganisms.

9.4.1 Coal Ash from Thermoelectric Power Plants

The coal ash is often disposed in landfills that are environmentally and financially unattractive (Mahmoud et al. 2017). Bottom ash is collected by gravity (except for fluidized bed furnaces), whereas fly ash requires filters to remove it from flue gas, the most abundant fraction of coal ash contains high added-value elements.

Table 9.3 shows the typical compositions of fly ash are given from different thermoelectric power plants: Maharashtra, India (Bankar et al. 2012), Candiota/RS, Brazil (Costa 2017), Haramachi, Japan (Park et al. 2009), South Africa (Mashau et al. 2018), Spain (Leiva et al. 2018), Texas, United States (Du et al. 2013) and La Trobe Valley/Victoria-Australia (Tennakoon et al. 2015). Depending on their process, thermoelectric power plants generate a variable fly ash composition. Obviously, the source and quality of coal (bituminous, sub-bituminous or lignite) is also crucial for this variation, which is related to the respective geological formation and geographical location.

Even different temperatures will affect fly ash composition, as demonstrated by Kazi et al. (2019). Nevertheless, SiO_2 and Al_2O_3 are the most abundant oxides in coal fly ash. A significant variation in composition is observed, for instance, SiO_2 ranges from 30 to 65.8%.Thus, according to the coal fly ash classification (Table 9.2), all thermal power plants have produced coal fly ash (class F), i.e., sum of SiO_2 + Al_2O_3 + $Fe_2O_3 \geq 70\%$, except in the United States (class C, <50%). In addition, the high amount of coal ash generated and the presence of highly toxic elements as As, Cr, and Pb make coal ash a serious environmental problem.

9.4.2 Bioleaching of Coal Ash by Bacteria

Chemolithoautotrophic bacteria can use inorganic compounds as electron donors and a source of electrons for growth, as *Leptospirillum ferriphilum* and *Acidithiobacillus ferrooxidans* for example, and can be applied for bioleaching (Yousuf et al. 2012).

The most accepted mechanism for bioleaching by chemolithoautotrophic bacteria is related to the intercession of ferric ions (Fe^{3+}) that were generated from the microbial oxidation of ferrous ion (Fe^{2+}) compounds present in the mineral. Then, Fe^{3+} acts as an oxidizing agent of metal sulfides, which leads to iron reduction (Fe^{2+}). Fe^{2+} can subsequently be oxidized over again to Fe^{3+} (Eqs. (9.1–(9.3). In this case, iron has a role as electron carrier (Mahmoud et al. 2017):

$$S + Fe^{3+} + H^{+} \rightarrow S^{2+} + 0.5H_2S_2 + Fe^{2+} \tag{9.1}$$

$$0.5H_2S_2 + Fe^{3+} \rightarrow 0.125S_8 + Fe^{2+} + H^{+} \tag{9.2}$$

$$0.125S_8 + 1.5O_2 + H_2O \rightarrow SO_4^{-} + 2H^{+} \tag{9.3}$$

Table 9.3 Typical composition of coal fly ash from different regions

	Bankar et al. (2012)	Costa (2017)	Park et al. (2009)	Mashau et al. (2018)	Leiva et al. (2018)	Du et al. (2013)		Tennakoon et al. (2015)
	India	Brazil	Japan	South Africa	Spain	United States		Australia
Chemical composition (wt.%)	[a](B/S-B)	[a](B/S-B)	[a](B/S-B)	[a](B/S-B)	[a](B/S-B)	[a](B/S-B)[b]	[a](B/S-B)[c]	[a](B/S-B)
Major elements (as oxides)								
Al_2O_3	29	22	16	23	23	19	22	26
CaO	2	2	1	7	4	26	9	4
Cr_2O_3	–	–	–	–	–	–	–	–
CuO	–	–	–	<0.01	–	–	–	–
Fe_2O_3	4	5	3	11	7	6	6	13
K_2O	1	2	1	1	4	–	1	1
MgO	–	1	–	2	3	6	2	2
MnO	–	–	1	1	–	–	–	–
Na_2O	–	–	–	7	1	1	1	1
P_2O_5	–	–	–	1	–	–	–	–
SiO_2	64	66	75	44	54	34	55	52
SO_3	–	–	–	–	–	2	1	–
TiO_2	2	1	1	1	1	–	–	1
V_2O_5	–	–	–	–	–	–	–	–
ZrO_2	–	–	–	0.1	–	–	–	–
Trace elements[d]								
As	4	–	–	22	–	–	–	–
Cd	2	–	–	–	–	–	–	–
Cr	135	69	119	–	–	–	–	–
Co	32	18	118	–	–	–	–	–
Cu	82	32	92	57	–	–	–	–

(continued)

Table 9.3 (continued)

Chemical composition (wt.%)	Bankar et al. (2012)	Costa (2017)	Park et al. (2009)	Mashau et al. (2018)	Leiva et al. (2018)	Du et al. (2013)		Tennakoon et al. (2015)
	India	Brazil	Japan	South Africa	Spain	United States		Australia
	[a](B/S-B)	[a](B/S-B)	[a](B/S-B)	[a](B/S-B)	[a](B/S-B)	[a](B/S-B)[b]	[a](B/S-B)[c]	[a](B/S-B)
Ga	–	–	–	35	–	–	–	–
Mn	–	183	297	–	–	–	–	–
Mo	–	–	–	9	–	–	–	–
Nb	–	–	–	31	–	–	–	–
Ni	72	13	88	63	–	–	–	–
Pb	51	21	<10	29	–	–	–	–
Rb	–	–	–	39	–	–	–	–
Sr	–	–	–	2.001	–	–	–	–
Th	–	–	–	41	–	–	–	–
U	–	–	–	28	–	–	–	–
W	–	–	–	48	–	–	–	–
Y	–	–	–	45	–	–	–	–
Zn	123	104	–	40	–	–	–	–
Zr	–	–	–	507	–	–	–	–
[e]SUM	97	93	94	78	84	59	83	91
[f]LOI	–	–	2.2	9.5	3.5	–	–	0.55

[a]Probably, bituminous or sub-bituminous (B/S)
[b]Class C – coal fly ash
[c]Class F—coal fly ash
[d]mg/kg
[e]SUM—Sum of SiO_2 + Al_2O_3 + Fe_2O_3
[f]*LOI* loss on ignition

Table 9.4 Selection of chemolithoautotrophic bacteria applied for bioleaching (Mahmoud et al. 2017; Schippers et al. 2013)

Autotrophic bacteria	Facultative autotrophic bacteria
Acidianus infernus	*Acidimicrobium ferrooxidans*
Acidithiobacillus ferrooxidans	*Acidithiobacillus caldus*
Acidithiobacillus thiooxidans	*Alicyclobacillus tolerans*
Leptospirillum ferriphilum	*Metallosphaera sedula*
Leptospirillum ferrooxidans	*Sulfobacillus acidophilus*
Sulfolobus metallicus	*Sulfobacillus thermosulfidooxidans*

Regarding bioleaching, chemolithoautotrophic bacteria have also a catalytic function ~105 times faster than the chemical oxidation, since they accelerate the (re)oxidation of Fe^{2+}, which would otherwise occur slowly due to the absence of oxygen (Rawlings 2005). Nevertheless, it is worth noting that some of them are facultative autotrophic (mixotroph), that is, these microorganisms have both autotrophic and heterotroph metabolisms (Table 9.4).

Regarding coal-fired thermoelectric power plants, bioleaching can be applied for both enrichment of coal and coal ash treatment. The bioleaching of coal ash aims to recover specific elements or oxides from coal ash such as Al_2O_3, TiO_2 (high added-value) or Pb (toxic element).

Jekic et al. (2007) studied the bioleaching of lignite ash by using *Acidithiobacillus ferrooxidans*. The authors have drawn attention to Cu that was leached from pyrite. The bioleaching reached (in weight percent of the element original amount) 27% Cu; 20% Cd; 15% Zn; 15% Mn; 11% Ni; 7% Cr; and 2% Pb. *Thiobacillus thiooxidans* was used for the bioleaching of coal fly ash (10% w/v) that lasted 3 weeks with a lag phase of 10 days. The bioleaching reached ~3000 and 400 mg/L for aluminum and iron, respectively. In addition, it was observed that coal fly ash enhanced the excretion of extracellular polymeric substances (Seidel et al. 2001). Similarly, *Acidiothiobacillus ferrooxidans* and *Acidiothiobacillus thiooxidans* (mixed culture) were applied for bioleaching of coal ash (fly and bottom). The bioleaching lead to (in weight percent of the element original amount): 57% Zn, 71% Mn, 85%, Cr, 74% Fe, and 74% Cu (Pangayao et al. 2015).

Therefore, bioleaching of coal ash, in particular fly ash, is promising due to the high level of leaching (>70%) that might be reached. However, chemolithoautotrophic bacteria are significantly affected by the toxicity of the coal ash elements, regarding delayed lag phase, which is a hindrance for bioleaching on an industrial scale. Thus, a consortium of chemolithoautotrophic bacteria and heterotrophic microorganisms may be an attractive alternative.

9.4.3 Bioleaching of Coal Ash by Fungi

Fungi (heterotrophic microorganisms) are also able to bioleach hydrocarbons in coal and coal residues due to their biosynthesis of anthropogenic chemicals, via the action

of cytochrome p-450, and extracellular enzymes (Xu and Ting 2009). Among the latest, lignolytic enzymes (manganese peroxidase, lignin peroxidase), phenol oxidase (laccase), organic acids (gluconic acid, oxalic acid, α-ketoglutaric acid, 3-hydroxybenzoic acid, and citric acid), may be mentioned (Deska et al. 2018).

The fungal bioleaching can be conducted by in situ or ex situ procedures (Xu et al. 2014). In situ strategy is less expensive, but ex situ strategy reaches, in general, higher yields. Khan et al. (2014) reported the bioleaching of coal fly ash using *Fusarium oxysporum*, a mesophilic fungus, in which the specific enzymes lead to silica nanoparticles. Chemolithoautotrophic bacteria have, inherently, the metabolic ability to bioleaching. Nevertheless, heterotrophic microorganisms (bacteria and fungi) can also bioleach, in general, at a faster grow rate and supporting higher concentrations of coal ash.

Thus, a synergic bioleaching between chemoautotrophic bacteria and heterotrophic microorganisms could improve significantly the leaching yields (Mahmoud et al. 2017). Santhiya and Ting (2006) adapted *Aspergillus niger* to single metal ions Ni, Mo or Al and then to a mixture of Ni, Mo, and Al. The authors reported an adaptation with single metals showed that the fungus could tolerate up to 1000 mg/L Ni, 1200 mg/L Mo and 2000 mg/L Al. In the presence of a mixture of these metals, the fungus was able to tolerate up to 100 mg/L Ni, 200 mg/L Mo and 600 mg/L Al (Santhiya and Ting 2006).

For instance, Ertit Taştan (2017) described fungal bioleaching with *Fusarium oxysporum or* and *Penicillium glabrum*, in which the microbial cells were able to grow in medium with high density of fly ash (10%). In addition, when compared to *P. glabrum*, *F. oxysporum* showed better results of bioleaching (weight percentage related to original amount): Mo (100%), S (64.36%), Ni (50%), and Cu (33.33%). Another advantage of bioleaching, mainly by fungi, is the production of metal nanoparticles, which possess a high added value.

9.5 Conclusions and Perspectives

Coal mining plays a key fundamental role as economic development parameter. It is expected that the global coal consumption will be stabilized at ~160 quadrillion Btu from 2015 to 2040. Coal-fired power plants produce per year ~750 million tons of bottom and fly ash, which is the world's fifth largest raw material resource but it is only partially used. Regarding the treatments of liquid coal wastes, pyrometallurgy and hydrometallurgy processes are traditionally applied. However, these processes lead to uncontrolled harmful products. Therefore, it is essential to develop technological alternatives for coal ash and liquid coal wastes.

A relative well-developed process is the bioleaching using autotrophic bacteria, e.g., *A. ferroxidans* due to its high growth rate and metabolic versatility to oxidize both iron and sulfur. Nevertheless, heterotrophic microorganisms as *Bacillus* ssp. and *Pseudomonas* ssp. are able to bioleach silver, copper, lead, and manganese. During bioleaching, a simultaneous production of metallic nanoparticles can be identified, however only a few papers have described the production of nanoparticles

by microorganisms from mining tailings, in particular by *Actinobacter* ssp., *Aquaspirillum magnetotacticum*, among others. Heterotrophic microorganisms as *Fusarium oxysporum* are able to produce unique metallic nanoparticles.

In conclusion, the bioleaching of mining tailings should be deeply investigated, including microbial consortium, reaction mechanisms and their relation to specific biomolecules. In addition, integrated biotechnological processes as bioleaching using A. *ferroxidans* followed to the metallic nanoparticle production by *Fusarium oxysporum* might produce synergistic effects.

References

Ahmaruzzaman M (2010) A review on the utilization of fly ash. Prog Energy Combust Sci 36:327–363

Anjum F, Shahid M, Akcil A (2012) Biohydrometallurgy techniques of low grade ores: a review on black shale. Hydrometallurgy 117–118:1–12

Banerjee I, Burrell B, Reed C, West AC, Banta S (2017) Metals and minerals as a biotechnology feedstock: engineering biomining microbiology for bioenergy applications. Curr Opin Biotechnol 45:144–155

Bankar A, Winey M, Prakash D, Kumar AR, Gosavi S, Kapadnis B, Zinjarde S (2012) Bioleaching of fly ash by the tropical marine yeast, Yarrowia lipolytica NCIM 3589. Appl Biochem Biotechnol 168:2205–2217

Bharadwaj A, Ting Y-P (2011) From biomining of mineral and ores to bio-urban mining of Industrial waste. In: Fourth environmental technology and management conference, Bandung, Indonesia, 3–4 Nov 2011. https://doi.org/10.13140/2.1.2904.1605

Bosecker K (1997) Bioleaching: metal solubilization by microorganisms. FEMS Microbiol Rev 20:591–604

Brierley CL (2008) How will biomining be applied in future? Trans Nonferrous Met Soc Chin 18:1302–1310

Brierley J, Brierley C (2001) Present and future commercial applications of biohydrometallurgy. Hydrometallurgy 59:233–239

Brierley CL, Brierley JA (2013) Progress in bioleaching: part B: applications of microbial processes by the minerals industries. Appl Microbiol Biotechnol 97:7543–7552

Brombacher C, Bachofen R, Brandl H (1997) Biohydrometallurgical processing of solids: a patent review. Appl Microbiol Biotechnol 48:577–587

Burchart-Korol D, Krawczyk P, Czaplicka-Kolarz K, Turek M, Borkowski W (2014) Development of sustainability assessment method of coal mines. J Sustain Min 13:5–11

Campodonico M, Vaisman D, Castro J, Razmilic V, Mercado F, Andrews B, Feist A, Asenjo J (2016) Acidithiobacillus ferrooxidans's comprehensive model driven analysis of the electron transfer metabolism and synthetic strain design for biomining applications. Metab Eng Commun 3:84–96. https://doi.org/10.1016/j.meteno.2016.03.003

Clark ME, Batty JD, van Buuren CB, Dew DW, Eamon MA (2006) Biotechnology in minerals processing: technological breakthroughs creating value. Hydrometallurgy 83:3–9

Costa JHDJAV (2017) Synechococcus nidulans from a thermoelectric coal power plant as a potential CO2 mitigation in culture medium containing flue gas wastes. Bioresour Technol 241:21–24

Das AP, Sukla LB, Pradhan N, Nayak S (2011) Manganese biomining: a review. Bioresour Technol 102:7381–7387

Demirbaş A, Balat M (2004) Coal desulfurization via different methods. Energy Source 26:541–550

Deska M, Głodniok M, Ulfig K (2018) Coal enrichment methods by using microorganisms and their metabolites. J Ecol Eng 19(2):213–220
Dickson DP (1999) Nanostructured magnetism in living systems. J Magn Magn Mater 203:46–49
Du L, Lukefahr E, Naranjo A (2013) Texas Department of Transportation fly ash database and the development of chemical composition–based fly ash alkali-silica reaction durability index. J Mater Civ Eng 25:70–77
Dwivedi A, Jain MK (2014) Fly ash-waste management and overview: a review. Recent Res Sci Technol 6(1):30–35
Ertit Taştan B (2017) Clean up fly ash from coal burning plants by new isolated fungi Fusarium oxysporum and Penicillium glabrum. J Environ Manage 200:46–52. https://doi.org/10.1016/j.jenvman.2017.05.062
Erüst C, Akcil A, Gahan C, Tuncuk A, Deveci H (2013) Biohydrometallurgy of secondary metal resources: a potential alternative approach for metal recovery. J Chem Technol Biotechnol 88:2115–2132. https://doi.org/10.1002/jctb.4164
Ghosh S, Mohanty S, Akcil A, Sukla LB, Das AP (2016) A greener approach for resource recycling: manganese bioleaching. Chemosphere 154:628–639
Gonçalves C d S, Bernardin AM, Boca Santa RAA, Leoni C, Martins GJM, Kniess CT, Riella HG (2018) Production of vitreous materials from mineral coal bottom ash to minimize the pollution resulting from the waste generated by the thermoelectrical industry. Boletín la Soc Española Cerámica y Vidr 57:142–150
Guo J, Guo J, Xu Z (2009) Recycling of non-metallic fractions from waste printed circuit boards: a review. J Hazard Mater 168:567–590
Hoque ME, Philip OJ (2011) Biotechnological recovery of heavy metals from secondary sources—an overview. Mater Sci Eng C 31:57–66
Jain N, Sharma D (2004) Biohydrometallurgy for nonsulfidic minerals—a review. Geomicrobiol J 21:135–144. https://doi.org/10.1080/01490450490275271
Jeevanandam J, Barhoum A, Chan YS, Dufresne A, Danquah MK (2018) Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J Nanotechnol 9:1050–1074
Jekic J, Beskoski V, Gojgic-Cvijovic G, Grbavcic M, Vrvic M (2007) Bacterially generated Fe2 (SO4)3 from pyrite, as a leaching agent for heavy metals from lignite ash. J Serb Chem Soc 72:615–619
Jerez CA (2017a) Metal extraction and biomining. In: Reference module in life sciences. Elsevier, Amsterdam, pp 1–14. https://doi.org/10.1016/b978-0-12-809,633-8.13077-8
Jerez CA (2017b) Bioleaching and biomining for the industrial recovery of metals. In: Reference module in life sciences. Elsevier, Amsterdam, pp 1–14. ISBN: 978-0-12-809633-8. https://doi.org/10.1016/B978-0-12-809633-8.09185-8
Johnson DB (2006) Biohydrometallurgy and the environment: Intimate and important interplay. Hydrometallurgy 83:153–166
Johnson DB (2014) Biomining-biotechnologies for extracting and recovering metals from ores and waste materials. Curr Opin Biotechnol 30:24–31
Johnson D (2018) The evolution, current status, and future prospects of using biotechnologies in the mineral extraction and metal recovery sectors. Minerals 8:343
Johnson DB, du Plessis CA (2015) Biomining in reverse gear: using bacteria to extract metals from oxidised ores. Miner Eng 75:2–5
Kazi TG, Lashari AA, Ali J, Baig JA, Afridi HI (2019) Volatilization of toxic elements from coal samples of Thar coal field, after burning at different temperature and their mobility from ash: risk assessment. Chemosphere 217:35–41
Khan SA, Uddin I, Moeez S, Ahmad A (2014) Fungus-mediated preferential bioleaching of waste material such as fly - ash as a means of producing extracellular, protein capped, fluorescent and water soluble silica nanoparticles. PLoS One 9:e107597

Leiva C, Arenas C, Vilches LF, Arroyo F, Luna-Galiano Y, Villegas R, Fernández-Pereira C (2018) Use of zeolitized coal fly ash as main component in panels with high fire resistance. ACI Mater J 115(3):393–399. https://doi.org/10.14359/51702009

Lovley DR, Stolz JF, Nord GL Jr, Phillips EJP (1987) Anaerobic production of magnetite by a dissimilatory iron-reducing microorganism. Nature 330:252–254

Lyddy R (2009) Nanotechnology. In: Information resources in toxicology, 4th edn. Elsevier, Amsterdam, pp 321–328. https://doi.org/10.1016/b978-0-12-373,593-5.00036-7

Maass D, de Medeiros Machado M, Rovaris B, Bernardin A, de Oliveira D, Hotza D (2019a) Biomining of iron-containing nanoparticles from coal tailings. Appl Microbiol Biotechnol 103:7231–7240. https://doi.org/10.1007/s00253-019-10,001-2

Maass D, Valério A, Lourenço L, de Oliveira D, Hotza D (2019b) Biosynthesis of iron oxide nanoparticles from mineral coal tailings in a stirred tank reactor. Hydrometallurgy 184:199–205. https://doi.org/10.1016/j.hydromet.2019.01.010

Mahmoud A, Cézac P, Hoadley AFA, Contamine F, D'Hugues P (2017) A review of sulfide minerals microbially assisted leaching in stirred tank reactors. Int Biodeter Biodegr 119:118–146

Mani D, Kumar C (2014) Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. Int J Environ Sci Technol 11:843–872

Mashau A, Gitari M, Akinyemi S (2018) Evaluation of the bioavailability and translocation of selected heavy metals by Brassica juncea and Spinacea oleracea L for a South African power utility coal fly ash. Int J Environ Res Public Health 15:2841

Narayanan KB, Sakthivel N (2010) Biological synthesis of metal nanoparticles by microbes. Adv Colloid Interface Sci 156:1–13

Natarajan KA (2018) Biotechnology of metals. Principles, recovery methods and environmental concerns. Elsevier, Amsterdam

Pangayao DC, Van Hullebusch ED, Gallardo SM, Bacani F (2015) Bioleaching of trace metals from coal ash using mixed culture of *Acidithiobacillus albertensis* and *Acidithiobacillus Thiooxidans*. J Eng Sci Technol 10:36–45

Park JS, Taniguchi S, Park YJ (2009) Alkali borosilicate glass by fly ash from a coal-fired power plant. Chemosphere 74:320–324

Prasad R (2014) Synthesis of silver nanoparticles in photosynthetic plants. Journal of Nanoparticles 2014:963961. https://doi.org/10.1155/2014/963961

Prasad R (ed) (2017) Fungal nanotechnology, fungal biology. Springer, Cham

Prasad R (2019a) Microbial nanobionics: basic research and applications. Springer, Berlin. ISBN 978-3-030-16534-5. https://www.springer.com/gp/book/9783030165338

Prasad R (2019b) Microbial nanobionics: state of art. Springer, Berlin. ISBN 978-3-030-16383-9. https://www.springer.com/gp/book/9783030163822

Prasad R, Kumar V, Prasad KS (2014) Nanotechnology in sustainable agriculture: present concerns and future aspects. Afr J Biotechnol 13(6):705–713

Prasad R, Pandey R, Barman I (2016) Engineering tailored nanoparticles with microbes: quo vadis. WIREs Nanomed Nanobiotechnol 8:316–330. https://doi.org/10.1002/wnan.1363

Prasad R, Bhattacharyya A, Nguyen QD (2017) Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives. Front Microbiol 8:1014. https://doi.org/10.3389/fmicb.2017.01014

Prasad R, Jha A, Prasad K (2018a) Exploring the realms of nature for nanosynthesis. Springer, Berlin. ISBN 978-3-319-99570-0. https://www.springer.com/978-3-319-99570-0

Prasad R, Kumar V, Kumar M, Wang S (2018b) Fungal nanobionics: principles and applications. Springer, Singapore. ISBN 978-981-10-8666-3. https://www.springer.com/gb/book/9789811086656

Rawlings DE (2005) Characteristics and adaptability of iron- and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates. Microb Cell Fact 4:13

Rohwerder MT (2003) Bioleaching review part A: progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. Appl Microbiol Biotechnol 63:239–248
Sand W, Gehrke T, Jozsa P-G, Schippers A (2001) (Bio)chemistry of bacterial leaching—direct vs. indirect bioleaching. Hydrometallurgy 59:159–175
Santhiya D, Ting Y-P (2006) Use of adapted Aspergillus niger in the bioleaching of spent refinery processing catalyst. J Biotechnol 121:62–74
Schippers A, Hedrich S, Vasters J, Drobe M, Sand W, Willscher S (2013) Biomining: metal recovery from ores with microorganisms. Adv Biochem Eng Biotechnol 141:1–47. https://doi.org/10.1007/10_2013_216
Seidel A, Zimmels Y, Armon R (2001) Mechanism of bioleaching of coal fly ash by Thiobacillus thiooxidans. Chem Eng J 83:123–130
Sinclair L, Thompson J (2015) In situ leaching of copper: challenges and future prospects. Hydrometallurgy 157:306–324
Srivastava S, Usmani Z, Atanasov AG, Singh VK, Singh NP, Abdel-Azeem AM, Prasad R, Gupta G, Sharma M, Bhargava A (2021) Biological nanofactories: using living forms for metal nanoparticle synthesis. Mini Rev Med Chem 21:245–265. https://doi.org/10.2174/1389557520999201116163012
Steinmann ZJN, Hauck M, Karuppiah R, Laurenzi IJ, Huijbregts MAJ (2014) A methodology for separating uncertainty and variability in the life cycle greenhouse gas emissions of coal-fueled power generation in the USA. Int J Life Cycle Assess 19:1146–1155
Temple KL, Le Roux NW (1964) Syngenesis of sulfide ores; desorption of adsorbed metal ions and their precipitation as sulfides. Econ Geol 59:647–655
Temuujin J, Surenjav E, Ruescher CH, Vahlbruch J (2019) Processing and uses of fly ash addressing radioactivity (critical review). Chemosphere 216:866–882
Tennakoon C, Sagoe-Crentsil K, San Nicolas R, Sanjayan JG (2015) Characteristics of Australian brown coal fly ash blended geopolymers. Construct Build Mater 101:396–409
Tributsch H, Rojas-Chapana J (2007) Bacterial strategies for obtaining chemical energy by degrading sulfide minerals. In: Biomining. Springer, Berlin, pp 263–280
Valério A, Conti D, Araújo P, Sayer C, Rocha S (2015) Synthesis of PEG-PCL-based polyurethane nanoparticles by miniemulsion polymerization. Colloids Surf B Biointerfaces 135:35–41. https://doi.org/10.1016/j.colsurfb.2015.07.044
Vera M, Schippers A, Sand W (2013) Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation—part A. Appl Microbiol Biotechnol 97:7529–7541
Wang C, Mu D, Mu D (2014) An LCA study of an electricity coal supply chain. J Ind Eng Manag 10:311–335
Xie Y, Xie S, Chen X, Gui W, Yang C, Caccetta L (2015) An integrated predictive model with an on-line updating strategy for iron precipitation in zinc hydrometallurgy. Hydrometallurgy 151:62–72
Xu T-J, Ting Y-P (2009) Fungal bioleaching of incineration fly ash: metal extraction and modeling growth kinetics. Enzyme Microb Technol 44:323–328
Xu T-J, Ramanathan T, Ting Y-P (2014) Bioleaching of incineration fly ash by *Aspergillus niger* – precipitation of metallic salt crystals and morphological alteration of the fungus. Biotechnol Rep 3:8–14
Yang H, Liu Q, Chen G, Tong L, Ali A (2018) Bio-dissolution of pyrite by *Phanerochaete chrysosporium*. Trans Nonferrous Met Soc Chin 28:766–774
Yeheyis MB, Shang JQ, Yanful EK (2009) Long-term evaluation of coal fly ash and mine tailings co-placement: a site-specific study. J Environ Manage 91:237–244
Yousuf B, Sanadhya P, Keshri J, Jha B (2012) Comparative molecular analysis of chemolithoautotrophic bacterial diversity and community structure from coastal saline soils, Gujarat, India. BMC Microbiol 12:150
Zhu L, Ji J, Wang S, Xu C, Yang K, Xu M (2018) Removal of Pb(II) from wastewater using Al2O3-NaA zeolite composite hollow fiber membranes synthesized from solid waste coal fly ash. Chemosphere 206:278–284

Recent Advances in Wastewater Sludge Valorization

10

Asmita Gupta, Madan Kumar, and Shaili Srivastava

Abstract

With a surge in the amount and complexity of wastewater in a rapidly urbanizing world, the challenge of maintaining an efficient treatment of wastewater in a cost-effective and environment-friendly way has to be met. Along with the generation of treated water, the wastewater treatment plant (WWTP) generates huge amount of sludge on daily basis. The handling and disposal of sludge is a major problem associated with WWTPs. The sewage sludge in rich in valuable resources and can also be used for the production of energy. The application of biorefinery concept to wastewater treatment will provide a renewable source for the production of value-added products and bioenergy production. The production of bioplastics, bioflocculant, biofertilizer, biodiesel, biogas, biohydrogen and recovery of phosphorus, enzymes and proteins can be done from sewage sludge. The current trends and challenges in sludge management and biovalorization have been discussed. The biovalorization of sludge will make the overall wastewater treatment process more economical and environmentally sustainable.

Keywords

Wastewater · Sewage sludge · Biovalorization · Bioflocculant · Incineration · Gasification · Pyrolysis

A. Gupta
Daulat Ram College, University of Delhi, New Delhi, India

M. Kumar
Centre for Rural Development, Indian Institute of Technology Delhi, New Delhi, India

S. Srivastava (✉)
Amity School of Earth and Environmental Sciences, Amity University, Gurgaon, Haryana, India

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_10

10.1 Introduction

Industrialization, globalization and urbanization, while on one hand has enabled human civilization expand and establish themselves world over, it has cost other species and the environment in general dearly. An expanding human population generates copious amounts of complex waste cocktail. While the solid waste is mostly sent to landfills, the wastewater is treated in WWTPs. In a typical WWTP, the raw wastewater (influent) undergoes various treatment stages for removal of contaminants and the result is treated water (effluent) and its solid settled part, the sewage sludge. The amount and characteristics of sludge generated in a WWTP is largely depended on the influent and type of treatment process employed (Suárez-Iglesias et al. 2017).

Initially, the focus of most WWTP research was on the wastewater treatment and reuse, and the generated sludge was either used as manure in agriculture or disposed off in landfills. With the advancement in knowledge about sludge characteristics, it was realized that sludge accumulates many inorganic and organic toxicants and therefore its proper treatment was necessary before its reuse or environmental disposal. Therefore, along with upgrading treatment facility, standards were also framed for safe environmental disposal of sludge (Karagiannidis et al. 2011; MoEF 2000).

To deal with the large amount of sludge that is produced in WWTPs, the need for proper treatment and resource recovery from sludge was also realized. Earlier, the wastewater treatment plant (WWTP) was viewed as a dedicated facility for wastewater treatment and maintaining sanitation and hygiene, but now days, it has been considered as a biorefinery plant. The aim of biorefinery is to minimize the waste generation during the entire process of wastewater treatment by recovering resource from waste so as to bring the concept of circular economy. The application of biorefinery model to WWTP would provide renewable alternative for valuable resources and energy.

The WWTP associated environmental concerns, government policies and growing interest from scientific and industrial set-up has pushed for the recovery of resource and energy from the wastewater and sludge. The wastewater and sludge are now used for the recovery of resources (Gupta and Thakur 2016; Kelessidis and Stasinakis 2012). The resources that are extracted from the sewage sludge are phosphorus, nitrogen, polyhydroxyalkanoates (PHAs), heavy metals, bioflocculant, biofertilizer, and biogas, biodiesel, biohydrogen and microbial fuel cell (MFC) as source of energy (Gherghel et al. 2019; Karn and Kumar 2019; Shi et al. 2018). Sludge valorization will address the problems associated with sludge treatment and recycling such as high sludge generation, pathogen removal and its disposal that will make the overall process more environmental friendly (Balasubramanian and Tyagi 2017).

The major challenges associated with the sludge biorefinery are improving the efficiency of the treatment and recovery process and reduction in the overall cost. With continuous research in sludge valorization and development of new

techniques, sludge treatment and resource recovery could become more economical in near future and sludge biorefinery could be adopted globally.

In light of the above, the present chapter focuses on the current scenario of sludge generation, treatment and management, with emphasis on sludge valorization and the associated challenges in the context of circular economy.

10.2 Scenario of Sludge Generation and Management

10.2.1 Sludge Generation

Sewage sludge is an inevitable by-product generated as a result of treatment of sewage or municipal wastewater at sewage treatment plants (STPs) (Singh et al. 2020). Concurrent with the escalating wastewater production volume is the sewage sludge generation owing to an exponential rise in human population and rapid urbanization. Globally, the per capita generation of sludge on a daily basis ranges from 35 to 85 g dry matter (dm), yearly average from a conventional wastewater treatment plant being approximately 10,000 ton (Ahmad et al. 2016; IWA 2019; Werle and Sobek 2019).

In India, the quantity of sewage and an equivalent amount of sludge generation has been immense and increasing with a fast growing population. According to one estimate, 38,354 million litres per day (MLD) of sewage with an equivalent amount of sludge is generated in India, whereas the existing treatment capacity is only 11,788 MLD, and the real-time treatment is even less at about 8251 MLD. Thus, only about 22% of the total waste generated in India is actually being treated (CSE 2016; Saha et al. 2018). While the direct environmental disposal of untreated sewage poses serious ecological and health consequences, the proper disposal and management of sewage sludge is also a matter of concern and needs to be well researched.

10.2.2 Sludge Management

With the incorporation of wastewater treatment and management strategies, the impulse for management of simultaneously generated sewage sludge was also felt. Several steps including upgrading existing treatment methods, designing and implementing new treatment plants, setting up quality standards and enforcing disposal laws, have been taken in order to manage the ever-increasing load of sewage sludge (Karagiannidis et al. 2011). Nevertheless, there are challenges associated with efficient real-time sludge management. Three major global challenges in sludge management today have been discussed as follows:

- One of the most prominent problems is the incessant increment in the sludge amount owing to an ever-increasing raw wastewater generation. Upgradation of existing set-up and addition of new and advanced treatment facilities is one of the effective solutions to cater to this problem.

- Second challenge is the high cost of sludge treatment. The high proportion of water present in sludge, even after drying (approximately 60–70% by weight) add immensely to the sludge volume. Treatment and disposal of this cocktail of watery bulky sludge is difficult and expensive (Gupta and Thakur 2015; Rizzardini and Goi 2009). In general, almost 50% of the total sewage treatment cost is due to the cost of sewage sludge treatment (Canales et al. 1994).
- The third challenge is the increasing complexity and thereby toxicity of sludge characteristics, untreated disposal of which may pose serious health and environmental consequences. With the augmentation in types and amount of contaminant load in wastewater, the composition of subsequently generated sludge is also getting complex, especially with respect to heavy metals and recalcitrant organic compounds, some of which are cytotoxic and genotoxic (Gupta and Thakur 2015). Removal of such harmful chemicals from sludge for its safe environmental disposal (either in landfills or in agricultural land) and meeting tightening quality standards needs implementation of advanced treatment strategies, which is again a cost and technology intensive process (Rulkens 2008).

A key solutions to tackle these challenges of sludge management is to move towards circular economy wherein the focus is not only on the use of sludge in arable lands as manure or in landfills (as is the practice in most of the countries), but also as energy, nutrient and mineral resource (Gherghel et al. 2019; Rulkens and Bien 2004).

10.3 Sludge Characteristics and Treatment

10.3.1 Sludge Characteristics

A municipal wastewater treatment plant (WWTP) generates various types of sludge depending upon its treatment process and stages (Fig. 10.1). In a conventional WWTP, there are two main stages: mechanical and followed by a subsequent biological stage. Untreated sewage first passes through a preliminary stage, wherein coarse solid particles, sand, grit, and grease are removed and sent to landfills. The liquid part then moves into the primary settling tank (mechanical stage) wherein fine suspended particles sediment. Subsequently, chemical dissolved pollutants and nutrients are biodegraded in the aeration tanks and the final (secondary) settling tanks (biological stage) (Manara and Zabaniotou 2012).

Thus, based on the stages at which it is generated, sludge can be characterized in the following types:

- *Primary sludge*: It is generated during the primary treatment process (mechanical stage) in the primary settling tank comprising of floatation and gravity separation, wherein coarse particles, oil and grease, and heavy solids are removed from raw sewage (Suárez-Iglesias et al. 2017; Karagiannidis et al. 2011). Main constituents

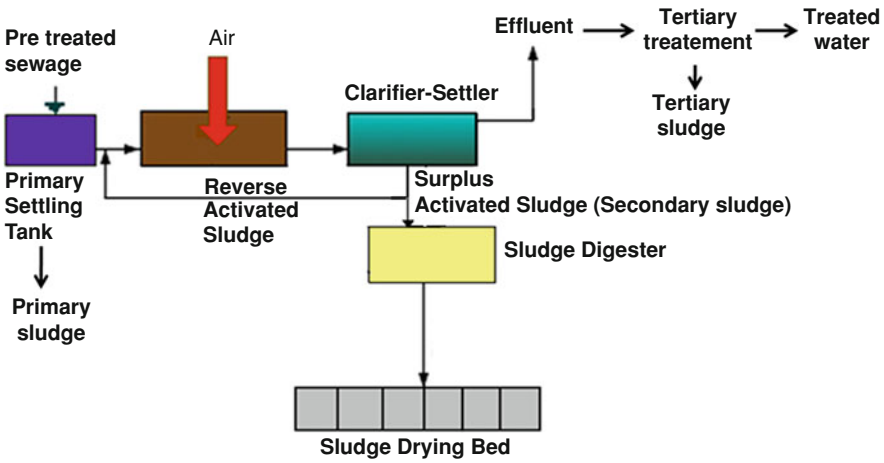


Fig. 10.1 Schematic representation of working of a conventional wastewater treatment plant showing the three types of sludge generation

of primary sludge are solids (5–9%) and water (90–99.5%) (Table 10.1) (Gherghel et al. 2019; Tyagi and Lo 2013).

- *Secondary sludge*: Also called waste activated sludge (WAS), it is generated during the biological stage in which microbial degradation of organic matter present in wastewater takes place. Secondary sludge contains 0.8 and 12% total solids (TS), rest being water. The concentration of total solid varies based on the treatment process used (Raheem et al. 2018; Tyagi and Lo 2013). The composition of WAS has been summarized in Table 10.1. Along with inorganic species (e.g. N, P, K, Ca, S and Mg), WAS also contains harmful contaminants like heavy metals, polyaromatic hydrocarbons, halogenated congeners, estrogens and microbes (pathogenic and nonpathogenic) and their decay products (Gupta and Thakur 2015; Wang et al. 2017). WAS that undergoes further treatment can be called aerobic or anaerobic stabilized sludge depending upon the stabilization process used. The treatment processes, stabilization methods and contaminant load of effluents strongly determine the characteristics of WAS including its structural composition and physicochemical properties.
- *Tertiary sludge:* Advanced sewage treatment processes mainly for nutrient (N and K) removal generate tertiary sludge (Manara and Zabaniotou 2012). Most often, organic matter and nutrient removal are done concurrently. The composition of tertiary sludge mainly depends on the treatment process and coagulant used for nutrient removal. In cases where poly aluminium chloride (PAC) coagulant ($(Al_n(OH)_mCl_{3n-m})x$) is used in advanced treatment stages, the sludge is aluminium-containing tertiary sludge and if Fe(III)-chloride coagulant ($FeCl_3 \cdot 6H_2O$) is used, sludge is iron-containing tertiary sludge (Monea et al. 2020).

Table 10.1 Characteristics of sewage sludge

Parameters	Primary sludge	Secondary sludge
Total solids (TS) %	5–9	0.8–12
Organic solids/volatile solids (VS) %	60–80	59–88
Total COD (g COD L^{-1})	36–144	10.4–15.1
pH	5–8	6.5–8
TKN (%)	2–5	2–5
C/N	13–34	11–22
Hydrogen (%)	7	6.7
Oxygen (%)	35.5	33
Nitrogen (%)	1.5–4	2.4–5
Phosphorus (%)	0.8–2.8	2.8–11
Potash (%)	0–1	0.5–0.7
Sulphur (%)	1.5	1
Cellulose (%)	8–15	7–9.7
Grease and fats (%)	7–35	5–12
Proteins (%)	20–30	32–41
Silica (%)	15–20	–
Alkalinity (mg L^{-1})	500–1500	580–1100
Organic acids (mg L^{-1})	200–2000	1100–1700
Fe (μg g^{-1} TS)	–	1000–154,000
Mg (μg g^{-1} TS)	–	300–20,000
Ni (μg g^{-1} TS)	–	2–5300
Mn (μg g^{-1} TS)	–	32–9870
Cd (μg g^{-1} TS)	–	1–3410
Cu (μg g^{-1} TS)	–	84–17,000
Pb (μg g^{-1} TS)	–	13–26,000
Zn (μg g^{-1} TS)	–	101–49,000
Mn (μg g^{-1} TS)	–	32–9870
Cr (μg g^{-1} TS)	–	10–99,000
Energy (MJ kg^{-1} TS)	23–29	16–23

Source: Adapted from Xiao and Zhou (2020), Elalami et al. (2019)

Primary and secondary sludge differ in their composition especially with respect to contaminant load, and nutrient and energy content (Gagliano et al. 2015; Nazari et al. 2017). While, total and volatile solids are present in higher amounts in the primary sludge, making it a potential resource for energy recovery through anaerobic digestion, the higher nutrients content in secondary sludge, makes it a valuable resource for material recovery and can be used as fertilizer. Therefore, in order to make treatment process more circular in its economy, separate collection and adequate utilization of the two types of sludge needs to be done as against the mixing of the primary and secondary sludge and their combined collection in the existing conventional wastewater treatment plants (Devi and Saroha 2017; Gherghel et al. 2019).

10.3.2 Sludge Treatment

Analysis of sludge characteristics reveal that it is not only loaded with nutrients and minerals but also with contaminants. Therefore, the proper treatment and resource recovery from sludge needs to be done before its environmental disposal. In order to treat and minimize sludge generation and meet safety standards (Table 10.2), several physical, chemical and biological methods are employed in the wastewater treatment process (Zhang et al. 2018; Gupta and Thakur 2015).

Onsite treatment of sludge is done by thickening, aerobic or anaerobic digestion, and dewatering, mostly done on drying beds (Karagiannidis et al. 2011). Dewatered sludge is further treated for removal of odour, heavy metals, organic pollutants and pathogens. The commonly used methods for the sludge treatment include thermochemical processes (incineration, gasification and pyrolysis) and biological process of anaerobic digestion (AD) (Raheem et al. 2018; Tyagi and Lo 2013).

10.3.2.1 Thermochemical Processes

A very effective way of removing contaminants and pathogens as well as recovering energy and value-added products from sludge is by its thermochemical processing (Jiang et al. 2016). Even nutrient and metal recovery from sludge may be achieved by incorporating thermochemical processes with other treatment techniques (Werle and Sobek 2019). Three most commonly used thermochemical processes are incineration, gasification and pyrolysis.

Incineration

Oxidation of sludge biosolids to flue gas (CO_2 and H_2O and ash) along with heat generation in an exothermic reaction is carried out by the process of incineration (Eq. 10.1).

$$\text{Sludge biosolids} + O_2(\text{excess}) \xrightarrow{\text{Incineration}} CO_2 + H_2O + \text{Heat} + \text{ash} \qquad (10.1)$$

Through the process of incineration, sludge volume is reduced by 90% and pathogenic contamination is totally removed. The ash (approximately 30 wt.%) that is generated can be either disposed off to landfills or used as construction material. The heat energy liberated during reaction is used for power generation through steam turbines (Tyagi and Lo 2013). Owing to the large volumes of sludge

Table 10.2 Sludge discharge standards for pH and heavy metals

Parameters	Sludge discharge standards[a]
pH	5.5–8.5
Cr	50.0
Cu	300.0
Ni	50.0
Zn	1000.0

Source: MoEF (2000)
[a]Sludge as compost, (mg kg^{-1} dry weight)

produced in treatment plants, limited sludge applicability and biorefinery options, incineration has become a fast and easy solution in many countries lately. Nevertheless, the dual problem of heavy metal accumulation in the residual ash and its low nutrient content, especially with respect to P has been the major drawback of the incineration process (Krüger et al. 2014). To cater to these issues, various approaches such as electrodialysis (Viader et al. 2015), acid leaching (Xu et al. 2012), thermal treatment with polyvinylchloride (PVC), MgO (Vogel et al. 2013), and recirculating biological sludge phosphorus directly, are being worked upon for improving the quality of incineration ash.

Gasification

Gasification is the incomplete oxidation of dried sludge under oxygen limiting conditions, at high temperature (700–1000 °C) to produce flammable gases, mainly comprising of a mixture of H_2, CO_2, CO, and CH_4, also known as "gasification gas" or syngas and a carbon-rich solid fraction (Skorek-Osikowska et al. 2017; Werle and Sobek 2019). The process of gasification results in volume reduction and removal of heavy metals from sludge biosolids (Peng et al. 2012). Syngas has a calorific value of 4–6 MJ Nm^{-3} and can be used in power generation or as fuel (Werle 2015). Gasification is considered to be one of the most advantageous methods of sludge treatment and valorization (Werle and Sobek 2019).

Pyrolysis

The thermal conversion of sludge to vapours or pyrolytic gases in an oxygen deficient environment is done by the process of pyrolysis. Once, the vapour is condensed through cooling, liquid or oil separates from the solid char (Tian et al. 2013). Parameters such as sludge characteristics, process temperature, pressure, and reaction time influence the quality and quantity of the solid char, liquid and gaseous products of pyrolysis. Pyrolysis proceeds through an endothermic reaction (consuming approximately 100 kJ kg^{-1} of energy) as compared to the exothermic incineration process. Pyrolysis is one of the most efficient ways of sludge valorization and the bio-products (gases, oils and chars) could be used as soil-amendments (Agrafioti et al. 2013), adsorbents of heavy metals (Ma et al. 2014; Qian et al. 2016), or in landfilling (Karayildirim et al. 2006).

10.3.2.2 Anaerobic Digestion (AD)

Sludge organic solids are transformed by the process of anaerobic digestion into biogas (relative density 0.85), which mainly comprises of methane (60–70%), carbon dioxide (30–40%), and other gases, e.g. nitrogen, hydrogen, and hydrogen sulphide (in trace amounts). The calorific value of this biogas is in the range of 13–21 MJ kg^{-3}, which is equivalent to that of lignite's (12–16 MJ kg^{-3}), although still lower than of coal's (15–27 MJ kg^{-3}) (Samolada and Zabaniotou 2012). Methane in biogas can be used as energy source in gas engines, electricity generation and heat production. Energy tapped from biogas can cover almost 50% operational costs of treatment plants (Deublein and Steinhauser 2011). The digestate remaining after anaerobic digestion can be used as fertilizer or compost as it is rich in nutrients

like N, P, K, which are essential for plant growth. Although, AD process cannot remove sludge contaminants as efficiently as in the thermochemical processes, yet its cost effectiveness and environment friendly stages make this process one of the most commonly employed sludge treatment technology world over.

A combination of two or more of these treatment processes is also being explored for approaching circular economy. One of the hybrid processes is combining AD with pyrolysis. The digestate obtained from AD is heated up to 800 °C under oxygen free conditions to get bio-oils, syngas, and biochars, their proportions depending on digestate composition and pyrolysis temperature. Biochar can be used in agriculture as a fertilizer or as adsorbent (activated biochar) (Fabbri and Torri 2016; Monlau et al. 2015; Samolada and Zabaniotou 2014).

10.4 Biovalorization of Sludge

Sludge is generated from wastewater treatment plant in huge quantities and it can be valorized for the recovery of resources and generation of energy (Fig. 10.2).

10.4.1 Resource Recovery

Several value-added products could be recovered from sludge. Some of them have been discussed below.

10.4.1.1 Polyhydroxyalkanaoates (PHAs)

Polyhydroxyalkanaoates (PHA) or bioplastic are being considered as green substitute to petroplastics which is currently being used worldwide. PHA is biodegradable

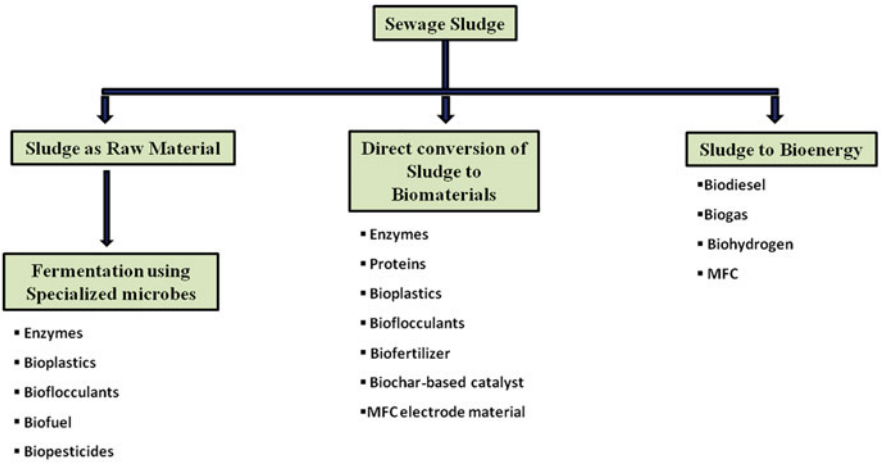


Fig. 10.2 Biovalorization of sewage sludge

microbial polymer accumulated as energy and carbon source inside the microorganisms. The bioplastics produced by microbes are similar in properties compared to plastics (Kumar et al. 2017). The major concern associated with its commercial production is high cost of raw material and process sterility. The production of PHA from sludge can overcome these two major limitations. The PHA can be produced by individual strains, mixed microbial culture and engineered strains using sewage and other wastes (Klai et al. 2016). In one study, the production of PHA from thermal hydrolyzed sludge using mixed microbial culture was reported and there was increase in PHA content (23–51 wt.%) when phosphorus was limited from 127.60 to 1.35 mg L^{-1} (Tu et al. 2019). Valentino et al. (2019) reported a combined approach where co-digestion of sewage sludge and organic fraction of municipal solid waste was investigated using mixed microbial culture for PHA production at pilot scale.

10.4.1.2 Proteins

The sewage sludge is mainly composed of microbial mass and around 40–50% of biomass is protein. Since, sludge is rich in proteinaceous matter so it will serve as low-cost substrate for protein recovery. The steps of protein recovery from sludge are screening, pretreatment, filtration, precipitation, separation and drying. The crude protein has wide applications such as animal feed, adhesive and fire-extinguishing foam. The process of sludge recovery, challenges and its application are discussed in detail (Xiao and Zhou 2020).

10.4.1.3 Enzymes

Sludge contains diverse microorganisms that are specialized in utilization of various substrates present in wastewater. Microbes are source of enzyme and sludge is rich in microbial mass so different type of enzyme can be extracted from the sludge. The enzymes extracted from the sludge are lipase, hydrolase, proteases, amylase that has got wide application in food, feed, and detergent, pharmaceutical and chemical sectors. Liu and Smith (2019) developed ultrasonic and protocol for extraction of protease and cellulose with 63.1 and 100% recovery rate. Similarly lipase amylase, alkaline phosphatase, glucosidase and protease were extracted from sludge and their application has been discussed (Karn and Kumar 2019). Production of enzymes such as alkaline protease, thermostable alkaline protease, lysozyme, lipase, protease, cellulase was reported using pretreated and raw sludge as carbon source using different strains of bacteria and fungi (Klai et al. 2016; Balasubramanian and Tyagi 2017).

10.4.1.4 Biofertilizer

The wastewater treatment plant generate huge amount of sewage sludge after treatment. The application of sewage sludge as biofertilizer in the agriculture field is one of the most common practice of sludge disposal. The sludge is rich in nutrient (N, P, K and organics) so its application can replace the use of chemical fertilizer. Before applying sludge as fertilizer, the contaminants (heavy metals, PAHs and other xenobiotics) present in sludge should be in safe limits. The studies reported

that application of sewage sludge to soil improves the chemical characteristics (nitrogen and carbon enrichment) and biological properties (enzyme activities) of the soil (Skowrońska et al. 2020).

10.4.1.5 Bioflocculant

The microorganisms present in sewage sludge are the natural source of bioflocculant production that are secreted outside microbe for effective growth and treatment of waste present in wastewater. The sewage sludge especially activated sewage sludge is a good source for bioflocculant extraction that is widely applied in wastewater treatment and other processes. The bioflocculant are mainly composed of polysaccharide with small amount of protein, lipid, glycoproteins and nucleic acids. There are several physical, chemical and their combination methods are available for bioflocculant extraction from sludge that is widely discussed (Shi et al. 2018).

10.4.1.6 Sewage Sludge to Biochar-Based Catalyst and MFC Electrode Material

The sludge disposal is a major challenge due to regular production at large scale. So, the research nowadays is focused on reduction of sludge volume and its value addition. Recently, a group reviewed conversion of sludge to sludge biochar-based catalyst (SBC) and microbial fuel cell (MFC) electrode through different processes such as pyrolysis, microwave digestion and hydrothermal carbonization (Mian et al. 2019). The SBC has been widely applied in removal of several organic pollutants from wastewater. The sludge based MFC electrode material has good electroconductive properties and provide cost-effective alternative to the existing electrode materials.

10.4.2 Energy Recovery

Sludge could also be used as source of energy generation. Some of the energy resources derived from sludge have been discussed as follows.

10.4.2.1 Biogas

The fresh sludge generated during the process contains pathogen and odour, hence direct application of fresh sludge is not recommended for use in field. The anaerobic digestion of sludge has several advantages such as biogas generation, reduction in sludge volume, pathogen removal and stabilizes the organic content of sludge (Balasubramanian and Tyagi 2017). The biogas generated from the sludge digestion is clean and renewable energy that can offset the demand of petroleum based fuel. The biogas is mainly composed of methane (50–70%) and carbon dioxide (30–50%) with traces of oxygen, nitrogen, hydrogen sulphide, water vapour and other impurities. The methane is the combustible component of the biogas and its composition is increased >97% by removing the other components through various available biogas upgrading technologies (Kapoor et al. 2019). The upgraded biogas can be used as fuel in household, and vehicles, boilers and can be injected into grid to

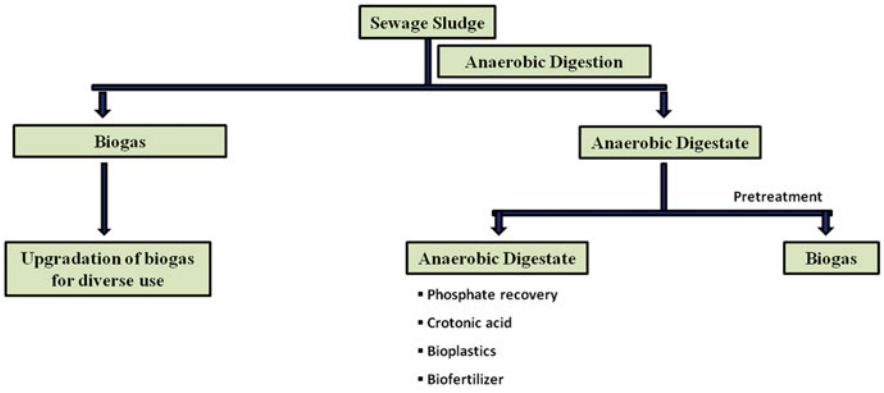


Fig. 10.3 Valorization of anaerobic digestate of sewage sludge

produce electricity. The production of biogas from sludge is the preferred technologies in wastewater treatment plants.

The research now a day is focused to improve to anaerobic digestibility, enhance biogas production and enhance dewatering of sludge through pretreatment and co-digestion. There are several physical (microwave, ultrasonic, electro-kinetic and homogenization), thermal, chemical (acid, alkali, ozonation and fenton), biological processes and their combinations are used for sludge pretreatment which is thoroughly discussed (Zhen et al. 2017). Co-digestion of sewage with other waste such as organic fraction of municipal solid waste and food waste, fatty wastes agricultural wastes, algal biomass and agro-industrial wastes which has been reviewed (Elalami et al. 2019). The sludge digestate left after anaerobic treatment can be further utilized for the recovery of resources and energy as represented in Fig. 10.3.

Energy and Phosphorus Recovery from Anaerobic Digested Sewage Sludge

The hydrothermal carbonization is an important process to extract energy and resource recovery from sludge. Hydrothermal carbonization of digested sludge was performed at temperature 180–240 °C (Merzari et al. 2019). A temperature range of 180–210 °C for sludge digestion was observed for enhanced phosphorus recovery. At this temperature the organic phosphorus associated with volatile carbon are converted to inorganic carbon on hydrochar, resulting in their smooth leaching of from the char surface which was later precipitated for its recovery. The process water generated during the phosphorus recovery with low refractory contaminants was further utilized for biogas production (325 CH_4 g^{-1} VS at 180 °C). The hydrothermal carbonization also yield upgraded lignite-like hydrochars as solid fuel with heating value range 20.5–23.1 MJ kg^{-1} (Marin-Batista et al. 2020).

Phosphorus Recovery from Anaerobic Digested Sewage Sludge

Phosphorus is one the essential nutrient that is mainly procured from the mining. The phosphorus is used as fertilizers, binder material and developing fire-resistant panels.

The reserve of phosphorus is limited on earth so the researchers are focusing on the recovery of phosphorus from renewable sources (Balasubramanian and Tyagi 2017). Sewage sludge contains 0.5–0.7% phosphorus so the focus has now been shifted to recovery of phosphorus from wastewater treatment plant in cost-effective ways. The phosphorus is recovered from the anaerobic digestate mainly in the form of struvite by crystallization and precipitation process. AshDec® is a thermochemical process for recovery of phosphorus from sludge ash. Struvite (magnesium ammonium phosphate hexahydrate) is produced commercially through AirPrex®, and PHOSPAQ® from sewage sludge (Gherghel et al. 2019). Research to develop economical and eco-friendly technology for the recovery of phosphorus is under progress.

PHA and Crotonic Acid Production from Anaerobic Digested Sewage Sludge
The digested sewage contains various forms of organics that can be further transformed into other value-added products. A study reported integration of thermochemical and biological processes to transform the digested organics to PHA and crotonic acid. The hydrothermal treatment increased the soluble COD load in aqueous phase that was further valorized through acidogenic fermentation, aerobic fermentation followed by thermochemical treatment to VFA, PHA and crotonic acid (Samorì et al. 2019).

10.4.2.2 Biodiesel

Sewage sludge contains significant amount of lipid that can be extracted and transesterified for the production of biodiesel. The biodiesel production from sewage sludge will provide renewable alternative to petrodiesel and also reduce pollution and greenhouse gas emission. The cost of biodiesel production from sludge will be much lower compared to the other lipid producing feedstocks considering the area involved for the production. There are several methods reported for biodiesel production from sludge (Capodaglio and Callegari 2018). A study reported supercritical methanol transesterification for methyl esters production from sewage sludge. A 24% yield of methyl ester was observed with more than 50% saturated fatty acids (Demirbas et al. 2017). In another study, biodiesel was extracted from wet urban sewage through liquid-liquid extraction technique and reported enhanced recovery compared to conventional hexane based extraction (Kech et al. 2018). di Bitonto et al. (2020) reported 36–50% and 20–24% lipid recovery from the total solids of sewage scum and primary sludge. The research to reduce the production cost is in progress (di Bitonto et al. 2020).

10.4.2.3 Biohydrogen

The biohydrogen is considered as one of the cleanest energy sources that has wide application across sectors. The production of hydrogen from anaerobic digestion of sewage sludge is considered as environment friendly technology. Hydrogen is mainly produced by facultative anaerobes (E. coli, *Paenibacillus* sp., *Bacillus* sp., *Aeromonas* sp.), obligate anaerobes (*Clostridium* sp., *Peptococcus*, *Methanobacterium*) and hydrogenogenic bacteria (*Desulfovibrio* sp.). The anaerobic

hydrogen production steps include hydrolysis, hydrogen and other acid production and methane production (Yao et al. 2018). The Hydrolysis is the limiting step and the hydrolysis of sludge can be improved by commonly used pretreatment methods such as acid and alkaline pretreatment, hydrothermal, microwave, ultrasonic and other pretreatment methods (Guo et al. 2008). The production of hydrogen is also affected by temperature, C/N ratio, pH value and Metals ions (Wang and Wan 2009). The hydrogen production can be further improved by inhibiting the growth of hydrogen consuming bacteria, co-digestion and further isolating novel high hydrogen producing strains or consortia.

10.4.2.4 Microbial Fuel Cell (MFC)

MFC is being used for energy and resource recovery from waste in sustainable ways (Shah et al. 2019). The research of application of MFC on liquid waste is plenty but application of MFC in solid waste management is limited. The sewage sludge is rich organics so it can be a suitable substrate for energy production. The MFC can be combined to biohydrogen production and anaerobic digestion/methanogenesis (Nastro et al. 2017). As discussed earlier, efficient hydrolysis of sludge through various pretreatment processes can improve the MFC performance. Further research is needed to improve the performance of MFC such as development of cost-effective electrode material, hydrolysis of organics, single chamber or dual chamber, integration of other processes to MFC.

10.5 Challenges Associated with Sludge Biovalorization

Sewage sludge treatment and disposal are expensive. It is environmentally sensitive and the problem will persist due to the increasing number of sewage treatment plants and more stringent environmental quality standards. The traditional methods of sewage disposal are not preferred. Sludge managers aspire to figure out cost-effective and innovative solutions. Recycling and use of wastes are much preferred strategy of waste management. However, with regard to sewage sludge management, perceptions over contaminants challenge recycling and reuse of waste.

10.5.1 Limitations of Sludge Valorization Due to Humic Substances

Organic matter in sewage sludge can be mainly divided into non-humic substances and humic substances. The non-humic acids include proteins polysaccharides, lignin and lipids. The humic acids include fulvic acids, humic acids and humin. Humic substances are regarded as non- or hardly biodegradable during conventional wastewater treatment processes, and the removal of humic substances is challenge in conventional activated sludge systems and is attributed to biosorption instead of biodegradation from wastewater.

Though the content of humic substances often accounts for as high as about 20–30% of the total organic matter in sewage sludge (Dignac et al. 1998; Li et al.

2013), their influence is always neglected in conventional digestion processes due to their low concentrations in the digestate. However, the implementation of pretreatments processes cause the dissolution, formation and shifts in structure characteristics of humic substance (Li et al. 2013), which could further enlarge their impacts on anaerobic digestions. Recent studies indicate that the abundant, distribution, composition and characteristics of humic substances were closely relative to sludge digestion process. Correspondingly, the operational conditions, such pH, humic substances concentrations, metal ions contents, redox potential, etc., are also crucial factors that contribute to and even determine the influence of humic substances on anaerobic digestion of sludge.

Some studies stated that humic substances inhibited sludge hydrolysis and further decreased methane yield by inactivating functional enzymes (Minderlein and Blodau 2010), while it is also found that humic substances acted like surfactants and accelerated sludge hydrolysis (Liu et al. 2015). Bhushan et al. (2006) reported that humic substances were good electron shuttles, and their presence enhanced the degradations of organic matter by transferring electrons to the organics (Bhushan et al. 2006). However, Davies et al. (2001) found that humic acids presented inhibitory effects on anaerobes and the presence of the humics altered the chemistry of the niche during anaerobic digestion (Davies et al. 2001). Despite of the divergence reported, it is consistent that the high concentrations of humic substances affect the conversion efficiency of sludge during anaerobic digestion.

10.5.2 Problems Related to Incineration of Sludge

The foremost issue related with incineration process is high amount of water content in sewage sludge. Further, incineration is not a complete waste disposal strategy. Incineration leaves behind about 30% of the dry solids as an ash. Water reduction requires high energy demand. Digested, oxidized and raw sludges have a natural water content around 92–99%. The liquid and solid component of sewage sludge can be separated by methods viz. physical, thermal, chemical and biological treatment. Among the methods, the dewatering rate is highest for thermal methods and is lowest for the biological methods. Further, the dewatering depends on the: (a) type of energy used (steam, electricity); (b) processing pressure and (c) reaction time.

The incineration process produces high amount of ash. The incineration ash is known to be hazardous in nature due to high content of heavy metals. It requires further high cost of its disposal in landfill sites. Now days, there are options and technology developed for utilization of ash such as for construction materials and bricks. In cement industry, sludge is used as a fuel and the ash is used as part of product itself. As regards the sludge disposal, landfill option is common and lowest option. However, the landfill disposal sewage sludge is considered unsustainable for reasons such as environmental pollution and loss of recyclable waste materials. Incineration technique has some other limitations such as it is energy deficient, SO_X and NO_X emissions problem, strict and expensive emission control. The technology has high cost demand of the flue gas cleaning and ash disposal problems.

There are some limitations in incineration, co-incineration and mono-incineration of sludge management. They are as follows:

- "*The water content of sewage sludge is the main problem for incineration. To burn water, it produces a negative energy balance. Pretreatment e.g. dewatering and/or drying device are needed.*
- *Dried sludge as powder is difficult to store (self-ignition) and could be explosive if the atmosphere is not inert.*
- *Mono-incineration of dewatered sludge is expensive and limited in its capacity.*
- *Co-incineration is depending on the kind of fuel and needs an optimal mixture.*
- *Sewage sludge is varying because of its pretreatment in the sewage plant—raw, oxidize digested sludge—with different composition, calorific value and pollutants.*
- *Ammonia from the sludge may cause problems in the bottom ash by increasing the leachability of copper.*
- *Phosphorus by sewage sludge needs a special care and a coating of cat-material for denitrification.*
- *Incineration is always an expensive solution, even if it is the most effective method to destroy critical organic pollutants and give the possibility to separate out salts and inorganic substances as well as heavy metals and to collect this substances separately in the residues of the flue gas cleaning system*" (Hall 2000).

10.5.3 Limitations in Pyrolysis of Sludge

Pyrolysis technology for sludge valorization have some limitations such as complexity in nature, air pollution, high investment cost and products produced in the process have not very well-established markets. Although, pyrolysis gas needs less treatment to meet emission limits than incineration.

10.5.4 Challenges in Sludge Gasification

Gasification converts dried activated sludge into combustible gases known as syngas which is mainly composed of H_2, CO, CO_2 and CH_4 at elevated temperatures of 700–1000 °C (Roche et al. 2014). Gas cleaning is required for syngas applications in heat and electricity generation.

However, implementation of waste activated sludge gasification confront several challenges such as higher moisture content of approximately 80 wt.% and lower heating value (LHV) of waste activated sludge are the major challenges, which results in lower gasification efficiency. Whereas the dewatering process is energy-intensive (i.e. kilogram of H_2O requires about 2260 kJ of unrecoverable energy) (Sikarwar et al. 2016) and thus, overall cost of sludge disposal becomes considerably higher. Moreover, high tar production from waste activated sludge gasification is also another main obstacle which requires additional treatment because it can

probably hinder the gasifier operation through blocking the tubing or fouling the downstream apparatus. Sludge water content required <10% dry solids (DS) content. In sludge gasification, dewatering and drying is essential, which required high investment and operation cost (Raheem et al. 2018).

10.5.5 Challenges in Anaerobic Digestion

Anaerobic digestion (AD) transforms sludge organic solids to biogas. Methane produced from waste sludge can be utilized for various applications such as gas engines, electricity and/or heat. Apart from benefits of AD, this method has some limitation such as lengthy reactions steps and low conversion efficiency by microbe and/or enzyme. Other challenges such as contamination of heavy metals and Persistent organic pollutants (POPs) contained in waste sludge cannot be alleviated via AD and would have impacts on the public health and environment if not treated properly (Raheem et al. 2018).

10.6 Emerging Approaches for Sludge Biovalorization

Landfilling and land application of sludge disposal methods have limitations due to the increasing ground water pollution problems. Many researchers have attempted reuse and recycling sludge as possible sustainable environmental options (Smol et al. 2015). In relation to this, the European Commission considers that "if waste is to become a resource to be fed back into the economy as a raw material, then, much higher priority needs to be given to reuse and recycling." Sludge reuse as raw material in different industries represents a good possibility of waste management considering the circular economy concept (Eliche-Quesada et al. 2011). Sludge is a rich source of organic components, which can be harnessed in terms of energy and nutrient. The International Solid Waste Association (ISWA), 2015, reported that, an important benefit of the energy and fuels obtained from the waste can replace other energy resources and limit the associated CO_2 emissions.

10.6.1 Hydrothermal Carbonization (HTC)

Hydrothermal carbonization (HTC) is an important thermochemical conversion process to treat low value biomass resources recovery from sewage sludge. HTC can be used as an energy efficient tool for CO_2 sequestration and alternative process to enhance the dewaterability of sewage sludge and meanwhile to convert sewage sludge into high value-added products, such as clean biofuel, organic fertilizer, hydrochar and liquid with a vast amount of organic compounds and gas by-product consisting mainly of CO_2 are produced under mild conditions (Wang et al. 2019; Titirici et al. 2007). Hydrothermal carbonization (HTC) is also used for the synthesis of Sludge Biochar based catalyst (SBCs).

Sewage Sludge conversion into biochar as an efficient catalyst for environmental application, shows great promise to sludge valorization. Generally, temperature 150–250 °C, retention time 1–24 h, and pressure 1–1.5 Ba operating condition employed in the HTC method. Previously, various TiO_2-integrated SBCs were synthesized using the HTC method (Zhang et al. 2018). The typical hydrothermal synthesis process followed two-steps heating route. At the first step, catalytic nanoparticles were combined with sewage sludge in alkaline media and then converted into hydrochar through high pressure and heat as a result of the HTC.

10.6.2 Organo-Mineral Fertilization Production by Sewage Sludge

Sewage sludge contain huge amount of organic matter, nutrients which has the potential to improve soil structure. So, sewage sludge is used to produce organo-mineral fertilizers. Nevertheless, direct application of sewage sludge is prohibited due to the presence of heavy metals, pathogens and toxic substances. Sludge must be treated and disinfect for removal of toxins and pathogenic organisms. To disinfect and deodorize the sewage sludge, different alkali compounds (lime, kiln dust, potassium hydroxide, and sodium hydroxide) and acidic compounds (sulphuric acid, phosphoric acid or its mixture) are used in the production of fertilizers. In the organo-mineral fertilizer production process, generally a mineral fertilizer or other wastes like cement kiln dust, lime kiln dust, etc. are used so that the organo-mineral fertilizer will be rich in micronutrients. To adsorb heavy metals, basaltic detritus and coal wastes are added to the sewage sludge. Sterilization, granulation and drying are important steps in organo-mineral fertilizer production from sewage sludge. Further, the granulated fertilizers are easy to transport, store and spread (Kominko et al. 2017).

10.6.3 Microbial Fuel Cell

Microbial fuel cell (MFC) technology, which uses microorganisms to transform chemical energy of organic compounds into electricity is considered a promising alternative. Extensive studies have corroborated new insights into MFC, which show that a wide array of carbon sources including wastes can be employed by using a variety of microbes. Consequently, microbial transformation of wastes using novel bioremediation strategies such as MFC for energy generation is considered as an efficient and environmentally benign approach (Logan et al. 2006; Shah et al. 2019). Many studies have been conducted using MFC to generate electricity from wastewater or waste biomass (including food waste and animal waste) (Zhao et al. 2012). The use of microbial fuel cells (MFC) for electricity production is considered a sustainable solution for different problems such as excess sludge and water-energy crisis (Nikhil et al. 2018). Also, MFC advances in sewage treatment can improve its energy use and resource recovery. Besides wastewater, sludge can be used as an efficient feedstock for energy generation from MFCs.

10.7 Conclusion

One of the solutions is to follow biological treatment technologies which not only provide low-cost and efficient treatment of wastewater but has also opened several new routes of wastewater and sludge valorization, making the entire process economically favourable. The application of biorefinery concept to WWTP will reduce the major problem associated sludge handling and disposal. Several value-added products and energy in the form of biogas, biodiesel and biohydrogen can be generated from the sludge are discussed and further improvement in these technologies will further reduce the cost of WWTPs and will make the overall process eco-friendly.

References

Agrafioti E, Bouras G, Kalderis D, Diamadopoulos E (2013) Biochar production by sewage sludge pyrolysis. J Anal Appl Pyrolysis 101:72–78

Ahmad T, Ahmad K, Alam M (2016) Sustainable management of water treatment sludge through 3 'R'concept. J Clean Prod 124:1–13

Balasubramanian S, Tyagi RD (2017) Value-added bio-products from sewage sludge. In: Current developments in biotechnology and bioengineering. Elsevier, Amsterdam, pp 27–42

Bhushan B, Halasz A, Hawari J (2006) Effect of iron (III), humic acids and anthraquinone-2, 6-disulfonate on biodegradation of cyclic nitramines by Clostridium sp. EDB2. J Appl Microbiol 100(3):555–563

di Bitonto L, Todisco S, Gallo V, Pastore C (2020) Urban sewage scum and primary sludge as profitable sources of biodiesel and biolubricants of new generation. Bioresour Technol Rep 9:100382

Canales A, Pareilleux A, Rols JL, Huyard A (1994) Decreased sludge production strategy for domestic wastewater treatment. Water Sci Technol 30(8):97

Capodaglio AG, Callegari A (2018) Feedstock and process influence on biodiesel produced from waste sewage sludge. J Environ Manag 216:176–182

CSE (2016) Article by DTE staff, 6 April 2016. https://www.downtoearth.org.in/news/waste/-78-of-sewage-generated-in-india-remains-untreated%2D%2D53444

Davies G, Ghabbour EA, Steelink C (2001) Humic acids: marvelous products of soil chemistry. J Chem Educ 78(12):1609

Demirbas A, Bamufleh HS, Edris G, Al-Sasi BO (2017) Biodiesel production from lipids of municipal sewage sludge by direct methanol transesterification. Energy Sources A Recov Util Environ Effects 39(8):800–805

Deublein D, Steinhauser A (2011) Biogas from waste and renewable resources: an introduction. Wiley, Chichester

Devi P, Saroha AK (2017) Utilization of sludge based adsorbents for the removal of various pollutants: a review. Sci Total Environ 578:16–33

Dignac M-F, Urbain V, Rybacki D, Bruchet A, Snidaro D, Scribe P (1998) Chemical description of extracellular polymers: implication on activated sludge floc structure. Water Sci Technol 38 (8–9):45–53

Elalami D, Carrère H, Monlau F, Abdelouahdi K, Oukarroum A, Barakat A (2019) Pretreatment and co-digestion of wastewater sludge for biogas production: recent research advances and trends. Renew Sust Energ Rev 114:109287

Eliche-Quesada D, Martínez-García C, Martínez-Cartas M, Cotes-Palomino M, Pérez-Villarejo L, Cruz-Pérez N, Corpas-Iglesias F (2011) The use of different forms of waste in the manufacture of ceramic bricks. Appl Clay Sci 52(3):270–276

Fabbri D, Torri C (2016) Linking pyrolysis and anaerobic digestion (Py-AD) for the conversion of lignocellulosic biomass. Curr Opin Biotechnol 38:167–173
Gagliano M, Braguglia C, Gianico A, Mininni G, Nakamura K, Rossetti S (2015) Thermophilic anaerobic digestion of thermal pretreated sludge: role of microbial community structure and correlation with process performances. Water Res 68:498–509
Gherghel A, Teodosiu C, De Gisi S (2019) A review on wastewater sludge valorisation and its challenges in the context of circular economy. J Clean Prod 228:244
Guo L, Li X-M, Bo X, Yang Q, Zeng G-M, Liao D-x, Liu J-J (2008) Impacts of sterilization, microwave and ultrasonication pretreatment on hydrogen producing using waste sludge. Bioresour Technol 99(9):3651–3658
Gupta A, Thakur IS (2015) Biodegradation of wastewater organic contaminants using Serratia sp. ISTVKR1 isolated from sewage sludge. Biochem Eng J 102:115–124
Gupta A, Thakur IS (2016) Study of optimization of wastewater contaminant removal along with extracellular polymeric substances (EPS) production by a thermotolerant Bacillus sp. ISTVK1 isolated from heat shocked sewage sludge. Bioresour Technol 213:21–30
Hall J (2000) Ecological and economical balance for different sludge management options. In: Langenkamp H, Marmo L (eds) Proceedings of the workshop on problems around sludge, Stresa, 18–19 Nov 1999
IWA Publishing News (2019). https://www.iwapublishing.com/news/sludge-production
Jiang L-B, Yuan X-Z, Li H, Chen X-H, Xiao Z-H, Liang J, Leng L-J, Guo Z, Zeng G-M (2016) Co-pelletization of sewage sludge and biomass: thermogravimetric analysis and ash deposits. Fuel Process Technol 145:109–115
Kapoor R, Ghosh P, Kumar M, Vijay VK (2019) Evaluation of biogas upgrading technologies and future perspectives: a review. Environ Sci Pollut Res 26(12):11631–11661
Karagiannidis A, Samaras P, Kasampalis T, Perkoulidis G, Ziogas P, Zorpas A (2011) Evaluation of sewage sludge production and utilization in Greece in the frame of integrated energy recovery. Desalin Water Treat 33(1–3):185–193
Karayildirim T, Yanik J, Yuksel M, Bockhorn H (2006) Characterisation of products from pyrolysis of waste sludges. Fuel 85(10–11):1498–1508
Karn SK, Kumar A (2019) Sludge: next paradigm for enzyme extraction and energy generation. Prep Biochem Biotechnol 49(2):105–116
Kech C, Galloy A, Frippiat C, Piel A, Garot D (2018) Optimization of direct liquid-liquid extraction of lipids from wet urban sewage sludge for biodiesel production. Fuel 212:132–139
Kelessidis A, Stasinakis AS (2012) Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. Waste Manag 32(6):1186–1195
Klai N, Tyagi RD, Surampalli RY, Zhang TC (2016) Value-added products from sludge. In: Green technologies for sustainable water management. American Society of Civil Engineers (ASCE), pp 255–296
Kominko H, Gorazda K, Wzorek Z (2017) The possibility of organo-mineral fertilizer production from sewage sludge. Waste Biomass Valor 8(5):1781–1791
Krüger O, Grabner A, Adam C (2014) Complete survey of German sewage sludge ash. Environ Sci Technol 48(20):11811–11818
Kumar M, Singhal A, Verma PK, Thakur IS (2017) Production and characterization of polyhydroxyalkanoate from lignin derivatives by Pandoraea sp. ISTKB. ACS Omega 2 (12):9156–9163
Li H, Li Y, Li C (2013) Characterization of humic acids and fulvic acids derived from sewage sludge. Asian J Chem 25(18):10087–10091
Liu K, Chen Y, Xiao N, Zheng X, Li M (2015) Effect of humic acids with different characteristics on fermentative short-chain fatty acids production from waste activated sludge. Environ Sci Technol 49(8):4929–4936
Liu Z, Smith SR (2019) Enzyme activity of waste activated sludge extracts. Water Sci Technol 80 (10):1861–1869

Logan BE, Hamelers B, Rozendal R, Schröder U, Keller J, Freguia S, Aelterman P, Verstraete W, Rabaey K (2006) Microbial fuel cells: methodology and technology. Environ Sci Technol 40 (17):5181–5192

Ma Y, Liu W-J, Zhang N, Li Y-S, Jiang H, Sheng G-P (2014) Polyethylenimine modified biochar adsorbent for hexavalent chromium removal from the aqueous solution. Bioresour Technol 169:403–408

Manara P, Zabaniotou A (2012) Towards sewage sludge based biofuels via thermochemical conversion–a review. Renew Sust Energ Rev 16(5):2566–2582

Marin-Batista J, Mohedano A, Rodríguez J, de la Rubia M (2020) Energy and phosphorous recovery through hydrothermal carbonization of digested sewage sludge. Waste Manag 105:566–574

Merzari F, Langone M, Andreottola G, Fiori L (2019) Methane production from process water of sewage sludge hydrothermal carbonization. A review. Valorising sludge through hydrothermal carbonization. Crit Rev Environ Sci Technol 49(11):947–988

Mian MM, Liu G, Fu B (2019) Conversion of sewage sludge into environmental catalyst and microbial fuel cell electrode material: a review. Sci Total Environ 666:525

Minderlein S, Blodau C (2010) Humic-rich peat extracts inhibit sulfate reduction, methanogenesis, and anaerobic respiration but not acetogenesis in peat soils of a temperate bog. Soil Biol Biochem 42(12):2078–2086

MoEF (2000) Ministry of Environment and Forests notification. http://www.moef.nic.in/legis/hsm/mswmhr.html. Accessed Dec 2019

Monea MC, Löhr DK, Meyer C, Preyl V, Xiao J, Steinmetz H, Schönberger H, Drenkova-Tuhtan A (2020) Comparing the leaching behavior of phosphorus, aluminum and iron from post-precipitated tertiary sludge and anaerobically digested sewage sludge aiming at phosphorus recovery. J Clean Prod 247:119129

Monlau F, Sambusiti C, Antoniou N, Barakat A, Zabaniotou A (2015) A new concept for enhancing energy recovery from agricultural residues by coupling anaerobic digestion and pyrolysis process. Appl Energy 148:32–38

Nastro R, Falcucci G, Minutillo M, Jannelli E (2017) Microbial fuel cells in solid waste valorization: trends and applications. In: Modelling trends in solid and hazardous waste management. Springer, New York, pp 159–171

Nazari L, Yuan Z, Santoro D, Sarathy S, Ho D, Batstone D, Xu CC, Ray MB (2017) Low-temperature thermal pre-treatment of municipal wastewater sludge: process optimization and effects on solubilization and anaerobic degradation. Water Res 113:111–123

Nikhil G, Chaitanya DK, Srikanth S, Swamy Y, Mohan SV (2018) Applied resistance for power generation and energy distribution in microbial fuel cells with rationale for maximum power point. Chem Eng J 335:267–274

Peng L, Wang Y, Lei Z, Cheng G (2012) Co-gasification of wet sewage sludge and forestry waste in situ steam agent. Bioresour Technol 114:698–702

Qian L, Zhang W, Yan J, Han L, Gao W, Liu R, Chen M (2016) Effective removal of heavy metal by biochar colloids under different pyrolysis temperatures. Bioresour Technol 206:217–224

Raheem A, Sikarwar VS, He J, Dastyar W, Dionysiou DD, Wang W, Zhao M (2018) Opportunities and challenges in sustainable treatment and resource reuse of sewage sludge: a review. Chem Eng J 337:616–641

Rizzardini CB, Goi D (2009) Considerations about European directives and italian regulation on sludge from municipal wastewater treatment plants: current status and future prospective. Open Waste Manage J 2:17–26

Roche E, de Andrés JM, Narros A, Rodríguez ME (2014) Air and air-steam gasification of sewage sludge. The influence of dolomite and throughput in tar production and composition. Fuel 115:54–61

Rulkens W (2008) Sewage sludge as a biomass resource for the production of energy: overview and assessment of the various options. Energy Fuel 22(1):9–15

Rulkens W, Bien J (2004) Recovery of energy from sludge–comparison of the various options. Water Sci Technol 50(9):213–221
Saha S, Saha B, Hazra G, Pati S, Pal B, Kundu D, Bag AG, Chatterjee N, Batabyal K (2018) Assessing the suitability of sewage-sludge produced in Kolkata, India for their agricultural use. Proc Indian Natl Sci Acad 84(3):781–792
Samolada M, Zabaniotou A (2012) Potential application of pyrolysis for the effective valorisation of the end of life tires in Greece. Environ Dev 4:73–87
Samolada M, Zabaniotou A (2014) Comparative assessment of municipal sewage sludge incineration, gasification and pyrolysis for a sustainable sludge-to-energy management in Greece. Waste Manag 34(2):411–420
Samorì C, Kiwan A, Torri C, Conti R, Galletti P, Tagliavini E (2019) Polyhydroxyalkanoates and Crotonic acid from anaerobically digested sewage sludge. ACS Sustain Chem Eng 7 (12):10266–10273
Shah S, Venkatramanan V, Prasad R (2019) Microbial fuel cell: sustainable green technology for bioelectricity generation and wastewater treatment. In: Shah S, Venkatramanan V, Prasad R (eds) Sustainable green technologies for environmental management. Springer, Singapore, pp 199–218
Shi S, Xu G, Yu H, Zhang Z (2018) Strategies of valorization of sludge from wastewater treatment. J Chem Technol Biotechnol 93(4):936–944
Sikarwar VS, Zhao M, Clough P, Yao J, Zhong X, Memon MZ, Shah N, Anthony EJ, Fennell PS (2016) An overview of advances in biomass gasification. Energy Environ Sci 9(10):2939–2977
Singh S, Kumar V, Dhanjal DS, Datta S, Bhatia D, Dhiman J, Samuela J, Prasad R, Singh J (2020) A sustainable paradigm of sewage sludge biochar: valorization, opportunities, challenges and future prospects. J Clean Prod 269:122259. https://doi.org/10.1016/j.jclepro.2020.122259
Skorek-Osikowska A, Uchman W, Werle S (2017) Modeling of energy crops gasification based on experimental data. Architect Civil Eng Environ 10(3):135–141. https://doi.org/10.21307/acee-2017-044
Skowrońska M, Bielińska EJ, Szymański K, Futa B, Antonkiewicz J, Kołodziej B (2020) An integrated assessment of the long-term impact of municipal sewage sludge on the chemical and biological properties of soil. Catena 189:104484
Smol M, Kulczycka J, Henclik A, Gorazda K, Wzorek Z (2015) The possible use of sewage sludge ash (SSA) in the construction industry as a way towards a circular economy. J Clean Prod 95:45–54
Suárez-Iglesias O, Urrea JL, Oulego P, Collado S, Díaz M (2017) Valuable compounds from sewage sludge by thermal hydrolysis and wet oxidation. A review. Sci Tot Environ 584:921–934
Tian Y, Zhang J, Zuo W, Chen L, Cui Y, Tan T (2013) Nitrogen conversion in relation to NH3 and HCN during microwave pyrolysis of sewage sludge. Environ Sci Technol 47(7):3498–3505
Titirici M-M, Thomas A, Antonietti M (2007) Back in the black: hydrothermal carbonization of plant material as an efficient chemical process to treat the CO_2 problem? New J Chem 31 (6):787–789
Tu W, Zhang D, Wang H (2019) Polyhydroxyalkanoates (PHA) production from fermented thermal-hydrolyzed sludge by mixed microbial cultures: the link between phosphorus and PHA yields. Waste Manag 96:149–157
Tyagi VK, Lo S-L (2013) Sludge: a waste or renewable source for energy and resources recovery? Renew Sust Energ Rev 25:708–728
Valentino F, Moretto G, Lorini L, Bolzonella D, Pavan P, Majone M (2019) Pilot-scale polyhydroxyalkanoate production from the combined treatment of organic fraction of municipal solid waste and sewage sludge. Ind Eng Chem Res 58(27):12149–12158
Viader RP, Jensen PE, Ottosen LM, Ahrenfeldt J, Hauggaard-Nielsen H (2015) Electrodialytic extraction of phosphorus from ash of low-temperature gasification of sewage sludge. Electrochim Acta 181:100–108

Vogel C, Exner RM, Adam C (2013) Heavy metal removal from sewage sludge ash by thermochemical treatment with polyvinylchloride. Environ Sci Technol 47(1):563–567
Wang J, Wan W (2009) Factors influencing fermentative hydrogen production: a review. Int J Hydrog Energy 34(2):799–811
Wang X, Li J, Liu R, Hai R, Zou D, Zhu X, Luo N (2017) Responses of bacterial communities to CuO nanoparticles in activated sludge system. Environ Sci Technol 51(10):5368–5376
Wang L, Chang Y, Li A (2019) Hydrothermal carbonization for energy-efficient processing of sewage sludge: a review. Renew Sust Energ Rev 108:423–440
Werle S (2015) Gasification of a dried sewage sludge in a laboratory scale fixed bed reactor. Energies 8(8):8562–8572
Werle S, Sobek S (2019) Gasification of sewage sludge within a circular economy perspective: a Polish case study. Environ Sci Pollut Res 26(35):35422–35432
Xiao K, Zhou Y (2020) Protein recovery from sludge: a review. J Clean Prod 249:119373
Xu H, He P, Gu W, Wang G, Shao L (2012) Recovery of phosphorus as struvite from sewage sludge ash. J Environ Sci 24(8):1533–1538
Yao Z, Su W, Wu D, Tang J, Wu W, Liu J, Han W (2018) A state-of-the-art review of biohydrogen producing from sewage sludge. Int J Energy Res 42(14):4301–4312
Zhang H, Xue G, Chen H, Li X (2018) Magnetic biochar catalyst derived from biological sludge and ferric sludge using hydrothermal carbonization: preparation, characterization and its circulation in Fenton process for dyeing wastewater treatment. Chemosphere 191:64–71
Zhao G, Ma F, Wei L, Chua H, Chang C-C, Zhang X-J (2012) Electricity generation from cattle dung using microbial fuel cell technology during anaerobic acidogenesis and the development of microbial populations. Waste Manag 32(9):1651–1658
Zhen G, Lu X, Kato H, Zhao Y, Li Y-Y (2017) Overview of pretreatment strategies for enhancing sewage sludge disintegration and subsequent anaerobic digestion: current advances, full-scale application and future perspectives. Renew Sust Energ Rev 69:559–577

Agricultural Waste Valorization: An Energy Production Perspective

11

Shiv Prasad, Dheeraj Rathore, and Anoop Singh

Abstract

The energy security, utilization of surplus agricultural waste, and environmental concerns lead to explore the opportunities for valorization of surplus agricultural waste. The agricultural waste is rich in lignocellulose, which can be converted into various forms of energy like ethanol, methane, hydrogen, etc. by adopting different technologies. Therefore, the available surplus agricultural residues can be utilized for sustainable energy production. This will not only produce energy from waste but also save the environment from emissions due to its disposal. The sustainable approach for valorization of agricultural waste can be found by employing the various modellings like life cycle assessment (LCA), life cycle costing (LCC), net energy ratio (NER), techno-economic assessment (TEA), etc.

Keywords

Agricultural waste · Lignocellulose · Bioenergy · Valorization · Sustainable bioeconomy · Torrefaction · Pyrolysis · Anaerobic digestion

S. Prasad
Centre for Environment Science and Climate Resilient Agriculture, Indian Agricultural Research Institute, New Delhi, India

D. Rathore
School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar, Gujarat, India

A. Singh (✉)
Department of Scientific and Industrial Research (DSIR), Ministry of Science and Technology, Government of India, Technology Bhawan, New Delhi, India

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_11

11.1 Introduction

Concerns over growing energy demand and energy security, together with the increase of CO_2 emissions due to fossil fuel utilization, contributing towards climate change, are driving the need to find sustainable energy sources (Prasad et al. 2012, 2019a; Behera and Prasad 2020; Venkatramanan et al. 2021a). The agricultural waste valorization for energy production could be one of the most efficient routes to minimize carbon emissions and dependency on fossil fuels (Prasad et al. 2020). Bioenergy or biofuels production from agricultural waste can counteract the accumulation of unrecycled products from various agrarian activities and agro-industrial sectors (Prasad et al. 2019b; Venkatramanan et al. 2021b); thus, addressing two environmental impacts concurrently, viz. disposal site requirement and emissions due to faulty disposal and reducing the dependency on the fossil fuels (Li and Yang 2016). Agricultural waste originates during different activities of crop production and its supply chain. It includes on-farm or off-farm wastes, post-harvest residues such as stalks, straw stalks, husk, bagasse, seed pods, waste fruit, vegetable peels, food processing and packaging wastes, waste from distribution, marketing and consumption sector (Prasad et al. 2007, 2020; Xu et al. 2018).

According to Food and Agriculture Organization (FAO), in 2016, agriculture corresponded to about one-third of total world land area and sugarcane, maize, wheat, rice, potatoes, etc. are among the most cultivated crops (FAO 2018). Deshavath et al. (2019) assessed that around 181.8 MT of agricultural residues (derived from rice, wheat, corn, and sugarcane crops) were burnt in open field in Brazil, China, India, and the United States in the year 2016, and the emissions were equivalent to 15.8 MT of CO_2 (Table 11.1).

FAO estimated that almost 1.3 billion tons of food are wasted every year. It is about one-third of the food produced globally. On the other hand, despite disposal and environmental challenges, agricultural waste can prove to be a sustainable source of energy. Agrarian waste has brought a lot of attention due to its rich organic composition, especially lignocellulose (Venkatramanan et al. 2021b). That can be turned into value-added products such as biochemicals, enzymes, and biofuels (Venkatramanan et al. 2021c). The valorization of agricultural waste into biofuel is in higher demand than its conversion to chemicals (Pham et al. 2015).

Table 11.1 Amount of crops residues burnt in open fields in the year of 2016 and their CO_2 emissions

Countries	Agriculture crop residues burnt (MT/year)				CO_2 emission (MT/year)
	Corn	Rice	Wheat	Sugarcane	
USA	35.11	0.07	7.10	0.24	3.73
India	10.20	23.63	12.09	3.22	4.25
China	38.98	16.75	9.74	1.09	5.75
Brazil	14.96	1.07	0.87	6.65	2.03
Total	*99.25*	*41.52*	*29.8*	*11.2*	*15.76*

Source: Adapted from Deshavath et al. (2019)

However, incorrect disposal of agricultural waste is becoming a severe and vexing problem. The current chapter is focusing on the sustainable use of agricultural wastes for the production of bioenergy.

11.2 Conversion Technologies

The term "Agricultural waste valorization" refers to processing activities aiming to reuse and recycle the wastes into useful products or energy and reduce the waste quantity. "*Waste valorization considers the processing of a large amount of production-related wastes and by-products, which are more homogeneous and abundant in magnitude. The types of wastes used in valorization usually are listed as non-hazardous according to environmental regulations in place*" (Kabongo 2013).

Conventional agricultural waste disposal methods include burning, dumping, landfilling, random piling, etc. Composting and anaerobic digestion are the two traditional waste management methods that keep agricultural waste out of landfills. Composting is popular because it provides biofertilizer for soil improvement. However, it also has shortcomings such as the release of leachate, NH_3, greenhouse gases, and odor (Liu et al. 2014). Currently, many modern conversion technologies are available, which can transform agricultural waste into biofuels for application in the residential, industrial, public transport, and power sectors. Figure 11.1 shows the common pathways for the production of liquid and gaseous fuels from agri-waste.

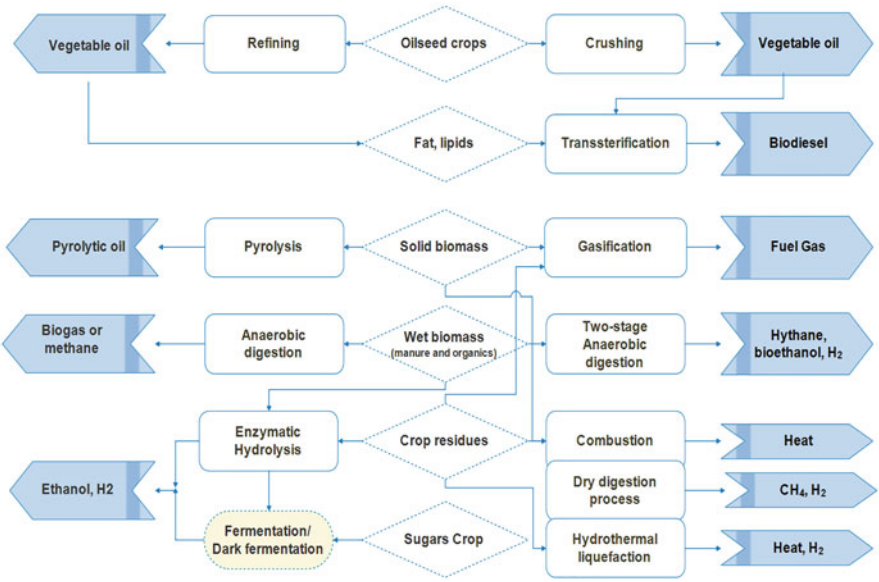


Fig. 11.1 Common pathways for valorization of agricultural waste to biofuels (Source: Prasad et al. 2020)

11.2.1 Thermal Conversion Technologies

The complete combustion of agricultural waste is called thermal conversion, which is to produce heat energy. The thermal combustion is achieved either by "direct combustion" or "incineration." Through the process of direct combustion, the agricultural waste is directly burned to transform into heat and electricity (Clini et al. 2008). The dry farm waste includes leaves, fibers, and stems. The dry farm waste is basically used as heat energy source and they are either directly burned or used as a raw material for the industrial boilers. The direct burning of dry farm waste for the purposes of cooking and lighting is in vogue globally. However, the open combustion of agricultural waste releases toxic emissions, namely dioxins, acid gases, and furans (Scarlat et al. 2015). Hence, direct combustion of agricultural waste is not a preferred option for energy generation. Agricultural waste is burnt in the industrial furnace to generate thermal energy. The thermal energy so generated is used to generate steam in a boiler. The steam turns the turbine that is attached to an electrical generator, which finally produces electricity (Chambers 2003).

Waste incineration is an oxidative combustion process to generate thermal energy under emission control. During the process of incineration, the farm wastes are converted either directly into CO_2 and water vapor or indirectly into CO, H_2, and char. In developed countries like EU, the USA, and Japan, where waste management policies check the land-based waste disposal, incineration is the preferred option (Scarlat et al. 2015). However, waste incineration technology is challenged by the factors like high capital, maintenance cost, and operation cost. These factors limit the energy recovery option (UNEP 2013; UN-HABITAT 2010). Further, the incineration process like the direct combustion process is influenced by the high moisture content of waste. It is also reported that the waste incineration without proper regulatory and control system is vulnerable to create harmful emissions like the dioxins and furans.

11.2.2 Thermochemical Conversion Technology

The thermochemical conversion process involves chemical reactions that occur at varying temperatures. These thermochemical conversion process may take place in the absence or presence of oxygen. While gasification process involves partial oxidation, the pyrolysis process occurs in the absence of oxygen. These processes require specialized reactors and process conditions. In the absence of such conditions, it may turn into an incineration or combustion process. It is important to note that the product of these processes end up either as fuel or as secondary feedstock (char) (Fig. 11.1). Similarly, incineration, pyrolysis, and gasification also release CO_2. The principles underlying the use of each of the thermochemical conversion technologies in harnessing energy from agricultural waste is here subsequently explained in particular.

Pyrolysis is one of the important thermochemical conversion technologies. It is basically a thermal degradation of biological materials in the absence of oxygen and

at a temperature of 400–900 °C (Bosmans et al. 2013). The pyrolysis process results in the production of pyrolysis oil, char, and syngas. The syngas is basically a mixture of carbon dioxide, carbon monoxide, hydrogen, water vapor, methane, and trace gases (Sahoo et al. 2021). Syngas can be used as a fuel. So pyrolysis process can be used for the production of heat, power, and chemicals. Despite the advantages of pyrolysis, agricultural waste with high ash content is not useful feedstocks for the pyrolysis process due to reactor blockage by ash accumulation.

Gasification is another thermochemical conversion process, which occurs in the partial presence of oxygen and at temperature 500–1800 °C to produce syngas. Syngas is used for heat production and electricity generation. In case of gasification process, generally wet agricultural waste is not preferred (Tock et al. 2010). However, supercritical water gasification (SCWG) technology is reported to be a promising technology to utilize wet agricultural waste. As regards the advantage of the supercritical water gasification technology, there is no need to dry the agricultural waste (Gasafi et al. 2008). The supercritical water gasification technology generates high amount of hydrogen and less of carbon monoxide. Further, the supercritical water gasification (SCWG) technology produces less tar and coke. Since the inorganics and salt compounds remained in the aqueous solution, the problem of corrosion can be controlled. However, the supercritical water gasification technology requires capital investment.

Plasma technology is gaining increasing interest for gas conversion applications, such as CO_2 conversion into value-added chemicals or renewable fuels. Plasma technology is based on a physical principle. Matter changes its state, when energy is supplied to it, solids become liquid, and liquids become gas. If even more power is provided to gas, it is ionized and goes into the energy-rich plasma state, the fourth state of matter (Nandkumar 2014). The initial energy essential to create plasma can either be thermal or electric current or electromagnetic radiation. The existence of charged gaseous species makes the plasma extremely reactive and causes it to behave differently from other gases, solids, and liquids. The peculiar benefit of plasma technology is that the energy confined in the plasma allows the usage of low energy biomass waste that would otherwise not be appropriate for energy production using gasification. The high-temperature conditions that are reached in plasma gasification result in the decomposition of organic compounds into their elemental constituents, forming a high-energy synthesis gas, consisting mainly of H_2 and CO_2.

Nevertheless, the application of plasma-based technology for energy generation is challenging. The use of electricity as a primary energy vector is expensive, turning the most potent barrier for plasma-based technology. Moreover, the inorganic fraction of glass, metals, and silicates that is melted and transformed into dense, inert, and non-leaching can be dangerous when released in the environment.

Torrefaction is a thermal process to convert biomass into a more homogeneous product that is densified through pelletization to produce a more energy-dense product called torrefied pellets (TOPs) or briquettes, with comparable properties to coal (Batidzirai et al. 2013). Torrefaction is referred to as mild pyrolysis and conducted in the temperature range at 200–300 °C under an inert air and low heating

rate (Medic et al. 2012). Torrefied biomass (briquettes), which retains up to 96% of its chemical energy, is hydrophobic and resistant to biodegradation. So, it is used as a substitute for coal, heating, co-firing power generation, and gasification (Prins et al. 2006). Briquettes formed from biomass waste is a cheap renewable energy source and eco-friendly. Despite the potential benefits of torrefaction technology, there are still some techno-economic challenges that need to be overcome before the technology is fully commercialized (Nordin 2012).

11.2.3 Biochemical Conversion Technologies

Biochemical conversion technologies of agricultural waste-to-energy are much more eco-friendly as compared to the thermal and thermochemical technologies. Biochemical conversion chiefly involves the action of enzymes to harness the energy from biomass. The technologies under this category are ethanol fermentation and anaerobic digestion for methane production.

11.2.3.1 Ethanol Production

Ethanol as an alternative fuel, is the most common biofuel worldwide. Currently, lignocellulosic biomass is being exploited extensively by many researchers across the globe for developing cleaner and sustainable energy as an alternative to the fossil fuel system (Prasad et al. 2007, 2019b). This ethanol is considered as a carbon-neutral biofuel due to the plant's origin of its carbon and, thus, when it is released during the combustion, it does not contribute to the increase in CO_2 emissions (Hsieh et al. 2002). Ethanol has been promoted as an alternative transportation fuel because of its antiknocking properties, which help to increase octane ratings and improve fuel efficiency (Prasad et al. 2014). The suitable feedstock for ethanol production are grouped into three: (a) directly fermentable sugar-containing materials such as starch; (b) lignocellulosic biomass; (c) urban/industrial organic residues. Reports are also available on direct fermentation of sugarcane, sugar beet, and sweet sorghum to ethanol (Prasad et al. 2009), which require the least costly pre-treatment, where starchy, lignocellulosic resources, and other wastes need expensive pre-treatment, to change into fermentable substrates. However, since maintaining the food security is given higher priority in our agrarian economy faced with rising population pressure, it is not possible to divert a fraction of the sugary or starchy food sources towards biofuel production. *Lignocellulosic material* is potentially the world's largest source of fermentable sugars. They can yield glucose after hydrolysis, which may further be fermented to ethanol. However, the recalcitrant structure of lignocellulose hinders the accessibility of carbohydrates to hydrolytic enzymes and prevents the release of fermentable sugars.

11.2.3.2 Anaerobic Digestion (AD)

Anaerobic digestion (AD) transforms agricultural waste into biogas and digested slurry in controlled anaerobic conditions. The resultant biogas is composed of

60–70% methane along with CO_2 (30–40%) and other traces, while digested sludge can act as a soil conditioner and plant nutrient (Prasad et al. 2017).

The AD process is driven by the concerted action of highly varied microbial populations, consisting of numerous groups of both strict and facultative anaerobes. The process is carried out in a well-designed anaerobic digester/bioreactor. The whole system consists of feedstock, digester, biogas holder, and digestate reservoir is named a biogas plant. The complete anaerobic digestion process can be separated into four main stages, namely hydrolysis, acidogenesis (or fermentation), acetogenesis, and methanogenesis. The anaerobic digestion process is performed in a properly designed bioreactor or anaerobic biodigester. The anaerobic digestion takes place due to the action of anaerobes and facultative anaerobes. The microorganisms involved in the anaerobic digestion process are diverse. The entire anaerobic digestion system is made up of "feedstock," "anaerobic digester," "digestate reservoir," and the "biogas holder." As regards the anaerobic digestion process, the stages are "hydrolysis," "acidogenesis," "acetogenesis," and "methanogenesis." In the hydrolysis process, the complex polysaccharides, biopolymers are converted into simpler substances by the synergistic action of mesophilic and thermophilic microorganisms. The complex polysaccharides include proteins and lipids. The products of hydrolysis are sugars, amino acids, fatty acids, and glycerol. The microorganisms involved in the hydrolysis process belong to the genera *Bacteroides, Butyrivibrio, Clostridium, Fusobacterium, Selenomonas,* and *Streptococcus* (Amani et al. 2010). In the process of acidogenesis, the fermentative organisms, anaerobic oxidizers convert the soluble monomers into organic acids. The microorganisms involved in acid generation are *Clostridium, Eubacterium,* etc. The products of acidogenesis are acetate, lactate, ethanol, CO_2, and H_2 (Insam et al. 2010). The intermediates such as propionate and butyrate are converted into carbon dioxide, hydrogen, and acetate by obligate hydrogen-producing acetogens (OHPA).

Syntrophic acetogenic bacteria such as *Syntrophomonas wolfei, Syntrophomonas sapovorans, Syntrophobacter wolinii*, *Syntrophobacter fumaroxidans, Desulfovibrio vulgaris*, *Thermoanaerobacterium brockii,* and *Pelobacter venetianus* are responsible for converting acetic acid into H_2 and CO_2. Methanogenesis is the last step in which acetate, H_2/CO_2, methanol, and formate are converted into methane (Gujer and Zehnder 1983). The genera *Methanosaeta* and *Methanosarcina* carry out the acetotrophic methanogenesis (Garcia et al. 2000). Other methanogenic groups, i.e., methylotrophic methanogens, utilize methane-containing compounds such as methanol, methylamine, and dimethyl sulfides (Deppenmeir et al. 1996). Although anaerobic digestion is an established waste management method for sewage sludge, wastewater, and animal manure, anaerobic digestion of agricultural waste has caused some operational insufficiencies. Furthermore, low temperature (<20 °C) limits methane yield, which is common during winter seasons.

11.3 Sustainability Assessment of Agricultural Waste Valorization

The technology search for sustainable valorization of agricultural waste for energy production is a great challenge, which can be met by conducting life cycle assessment (LCA) and by calculating the Net Energy Ratio (NER) because LCA is able to provide details of emissions/emission reduction, while NER tells about their energy balance, only positive energy balance processes can be considered for valorization purpose after conducting their LCA. The processes which shows positive energy balance and reduces the emission by replacing the existing/conventional energy source would be sustainable processes for conversion of agricultural waste valorization. The cost-effectiveness of the developed product is also very important to accept the product by the society. Therefore, life cycle costing (LCC) can be adopted for economic analysis of the developed product.

Duque-Acevedo et al. (2020) in a study mentioned that new and better techniques for the recovery of agricultural waste have been developed, based on industrial innovation and high technology, which has contributed to guaranteeing resource efficiency, sustainable production and consumption, and the reduction of negative environmental impact. Serna et al. (2016) in a techno-economic analysis of an specialized energy producing biorefinery to obtain bioethanol, biogas, and electricity taking advantage of agricultural wastes from Colombia concluded that the moisture content of agricultural waste is a challenge for waste valorization as it increases the production cost. They stated that such raw materials can be used under biorefinery scheme for some isolated zones for boosting rural areas or just to produce energy carriers as biogas or ethanol for transport.

Watson et al. (2020) review the impact of hydrothermal liquefaction (HTL) conditions and the feedstock composition on the energy and elemental distribution of process outputs with specific emphasis on the hydrothermal liquefaction aqueous phase (HTL-AP). They compare and contrast the current state of value-added products separation along with biological and thermochemical (gasification and HTL) pathways to valorize HTL-AP. They performed life cycle analysis (LCA) and techno-economic assessments (TEA) to appraise the environmental sustainability and economic implications of these different valorization techniques and presented the perspectives and challenges and the integration approaches of HTL-AP valorization pathways with HTL. Watson et al. (2020) mentioned that HTL-AP is a monetary and environmental burden to HTL biorefineries. Bio-conversion strategies may enable the valorization of HTL-AP, for the production of hydrogen, methane, electricity, and chemicals by employing different pathways, which would make HTL technology more energy productive and cost-effective.

Life cycle assessment (LCA) emerged as a powerful tool to assess the completed process chain and provide a better solution to reduce the emission and increase the net energy ratio. It involves the establishment of number of assumptions regarding the process and data. The results of LCA studies involve a high degree of uncertainty due to the lack of standardized assumptions, which make difficulties in the

assessment of the net energy production and GHG emissions amongst different studies (Quinn and Davis 2015). Techno-economic assessment (TEA) modeling has also emerged as an important tool to understand the commercial viability of biowaste-to-biofuel thermochemical techniques. Engineering related processing models with simulation software packages are generally used to estimate the investment and final selling prices of the final product (Onwudili et al. 2013; Quinn and Davis 2015). The biocrude oil yield, capacity for sustainable production, reducing the burden of the HTL process on the environment, and maximizing long-term profitability are instrumental factor influencing the optimism or pessimism of TEA studies (Juneja and Murthy 2017). Si et al. (2019) compared benchmark commercial applications of two different HTL-AP valorization methodologies, two-stage fermentation and catalytic hydrothermal gasification, to produce biohythane and suggested that two-stage fermentation with conventional reactors resulted in a higher net energy return than that of catalytic hydrothermal gasification.

The employment of various models such as LCA, LCC, NER, and TEA could give a better understanding for choice of processes to achieve a sustainable pathway for valorization of a particular agricultural waste.

11.4 SWOT Analysis of Agricultural Waste Valorization

The SWOT analysis is conducted basically to identify internal strengths and weaknesses, as well as external opportunities and threats of agri-waste valorization for energy production. The analysis is presented in Fig. 11.2. The analysis shows a strong strength for agricultural waste valorization because surplus agricultural waste is available in abundance, resolves the requirement of disposal site and also provides energy and not only reduces the dependency over fossil fuel but also reduces the environmental pollution. In spite of several strengths, it also has some weaknesses such as higher technology cost, absence of waste collection chain, higher capital investment, and lack of governmental support in terms of subsidy, compensations, etc. All this could lead to higher price of unit energy generated and/or negative net energy ratio. There is a number of opportunities available to improve the system and to attract the entrepreneurs and industrialists for valorization of agricultural waste for energy production. Entrepreneurs/industrialists can be attracted by showcasing the technologies at various platforms, by providing regular training and updating the new developments to them and also by developing policies to provide incentives, subsidies, etc. Some threats are also present like lack of specialized equipment manufacturer, entrepreneurs and policies, etc.

11.5 Conclusion

The valorization of agricultural waste for energy production can be considered as a best approach for utilization of agricultural waste as this will not only contribute in energy production but also help in reducing the emission generated due to its

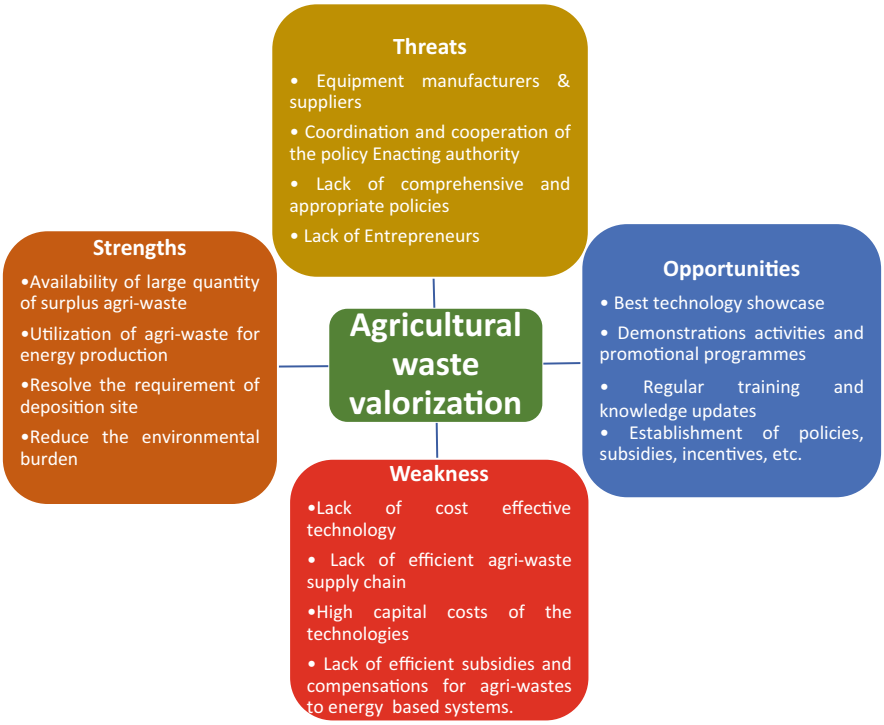


Fig. 11.2 SWOT analysis of agricultural waste valorization

disposal. The sustainable pathway can be achieved by employing various models like LCA, LCC, NER, TEA, etc. The valorization of agricultural waste has a number of opportunities and strengths like surplus agricultural waste, best technologies show-case, etc. but it also have some weaknesses and threats such as cost-effectiveness, efficient supply chain, equipments production, etc. The weaknesses and threats can be reduced by implementation of policies, incentives, subsidies, etc.

References

Amani T, Nosrati M, Sreekrishnan TR (2010) Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects-a review. Environ Rev 18:255–278

Batidzirai B, Mignot APR, Schakel WB, Junginger HM, Faaij APC (2013) Biomass torrefaction technology: techno-economic status and future prospects. Energy 62:196–214

Behera BK, Prasad R (2020) Environmental technology and sustainability. Elsevier, Amsterdam. ISBN: 9780128191033. https://www.elsevier.com/books/environmental-technology-and-sustainability/behera/978-0-12-819103-3

Bosmans A, Vanderreydt I, Geysen D, Helsen L (2013) The crucial role of waste to- energy technologies in enhanced landfill mining: a technology review. J Clean Prod 55:10–23

Chambers A (2003) Renewable energy in non-technical language. PennWell Books, Houston

Clini C, Musu I, Gullino ML (2008) Sustainable development and environmental management: experiences and case studies. Springer, Berlin, pp 228–229

Deppenmeir U, Müller V, Gottschalk G (1996) Pathways of energy conservation in methanogenic archaea. Archaea Microbiol 165:149–163

Deshavath NN, Veeranki VD, Goud VV (2019) Lignocellulosic feedstocks for the production of bioethanol: availability, structure, and composition. In: Rai M, Ingle AP (eds) Sustainable bioenergy advances and impacts. Elsevier, Netherlands, pp 1–19. https://doi.org/10.1016/B978-0-12-817654-2.00001-0

Duque-Acevedo M, Belmonte-Urena LJ, Cortes-García FJ, Camacho-Ferre F (2020) Agricultural waste: review of the evolution, approaches and perspectives on alternative uses. Glob Ecol Conserv 22:e00902

FAO (2018) Food and agriculture organization of the United Nations. In: World food and agriculture statistical pocketbook 2018. FAO, Rome

Garcia JL, Patel BKC, Ollivier B (2000) Taxonomic phylogenetic and ecological diversity of methanogenetic Archaea. Anaerobe 6:205–226

Gasafi E, Reinecke MY, Kruse A, Schebek L (2008) Economic analysis of sewage sludge gasification in supercritical water for hydrogen production. Biomass and Bioenergy 32(12):1085–1096

Gujer W, Zehnder AJB (1983) Conversion processes in anaerobic digestion. Water Sci Technol 15:127–167

Hsieh W, Chen R, Wu T, Lin T (2002) Engine performance and pollutant emission of an SI engine using ethanol-gasoline blended fuels. Atmos Environ 36:403–410

Insam H, Franke-Whittle I, Goberna M (2010) Microbes in aerobic and anaerobic waste treatment. In: Insam H, Franke-Whittle I, Goberna M (eds) Microbes at work. Springer, Berlin, pp 1–34

Juneja A, Murthy GS (2017) Evaluating the potential of renewable diesel production from algae cultured on wastewater: techno-economic analysis and life cycle assessment. AIMS Energy 5:239–257

Kabongo J (2013) Waste valorization. In: Encyclopedia of corporate social responsibility. Springer, Berlin, pp 2701–2706. https://doi.org/10.1007/978-3-642-28036-8_680

Li S, Yang X (2016) Biofuel production from food wastes. In: Luque R, CSK L, Wilson K, Clark J (eds) Handbook of biofuels production. Woodhead Publishing, Sawston, pp 617–653

Liu H, Ma X, Li L, Hu Z, Guo P, Jiang Y (2014) The catalytic pyrolysis of food waste by microwave heating. Bioresour Technol 166:45–50

Medic D, Darr M, Shah A, Potter B, Zimmerman J (2012) Effects of torrefaction process parameters on biomass feedstock upgrading. Fuel 91:147–154

Nandkumar N (2014) Plasma-the fourth state of matter. Int J Sci Technol Res 3(9):49–52

Nordin A (2012) The dawn of torrefaction BE-sustainable: the magazine of bioenergy and bioeconomy, pp 21–23. http://www.besustainablemagazine.Com/cms2/the-dawn-of-commercial-torrefaction

Onwudili JA, Lea-Langton AR, Ross AB, Williams PT (2013) Catalytic hydrothermal gasification of algae for hydrogen production: composition of reaction products and potential for nutrient recycling. Bioresour Technol 127:72–80

Pham TPT, Kaushik R, Parshetti GK, Mahmood R, Balasubramanian R (2015) Food waste-to-energy conversion technologies: current status and future directions. Waste Manag 38:399–408

Prasad S, Dhanya MS, Gupta N, Kumar A (2012) Biofuels from biomass: a sustainable alternative to energy and environment. Biochem Cell Arch 12(2):255–260

Prasad S, Kumar A, Muralikrishna KS (2014) Biofuels production: a sustainable solution to combat climate change. Indian J Agric Sci 84(12):1443–1452

Prasad S, Lata, Joshi HC (2009) Selection of efficient S. cerevisiae strain for ethanol production from Sorghum stalk juice. Curr Adv Agri Sci 1(2):70–73

Prasad S, Rathore D, Singh A (2017) Recent advances in biogas production. Chem Eng Process Tech 3(2):1038

Prasad S, Singh A, Joshi HC (2007) Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resour Conserv Recycl 50(1):1–39

Prasad S, Sheetal K, Venkatramanan V, Kumar S, Kannojia S (2019a) Sustainable energy: challenges and perspectives. In: Sustainable green technologies for environmental management, pp 175–197. https://doi.org/10.1007/978-981-13-2772-8_9

Prasad S, Venkatramanan V, Kumar S, Sheetal K (2019b) Biofuels: a clean technology for environment management. In: Sustainable green technologies for environmental management. Springer, Berlin, pp 219–240. https://doi.org/10.1007/978-981-13-2772-8_11

Prasad S, Singh A, Korres NE, Rathore D, Sevda S, Pant D (2020) Sustainable utilization of crop residues for energy generation: a life cycle assessment (LCA) perspective. Bioresour Technol 303:122964

Prins MJ, Ptasinski KJ, Janssen FJJG (2006) More efficient biomass gasification via torrefaction. Energy 31(15):3458–3470

Quinn JC, Davis R (2015) The potentials and challenges of algae based biofuels: a review of the techno-economic, life cycle, and resource assessment modeling. Bioresource Technol 184:444–452

Sahoo J, Patil P, Verma A, Lodh A, Khanna N, Prasad R, Pandit S, Fosso-Kankeu E (2021) Biochemical aspects of syngas fermentation. In: Prasad R, Singh J, Kumar V, Upadhyay CP (eds) Recent developments in microbial technologies. Springer Nature, Singapore, pp 395–424

Scarlat N, Motola V, Dallemand JF, Monforti-Ferrario F, Linus M (2015) Evaluation of energy potential of municipal solid waste from African urban areas. Renew Sust Energ Rev 50:1269–1286

Serna LVD, Toro JCS, Loaiza SS, Perez YC, Alzate CAC (2016) Agricultural waste management through energy producing biorefineries: the Colombian case. Waste Biomass Valor. https://doi.org/10.1007/s12649-016-9576-3

Si B, Watson J, Aierzhati A, Yang L, Liu Z, Zhang Y (2019) Biohythane production of post-hydrothermal liquefaction wastewater: a comparison of two-stage fermentation and catalytic hydrothermal gasification. Bioresour Technol 274:335–342

Tock JY, Lai CL, Lee KT, Tan KT, Bhatia S (2010) Banana biomass as a potential renewable energy resource: a Malaysian case study. Renew Sust Energ Rev 14(2):798–805

UNEP (2013) Municipal solid waste: is it garbage or gold? UNEP Global Environmental Alert Series. http://www.unep.org/pdf/UNEP_GEASoct_2013

UN-HABITAT (2010) Collection of municipal solid waste in developing countries. United Nations Human Settlements Programme (UN-HABITAT), Nairobi

Venkatramanan V, Shah S, Prasad R (eds) (2021a) Exploring synergies and trade-offs between climate change and the sustainable development goals. Springer, Singapore. https://doi.org/10.1007/978-981-15-7301-9

Venkatramanan V, Shah S, Rai AK, Prasad R (2021b) Nexus between crop residue burning, bioeconomy and sustainable development goals over North-Western India. Front Energy Res 8:614212. https://doi.org/10.3389/fenrg.2020.614212

Venkatramanan V, Shah S, Prasad R (eds) (2021c) Sustainable bioeconomy: pathways to sustainable development goals. Springer Nature, Singapore. https://doi.org/10.1007/978-981-15-7321-7

Watson J, Wang T, Si B, Chen W-T, Aierzhati A, Zhang Y (2020) Valorization of hydrothermal liquefaction aqueous phase: pathways towards commercial viability. Prog Energy Combust Sci 77:100819

Xu F, Li Y, Ge X, Yang L, Li Y (2018) Anaerobic digestion of food waste-challenges and opportunities. Bioresour Technol 247:1047–1058

Microbial Approach for Valorization of Mining Wastes and Tailings: An Overview

12

Fabíola Fernandes Costa, Érika Tallyta Leite Lima, Yrvana Pereira dos Santos Brito, Deborah Terra de Oliveira, Geraldo Narciso da Rocha Filho, and Luís Adriano Santos do Nascimento

Abstract

Mining is one of the most important economic activities on Earth and has played an important role in human existence. Minerals and metals are crucial for a large number of services and infrastructures that are used by society. However, extensive mining and industrial activities have led to production of large volumes of wastes and management of these materials, such as tailings and waste rock which is an environmental challenge. Moreover, the growing worldwide demand for ores has made developing processes for economic recovery from secondary sources increasingly important. In this scenario, the development of environmentally friendly technologies for valorizing mining wastes is mandatory. This chapter thus intends to provide a current overview of an alternative and green approach for valorization of mining waste and tailings by microbial means, that is, biomining.

Keywords

Biovalorization · Mining wastes and tailings · Biomining

12.1 Introduction

Minerals and metals have a vital role to play in the evolution of current and future civilizations, as has occurred in the past (Giurco and Cooper 2012). The extraction of such mineral substances existing underground has led to an activity that is necessary and indispensable for socio-economic development of a society: mining (Dubiński 2013).

F. F. Costa · É. T. L. Lima · Y. P. d. S. Brito · D. T. de Oliveira · G. N. da Rocha Filho · L. A. S. do Nascimento (✉)
Federal University of Pará, Belém, Pará, Brazil

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_12

Minerals are present in rocky bodies and are composed of several chemical elements; examples of minerals include hematite, pyrite, and bauxite. For their part, metals are the elements such as iron and aluminum extracted from these minerals (Karimi et al. 2012; Michel et al. 2018). Both are very useful to society through their final use, such as iron in construction, aluminum in electronics, gold for jewelry, and copper in wiring (Giurco and Cooper 2012). In several industrial processes, the raw materials come from minerals (Michel et al. 2018).

Mining activities have grown significantly over the last decade (Dubiński 2013). Worldwide, mineral extraction is growing rapidly (Henckens et al. 2016). Considering China alone, there has been a jump of from approximately 3 kg/person/year of metals to approximately 30 kg/person/year for the 2010–2014 period alone; obviously, this growth is proportional and intrinsically linked to the country's economic growth during that period.

The rapid growth of human population and the high rates of mineral consumption are leading to exhaustion of the planet's mineral resources, and that scarcity creates need to find new alternatives to meet the demands of worldwide progress (Giraldo and Tobón 2013). Although the mining activity is still mostly done through processing terrestrial minerals, minerals can also be obtained from oceanic resources and secondary sources such as scrap and mining sanitary landfills. Land-based mining has lower costs and environmental impacts than ocean-based mining; however, the latter option is becoming increasingly viable in order to meet future demands (Giurco and Cooper 2012; Behera and Prasad 2020).

Despite its crucial role in society's industrial and technological development, mining has frequently been associated with negative environmental impacts and social conflicts (Giurco and Cooper 2012; Caron et al. 2016).

Even with advances in the mining area, some procedures are still extensively used at many industrial plants. Mining activity commonly begins with open pit extraction of minerals, from rocks rich in the ore of interest. The material extracted then goes to the fragmentation, selection, and classification stages, and the product from those processes is presented in the desired granulometric range, while the undesirable portion is rejected close to the mining areas (Abed et al. 2008).

Thus, the extraction and beneficiation process produces residues that are stored in open pit containment basins, which, besides becoming an environmental liability for the companies, may become a threat to the environment (Lima et al. 2019). These waste (tailings) containment dams are some of the largest surface geotechnical structures built on Earth, and are frequently constructed with steep slopes using the coarse portion of the tailings with savings in cost, although keeping such structures standing is one of the most challenging tasks in mining residue management (Azam and Li 2010).

Considering the economic and environmental liabilities involved with those structures, the reuse of those industrial wastes in order to minimize impacts to the natural and human environment and near mining industries has been drawing increasing interest and bringing academic research and the mining industry together (Caron et al. 2016). Reusing these wastes is an advantageous alternative, because it reduces the worldwide stockpile of residues, reducing possible ground

contamination as well as providing economic value for the recycled wastes (Michel et al. 2018). Studies have reported applications of wastes as raw materials for producing cement and concrete (Nascimento et al. 2019), for geopolymer production (Capasso et al. 2019), and for producing catalysts.

With a view to a more sustainable future society, mining activity must become more creative and efficient, in order to achieve sustainability criteria and social and environmental goals (Giurco and Cooper 2012; Behera and Prasad 2020). One tendency that has been gaining ground in that regard is the use of biotechnology to exploit low quality ore deposits and mine tailings (Nascimento et al. 2019).

In this regard there is the bioleaching process, a biomining tool that involves processing and extraction of metals from their ores using microbial techniques. This method is currently used by the mining industry to extract copper, uranium, manganese, and gold from low-grade ores at an industrial scale. This extraction method uses the capacity that some microorganisms have for solubilizing some of the mineral components with a microbial attack that results in a solubilized metal (Das and Ghosh 2018), which enables it to be reused.

From that perspective, this chapter examines the important role that microorganisms can play in making use of mining industry wastes, thus reducing the environmental and economic liabilities that result from this activity that has a key role in economic, technological, and social development on a global scale.

12.2 The Mining Activity and Production of Wastes

Mining is an ancient industrial activity that is very profitable and provides a very large quantity of minerals and metals. Most giant world-class mineral deposits are formed under optimal physical-chemical processes, in high energy systems and under specific tectonic structures and/or magmatic environments (Groves 2008).

With the increase in the worldwide population as well as the advance of technologies, the use and refinement of metals has increased. This is because metals are the raw materials that have the widest range of applications, from use in electronic devices to the production of iron and its great number of uses. However, this practice has raised environmental concerns due to the large production of toxic wastes and their interaction with the environment, since the amount of these wastes that are recovered/reutilized does not equal the amount that has been discarded.

In ores there are two types of associated minerals: ore mineral, which has economic value, and gangue mineral, which is waste (Hudson-Edwards and Dold 2015). Mining residue is defined as all the original material, accumulated and present in mines, that is undesired and currently does not have economic value. Such residues may contain components in concentrations that may represent risks to the ecosystem and to human health, such as arsenic, lead, cyanide, silicates, oxides, hydroxides, carbonates, and sulfides (Hudson-Edwards et al. 2011).

At this point, it is necessary to distinguish between the two main types of mining wastes, those where the unwanted part has been separated from the ores ("tailings") and rocky material left after mining ("mine waste"). Tailings are the crushed rocks

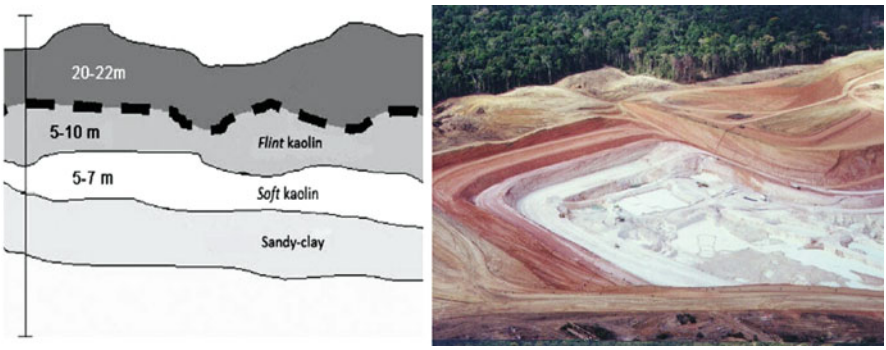


Fig. 12.1 Schematic geological profile structuration of Amazon kaolin. (Source: Adapted from (Carneiro et al. 2003))

left over after economically valuable metals, minerals, combustible minerals, or coal are removed, while mine waste comes from the materials above and below the ore bodies, which are removed during mining without being processed (Capasso et al. 2019).

One example is kaolin mining in the Amazon. In these formations, one may observe two main units of kaolin (Fig. 12.1). The first is the lower layer, made up mainly of soft kaolin (which is the kaolin that will be processed by industry) and the second is flint kaolin, which, because of its high iron content is not used by the kaolin industry and is thus a "mine waste" (Carneiro et al. 2003; Do Nascimento et al. 2011).

After being removed from the soil, soft kaolin is taken for processing, which consists of operations for centrifuging, magnetic separation, whitening, and filtering, which end up generating large volumes of tailings that are deposited in large settling ponds, which every year receive more than million tons of these materials (Figs. 12.2 and 12.3) (Pires et al. 2014; Lima et al. 2019).

In general, preparing metallic minerals such as Au, Cu, Pb, Zn and industrial mineral deposits such as phosphate and bauxite involves reduction in size and separation of those minerals. In the first stage of processing, blocks of rock crushed to break down the ore, enabling the minerals to be freed from the gangue phases, while at a later stage, the minerals in the ore are effectively separated from the gangue minerals. At this stage, several methods using gravimetric, magnetic, or electrical properties may be included (Lottermoser 2010). When the result is tailings, mainly due to the sulfide flotation process, it is very probable that acid mine drainage will result, which is one of the main causes of environmental problems in contemporary mining activity (Dold 2014).

Depositing tailings requires extensive surface areas and represents a potential risk of contaminating ground and surface waters (Smuda et al. 2014). In the past, those tailings were discharged into rivers or wetlands; but today they are used as underground fill, stored in open ponds, dried and stacked, or pumped into tailings

Fig. 12.2 Settling ponds for kaolin tailings. (Source: Barata et al. (2008))

Fig. 12.3 Satellite view (Google Maps) of the municipality of Barcarena, State of Pará-Brazil, where one can see settling ponds for tailings from the kaolin industry (yellow arrow) and "red mud" from aluminum (blue arrow)

reservoirs that can range in size from a few hectares to thousands of hectares, for later treatment and/or reprocessing (Lottermoser 2011).

These containment facilities are vulnerable due to several reasons, such as construction of dikes with waste materials from mining operations, lack of regulations on design criteria, especially in developing countries, and the high cost of maintenance after mine closings (Kossoff et al. 2014).

There are major socio-environmental concerns related to these constructions, mainly due to significant dam failures in the history of mining operations, such as at Merriespruit (South Africa), 1994; Omai (Guyana), 1994; Los Frailes (Spain), 1998; Baia Mare (Romania), 2000; and Aitik (Sweden), 2000; Mount Polley (Canada), 2014; Mariana (Brazil), 2015 and, more recently, Córrego do Feijão (Brazil), 2019 (Achterberg et al. 1999; David 2002; Bobos et al. 2006). However, the standard of public reports varies considerably around the world and many incidents of tailings-dam failures are underestimated, or may not even be reported, leading to serious losses for development of safety regulations in many regions of the world (Azam and Li 2010).

In order to reduce the volume of residues produced by mining activity, and thus minimize the possibility of new leaks from dams, a number of extraction methods have been implemented in some industrial plants, seeking more environmentally friendly processes. One example of that is treatment of mine wastes, with a view to reducing the toxicity or volume of wastes and reprocessing designed for using the residual material as raw material for producing valuable products such as minerals and recovered metals (Bobos et al. 2006).

However, considering future treatment mining wastes, one of the most promising alternative methods in environmental and economic terms consists of biomining and bioremediation, in other words, using biotechnological tools to attempt to recover ores from tailings or eliminate those toxic wastes released into the environment (Holmes 1988; Johnson 2013).

12.3 Biotechnology for Valorization of Mining Residues

"Biomining" is the main term used to define the use of microorganisms for extracting metals from ores. There are two main approaches in this field: (1) Bioleaching: where metals (for example zinc, nickel, copper, and nickel) are obtained in solubilized form after mineral dissolution; (2) Biooxidation: The target metal is exposed, making it more susceptible to chemical extraction (Johnson 2013).

The employment of microorganisms in biomining has a great appeal in scientific research, given that for meeting the enormous demand of the mining industry and its varied products there is a great variety of microorganisms that survive under varied environmental conditions and are capable of metabolizing different types of metals (Bindschedler et al. 2017; Pollmann et al. 2018).

The "in situ biomining" process has been used to recover metals since at least the Middle Ages. In locations remote from each other in Spain, China, and Great Britain, it was known that waters draining metal mines often contained soluble metals, and

that copper could be extracted from these waters by placing scrap iron into ponds around mine sites used to collect mine drainage (Johnson 2013). However, the pivotal role of bacteria in this process was not recognized until close to 1950. Mining exposes minerals to air and water, both of which are required by some specialized species of chemolithotrophic prokaryotes (bacteria and archaea) that can use energy derived from the oxidation of ferrous iron and/or reduced sulfur to sustain their growth (Rawlings and Johnson 2007).

This characteristic of some microorganisms has been shown to be an excellent alternative for recycling toxic metal wastes that are already in the environment such as wastes of copper, iron, uranium, nickel, and mercury, so that it is possible to recover some metals and reduce the release of toxic matter into the environment, making mining activity more ecologically responsible (Bindschedler et al. 2017; Pollmann et al. 2018).

Beyond the capacity to metabolize, transform, and even accumulate minerals using its own metabolism, it is also possible to induce a microorganism to absorb metals through genetic transformation techniques that enable it to metabolize wastes. It is also possible to reuse tailings from mining, making it a cleaner activity (Choudhary and Sar 2009; Anjum et al. 2011; Martínez-Bussenius et al. 2017).

The processes involved in biomining are heap leaching, dump leaching, and agitated leaching. In case of heap leaching, the freshly mined materials are heaped and then bioleached. With regard to dump leaching, the low-value ore or waste rock materials are placed in a sealed pit and through the bioleaching process, the valuable metals are extracted. In the case of agitated leaching, the crushed ore materials are placed in a vat, and the vat is shaken so as to distribute evenly the microbes and the crushed material. This process will fasten the bioleaching process. The leaching duration may take days or months, and thus, this can be slower than conventional mineral extraction techniques (Johnson 2014).

The biomining technology was established in the 1960s by the Kennecott Copper Corporation to extract copper from waste rock dumps at the Bingham Canyon mine in Utah, some years after the discovery of the first bacterium that was shown to be able to catalyze the dissimilatory oxidation of ferrous iron in low pH liquors, *Thiobacillus* (now *Acidithiobacillus*) *ferrooxidans*. The abilities of the iron-oxidizing acidithiobacilli to generate both ferric iron (the main oxidant of sulfide minerals) and sulfuric acid creates an environment in which they promote mineral dissolution. The extreme acidity of these bacteria is also important because it causes the retainment in solution of most of the metals released from the degraded sulfide minerals (Johnson 2013, 2014).

Obviously, after these events, the research on microorganisms for biomining has increased considerably. The thermophilic, mesophilic bacteria and archaea involved in biohydrometallurgy (Brierley and Brierley 2013) can be divided into those that can be used to process minerals and those that have potential for pollution control and metal recovery (Johnson 2013).

With regard to the microorganisms studied in the context of biomining, there are two major groups: autotrophic and heterotrophic. Some heterotrophic bacteria and fungi are known for their ability to leach metals, especially from oxidic, siliceous,

and carbonaceous materials. Unlike autotrophs, heterotrophic microorganisms utilize organic substances such as carbon and energy source, which improves costs. However, there are disadvantages such as possible contamination with undesired microorganisms and enhanced safety measures to avoid the occurrence of pathogenic organisms. This scenario makes their large industrial application on leaching processes a difficult undertaking (Schippers et al. 2013).

However, much knowledge about the interaction of microbe-mineral particles remains to be discovered, with a view to increasing metal production from biohydrometallurgy. Because biological processing would need to demonstrate a greater than 20% advantage over conventional pyrometallurgical processing in order to be interesting to the mining industry, fundamental research in developing the indispensable process for applying the organisms and optimizing their performance in engineered systems as well as the detailed engineering of heaps and stirred tanks to accommodate the microorganisms and their functions are crucial for improving the role of biomining (Brierley and Brierley 2013; Watling 2016).

The commercial application of bioleaching and mineral biooxidation is strongly dependent on improvement in engineering systems for heaps and stirred tanks. Considering the advantage of reducing the amount of acid metals by oxidative dissolution instead of leaching from the rocks in mining operations, it is understandable that dump leaching operations are found in many parts of the world (Johnson 2013).

The most widespread application of heap leaching is for bioleaching copper, although other base metals and gold have also been recovered using bioheap technology. The main advantage of using heaps as opposed to dumps is in the time required for extracting target metals, which is generally ~1 year in heaps and several years in dump operations (Johnson 2014).

Concerning aerated stirred tanks to process mineral concentrates, their main application is related to the biooxidation of gold concentrates (Rawlings 2002), although a notable exception has been found in a mining operation at Kasese, Uganda. This is a particularly good example of biomining technology being used to recover a valuable product from what was previously considered to be a waste material. In stirred-tank biohydrometallurgical applications, the principal problems encountered by the microorganisms are the buildup of potentially toxic components, physical damage to the cells as a result of vigorous agitation and aeration, and stresses due to loading solids into the reactors (Johnson 2013).

In a significant discovery, a new archaean species, *Acidianus sulfidivorans* sp. described by Plumb et al. (2007), shows a capacity for extracting metal under extreme conditions. *A. sulfidivorans* has a range of growth of pH 0.35 to 3.0 at 45 to 83 °C and may make bioleaching feasible under these conditions (Brierley and Brierley 2013).

Currently, bioleaching and mineral biooxidation are employed in heaps and stirred tanks. Both of these have been extensively used for well over a 100 years in the mining industry for traditional metallurgical processing; however, since the mid-1980s, they have been adapted for biohydrometallurgical processing. Heap bioleaching is used around the world for copper extraction, accounting for close to

15% of copper global production, while stirred-tank bioleaching and mineral biooxidation are typically reserved for the processing of mineral concentrates (Brierley and Brierley 2013).

In a paper on treatment methods for refractory gold-bearing ores (Iglesias and Carranza 1994), bioleaching is mentioned as a pre-treatment based on the action of bacteria (mainly *Thiobacillus, Sulfolobus,* and *Acidianus*) through direct mechanism biochemical reactions (by physical contact between bacteria and the mineral surface) or indirectly, for oxidizing reduced sulfur species and ferrous iron to sulfate and ferric iron, respectively.

$$4FeS_2 + 15O_2 + 2H_2O \overset{\text{bacteria}}{\rightarrow} Fe_2(SO_4)_3 + 2H_2SO_4 (\text{direct mechanism})$$

Reactions involved in indirect mechanism:

$$4FeAsS + 13O_2 + 16H_2 \overset{\text{bacteria}}{\rightarrow} 4H_3AsO_4 + 4FeSO_4$$

$$4FeAsS + 11O_2 + 2H_2O. \overset{\text{bacteria}}{\rightarrow} 4H_3AsO_3 + 4FeSO_4$$

$$H_3AsO_3 + 2Fe_2(SO_4)_3 + H_2O. \overset{\text{bacteria}}{\rightarrow} H_3AsO_4 + 2FeSO_4 + H_2SO_4$$

$$FeAsS + 2Fe_2(SO_4)_3 + 3H_2O + 5/2O_2 \rightarrow 2H_3AsO_4 + 6FeSO_4 + 2S$$

$$2FeAsS + 2Fe_2(SO_4)_3 + 4H_2O + 6O_2. \rightarrow 2H_3AsO_4 + 4FeSO_4 + H2SO_4$$

$$4FeSO_4 + O_2 + 2H_2SO_4 \overset{\text{bacteria}}{\rightarrow} 2Fe_2(SO_4)_3 + 2H_2O$$

$$2S + 2H_2O + 3O_2 \overset{\text{bacteria}}{\rightarrow} 2H_2SO_4$$

For the paper "Beneficiation of iron ore slime using *Aspergillus niger* and *Bacillus circulans*" experiments were performed for removing alumina from iron ore slime. After in situ leaching, *A. niger* removed about 38% of alumina in 15 days, while *B. circulans* was able to remove 39% of alumina after 6 days at 10% pulp density. When culture filtrate leaching was tested, *A. niger* removed 20% alumina with a 13-day culture filtrate at 2% pulp density (Pradhan et al. 2006).

With regard to biooxidation, gold is the main metal recovered on a commercial scale using this method. When comparing bioprocessing of ores and concentrates with smelting, it is possible to observe some advantages with the former, including both environmental and economic aspects. Biomining operates at far lower temperatures (generally 30–50 °C, though up to ~80 °C is possible) and pressures than alternative processing technologies and involves less energy consumption (cooling, rather than heating, is required, for example, in stirred-tank operations) and lower CO_2 emissions (Rawlings and Johnson 2007).

Donati et al. (2016) described how some microorganisms mostly belonging to the *Bacteria* and *Archaea* domains are capable of metabolizing metals and thus recover

them from waste materials released into the environment. Normally those microorganisms perform oxidation processes to process those minerals under aerobic conditions with oxygen acting as the acceptor of electrons. In the biooxidation process, what is most important is the recovery of compounds that contain iron and sulfur. Pollmann et al. (2018) also explain that microorganisms such as fungi and bacteria can be used in the process of bioleaching minerals, solubilizing them through reduction reactions in their metabolisms and forming intermediaries such as magnetite during the process. Among the processes for metabolizing minerals performed by microorganisms, there is also a procedure known as bioflocculation.

Ayangbenro et al. (2019) have confirmed the production of bioflocculants by bacteria identified as *Pseudomonas koreensis* and *Pantoea* sp. capable of aggregating kaolin particles and thus making them easier to remove from the medium.

Besides the use of individual microorganisms for recovering mineral residues, there are also studies in the use of consortia of microorganisms for that purpose, such as the work of Subedi et al. (2017) who reported using a consortium composed of *Pseudomonas, Lysinibacillus,* and species of *Thauera* microorganisms that work on simultaneously reducing nitrates and selenate. In all of those, bacteria genes for assimilating nitrate or selenate were identified, which reveals their potential for recovering those compounds in mining residues. Roh et al. (2002) isolated and identified 5 bacteria from an anaerobic environment that are capable of reducing minerals such as manganese, cobalt, and iron with their metabolism, showing their potential application as bioleachers of minerals and excellent tools for reuse of mining wastes.

12.4 Biomining of Important Elements and Materials

12.4.1 Copper

Copper, zinc, and nickel ores exist largely in the form of sulfides. These compounds are insoluble under normal environmental conditions as well as in weak acids, in contrast to oxidic minerals of these metals. Therefore, sulfide ores are concentrated by flotation and after an extensive process, a final product is obtained with a quality of 99.99% (Schippers et al. 2013).

Copper production from low-grade ores contributes significantly to world copper production. Further, heap and dump bioleaching contribute significantly to world copper production. In effect, biohydrometallurgy is gaining importance in the processing of low-grade copper sulfide ores, particularly in Chile which accounts for about 20% of world copper production. In Peru about 15% of the produced copper originates from leaching processes, and of that amount, approximately one-third is produced from bioleaching (Watling 2016; Schippers et al. 2013).

12.4.2 Gold

Bioleaching is not available for biomining gold. Sulfidic iron and, perhaps, the arsenic matrix, in which the gold is either bound in the crystal lattice or enclosed as a particle, is biologically oxidized (biooxidation). After that, the refractory gold is easily released by extracting solubilized oxidized mineral components. Therefore, pre-treatment of refractory gold concentrates in agitated tanks at a commercial scale has a long and successful history. It was possible to identify at least 14 active gold projects with biooxidation in the commercial project databank of the Minerals Economic Group (www.metalseconomics.com) and in other sources (BGR databanks). These projects produced at least 84 t of gold and 161 t of silver in 2010. Biooxidized gold corresponds to about 3.3% of the global production (Schippers et al. 2013; Watling 2016).

Looking to the future, Natarajan (2018) reports that the use of specific biosensors and bioindicators can be an important tool for discovering new gold deposits; gold-solubilizing microorganisms can be used for replacing toxic cyanides; gold extraction methods "in situ" should be developed; and microbiological and biotechnological processes will be created/improved for safe environmental disposal of processed wastes.

Natarajan (2018) describes the potential of biotechnology in gold mining for the present and future as a result of several research activities, mainly in the following aspects:

- Biogenesis and biomineralization of gold deposits.
- Bioindicators and biosensors for gold exploration.
- Biooxidation of gold-encapsulated sulfide mineral concentrates to free gold.
- Direct dissolution and complexation of gold (amino acids produced by *Bacillus* sp., cyanogenic organisms producing cyanides).
- Production of gold nanoparticles (*Bacillus subtilis, Shewanella, Rhodococcus,* and *Chlorella vulgaris*).
- Bioaccumulation and sorption of gold (fungi, yeasts, and bacterial biomass), gold removal from waste solutions.

12.4.3 Manganese

Manganese (Mn) is rarely found in its elemental state, being encountered mainly in its oxide form as a key constituent in naturally appearing minerals such as manganite (MnOOH) and birnessite ($dMnO_2$) (Das et al. 2011; Ghosh et al. 2016). Mn has fundamental applications in industries such as steel, glass, and batteries (Das et al. 2012) and its reserves are spread out over in many countries.

Extensive industrial activities lead to the generation of huge amount of Mn wastes (Ghosh et al. 2015). For instance, when lean grade ore containing 20% Mn is used, up to 7 tons of mining residues are generated for each ton of Mn produced (Toro et al. 1993). About 20% of untreated mining wastes are discharged into the

environment which leads to the contamination of both terrestrial and aquatic ecosystems (Liu et al. 2004). This contamination is an important concern, given that Mn wastes have several toxic effects on human health (Das and Singh 2011; Das et al. 2014).

In terms of economic aspects, there is an enormous loss of a valuable element in these wastes, which could be recovered. Moreover, recovery and reuse from Mn waste slag has been considered due to role of Mn extraction in depleting bioresources. It is therefore important to develop a technology for the recovery and reuse of these metallic pollutants (Ismail et al. 2004). Among various techniques that are under investigation to promote this is bioleaching.

Some reports about the biological process for this purpose have been found. For instance, Li et al. (2009) reported Mn biorecovery from electrolytic Mn slag, while Mn bioleaching from ores with *Penicillium citrinum* was described by Sukla et al. (1993) and Acharya et al. (2002). *Staphylococcus epidermis* recovered up to 80% of Mn, showing that the biological approach is a good option for recovery of metals from low-grade Mn ore and mine wastes. *Bacillus* sp. were described by Das and Ghosh (2018) as capable of solubilizing mine residues that contained manganese (Mn) through modifying parameters such as pH and the temperature at which those bacteria grew; they achieved high rates in bioconverting the mineral.

12.4.4 Other Elements

Compared with biooxidation of refractory gold and also with copper leaching, bioprocessing for other metals remains an exception. Nickel, cobalt, and zinc are biorecovered for use only when the framework conditions (low-grade or refractory character of the ore, remoteness of the production plant) exclude conventional ore-processing. In Uganda, a bioleaching plant at Kasese oxidizes 240 t of pyrite concentrate/day for the production of cobalt, copper, nickel, and zinc; Kasese accounts for only 1.25% of world cobalt production (Schippers et al. 2013).

For the case of uranium, an acid or alkaline digestion is applied for in situ leaching (a procedure in which insoluble UO_2 is oxidized to water-soluble uranyl ions $(UO_2)^{2+}$ by means of microorganisms such as *A. ferrooxidans*) considering the characteristics of the rocks. Here, the ore deposit receives an oxidizing solution with complexing agents via bore holes. Next, the uranium-enriched solution is pumped to the surface to be processing posteriorly. At present, the worldwide capacity of about 30 active uranium in situ leaching projects is about 34,000 t of uranium contents (Schippers et al. 2013).

12.4.5 Mine-Impacted Waters and Red Mud

Aqueous wastes are generally either streams that drain abandoned mines, mine tailings, and waste rocks (commonly called as “acid mine drainage”—AMD) or pit lakes that are (extremely) acidic (frequently pH < 3) and rich in various transition

metals, (mainly iron, copper, zinc), aluminum and metalloids such as arsenic, and sulfate ion (Nordstrom 2000). The acidic character of the waters allows these elements to stay in solution until further processing. Conventional remediation of acidic mine waters by aeration and neutralization (by addicting alkaline chemicals) results in a mixed metal sludge, which requires special deposition in designated landfill sites (Johnson and Hallberg 2005).

Despite major drawbacks, including high costs for reagents and operation, conventional chemical approaches fail to recover valuable metals. Approaches using hydrogen sulfide, produced by neutrophilic, sulfate-reducing bacteria, for offline metal sulfide precipitation (in a separate vessel) have been described as an alternative method to hydroxide precipitation (Tabak and Govind 2003).

As new approach, "passive biological treatment" uses organic composts either placed below the land surface to act as permeable reactive barriers or acting as bioreactors when constructed as flow-through systems for surface mine waters. Mixtures of organic materials relatively degradable and recalcitrant and are anaerobically degraded producing small molecular weight organic compounds that generates alkalinity and immobilizing some metals present in AMD (Schippers et al. 2013).

A very abundant side-product generated by the Bayer processing (estimated global production of 100–150 million tons per year) (Klauber et al. 2011; Power et al. 2011), red mud is a waste obtained during the extraction of alumina from bauxite ore. High alkalinity of red mud makes disposal into the environment a great challenge (Vachon et al. 1994; Pyasi and Smarajit 2014).

Red mud consists of Al, Fe, Si, and Ti bearing mineral phases such as gibbsite, kaolinite, hematite, anatase, quartz mineral, and others (Pyasi and Smarajit 2014). In the literature, there are few studies over biohydrometallurgical extraction of Al from red mud. For instance, pure cultures of *A. niger, P. notatum, P. Simplicissimum,* and *Trichoderma viride* and indigenous *Acidithiobacilli* enriched from sewage sludge been used for Al bioleaching, the adapted *Acidithiobacilli* was able to leach 47% of Al from the red mud versus 10% using non-adapted *Acidithiobacilli*. In another experiment, complexolysis leaching of Al from red mud allows a maximum of 75% of Al being bio-solubilized by *Penicillium simplicissimum* (Vachon et al. 1994). A maximum of 80% of Pb, 80% of Zn, and 67% of Cu was bioleached by *A. niger* within 30 days as well as 69.8% of Al and 60% of Ti were solubilized by *A. niger* within 40 days (Qu et al. 2013; Vakilchap et al. 2016).

Extremophiles play a major role in escalating the rates of metal recoveries, thereby making the treatment of recalcitrant ores possible. Commercial bioprocessing systems involve the use of complex microflora, comprising of both acidophilic heterotrophic and autotrophic microorganisms.

12.4.6 Biomining of e-Wastes

In the present day, electronic wastes (e-wastes) are one of the most problematic wastes. Its generation is increasing rapidly due to the increasing population and

development of Information and Technology sector. In European Union, it is estimated that the electronic wastes generation would be 12.3 million tons per year by the end of 2020 (Huisman et al. 2008). Further, recovery of metals from e-waste is potentially more efficient. Nevertheless, it is reported that the electronic wastes are complex both in terms of structure and composition and so recovery of metals is difficult (Guezennec et al. 2015). The printed circuit boards (PCBs) are the main valuable e-waste, once about 90% of its economic value is from the precious and/or critical metals present in its composition (Cui and Zhang 2008). For example, 65% of gallium produced globally are used in the manufacture of PCBs. Copper is another important element which is abundant in the electronic wastes. It is important to extract these valuable elements from the electronic wastes, considering its capacity and demand in 2015 (Guezennec et al. 2015).

Regarding its importance, the majority of the studies related to leach e-wastes under a biological approach involves the treatment of printed circuit boards (PCBs). There are some reports related to recover gold and other noble metals using various fungi and the organic acids produced by them or cyanide (Brandl et al. 2001, 2008; Faramarzi et al. 2004; Chi et al. 2011).

However, there is an important issue to be overcome and improve the efficiency of e-wastes bioleaching and it is their toxicity on the microorganisms. One possible way is to stagger the production of the lixiviant and the addition of the e-waste in a two-step process. This process has been shown to be efficient for improving leaching rates. Moreover, there are extra costs due to chemical products added to maintain the pH required for the microbial action (Guezennec et al. 2015).

According to results from Zhu et al. (2011), biohydrometallurgical reprocessing of e-wastes and mining wastes are technically possible, since they extracted copper (in laboratory-scale) with a yield above 90% after 3 days of one-step leaching. On the other hand, Guezennec et al. (2015) reported that the lixiviant solutions obtained from the bioleaching of sulfidic mining wastes using microbial consortium showed a good performance for base metal recovery from waste PCBs (Cu, Ni, Zn, Pb, Sn, Ga) (Guezennec et al. 2015).

12.5 The Fundamental Role of OMICS for the Future of Biomining Process

The future of bioleaching is related to obtaining an understanding of the molecular level of living organisms aided by genomics as well as by mRNA transcript level measurement through transcriptomics; protein abundance quantification using proteomics; measurement of cellular metabolites through metabolomics; and understanding cellular interactions through interactomics (Jerez 2008), which are being more and more used to study the microbial communities.

In general, these technologies may be used to discover new non-cultivable microorganisms and explore the new properties of microorganisms that arise from the interplay of genes, proteins, other macromolecules, small molecules, and the environment. This is particularly possible today due to the large numbers of genomic

sequences which are becoming increasingly available. However, additional genomic sequences of the different biomining microorganisms will be required to define the molecular adaptations to their environment and the interactions between the members of the community. The techniques adopted to study bacterial diversity use 16S rRNA and rDNA profiles. Since the genome sequencing of bioleaching microorganisms (*A. ferrooxidans*), genomes of many acidophilic microorganisms, plasmids and viruses from acidic environments have been deciphered (Cárdenas et al. 2010). Studies on microbial functions, microbial physiology, and microbial growth conditions are important as it aids in their application to bioleaching (Auernik et al. 2008; Aliaga Goltsman et al. 2009; Justice et al. 2014). Jerez (2008) stressed the importance of genomics, proteomics, and other OMICS technologies for biomining evolution. From questions as "How can molecular biology help to improve this complex community of microorganisms to make them more efficient in biomining?" or "How can the OMICS approach help to improve the efficiency of biomining?", Jerez (2008) reports that the main focus of research has been the energetic metabolism which is directly responsible for bioleaching. Genes involved in phosphate, sulfur and iron metabolism, quorum sensing, those potentially involved in several other functions such as metal resistance, amino acid biosynthesis pathways and those involved in the formation of extracellular polysaccharide (EPS) precursors have been studied extensively in the recent past. For instance, it could be cited:

- The great importance of being able to introduce DNA in biomining acidophiles is that such a system could be used to generate knock-out mutations in the genes of interest to do functional genomics and have an experimental proof of the supposed or predicted function of the corresponding gene product. In the case of a moderate thermophilic microorganism such as *Acidithiobacillus caldus* MTH-04, the construction of a conjugative transfer system between this extreme acidophile and *Escherichia coli* has recently been reported (Lin et al. 2007). This system will not only greatly facilitate the genetic and functional study of an important biomining bacterium but may eventually allow improving a strain by using a genetic engineering approach.
- The sulfur oxidation enzymatic activity structure from *Acidianus ambivalens* has detailed characterization (Urich et al. 2004). Although this microorganism is known to oxidize sulfur, its role in bioleaching is not clearly defined. On the other hand, the genetic basis for iron oxidation in the *Sulfolobus metallicus* has been described for the first time using a cDNA substrative hybridization approach (Bathe and Norris 2007). Several iron-responsive genes were identified and their expression was characterized under different growth conditions. These authors also reported preliminary proteomic evidence indicating that some of these genes may form a respiratory oxidase complex in the membrane of *S. metallicus*.
- A Chilean institution has already sequenced the genomes of *A. thiooxidans* and *A. caldus* and the company Biosigma S.A. has isolated some new bacteria from biomining operations and has entirely sequenced their genomes.

Having the genomic sequence of a given biomining microorganism is very important, since it is then possible to formulate hypothesis about the regulation of the expression of most of these genes under different environmental conditions. Metabolic reconstruction and modeling provides an important preliminary step in understanding the unusual physiology of this extremophile especially given the severe difficulties involved in its genetic manipulation and biochemical analysis. However, all these bioinformatic predictions will have to be demonstrated experimentally by using functional genomics, proteomics, and other approaches (Jerez 2008). It is possible to affirm that new OMICS methods are essential to provide appropriated chemical and/or physical manipulations of the bacterial environment and improve bioleaching rates in biomining operations through an accurate monitoring of the biomining consortia.

12.6 Conclusion

The mining industry still has many challenges to overcome in the coming years, but one may clearly observe the importance of the role that microorganisms have developed in recovering wastes from mine work. The enhancement of industrial application of bioprocessing systems will require the use of complex microflora, involving acidophilic, heterotrophic, and autotrophic microorganisms. Then, a better knowledge about interactions among the components of the microbial population is entirely needed once these advanced microorganisms can provide reuse for toxic tailings that are continuously being released into the environment and cause damage to biodiversity and to human populations. Several research activities (including OMICS studies) are being developed in that regard, applying biotechnology techniques for use and valorization of microorganisms that are capable of making use of those metals contained in tailings, increasing the yield obtained from refining minerals and reducing environmental pollution.

References

Abed AM, Sadaqah R, Kuisi MA (2008) Uranium and potentially toxic metals during the mining, beneficiation, and processing of phosphorite and their effects on ground water in Jordan. Mine Water Environ 27:171–182. https://doi.org/10.1007/s10230-008-0039-3

Acharya C, Kar RN, Sukla LB (2002) Bioleaching of low grade manganese ore with Penicillium citrinum. Eur J Miner Process Environ Prot 2:197–204

Achterberg EP, Braungardt C, Morley NH et al (1999) Impact of Los Frailes mine spill on riverine, estuarine and coastal waters in southern Spain. Water Res 33:3387–3394. https://doi.org/10.1016/S0043-1354(99)00282-1

Aliaga Goltsman DS, Denef VJ, Singer SW et al (2009) Community genomic and proteomic analyses of chemoautotrophic iron-oxidizing "Leptospirillum rubarum" (group II) and "Leptospirillum ferrodiazotrophum" (group III) bacteria in acid mine drainage biofilms. Appl Environ Microbiol 75:4599–4615. https://doi.org/10.1128/AEM.02943-08

Anjum R, Grohmann E, Malik A (2011) Molecular characterization of conjugative plasmids in pesticide tolerant and multi-resistant bacterial isolates from contaminated alluvial soil. Chemosphere 84:175–181. https://doi.org/10.1016/j.chemosphere.2011.02.002

Auernik KS, Cooper CR, Kelly RM (2008) Life in hot acid: pathway analyses in extremely thermoacidophilic archaea. Curr Opin Biotechnol 19:445–453. https://doi.org/10.1016/j.copbio.2008.08.001

Ayangbenro AS, Babalola OO, Aremu SO (2019) Bioflocculant production and heavy metal sorption by metal resistant bacterial isolates from gold mining soil. Chemosphere 231:113. https://doi.org/10.1016/j.chemosphere.2019.05.092

Azam S, Li Q (2010) Tailings dam failures: a review of the last one hundred years. Geotech News 28:50–53

Barata MS, Carpena D, Dal C (2008) Avaliação preliminar do resíduo caulinítico das indústrias de beneficiamento de caulim como matéria-prima na produção de uma metacaulinita altamente reativa. Ambient Construìdo 2:69–78

Bathe S, Norris PR (2007) Ferrous iron- and sulfur-induced genes in Sulfolobus metallicus. Appl Environ Microbiol 73:2491–2497. https://doi.org/10.1128/AEM.02589-06

Behera BK, Prasad R (2020) Environmental technology and sustainability. Elsevier, Amsterdam. (ISBN: 9780128191033) https://www.elsevier.com/books/environmental-technology-and-sustainability/behera/978-0-12-819103-3

Bindschedler S, Vu Bouquet TQT, Job D et al (2017) Fungal biorecovery of gold from E-waste. Adv Appl Microbiol 99:53–81. https://doi.org/10.1016/bs.aambs.2017.02.002

Bobos I, Durães N, Noronha F (2006) Mineralogy and geochemistry of mill tailings impoundments from Algares (Aljustrel), Portugal: implications for acid sulfate mine waters formation. J Geochem Explor 88:1–5. https://doi.org/10.1016/j.gexplo.2005.08.004

Brandl H, Bosshard R, Wegmann M (2001) Computer-munching microbes: metal leaching from electronic scrap by bacteria and fungi. Hydrometallurgy 59:319–326. https://doi.org/10.1016/S1572-4409(99)80146-1

Brandl H, Lehmann S, Faramarzi M, Martinelli D (2008) Biomobilization of silver, gold, and platinum from solid waste materials by HCN-forming microorganisms. Hydrometallurgy 94:14–17. https://doi.org/10.1016/j.hydromet.2008.05.016

Brierley CL, Brierley JA (2013) Progress in bioleaching: part B: applications of microbial processes by the minerals industries. Appl Microbiol Biotechnol 97:7543–7552. https://doi.org/10.1007/s00253-013-5095-3

Capasso I, Lirer S, Flora A et al (2019) Reuse of mining waste as aggregates in fly ash-based geopolymers. J Clean Prod 220. https://doi.org/10.1016/j.jclepro.2019.02.164

Cárdenas JP, Valdés J, Quatrini R et al (2010) Lessons from the genomes of extremely acidophilic bacteria and archaea with special emphasis on bioleaching microorganisms. Appl Microbiol Biotechnol 88:605–620. https://doi.org/10.1007/s00253-010-2795-9

Carneiro BS, Scheller T, De Castro EAS, Neves RF (2003) Caracterização mineralógica e geoquímica e estudo das transformações de fase do caulim duro da região do Rio Capim, Pará (Mineralogical and geochemical characterization of the hard kaolin from the Capim region, Pará, northern Brazil). Cerâmica 49:237–244

Caron J, Durand S, Asselin H (2016) Principles and criteria of sustainable development for the mineral exploration industry. J Clean Prod 119:215–222. https://doi.org/10.1016/j.jclepro.2016.01.073

Chi TD, Lee JC, Pandey BD et al (2011) Bioleaching of gold and copper from waste mobile phone PCBs by using a cyanogenic bacterium. Miner Eng 24:1219–1222. https://doi.org/10.1016/j.mineng.2011.05.009

Choudhary S, Sar P (2009) Characterization of a metal resistant Pseudomonas sp. isolated from uranium mine for its potential in heavy metal (Ni2+, Co2+, Cu2+, and Cd2+) sequestration. Bioresour Technol 100:2482–2492. https://doi.org/10.1016/j.biortech.2008.12.015

Cui J, Zhang L (2008) Metallurgical recovery of metals from electronic waste: a review. J Hazard Mater 158:228–256. https://doi.org/10.1016/j.jhazmat.2008.02.001

Das AP, Ghosh S (2018) Bioleaching of manganese from mining waste materials. Mater Today Proc 5:2381–2390. https://doi.org/10.1016/j.matpr.2017.11.459
Das AP, Singh S (2011) Occupational health assessment of chromite toxicity among Indian miners. Indian J Occup Environ Med 15:6–13. https://doi.org/10.4103/0019-5278.82998
Das AP, Sukla LB, Pradhan N, Nayak S (2011) Manganese biomining: a review. Bioresour Technol 102:7381–7387. https://doi.org/10.1016/j.biortech.2011.05.018
Das AP, Sukla LB, Pradhan N (2012) Microbial recovery of manganese using Staphylococcus epidermidis. Int J Nonferrous Metall 01:9–12. https://doi.org/10.4236/ijnm.2012.12002
Das AP, Ghosh S, Mohanty S, Sukla LB (2014) Consequences of manganese compounds: a review. Toxicol Environ Chem 96:981–997. https://doi.org/10.1080/02772248.2015.1005428
David CP (2002) Heavy metal concentrations in marine sediments impacted by a mine-tailings spill, Marinduque Island, Philippines. Environ Geol 42:955–965. https://doi.org/10.1007/s00254-002-0601-4
Do Nascimento LAS, Angélica RS, da Costa CEF et al (2011) Comparative study between catalysts for esterification prepared from kaolins. Appl Clay Sci 51:267–273. https://doi.org/10.1016/j.clay.2010.11.030
Dold B (2014) Evolution of acid mine drainage formation in Sulphidic mine tailings. Fortschr Miner 4:621–641. https://doi.org/10.3390/min4030621
Donati ER, Castro C, Urbieta MS (2016) Thermophilic microorganisms in biomining. World J Microbiol Biotechnol 32:179. https://doi.org/10.1007/s11274-016-2140-2
Dubiński J (2013) Sustainable development of mining mineral resources. J Sustain Min 12:1–6. https://doi.org/10.1007/978-981-10-4564-6_9
Faramarzi MA, Stagars M, Pensini E et al (2004) Metal solubilization from metal-containing solid materials by cyanogenic Chromobacterium violaceum. J Biotechnol 113:321–326. https://doi.org/10.1016/j.jbiotec.2004.03.031
Ghosh S, Pandey NK, Bhattacharya S et al (2015) Evidence of two oxidation states of copper during aggregation of hen egg white lysozyme (HEWL). Int J Biol Macromol 76:1–9. https://doi.org/10.1016/j.ijbiomac.2015.02.014
Ghosh S, Mohanty S, Akcil A et al (2016) A greener approach for resource recycling: manganese bioleaching. Chemosphere 154:628–639. https://doi.org/10.1016/j.chemosphere.2016.04.028
Giraldo W, Tobón JI (2013) Extraterrestrial minerals and future frontiers in mineral exploration. DYNA 80:83–87
Giurco D, Cooper C (2012) Mining and sustainability: asking the right questions. Miner Eng 29:3–12. https://doi.org/10.1016/j.mineng.2012.01.006
Groves DI (2008) Conceptual mineral exploration. Aust J Earth Sci 55:1–2. https://doi.org/10.1080/08120090701673310
Guezennec AG, Bru K, Jacob J, D'Hugues P (2015) Co-processing of sulfidic mining wastes and metal-rich post-consumer wastes by biohydrometallurgy. Miner Eng 75:45–53. https://doi.org/10.1016/j.mineng.2014.12.033
Henckens MLCM, van Ierland EC, Driessen PPJ, Worrell E (2016) Mineral resources: geological scarcity, market price trends, and future generations. Resour Policy 49:102–111. https://doi.org/10.1016/j.resourpol.2016.04.012
Holmes DS (1988) Biotechnology in the mining and metal processing industries: challenges and opportunities. Mining Metall Explor 5:49–56. https://doi.org/10.1007/bf03402490
Hudson-Edwards K, Dold B (2015) Mine waste characterization, management and remediation. Fortschr Miner 5:82–85. https://doi.org/10.3390/min5010082
Hudson-Edwards KA, Jamieson HE, Lottermoser BG (2011) Mine wastes: past, present, future. Elements 7:375–380. https://doi.org/10.2113/gselements.7.6.375
Huisman J et al (2008) 2008 Review of Directive 2002/96 on Waste Electrical and Electronic Equipment (WEEE). https://ec.europa.eu/environment/waste/weee/pdf/final_rep_unu_annexes.pdf

Iglesias N, Carranza F (1994) Refractory gold-bearing ores: a review of treatment methods and recent advances in biotechnological techniques. Hydrometallurgy 34:383–395. https://doi.org/10.1016/0304-386X(94)90074-4

Ismail AA, Ali EA, Ibrahim IA, Ahmed MS (2004) A comparative study on acid leaching of low grade manganese ore using some industrial wastes as reductants. Can J Chem Eng 82:1296–1300. https://doi.org/10.1002/cjce.5450820618

Jerez CA (2008) The use of genomics, proteomics and other OMICS technologies for the global understanding of biomining microorganisms. Hydrometallurgy 94:162–169. https://doi.org/10.1016/j.hydromet.2008.05.032

Johnson DB (2013) Development and application of biotechnologies in the metal mining industry. Environ Sci Pollut Res 20:7768–7776. https://doi.org/10.1007/s11356-013-1482-7

Johnson DB (2014) Biomining-biotechnologies for extracting and recovering metals from ores and waste materials. Curr Opin Biotechnol 30:24–31. https://doi.org/10.1016/j.copbio.2014.04.008

Johnson DB, Hallberg KB (2005) Acid mine drainage remediation options: a review. Sci Total Environ 338:3–14. https://doi.org/10.1016/j.scitotenv.2004.09.002

Justice NB, Norman A, Brown CT et al (2014) Comparison of environmental and isolate Sulfobacillus genomes reveals diverse carbon, sulfur, nitrogen, and hydrogen metabolisms. BMC Genomics 15:1–17. https://doi.org/10.1186/1471-2164-15-1107

Karimi E, Teixeira IF, Ribeiro LP et al (2012) Ketonization and deoxygenation of alkanoic acids and conversion of levulinic acid to hydrocarbons using a red mud bauxite mining waste as the catalyst. Catal Today 190:73–88. https://doi.org/10.1016/j.cattod.2011.11.028

Klauber C, Gräfe M, Power G (2011) Bauxite residue issues: II. options for residue utilization. Hydrometallurgy 108:11–32. https://doi.org/10.1016/j.hydromet.2011.02.007

Kossoff D, Dubbin WE, Alfredsson M et al (2014) Mine tailings dams: characteristics, failure, environmental impacts, and remediation. Appl Geochem 51:229–245. https://doi.org/10.1016/j.apgeochem.2014.09.010

Li HL, Li AM, Cao CM, Yang JB, Zeng Q, Liu GM (2009) Study on bioleaching of manganese from electrolytic manganese residue. Chin J Environ Eng 3:1667e1672

Lima ETL, Queiroz LS, Pires LHDO et al (2019) Valorization of mining waste in the synthesis of organofunctionalized aluminosilicates for the esterification of waste from palm oil deodorization. ACS Sustain Chem Eng 7:7543–7551. https://doi.org/10.1021/acssuschemeng.8b05484

Lin J, Zhang Z, Bian J et al (2007) Construction of conjugative gene transfer system between Escherichia coli and moderately thermophilic, extremely acidophilic Acidithiobacillus caldus MTH-04. J Microbiol Biotechnol 17:162–167

Liu HL, Teng CH, Cheng YC (2004) A semiempirical model for bacterial growth and bioleaching of Acidithiobacillus spp. Chem Eng J 99:77–87. https://doi.org/10.1016/j.cej.2003.10.010

Lottermoser B (2010) Mine wastes. Springer, Berlin

Lottermoser BG (2011) Recycling, reuse and rehabilitation of mine wastes. Elements 7:405–410. https://doi.org/10.2113/gselements.7.6.405

Martínez-Bussenius C, Navarro CA, Jerez CA (2017) Microbial copper resistance: importance in biohydrometallurgy. J Microbial Biotechnol 10:279–295. https://doi.org/10.1111/1751-7915.12450

Michel R, de Bilbao E, Poirier J (2018) Recycling bauxite waste for the mineral industry: phase transformations and microstructure during sintering. Waste Biomass Valor 9:1261–1271. https://doi.org/10.1007/s12649-016-9775-y

Nascimento D, Lucheta A, Palmieri M et al (2019) Bioleaching for copper extraction of marginal ores from the Brazilian Amazon region. Metals (Basel) 9:81. https://doi.org/10.3390/met9010081

Natarajan KA (2018) Biotechnology of metals. Principles, recovery methods and environmental concerns. Elsevier, Amsterdam

Nordstrom DK (2000) Advances in the hydrogeochemistry and microbiology of acid mine waters. Int Geol Rev 42:499–515. https://doi.org/10.1080/00206810009465095

Pires LHO, de Oliveira AN, Monteiro OV Jr et al (2014) Esterification of a waste produced from the palm oil industry over 12-tungstophosforic acid supported on kaolin waste and mesoporous materials. Appl Catal Environ 160–161:122–128. https://doi.org/10.1016/j.apcatb.2014.04.039

Plumb JJ, Haddad CM, Gibson JAE, Franzmann PD (2007) Acidianus sulfidivorans sp. nov., an extremely acidophilic, thermophilic archaeon isolated from a solfatara on Lihir Island, Papua New Guinea, and emendation of the genus description. Int J Syst Evol Microbiol 57:1418–1423. https://doi.org/10.1099/ijs.0.64846-0

Pollmann K, Kutschke S, Matys S et al (2018) Bio-recycling of metals: recycling of technical products using biological applications. Biotechnol Adv 36:1048–1062. https://doi.org/10.1016/j.biotechadv.2018.03.006

Power G, Gräfe M, Klauber C et al (2011) Bauxite residue issues: I. Current management, disposal and storage practices. Hydrometallurgy 108:33–45. https://doi.org/10.1016/j.hydromet.2011.02.006

Pradhan N, Das B, Gahan CS et al (2006) Beneficiation of iron ore slime using Aspergillus Niger and Bacillus circulans. Bioresour Technol 97:1876–1879. https://doi.org/10.1016/j.biortech.2005.08.010

Pyasi A, Smarajit S (2014) Value added metal extraction from red mud. Dissertation, National Institute of Technology, Rourkela. http://ethesis.nitrkl.ac.in/5744/1/E-94.pdf

Qu Y, Lian B, Mo B, Liu C (2013) Bioleaching of heavy metals from red mud using Aspergillus Niger. Hydrometallurgy 136:71–77. https://doi.org/10.1016/j.hydromet.2013.03.006

Rawlings DE (2002) Heavy metal mining using microbes. Annu Rev Microbiol 56:65–91. https://doi.org/10.1146/annurev.micro.56.012302.161052

Rawlings DE, Johnson DB (2007) The microbiology of biomining: development and optimization of mineral-oxidizing microbial consortia. Microbiology 153:315–324. https://doi.org/10.1099/mic.0.2006/001206-0

Roh Y, Liu SV, Li G et al (2002) Isolation and characterization of metal-reducing Thermoanaerobacter strains from deep subsurface environments of the Piceance Basin, Colorado. Appl Environ Microbiol 68:6013–6020. https://doi.org/10.1128/AEM.68.12.6013-6020.2002

Schippers A, Hedrich S, Vasters J, Drobe M, Sand W, Willscher S (2013) Biomining: metal recovery from ores with microorganisms. In: Schippers A, Glombitza F, Sand W (eds) Geobiotechnology I. Advances in biochemical engineering/biotechnology, vol 141. Springer, Berlin, Heidelberg, pp 1–47. https://doi.org/10.1007/10_2013_216

Smuda J, Dold B, Spangenberg JE et al (2014) Element cycling during the transition from alkaline to acidic environment in an active porphyry copper tailings impoundment, Chuquicamata, Chile. J Geochem Explor 140:23–40. https://doi.org/10.1016/j.gexplo.2014.01.013

Subedi G, Taylor J, Hatam I, Baldwin SA (2017) Simultaneous selenate reduction and denitrification by a consortium of enriched mine site bacteria. Chemosphere 183:536–545. https://doi.org/10.1016/j.chemosphere.2017.05.144

Sukla LB, Panchanadikar VV, Kar RN (1993) Microbial leaching of lateritic nickel ore. World J Microbiol Biotechnol 9:255–257. https://doi.org/10.1007/BF00327850

Tabak HH, Govind R (2003) Advances in biotreatment of acid mine drainage and biorecovery of metals: 2. Membrane bioreactor system for sulfate reduction. Biodegradation 14:437–452. https://doi.org/10.1023/A:1027332918844

Toro L, Vegli F, Terreri M, Ercole C, Lepidi A (1993) Manganese bioleaching from pyrolusite: bacterial properties reliable for the process. FEMS Microbiol Rev 11:103–108. https://doi.org/10.1111/j.1574-6976.1993.tb00273.x

Urich T, Bandeiras TM, Leal SS et al (2004) The Sulphur oxygenase reductase from Acidianus ambivalens is a multimeric protein containing a low-potential mononuclear non-haem iron Centre. Biochem J 381:137–146. https://doi.org/10.1042/BJ20040003

Vachon P, Tyagi RD, Auclair JC, Wilkinson K (1994) Chemical and biological leaching of aluminum from red mud. Environ Sci Technol 28:26–30. https://doi.org/10.1021/es00050a005

Vakilchap F, Mousavi SM, Shojaosadati SA (2016) Role of Aspergillus Niger in recovery enhancement of valuable metals from produced red mud in Bayer process. Bioresour Technol 218:991–998. https://doi.org/10.1016/j.biortech.2016.07.059

Watling H (2016) Microbiological advances in biohydrometallurgy. Fortschr Miner 6:49. https://doi.org/10.3390/min6020049

Zhu N, Xiang Y, Zhang T et al (2011) Bioleaching of metal concentrates of waste printed circuit boards by mixed culture of acidophilic bacteria. J Hazard Mater 192:614–619. https://doi.org/10.1016/j.jhazmat.2011.05.062

Microbial Degradation of Lignocellulosic Biomass to Obtain High Value-Added Products

13

J. A. Cecilia, C. P. Jiménez-Gómez, C. García-Sancho, and P. Maireles-Torres

Abstract

The depletion of the fossil fuels has led to the search for and development of more sustainable and environmental benign energy source. In this sense, the biomass has emerged as alternative resource since biomass is the only source from which it is possible to obtain energy and mainly chemicals that, currently, are obtained from the traditional fossil fuels. The selection of the biomass source is a key parameter to reach a sustainable process. Considering these premises, the lignocellulosic biomass has emerged as a potential alternative source due to its non-edible character as well as its high availability throughout the Earth. Traditionally, the lignocellulosic biomass has been treated thermochemically to obtain high value-added products and energy. However, in the recent past, the microbial treatment of the lignocellulosic biomass has emerged as efficient methodology to solve the problems of energy shortage and the synthesis of valuable products. The aim of this chapter is to evaluate the metabolic process involved in the microbial degradation of lignocellulosic biomass as well as highlight the valuable products obtained through this microbial treatment by alcoholic fermentation and anaerobic digestion.

Keywords

Lignocellulosic wastes · Microbial degradation · Value products

J. A. Cecilia (✉) · C. P. Jiménez-Gómez · C. García-Sancho · P. Maireles-Torres
Departamento de Química Inorgánica, Cristalografía y Mineralogía (Unidad Asociada al ICP-CSIC), Facultad de Ciencias, Universidad de Málaga, Málaga, Spain
e-mail: jacecilia@uma.es

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_13

13.1 Introduction

In the last two centuries, the industrial revolution has led to an improvement of life quality, which has implied a growing of the world population, but an excessive consumption of the planet's resources led to global climate change (Venkatramanan et al. 2020; 2021a). From the energetic point of view, fossil fuel reserves are gradually diminishing, so that they will be depleted in mid-term. This problematic situation, together with more stringent environmental laws, has prompted the search and development of alternative energy sources to replace the traditional fossil fuels (Prasad et al. 2019). Several alternative energy sources such as solar, geothermal, wind, tidal, or biomass have emerged as an environmental benign energy source (Prasad et al. 2021). However, a sole energy source is not enough to satisfy the global demand, so it is necessary to use several complementary energy sources. Among them, in the last decade, much attention has been paid to biomass, because it is the only source from which both energy and chemicals can be obtained (Venkatramanan et al. 2021b).

Biomass has been used since time immemorial. Biomass energy sources include woody plants, food crops, agricultural crop residues, forest wastes, etc. (Shah and Venkatramanan 2019). The selection of the biomass source is a key parameter that must be considered to reach a long-term sustainable green technology (Shah et al. 2019). It is necessary to select a non-edible biomass to avoid its interference with the food chain, since this would cause an increase in the price of the edible biomass as a consequence of the economic speculation, which would provoke an increase in social imbalances. Lignocellulose is a potential biomass source (Shah and Venkatramanan 2019) due to its worldwide availability and, in many cases, no interference with the food chain, being also considered a low-value waste (Huber et al. 2006). Lignocellulose is composed by lignin (15–25%), cellulose (40–50%), and hemicellulose (20–35%) (Huber et al. 2006; Mariscal et al. 2016). Each fraction can be selectively extracted with relatively high purity. Then, these fractions are undergone to thermochemical treatment to obtain both high value-added chemicals and energy (Adrio and Demain 2014). Nevertheless, these treatments may require severe pressure and temperature conditions, as well as the design of expensive catalyst.

The biodegradation of lignocellulosic biomass with microbes has emerged as an alternative to the chemical degradation. In fact, it is well known that the bacterial degradation of several plants, fruits, or seeds has been employed to obtain products such as wine, beer, vinegar, cheese, or bread since many centuries ago (Adrio and Demain 2014).

Focusing on lignocellulosic biomass, cellulose can be fractionated into their respective monomers (glucose) by the action of cellulase enzymes. Then, glucose is usually transformed into bio-alcohols, used as biofuels (Ahmad et al. 2010). In the case of hemicellulose, several enzymes such as β-xylosidase, endo-1,4-xylanase, acetylxylan esterase, α-L-arabinofuranosidase, and α-glucuronidase degrade the xylan-polymer to give rise to monomers, mainly xylose and mannose. The obtained liquor is considered as feedstock for the synthesis of valuable products, which can be

used from fuel additives to the polymer field (Kumar et al. 2008; Carvalheiro et al. 2008). With regard to the lignin, this fraction is formed by a heterogeneous polymer composed by aromatic units of p-hydroxyphenyl, guaiacyl, and syringyl. Generally, the white-rot fungi and lignin-enzymes (lignin peroxidase, manganese peroxidase, laccase) can degrade the lignin fraction into bioproducts with interesting applications, like bioremediation (Beltz et al. 2001) or biobleaching (Takano et al. 2001). Similarly, several kinds of bacteria such as *Aneurinibacillus aneurinilyticus*, *Bacillus subtilis*, and *Paenibacillus subtilis* have shown a great potential for the degradation of lignin into lighter polymerization fractions (Raj et al. 2007; Chandra and Bharagava 2013).

In summary, it has been reported that a wide variety of microorganisms, such as fungi, bacteria, or actinomycetes are able to degrade both hemicellulose and cellulose into other carbonaceous species with value addition. In the case of lignin, this fraction is the most of recalcitrant of lignocellulosic biomass, in such a way that the number of microorganisms that can degrade it is more limited. As representative example of the selective degradation of a specific lignocellulosic fraction, the white-rot fungi possess enzymes with strong oxidative activity to degrade the lignin fraction into CO_2 (Sánchez 2009). In the same way, brown-rot fungi are able to depolymerize the cellulose fraction, while the lignin fraction is only slightly modified.

Nowadays, the development of microbial field for the environmental biotechnology is considered as a challenge to detect and isolate ligninolytic enzymes. However, this protocol can vary depending on the starting microorganism, as well as possible contaminants, mainly organic pollutants, since these organic compounds can interfere in the purification steps (Chandra and Bharagava 2013). Despite this drawback, the large amount of lignocellulosic wastes generated worldwide could be bio-converted into valuable and necessary compounds for society. Among the advantages related to the use of microorganisms to valorize the lignocellulosic biomass, it can be highlighted that milder experimental conditions are required in comparison to non-microbiological procedures. Other advantages are associated to the absence of solvent, so the wastes or by-products obtained in these microbiological processes are practically negligible (Zimbardi et al. 1999; Howard et al. 2003). The main drawbacks of these enzymatic processes are associated to the heterogeneity of lignocellulosic residue and the scarce development of analytical tools to follow enzymatic cellulose degradation.

Considering these premises, this chapter pursues to carry out a detailed study of lignocellulosic biomass and its enzymatic degradation. In addition, this chapter aims to highlight the methods of analysis and the products obtained after enzymatic degradation.

13.2 Composition of the Lignocellulosic Biomass

As stated above, plant cell walls are composed of three fractions (cellulose, hemicellulose, and lignin), whose composition and percentage depend on plant species, age, or growth stage (Jeffries 1994) (Fig. 13.1; Table 13.1). Cellulose is the main constituent of plant cell walls, providing its structural support (Table 13.2), being a

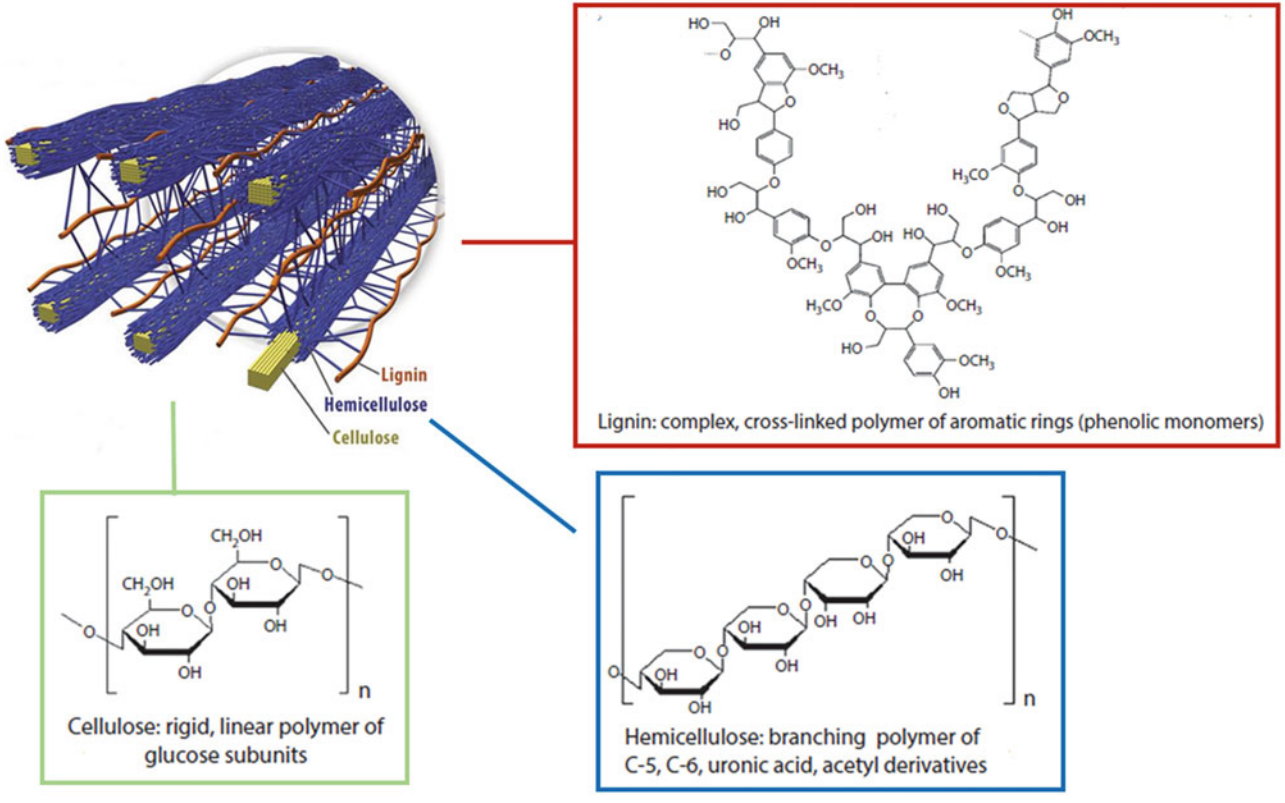


Fig. 13.1 Structure of lignocellulosic biomass. (Source: https://science.osti.gov/ber/Highlights/2014/BER-2014-07-p)

Table 13.1 Percentage of cellulose, hemicellulose, and lignin of common agricultural residues and wastes

	Percentage (%)		
Lignocellulose source	Cellulose	Hemicellulose	Lignin
Coastal Bermuda grass	25	35.7	6.4
Corn cobs	45	35	15
Cotton seeds hair	80–95	5–20	0
Fresh bagasse	33	30	19
Hardwood stems	40–55	24–40	18–25
Leaves	15–20	80–85	0
Newspaper	40–55	25–40	18–30
Nut shells	25–30	25–30	30–40
Paper	85–99	0	1–15
Primary wastewater solids	8–15	NA	24–29
Rice straw	32	24	18
Softwood steams	45–50	25–35	25–35
Solid cattle manure	2–5	1–3	3–6
Sorted refuse	60	20	20
Swine waste	6	28	NA
Switch grass	45	31	12
Wastepaper from chemical pulps	60–70	10–20	5–10

polymer of β-D-glucopyranose moieties linked via β-1,4-glycosidic bonds, forming long and linear chains (microfibrils) interconnected between them by intermolecular hydrogen bonding, obtaining a crystalline structure with high resistance to hydrolysis, as well as amorphous regions prone to enzymatic degradation (Beguin and Aubert 1994).

Hemicellulose is a complex polymer of heteropolysaccharides coating the cellulose microfibrils, composed of pentoses (D-xylose and L-arabinose) and hexoses (D-glucose, D-mannose and D-galactose), which form branched chains linked by β-(1,4)-glycosidic bonds and occasionally β-(1,3)-glycosidic (Table 13.2). Generally, hemicellulose possesses a lower molecular weight, in comparison to cellulose, while branches with short lateral chains are easily hydrolyzed (Saha 2003), being this fraction the most thermochemical sensitive of the lignocellulosic biomass (Hendriks and Zeeman 2009).

Lignin is a complex fraction formed by cross-linked polymers of phenolic monomers (coniferyl, coumaryl, and sinapyl alcohols) (Table 13.2), which confers rigidity in plant cell walls, as well as resistance to oxidative stress and microbial attack. Lignin is considered the "glue," joining the different fractions of lignocellulose, making it insoluble in water (Hendriks and Zeeman 2009). Lignin is also in close association with the cellulose microfibrils, in such a way that lignocellulose is less prone to undergo enzymatic and microbial hydrolysis (Chang and Holtzapple 2000).

Table 13.2 Chemical composition and elemental structure of cellulose, hemicellulose, and lignin in cell walls of plants

	Cellulose	Hemicellulose	Lignin
Subunit	D-pyran glucose units	D-xylose, L-arabinose, mannose, galactose, glucuronic acid	Guaiacylpropane (G), syringylpropane (S), phydroxyphenylpropane (H)
Sugar units	2000–14,000	500–3000	>10,000
Bonds between the subunits	β-1,4-Glycosidic bonds	β-1,4-Glycosidic bonds in main chains; β-1.2-, β-1.3-, β-1.6-glycosidic bonds in side chains	Various ether and C-C bonds, mainly β-O-4 ether
Polymer	β-Glucan	Polyxylose, galactoglucomannan (gal-Glu-man), glucomannan (Glu-man)	G lignin, GS lignin, GSH lignin
Composition	Three-dimensional linear molecular composed of crystalline and amorphous regions	Three-dimensional heterogeneous molecule with small crystalline regions	Amorphous, heterogeneous, nonlinear three-dimensional polymer
Bonds between three components	Bonds between three components	Contains chemical bond with lignin	Contain chemical bond with hemicellulose

Source: Yang (2008)

13.3 Cellulose

13.3.1 Structure and Properties of Cellulose

Cellulose is the most abundant biopolymers in nature, accounting for an annual biomass production of 1.5×10^{12} tons through photosynthesis processes. This polymer is a linear polysaccharide polymer, formed mainly by monomers of glucose, forming a fibrous structure, which cannot be digested easily (Brown 1996). Cellulose is an insoluble polymer formed by 2000–14,000 units, which appear in plant cell walls as microfibrils with a diameter of 2–20 nm and a length of 100–40,000 nm. Cellulose is also generated as a highly hydrated form by the bacterium *Acetobacter xylinum*.

The framework type of cellulose, arrangement in fibrils, and interactions between them are indicated in Fig. 13.2. The cellulose displays a complex structure, though the smallest details are difficult to observe (O'Sullivan 1997). Generally, the cellulose is a relatively simple polymer formed by D-glucose linked by β-1,4-glycosidic bonds to generate a linear polymeric chain formed by D-glucose residues. In turn, the individual fibers are interconnected between them by hydrogen bonding and Van der Waals forces, generating a crystalline structure.

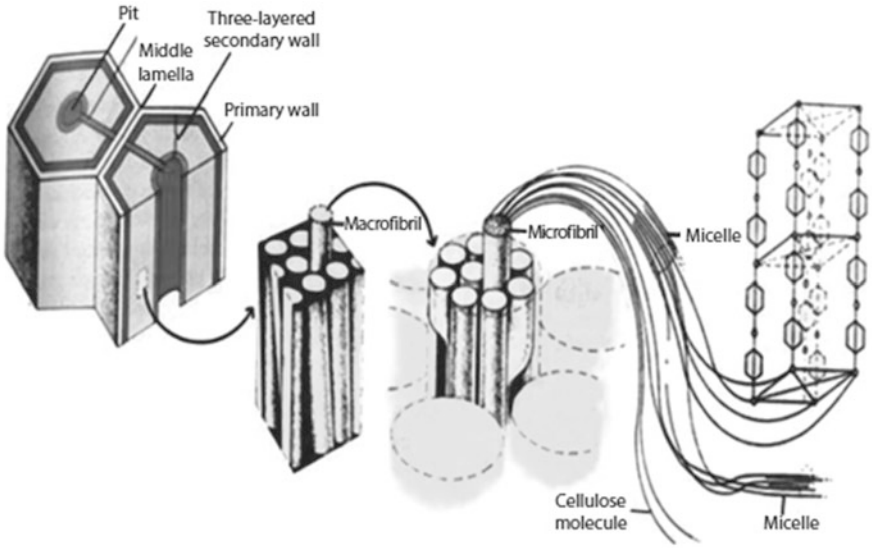


Fig. 13.2 Elemental framework of cellulose and arrangement of macro- and microfibrils

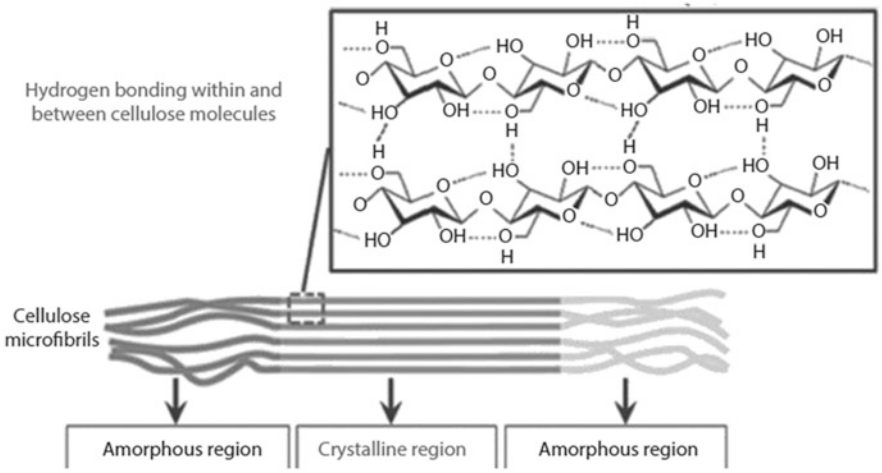


Fig. 13.3 Chemical structure and interaction of cellulose units. Amorphous and crystalline region of cellulose fibers

In spite of cellulose being highly crystalline, this fraction is not uniform, since less ordered sections and other sections with high crystallinity are present (Hon 1994) (Fig. 13.3). The structural complexity increases even more, since crystalline cellulose also interacts with hemicellulose and lignin fractions, in variable proportions depending on the species, resulting in very complex morphologies.

The biosynthesis of cellulose is not clear yet. It would involve the polymerization of glucose units from a substrate denoted uridine diphosphate (UDP) glucose to obtain *p*-1,4-D-glucan, using cellulose synthase as enzyme. UDP glucose can also be obtained from sucrose synthase enzyme, which is related to the plasma membrane. On the other hand, it has been reported that the feeding of glucose to bacteria *Gluconacetobacter xylinum* can synthesize modified cellulose (Bauchop 1979).

13.3.2 Cellulose Biodegradation

Several microorganisms such as bacteria, fungi, some anaerobic protozoa and slime molds can degrade cellulose into simpler structures, form three types of cellulases (exo-1,4-β-D-glucanase, endo-1,4-β-D-glucanase, and β-glucosidase) either in the form of complex or separately. These microorganisms can interact with the cellulose wastes, releasing CH_4, CO_2 and H_2O under anaerobic conditions, while aerobic conditions only lead to the formation of CO_2 and H_2O (Leschine 1995).

Cellulases, which hydrolyze the β-1,4-glycosidic bonds, can be classified into endoglucanases, exoglucanases, and cellobiohydrolases. Endoglucanases hydrolyze the internal bonds, the amorphous cellulose and the termination of fibers, while exoglucanases cleave the polymeric structure to produce tetrasaccharides, or disaccharides such as cellobiose. In the case of the cellobiohydrolases, this can hydrolyze the cellobiose to form D-glucose units. Itself, each enzyme does not have enough potential to completely degrade cellulose in its respective monomers. On the other hand, the joint work of all of them exerts a synergistic effect that increases the efficiency of the hydrolysis step (Fig. 13.4).

13.3.2.1 Biodegradation of Cellulose by Fungi

The degradation of cellulose using aerobic fungi is the best characterized cellulase system, as inferred from the studies carried out with *Trichoderma koningii*, *Trichoderma reesei*, *Trichoderma viride*, *Penicillium funiculosum/pinophilum*, *Sporotrichum pulverulentum,* or *Talaromyces emersonii*. In all cases, the endoglucanases (1,4-β-D-glucan glucanohydrolase) attack the H_3PO_4-swollen cellulose, releasing cello-oligosaccharides. The exo-l,4-β-D-glucanase (1,4-β-D-glucan cellobiohydrolase) hydrolyzes H_3PO_4-swollen cellulose, removing the cellobiose units from the end of the cellulose chain. The synergistic effect of endoglucanase and exoglucanase improves the hydrolysis degree of crystalline cellulose. Then, β-glucosidase (cellobiase or β-D-glucoside glucohydrolase) hydrolyzes the cellobiose into glucose.

Most fungal cellulases are formed by glycoproteins in different forms. For example, *Trichoderma viride* contains four exoglucanases with different composition related between them, which interact covalently with the cellulose. In the same way, *Talaromyces emersonii* also presents four endoglucanases, which differ in their glycosylation degree.

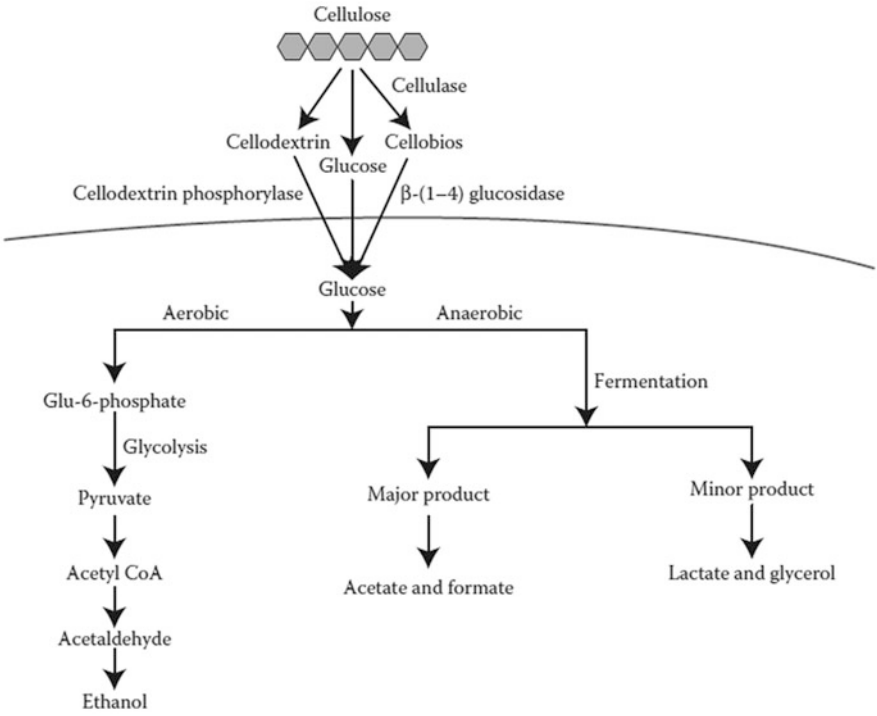


Fig. 13.4 Pathway of the degradation of cellulose

In most cases, the extracellular cellulase of fungi appears as individual entities, although there are some exceptions. Thus, *Trichoderma reesei* possesses aggregates of extracellular enzyme formed by six proteins with cellulase, β-glucosidase, and xylanase activities. These enzymes are aggregated in the cell wall through Ca^{2+} species. However, it is not clear how the crystalline cellulose interacts with these components and suffers the subsequent hydrolysis. Similar results were obtained on rumen anaerobic fungi, since this microbe produces extracellular cellulases and xylanases, which favor the depolymerization of the cellulose fraction and its subsequent fermentation in the rumen (Bauchop 1979).

Several fungi as *Orpinomyces*, *Piromyces*, *Coccomyces*, *Neocallimastix*, and *Rurainorayces* have shown activity in the anaerobic digestion of cellulose. These fungi have been ingested by herbivorous and omnivorous animals, being these anaerobic fungi detected in saliva, alimentary tracts, and feces, which can help to a partial depolymerization of cellulose. Between these species, most of the studies were carried out with the species *Neocallimastix frontalis*, which is formed by a multicomponent enzyme complex with a specific activity of the crystalline cellulose solubilizing factor in degrading fibers higher than other cellulases reported in the literature (Wilson and Wood 1992), so this species plays an important role in the anaerobic digestion of cellulose in the rumen.

13.3.2.2 Biodegradation of Cellulose by Bacteria

Most of the aerobic and anaerobic bacteria produce endoglucanases. In the case of aerobic processes, it has been reported that actinomycete *Microbispora bispora* can hydrolyze the crystalline cellulose through a cellobiohydrolase enzyme (Table 13.3). The degradation of crystalline cellulose is more effective using anaerobic bacteria, such as *Acetivibrio cellulolyticus*, *Clostridium cellulovorans, Ruminococcus albus and Fibrobacter succinogenes* or *Clostridium thermocellum,* being involved a high molecular weight enzyme complex called cellulosome, which is formed by several endoglucanases and exoglucanases. In this sense, some bacteria, such as *Clostridium thermocellum*, *Ruminococcus flavefaciens* or *Acetivibrio cellulolyticus*, improve the efficiency of the hydrolysis in the presence of Mg^{2+}, Ca^{2+} or thiol-species (Eriksson

Table 13.3 Degradation of cellulose using bacteria

Microorganism	Enzyme	Starting action
Aerobic bacteria		
Bacillus licheniformis 1	Endoglucanase	Cleaves linkages at random
Bacillus sp. 1139	Endoglucanase	Cleaves linkages at random
Bacillus sp. N-4	Endoglucanase cel A	
	Endoglucanase cel B	
	Endoglucanase cel C	
Bacillus sp. KSM-522	Endoglucanase	
Bacillus subtilis	Endoglucanase	
Microbispora bispora	Endoglucanase I	Hydrolysis of 1,4-β-D-glucosidic linkages in cellulose and cellotetraose, releasing cellobiose from the nonreducing ends of the chains
	Endoglucanase II	
	Exoglucanase I	
	Exoglucanase II	
	β-Glucosidase	Releases glucose from cellobiose and short chain cellooligosaccharides
Anaerobic bacteria		
Acetivibrio cellulolyticus	Exoglucanase C1	Hydrolysis of 1,4-beta-D-glucosidic linkages in cellulose and cellotetraose, releasing cellobiose from the nonreducing ends of the chains
	Endoglucanase C2	Cleaves linkages at random
	Endoglucanase C3	Cleaves linkages at random
	β-Glucosidase B1	Releases glucose from cellobiose and short chain cellooligosaccharides
Mesophilic actinomycetes		
Streptomyces antibiotics	Complete cellulase system	Catalyzes extensive hydrolysis of crystalline cellulose
S. flavogriseus		
S. Viridosporus		
S. Nitrosporeus		
S. Albogriseolus		
Micromonospora melanosporea		

and Wood 1985). Several authors have isolated individual subunits of these bacteria, but the mechanism of the cellulose degradation is not fully understood (Eriksson and Wood 1985). Thus, the isolation of some subunits of *Clostridium thermocellum* cellulosome and its interaction with a commercial cellulose (Avicel) has been reported, but its solubilization was low, so the degradation is not very efficient. Other authors isolated four subunits of *C. thermocellum* cellulosome and also studied the solubilization of crystalline cellulose (Wood 1985; Bhat and Bhat 1997).

In spite of the fact that anaerobic bacteria digest the crystalline cellulose, it is not clear that exoglucanase enzymes are involved in the hydrolysis of this lignocellulosic fraction. As example, the bacteria *Bacteroides cellulosolvens* have the enzyme cellobiose phosphorylase, while other bacteria as *Clostridium thermocellum* or *Ruminococcus flavefaciens* generate both β-glucosidase and cellobiose phosphorylase (Wood 1985; Bhat and Bhat 1997). The kinetic data established that cellobiose phosphorylase shows a higher affinity by the cellobiose, although the mechanism is not well described yet.

Nowadays, the main challenge is related to the isolation of all the subunits of the bacteria involved in cellulose degradation to understand the hydrolysis mechanism associated to these cellulases enzymes.

The anaerobic digestion of cellulose takes place through a fermentation process carried out by microorganisms (Vogels 1979). This process can occur on the surface of soils or sediments, as well as in river or marine sediments, in such a way that these zones are considered a reservoir in the carbon cycle. Thus, it has been reported that the top meter of soil can contain a carbon content two times higher than that found in the atmosphere, so this source could contribute to abate climate change and as alternative feedstock to obtain high value-added products (Jenkinson et al. 1991).

The anaerobic digestion of cellulose occurs through several fermentation and respiration steps, using electron acceptors such as CO_2 or nitrate, Mn (IV), Fe (III), and sulfate, which can decompose the cellulose into CH_4, CO_2, and H_2O, as Fig. 13.5 shows.

In most soils, the anaerobic digestion is similar. Some microbes (fungi or bacteria) generate enzymes, which depolymerize cellulose into cellodextrins, cellobiose, and glucose. Then, these sugars are fermented by saccharolytic and cellulolytic microorganisms producing CO_2, H_2, alcohols, and organic acids, such as acetic, propionic, and butyric. Most of the H_2 produced is consumed by methanogens and homoacetogens to reduce CO_2 into methane and acetate, respectively. On the other hand, some methanogens also use acetate to produce fermenters or homoacetogens by the acetoclastic scission to CH_4 and CO_2 (Mah 1981). The presence of syntrophic bacteria also plays a key role in the cellulose conversion into CH_4 and CO_2, since this bacteria ferments carboxylic acids (propionate and butyrate) or alcohols into acetate, H_2, and CO_2. Syntrophic bacteria only grow in the presence of H_2-consuming organisms, so they take advantage of the H_2 generated in their growth (Wolin and Miller 1987).

In summary, the synergistic effect of several microbes can lead to the total digestion of cellulose into CO_2 and CH_4 under anaerobic conditions. As cellulose is a very abundant carbon source on the planet, the formation of both CO_2 and CH_4

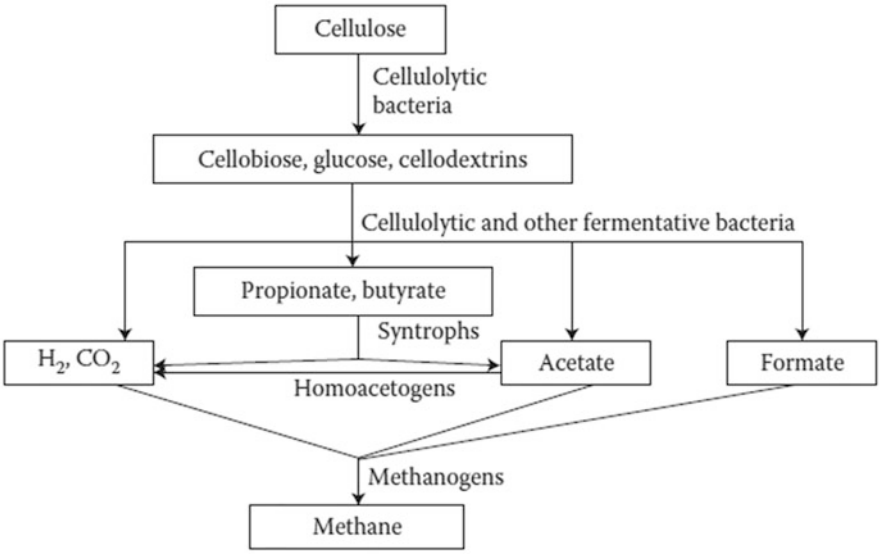


Fig. 13.5 Anaerobic cellulose degradation by microbial communities in soils and sediments

plays a major role in carbon cycling. In marine environments, sulfate can be reduced by sulfate-reducing bacteria, in such a way that the anaerobic degradation of cellulose in marine systems can lead to H_2S as a major product.

13.4 Hemicellulose

13.4.1 Structure and Properties of Hemicellulose

The term hemicellulose is formed by *hemi*, whose meaning is peripheral, and *cellulose* such that hemicellulose is peripheral to cellulose structure. This lignocellulose fraction is composed by polysaccharides located in the cell walls of all terrestrial plants, being formed by xyloglucans, xylans, mannans, and glucomannans linked by equatorial β-(1,4) bonds. Other polysaccharides, such as arabinans, galactans, and arabinogalactans, appear in this fraction, although these polysaccharides seem to come from pectin. In addition, another polymer that can be present in cellulose is callose, which is formed by glucose residues linked by β-(1,3) bonds (Scheller and Ulvskov 2010).

The most important biological role of hemicellulose is to contribute to strengthening cell walls by interaction with cellulose through hydrogen bonding and, in some walls, with lignin (Zhang et al. 2015). Some hemicelluloses, such as glucomannans, can also participate as extracellular carbohydrate storage in secondary cell wall of softwood. In some cases, hemicellulose is also linked with pectin to cellulose, forming cross-linked fibers, as indicated in Fig. 13.6. The proportion of

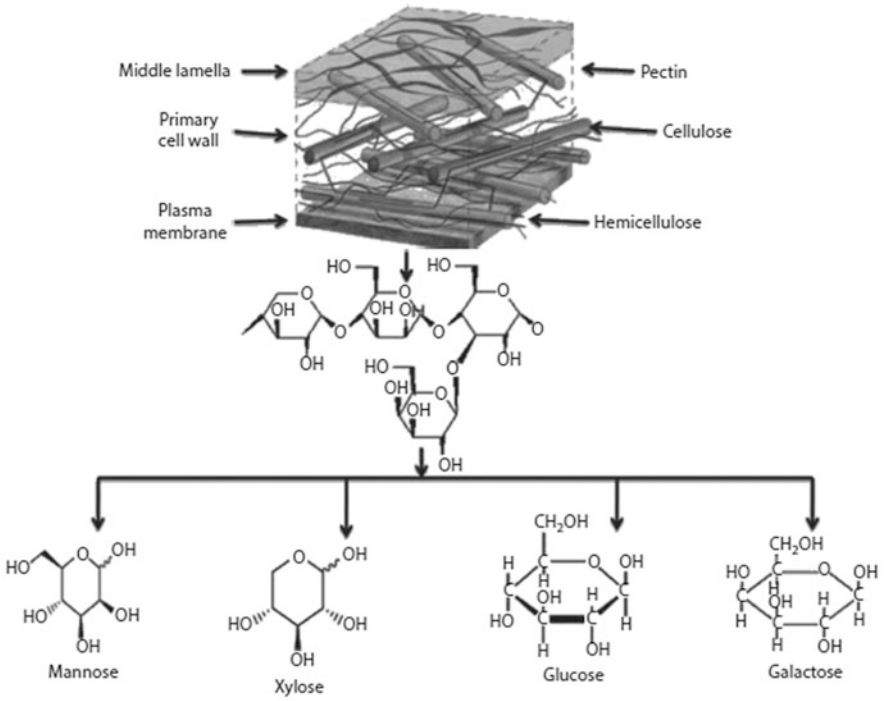


Fig. 13.6 Composition and structure of hemicellulose fraction

hemicellulose is variable depending on the species as well as the cell types within plants. This fraction is formed in the Golgi membrane through glycosyl transferases.

Contrary to cellulose, that possesses a fibrous and crystalline structure very resistant to hydrolysis, hemicellulose presents an amorphous and random structure with lower strength. This fact implies that hemicellulose is more prone to be enzymatically hydrolyzed by hemicellulase enzymes, or chemically in the presence of acids or bases. Hemicellulose exhibits chains shorter than cellulose (Table 13.2) and, in addition, presents a branched structure, while cellulose acquires a fibrous and linear structure.

Biomass present in trees can be classified basically into soft and hardwoods. The main hemicellulose component in softwood is O-acetyl-galactoglucomannan (~20%) of the dry weight (Lundqvist et al. 2003), which exhibits a polymerization degree of 100–150, which implies a molecular weight of 16,000–24,000. Generally, O-acetyl-galactoglucomannan contains a glucose: mannose ratio of about 1:3, while the galactose:glucose ratio can vary from 1:1 to 1:10 (Hakkila 1989). In the case of the hardwoods, the main hemicellulose component is glucuronoxylans (~ 15–30%) of the dry weight, being O-acetyl-(4-O-methylglucurono)-b-D-xylan. These glucuronoxylans can be partially acetylated in the 2,3 positions of xylose monomers, while the softwood cannot be acetylated.

13.4.2 Types of Hemicellulose

Hemicelluloses contain most of the D-pentose sugars, and also small amounts of L-sugars occasionally. This fraction is mainly composed of xylose, while galacturonic and mannuronic acids are also present in smaller proportions. The most common hemicelluloses are xylan, xyloglucan, glucuronoxylan, arabinoxylan, and glucomannan, as will be described in the following sub-sections.

13.4.2.1 Xylans

Xylan is the predominant hemicellulose in most plant cell walls (about a third of the total plant biomass). This is formed by α-(1,4)-D-xylopyranose backbone with a variety of side chains. The degradation of the xylan is inversely related to the branching degree, so the removal of these ramifications improves the degradation rate by endoxylanase enzymes.

Xylans of grasses contain a high content of arabinoxylans, which are composed of two pentose sugars (arabinose and xylose). Arabinoxylans are present in the primary and secondary plant cell walls, such as seeds and woods. Besides arabinoxylans, large amount of ferulic acid and other phenolic acids can appear interacting covalently. The presence of these phenolic acids provides interesting antioxidant properties to arabinoxylans (Wyman et al. 2004).

In hardwoods, xylans are highly substituted with acetyl or 4-O-methyl glucuronic acid, being named glucuronoxylans, mainly formed by xylose and glucuronic acid. This polysaccharide contains linear chains of α-(1,4)-xylan backbone with side chains of 4-O-methylglucuronic acid linked by α-(1,2)-glycosidic bonds, while the C2 and C3 positions of xylan are esterified with acetic acid. The molar ratio xylose/glucuronic acid/acetyl reported in the literature is 10:1:7 (Awano et al. 2002) (Fig. 13.7).

13.4.2.2 Glucans

Both glucan and xyloglucan display a similar structure to cellulose. Xyloglucan has a backbone of β-(1,4)-linked glucose residues, most of which are substituted with

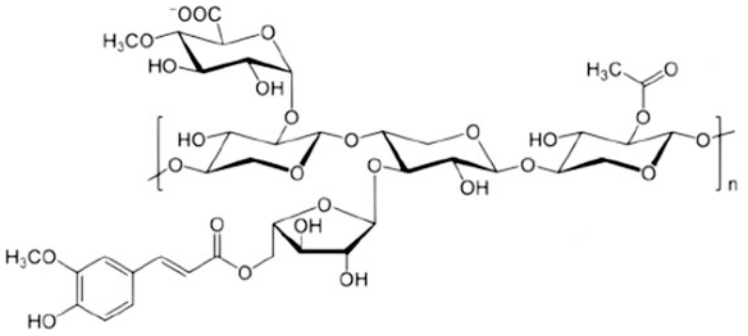


Fig. 13.7 Schematic structure of xylan

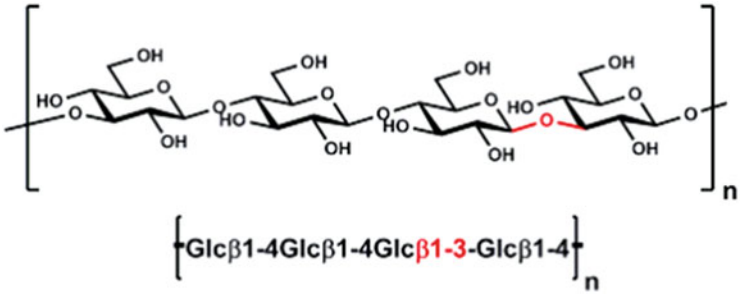


Fig. 13.8 Schematic structure of β-Glucan

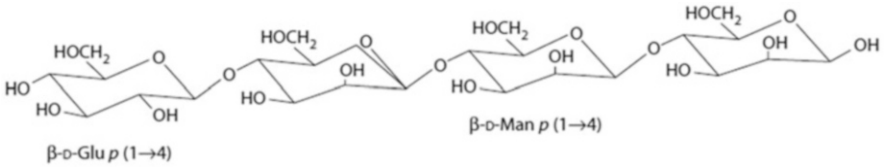


Fig. 13.9 Schematic structure of glucomannoxylans

1,6-linked xylose sidechains. This type of hemicellulose is present in the walls of numerous higher plants, such as dicots and conifers. β-Glucan consists of a mixture of β-(1,3)- and β-(1,4)-linked glucose residues, where the (1,4) to (1,3) linkage ratio varies as a function of the species (Fig. 13.8).

13.4.2.3 Mannans

Mannan is a linear polymer of the sugar mannose linked by β-(1,4) bonds. Mannan possesses α-(1,6)-linked backbones and α-(1,2)- and α-(1,3)-linked branches. Other mannose-based polysaccharides that are present in walls of many plants are glucomannoxylans and galactoglucomannans (Fig. 13.9). Galactoglucomannans are formed by β-D-mannopyranose and β-(1,4)-D-glucopyranose residues in linear chains, which are used as prebiotics in nutritional supplements.

13.4.3 Hemicellulose Biodegradation

Hemicelluloses are enzymatically degraded into monomeric sugars and acetic acid through hydrolytic enzymes denoted as hemicellulases. Among them, the main enzymes are endo-1,4-β-xylanase, which hydrolyzes xylans in their respective oligosaccharides, and xylan 1,4-β-xylosidase, which produces xylose from xylan oligosaccharides (Jeffries 1994; Kirk and Cullen 1998) (Fig. 13.10).

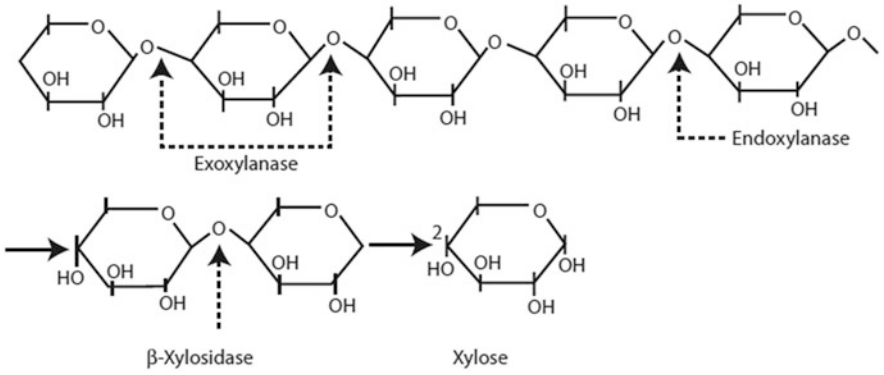


Fig. 13.10 Enzymatic cleavage of hemicellulose

As the hemicellulose can suffer esterification reactions in the C2 and C3 positions of furanose units, the biodegradation of hemicellulose requires esterases enzymes, such as xylan esterases, *p*-coumaric esterases, ferulic esterases, α-L-arabinofuranosidases, and α-4-O-methyl glucuronosidases. Hemicellulose can be biologically degraded by various bacteria and fungi.

13.4.3.1 Biodegradation of Hemicellulose by Fungi

As in the case of cellulose fraction, fungi play a determinant role in the degradation of polysaccharides of vegetable biomass. The enzyme type involved in the hemicellulose degradation depends on fungal species, so the understanding of fungal diversity is an important issue for the development of these enzymatic processes in industrial applications. In this sense, it has been reported that *Trichoderma reesei* and *Aspergillus niger* produce endo-1,4-β-xylanase, which can cleave endo-β-1,4-glycosidic bonds, while *Aspergillus awamori* generates β-D-xylosidase that breaks exo-β-1,4-glycosidic bonds. On the other hand, *Phanerochaete chrysosporium* have also shown the ability to synthesize endoxylanases. In all cases, the optimum temperature for xylanases is between 40 and 60 °C, being less thermostable than bacterial xylanases. Table 13.4 compiles the enzymes secreted by fungi involved in the depolymerization of hemicellulose.

13.4.3.2 Biodegradation of Hemicellulose by Bacteria

The decomposition of hemicellulose in bacterial medium is similar to that observed for cellulose. In both cases, the depolymerization starts outside of the cell, and the sugars produced are then transported into the cell for catabolism or anabolism. Generally, the hemicellulose decomposition is faster than cellulose due to hemicellulose is more branched and also displays a lower number of monosaccharides units. Bacteria responsible for degrading xylanes have been isolated in compost, soils, litter, and sludges, the rumen systems and other gastro-intestinal tracts. In the case of the human intestine, several hemicellulolytic bacteria such as *Aeromonas*,

Table 13.4 Enzymes secreted by several fungi

Fungi	Enzymes	Substrate	Mode of action
Aspergillus niger, *Trichoderma reesei*	Endo-1,4 β-xylanase	Xylan backbone	Endo-β-1,4- glycosidic bond
Aspergillus awamori	β-D-xylosidase	Xylan oligomer	Exo-β-1,4-glycosidic bond
A. niger, T. reesei	α-L-Arabinofuranosidase	Arabinose group	α-1,2-glycosidic bond, α-1,3-glycosidic bond, α-1,5-glycosidic bond
A. niger	Endo-1,5-α-arabinases	Arabinose group	α-1,5-glycosidic bond
A. awamori	Arabino Xylan-arabinofuranohydrolases	Arabinose group	α-1,4-glycosidic bond
A. niger	α-Glucuronidase	Glucuronic acid group	α-1,2-glycosidic bond
A. niger, *T. reesei*	Acetyl xylan esterase	Acetyl group	Ester bonds
A. niger, *T. reesei*	Acetyl esterases	Feruloyl group	Ester bonds
A. niger, *T. reesei*	Feruloyl esterase	*p*-Coumaroyl group	Ester bonds

Bacteroides sp., *Butyrivibrio* sp., *Clostridium* sp., and *Ruminococcus* have been isolated. For the enzymatic production of mannose, several bacteria such as *Aeromonas hydrophila*, *Bacteroides* sp., *Butyrivibrio* sp., *Cellulomonas* sp., *Clostridium* sp., *Polyporous versicolor*, *Streptomyces olivochromogenes,* or *Trichoderma harzianum* have shown to be efficient in the hemicellulose degradation (Blanco et al. 1999).

In all cases, these bacteria accelerate the hydrolysis of hemicellulose through hemicellulases consisting of three enzymes (exoxylanase, endoxylanase, and β-xylosidase), which hydrolyze the polysaccharides in short oligomers of xylobioses and xylose. Then, in a final step, these monomers or oligosaccharides are converted into ethanol, CO_2, and H_2O (Fig. 13.11). Under aerobic conditions, some ruminal species have shown the presence of bacterial xylanases (Blanco et al. 1999).

The anaerobic hydrolysis of hemicellulose and cellulose by bacteria is analogous. In the first step, hemicellulose is hydrolyzed into their respective monomers. Then, these monomers are fermented into organic acids, CO_2 and H_2, which are finally converted into CH_4 (Fig. 13.12). In this sense, *Butyrivibrio fibrisolvens* are anaerobic bacteria with a thin cell wall. Most strains ferment xylan, pectin, arabinose, glucose, fructose, and galactose, whose genetic code is analogous to endoglucanase, cellodextrinase, β-glucosidase, xylanase, and β-xylosidase/α-L-arabinofuranosidase (Lin and Thomson 1991). *Prevotella (Bacteroides) ruminicola* are other anaerobic bacteria with several genetic sequences of endoglucanase, β-xylosidase, α-L-arabinofuranosidase, and xylanase being cloned. Thus, these bacteria can ferment starch, xylan, pectin, maltose, cellobiose, xylose, and arabinose into glucose, fructose, galactose, and lactose. Then, these monomers are fermented into formate, acetate, propionate, and succinate. Likewise, *Clostridium thermolacticum* can also

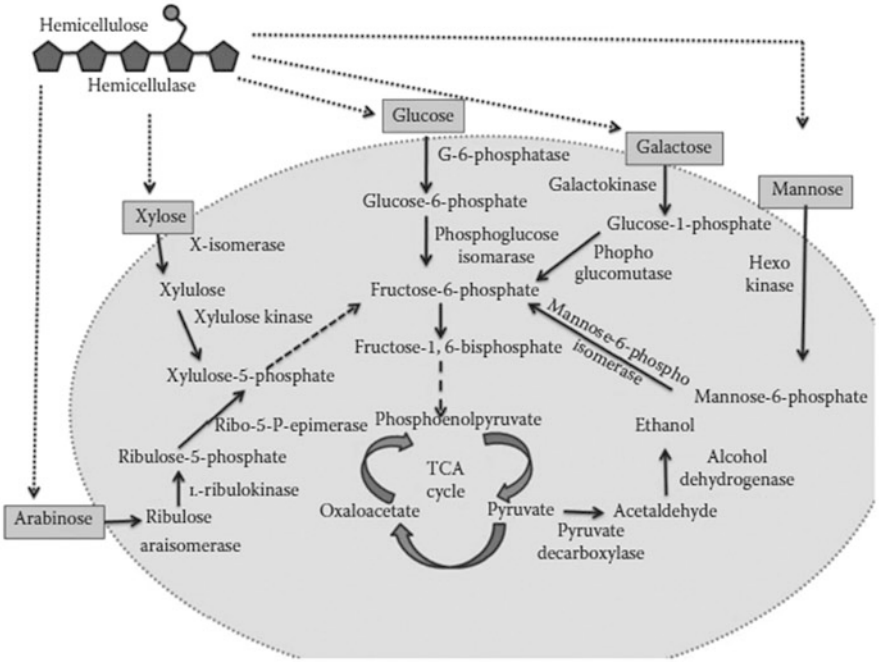


Fig. 13.11 Enzymatic degradation of arabinose, xylose, glucose, galactose, and mannose

ferment xylan, starch, cellobiose, glucose, and xylose. Several hemicellulolytic enzymes (two xylanase, six β-xylosidase, and α-L-arabinofuranosidase) have been isolated and sequenced from *Clostridium stercorarium* (Lin and Thomson 1991). In the same way, from *Thermoanaerobacterium saccharolyticum*, several enzymes (xylanase, two xylosidases, two acetyl xylanesterases, and α-glucuronidase) were isolated and cloned. In the case of actinomycetes, several bacteria, such as *Thermomonospora* or *Actinomadura* can produce xylanases, which can also degrade the hemicellulose. Table 13.5 summarizes the enzymes secreted by bacteria involved in the depolymerization of hemicellulose.

13.5 Lignin

13.5.1 Structure and Properties of Lignin

Lignin is a water insoluble and optically inactive molecule present in lignocellulosic biomass (Tuomela et al. 2000). This fraction provides support, impermeability, as well as resistance against microbial attack and oxidative stress to the plant cell walls. Generally, lignin is a complex, irregular, heterogeneous and tridimensional fraction of cross-linked, phenolic (aromatic) biopolymers. The molecular weight of lignin

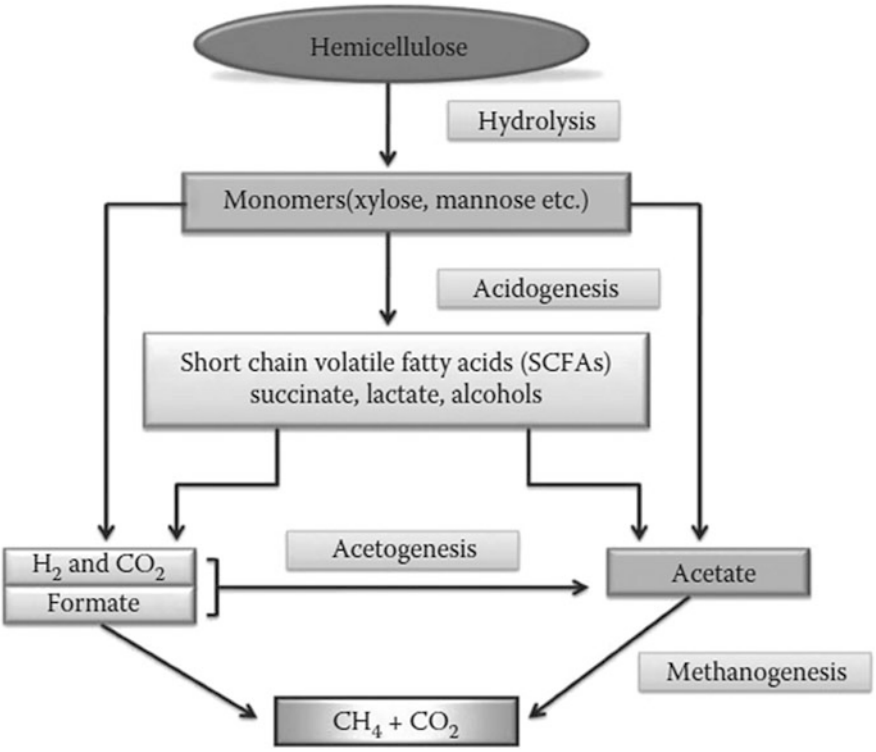


Fig. 13.12 Degradation of hemicellulose through anaerobic bacteria

Table 13.5 Enzymes secreted by several bacteria

Bacteria	Enzyme	Substrate	Mode of action
Fibrobacter succinogenes	β-Xylosidases	Xylan oligomer	Exo-β-1,4-glycosidic bonds
Ruminococcus albus	β-Xylosidases	Xylan oligomer	Exo-β-1,4-glycosidic bonds
Prevotella (Bacteroides) ruminicola	β-Xylosidases	Xylan oligomer	Exo-β-1,4-glycosidic bonds

can vary from tens of thousands of Daltons to infinite. This fraction is composed by the combination of three units, guaiacyl alcohol (G unit), syringyl alcohol (S unit), and *p*-hydroxyphenyl alcohol (H unit) (Fig. 13.13). The composition of lignin depends on the stage of the plant, species, tissues, cells, and environmental conditions. Thus, the S/G units ratio in lignin increases as the plant reaches maturity (Dixon et al. 2001). Lignin also varies its chemical composition in response to artificial stressors, or natural or external modifications, such as temperature, irradiation temperature, drought, mechanical wounding, ultraviolet degradation, or the

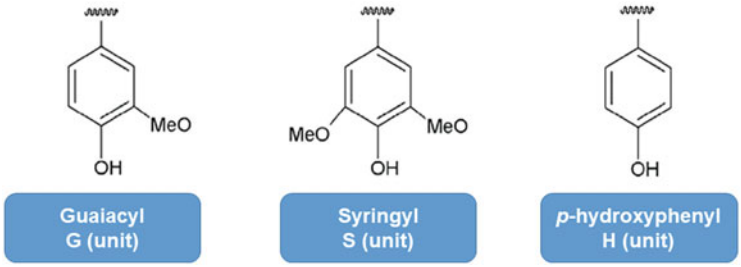


Fig. 13.13 Monomers of lignin

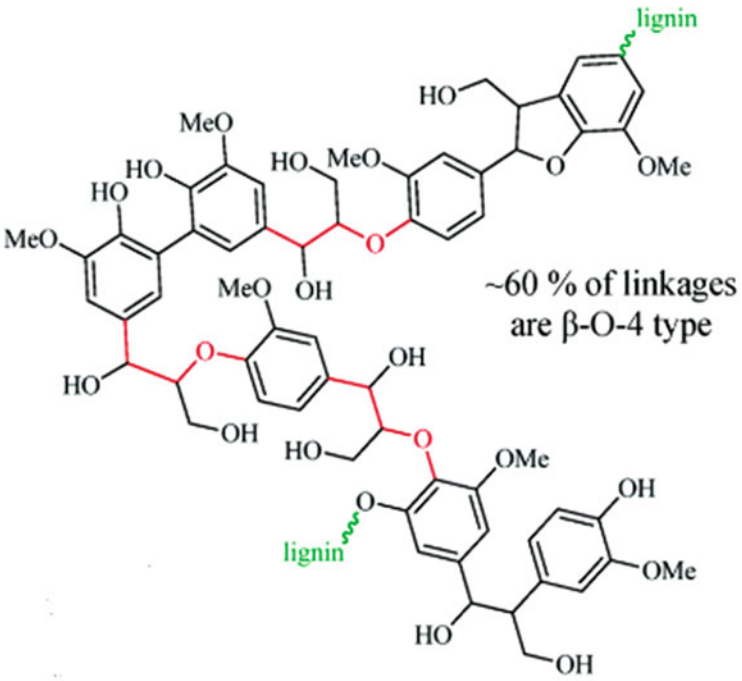


Fig. 13.14 Elemental structure of the lignin (β-aryl-ether bond in red marks)

presence of pathogens and pests (Moura et al. 2010). On the other hand, it has been reported that plants can generate H units at the injury sites.

After cellulose, lignin is the second most abundant organic compound on earth. This fraction is a polymeric structure of phenylpropane units randomly linked through ether, ester, and C–C bonds. An important and typical structural sequence of lignin is phenylpropanoid with methoxyl substitutes (Fig. 13.14). In spite of elemental units of lignin mainly linked by β-aryl-ether bond, it is impossible to

identify a typical structure, since both the polymerization and its fragmentation are random.

Lignin is found in cell walls forming a complex with cellulose and hemicellulose, providing rigidity to the framework, as well as protection to these carbohydrates against biological degradation (Fig. 13.1). On the other hand, lignin also allows the H_2O transport from the roots to the leaves by the xylem vasculature.

Essentially, the lignin in cell walls is formed by dead cells linked between them in such a way that long hollow tubes are formed, and, then, the lignin is deposited in these hollow sites, which hinders the isolation and characterization of the lignin fraction. In addition, lignin improves the cation exchange capacity in soils, which increases the photosynthetic productivity.

The synthesis of lignin is a complex process that starts in the cytosol, where, from the amino acid phenylalanine, glycosylated monolignols are synthesized. The polymerization reaction begins with the enzymatic deamination of phenylalanine by phenylalanine ammonia lyase, generating monolignol (Fig. 13.15). Then, the monolignol is transported to the plant cell walls where the lignification occurs (Samuels et al. 2002). In the next step, β-glucosidases release monolignols for subsequent polymerization during lignin synthesis. The polymerization initiates with an electron oxidation of phenylpropanoid precursor to phenoxy radicals, via peroxidases, phenol oxidases, and laccases (Boerjan et al. 2003; Vanholme et al. 2010). This polymerization leads to a heterogeneous structure, whose elementary units are linked by aryl-ether C-C and mainly aryl-glycerol β-aryl-ether bonds (Fig. 13.16) (Bugg et al. 2011).

13.5.2 Microbial Lignin Depolymerization

The degradation of lignin present in plant cell walls is a challenge, though, nowadays, some fungi enzymes are able to degrade the lignin. More recently, novel lignin-degrading enzymes from bacteria have been discovered, which are more efficient than those coming from fungi. Lignin exhibits a higher complexity than hemicellulose and cellulose, which renders its degradation difficult. Thus, one of the main challenges is to develop enzymatic systems, where microorganisms may accelerate the degradation of lignin.

13.5.2.1 Biodegradation of Lignin by Fungi

Fungi are the most studied microorganisms for lignin degradation. These fungi secrete enzymes, which are involved in the lignin depolymerization. Among all the fungi studied, white-rot fungi are the most active species. The lignin depolymerization takes place through the enzymatic oxidation of phenolic compounds (via cation radicals), producing their respective phenoxy radicals. Based on enzyme production, the enzymes of white-rot fungi can be classified into three categories: (a) the lignin-manganese peroxidase group (*Phlebia radiata*, *Phanerochaete chrysosporium*), (b) manganese peroxidase–laccase group (*Rigidoporus lignosus*,

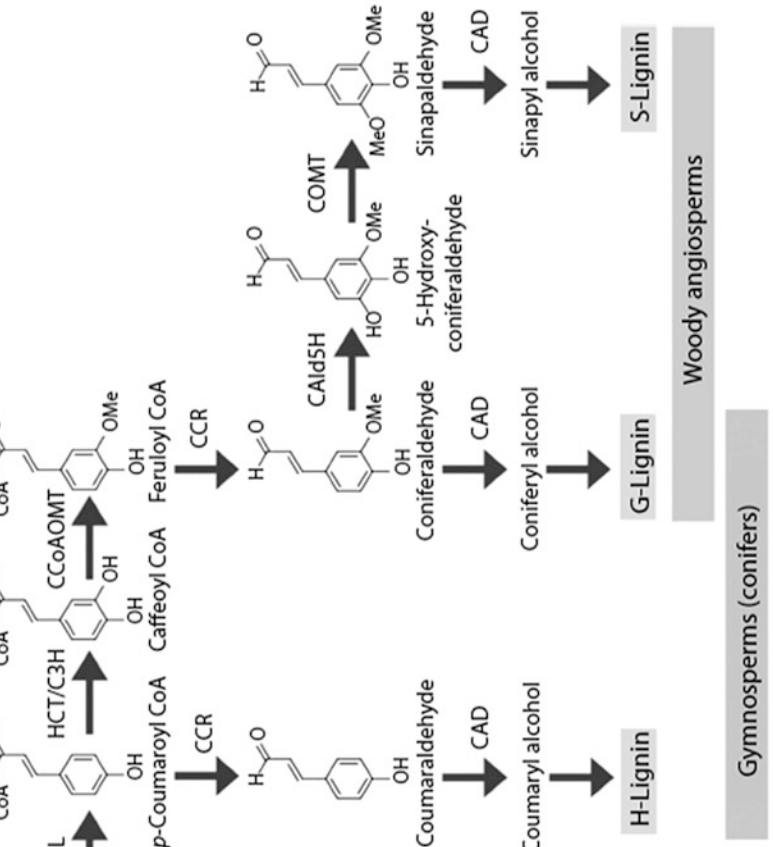


Fig. 13.15 Biosynthesis of the elementary units of lignin

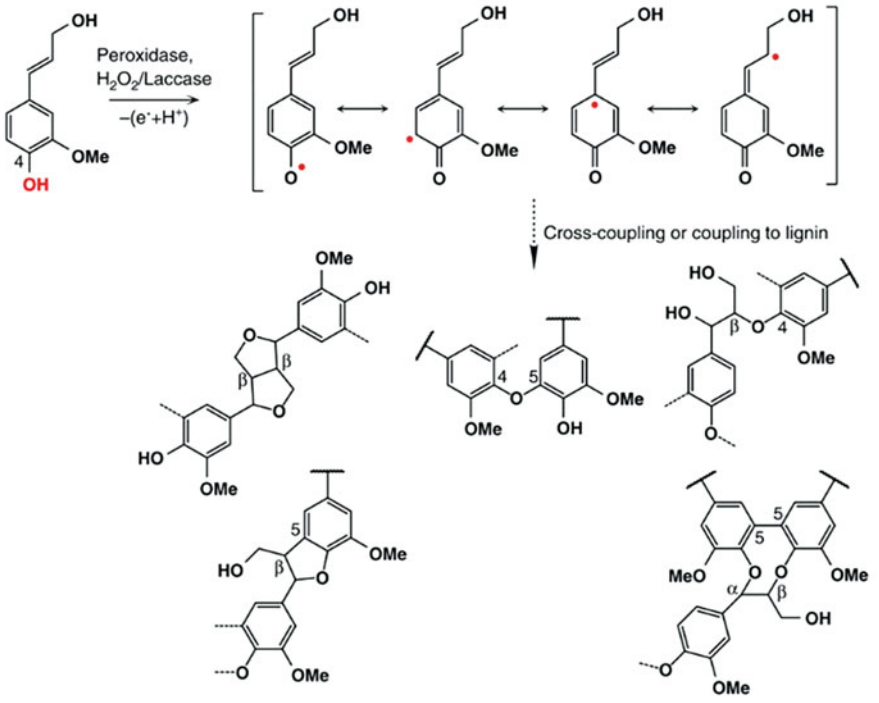


Fig. 13.16 Scheme for monolignol oxidative dehydrogenation and polymerization processes

Dichomitus squalens), and (c) lignin peroxidase–laccase group (*Junghuhnia separabilima*, *Phlebia ochraceofulva*) (Hatakka (1994).

Lignin Peroxidases

Most of the lignin peroxidases are produced by white-rot fungi (*Phlebia radiate*, *Phlebia ochraceofulva*, *Phanerochaete chrysosporium*, *Phanerochaete flavido-alba*, *Trametes trogii*). Lignin peroxidases have a molecular weight of about 40 kDa, an optimum pH of 2.5–3.0, acid isoelectric point, and high redox potential. The structure of this class of enzymes reveals that the heme access channel is the substrate-binding site. The bio-catalytic cycle of these enzymes is similar to that of other peroxidases, as shown in Fig. 13.17.

Lignin peroxidases can depolymerize a wide range of lignin model compounds, which suggests a non-specific depolymerization process. In general, it is assumed that the depolymerization happens by the oxidative cleavage of β-O-4 and C_α-C_β linkages. In addition, this group of enzymes is involved in the side-chain cleavages, demethoxylations, benzyl alcohol oxidations, and ring-opening reactions. However, lignin peroxidases do not have enough activity to degrade lignin into CO_2.

The mechanism of oxidative-lignin depolymerization is very complex and, although it is not clear, it is known that the interaction of lignin peroxidases with the lignin polymer involves veratryl alcohol (Valc), which is a secondary metabolite

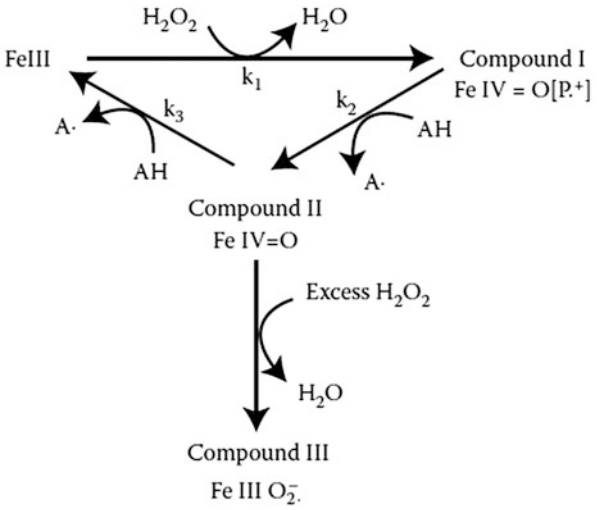


Fig. 13.17 Lignin peroxidase (catalytic cycle)

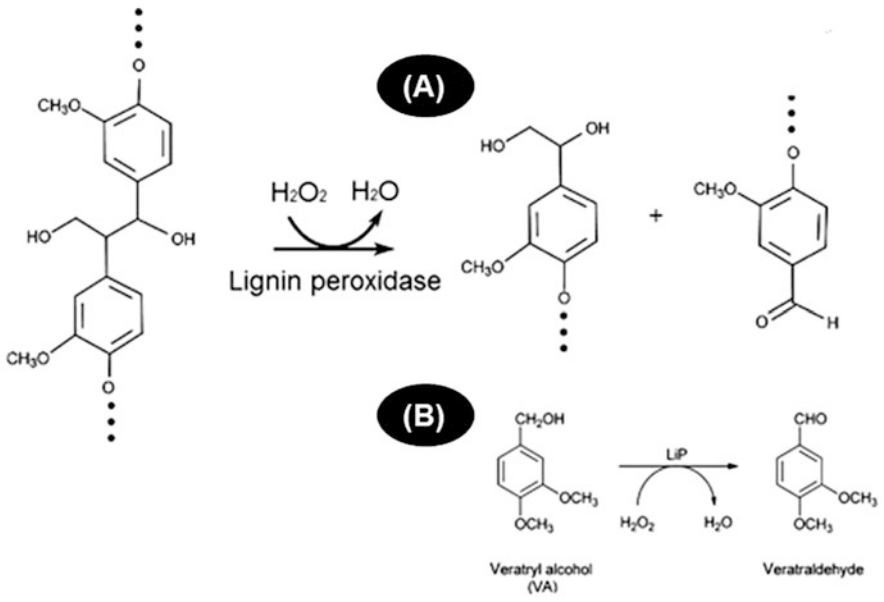


Fig. 13.18 Cleavage reaction catalyzed by lignin peroxidases (**a**). Oxidation of veratryl alcohol is generally used to estimate the lignin peroxidase activity (**b**)

of white-rot fungi that acts as a cofactor for the enzyme (Fig. 13.18). Other compounds, such as 3,4-dimethoxytoluene, 1,4-dimethoxybenzene (1,4-DMB), and 3,4,5-trimethoxybenzyl alcohol have been found as alternative cofactors of lignin peroxidase (Mester and Tien 2000).

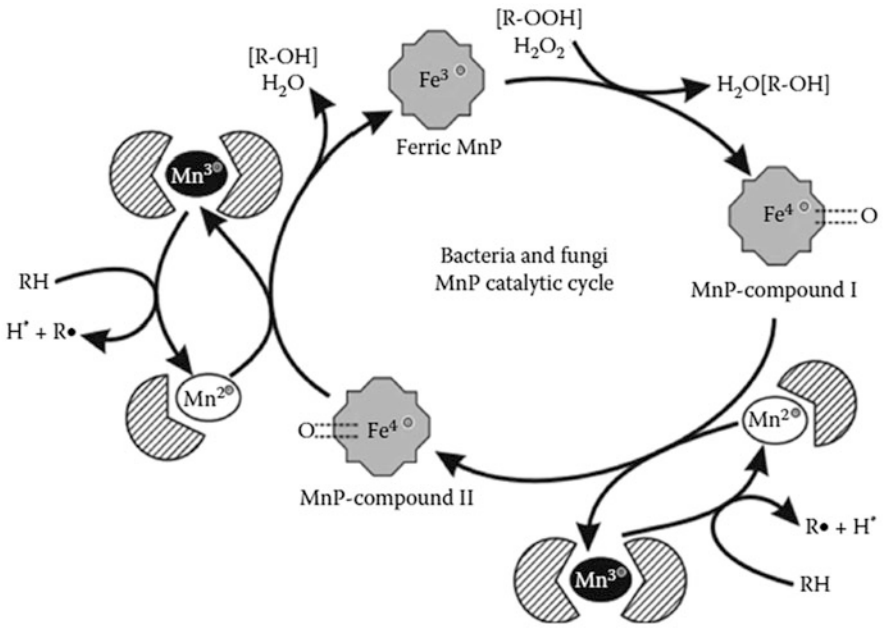


Fig. 13.19 Manganese peroxidase (catalytic cycle)

Manganese Peroxidases

Manganese peroxidases are also secreted by white-rot fungi (*Coriolaceae*, *Meruliaceae*, *Polyporaceae*) and some fungi which decomposes the soil litter (*Strophariaceae*, *Tricholomataceae*). Like lignin peroxidases, manganese peroxidases are also glycosylated heme-containing extracellular peroxidase, and Mn(II), used a substrate, is widespread in lignocellulose and soil. The enzymatic cycle begins by binding H_2O_2, or an organic peroxide, to the native Fe (III) enzyme, forming an iron-peroxide complex (Fig. 13.19). Oxygen peroxide–oxygen bond cleaves to produce a Fe^{4+}-oxo-porphyrin-radical complex. Then, a reduction occurs through the Fe^{4+}-oxo-porphyrin complex, while Mn(II) is oxidized to Mn(III) and this continues with the generation of the native enzyme and release of a water molecule. The presence of a high H_2O_2 concentration causes a reversible deactivation of the manganese peroxidase by the formation of the inactive Mn (III) oxidation state (Hofrichter 2002).

Laccases

Laccases are widely distributed in white-rot basidiomycetes. These enzymes are involved in the lignin depolymerization, besides carrying out other functions in fructification formation, fungal pigmentation, detoxification, pathogenicity, detoxification, and sporulation. Laccases contain four copper centers per enzyme and three different types (CuT1, CuT2 and CuT3) have been identified, where each one has a different role in the oxidation of laccase substrates. The optimum conditions of laccases are a pH of 3.0–5.7 and a temperature as high as 75 °C (Dedeyan et al. 2000).

Table 13.6 Enzymes secreted by fungi involved in the lignin depolymerization

Enzyme activity	Cofactor or substrate	Main effect or reaction
Lignin peroxidase	H_2O_2, veratryl alcohol	Aromatic ring oxidized to cation radical
Manganese peroxidase	H_2O_2, Mn, organic acid as chelator, thiols, unsaturated lipids	Mn(II) oxidized to Mn(III); chelated Mn(III) oxidizes phenolic compounds to phenoxyl radicals; other reactions in the presence of additional compounds
Laccase	O_2; mediators, for example, hydroxybenzotriazole or ABTS	Phenols are oxidized to phenoxyl radicals; other reactions in the presence of mediators
Glyoxal oxidase	Glyoxal, methyl glyoxal	Glyoxal oxidized to glyoxylic acid; H_2O_2 production
Aryl alcohol oxidase	Aromatic alcohols (anisyl, veratryl alcohol)	Aromatic alcohols oxidized to aldehydes; H_2O_2 production

Table 13.7 List of fungi with ligninolytic enzyme activity

	Ligninolytic enzyme		
Fungi	Lignin peroxidase	Manganese peroxidase	Laccase
Aspergillus oryzae	☺	☺	☺
Bjerkandera adusta	☺	☺	☺
Bjerkandera sp.	☺	☺	☺
Ceriporiopsis subvermispora	☺	☺	☺
Coprinus cinereus	☺	☺	☺
Marasmius quercophilus	☺	☺	☺
Phanerochaete chrysosporium	☺	☺	☺
P. flavido-alba	☺	☺	☺
Phlebia ochraceofulva	☺	☺	☺
P. radiata	☺	☺	☺
P. tremellosa	☺	☺	☺
Pleurotus eryngii	☺	☺	☺
Trametes trogii	☺	☺	☺
T. versicolor	☺	☺	☺

Other Enzymes

Both fungal aryl-alcohol dehydrogenases and quinone reductases are also involved in lignin degradation. These evolve in different non-enzymatic reactions, such as C-4-ether breakdown, Cα–Cβ breakdown, aromatic ring cleavage, or demethoxylation. While enzymes secreted by fungi, involved in the lignin depolymerization are compiled in Table 13.6, Table 13.7 presents a list of fungi with ligninolytic enzyme activity.

13.5.2.2 Biodegradation of Lignin by Bacteria

Several bacteria have shown their ability to cleave the lignin bonds (Table 13.8). The enzymes involved in this lignin degradation exhibit the same mechanism to those

Table 13.8 List of bacteria with ligninolytic enzyme activity

Bacteria	Ligninolytic enzyme		
	Lignin peroxidase	Manganese peroxidase	Laccase
Aneurinibacillus aneurinilyticus		☺	☺
Bacillus cereus	☺	☺	☺
Campylobacter		☺	
Citrobacter freundii		☺	
Geobacter		☺	
Methanobacterium		☺	
Nitrosomonas		☺	
Pseudochrobactrum glaciale	☺	☺	☺
Pseudomonas putida		☺	☺
Providencia rettgeri	☺	☺	☺
Rhodococcus jostii		☺	☺
Serratia liquefaciens	☺	☺	☺
S. marcescens	☺	☺	☺
Sphingomonas paucimobilis	☺	☺	☺
Streptomyces viridosporus	☺	☺	☺

reported for fungi in the previous section (peroxidase and laccases). In this sense, it has been reported that both *Citrobacter freundii* and *Citrobacter* sp. secrete enzymes with peroxidase activity, although their activity depends on the presence of heavy metals, which seems to have an adverse effect in the lignin depolymerization (Chandra and Bharagava 2013). The lignin degradation was also performed by the inoculation of pre-grown seed culture of purified bacteria. The reported data revealed the growth of the inoculated bacteria and the degradation of the lignin after long incubation period (216 h) (Raj et al. 2007; Chandra et al. 2007). In order to simulate easier target molecules, lignin peroxidases, manganese peroxides, and laccases were evaluated in the oxidation of pigment and dyes in the presence of H_2O_2, obtaining better results than those attained in the lignin depolymerization, although the activity is lower than those reported by white-rot fungi (de Oliveira et al. 2009). Moreover, the laccase activity was noted to be significantly higher in subsequent stages of bacterial growth in comparison to peroxidases.

13.6 Future Perspectives

Future perspectives for the bioconversion of lignocellulosic wastes into high value-added chemicals must be focused on the improvement of microorganisms and enzyme performances, considering that the catalyst must be economically competitive and should not cause negative environmental impact.

As it is well known, the use of microorganism in the lignocellulosic degradation is limited to the optimum conditions of the culture (pH, temperature, substrate concentration, sterilization), which hinder the industrial implementation.

Considering these drawbacks, the main efforts are focused on two lines: i) the search and development of new lignocellulolytic microorganisms, as well as the use of co-culture systems, which improve the depolymerization in shorter times under broader reaction conditions, and ii) the experimental parameters must be optimized to enhance the efficiency of these bio-processes (Agler et al. (2011). Another disadvantage of the biodegradation of lignocellulose is related to the generation of several by-products, derived from the depolymerization of lignin, or other compounds coming from sugar degradation, which can inhibit the subsequent processes of fermentation and saccharification (Allen et al. (2010), so the challenge is aimed at identifying and minimizing those compounds that poison biochemically active sites.

An advanced methodology that detects and improves the fermentation performance of lignocellulosic feedstock is the phenotypic microarray. This methodology allows to carry out a global overview of genes differentially expressed, when several growth conditions are compared (Greetham 2014), providing interesting information about the panel of genes that are expressed under the studied bio-catalytic conditions.

With regard to the enzymatic treatment, the main disadvantage is related to the high cost of enzymes and their large amounts required for industrial applications. In this sense, both bacteria and fungi are highly used in a wide range of enzymatic processes, which can diminish the cost of processes. However, the enzymes secreted by fungi and bacteria often display low performance in long times, high biodegradability, and restricted pH and temperature, so increasing the scale to be used in industrial applications can be a very complicated challenge. In the recent years, several advanced techniques have been focused on the development of enzymes that improve the yield in the lignocellulose depolymerization, in such a way that the implementation of these enzymes in second-generation biorefineries could be feasible (Heux et al. 2015).

Metagenomics is another advanced methodology, which provides direct access to the DNA of microorganisms. From this technique, it is possible to discover a greater number of enzymes capable of degrading lignocellulosic biomass (Hess et al. 2011). However, this field is still under development, since the genetic sequences to be cloned are limited. As an alternative, it has been proposed a metagenomic study that facilitates cloning genes in appropriate hosts, which are suitable for the expression and secretion of enzymes of interest to elucidate the relationship between structure and function (Heux et al. 2015).

The in vitro enzymes engineering is another strategy to develop enzymes that can degrade lignocellulosic biomass with higher efficiency to be industrially implemented (Heux et al. 2015). However, this technology is still incipient and requires significant advances due to the complexity of lignocellulose. There are only few studies on cellulases and cellobiohydrolases (Heinzelman et al. 2010). In the recent years, several authors have developed the expression of in vitro-optimized enzymes in a cell factory. The use of several sources to design the microorganism can reduce the cost of microorganism synthesis. Most of the research is focused on the development of recombinant fermenting strains able to hydrolyze

polysaccharides (Kondo et al. 2013). Among these fermenting strains, the main strain studied is *Saccharomyces cerevisiae*, which is highly used in the beer industry, so its cost is much lower than that of other microorganisms, being resistant at low pH and high sugar consumption rate.

The metabolic engineering is employed to elucidate the genes responsible for tolerance to enhance the growth and fermentation capability of yeasts, even with high concentrations of inhibitors. On the other hand, the evolutionary engineering is used, in combination with advanced techniques, to program the yeasts genetic pathway and minimize the inhibitor effects (Almario et al. 2013). The use of genetically modified organisms in the waste valorization displays serious difficulties associated to ethical issues, such as the detrimental effect on humans and environment, as well as the controversy related to the use and manipulation of microorganisms.

References

Adrio J, Demain A (2014) Microbial enzymes: tools for biotechnological processes. Biomol Ther 4:117–139. https://doi.org/10.3390/biom4010117

Agler M, Wrenn B, Zinder S, Angenent L (2011) Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. Trends Biotechnol 29:70–78. https://doi.org/10.1016/j.tibtech.2010.11.006

Ahmad M, Taylor C, Pink D, Burton K, Eastwood D, Bending G, Bugg T (2010) Development of novel assays for lignin degradation: comparative analysis of bacterial and fungal lignin degraders. Mol Biosyst 6:815. https://doi.org/10.1039/b908966g

Allen S, Clark W, McCaffery J, Cai Z, Lanctot A, Slininger P, Liu Z, Gorsich S (2010) Furfural induces reactive oxygen species accumulation and cellular damage in Saccharomyces cerevisiae. Biotechnol Biofuels 3:2. https://doi.org/10.1186/1754-6834-3-2

Almario M, Reyes L, Kao K (2013) Evolutionary engineering of Saccharomyces cerevisiae for enhanced tolerance to hydrolysates of lignocellulosic biomass. Biotechnol Bioeng 110:2616–2623. https://doi.org/10.1002/bit.24938

Awano T, Takabe K, Fujita M (2002) Xylan deposition on secondary wall of Fagus crenata fiber. Protoplasma 219:106–115. https://doi.org/10.1007/s007090200011

Bauchop T (1979) Rumen anaerobic fungi of cattle and sheep. Appl Environ Microbiol 38:148–158

Beguin P, Aubert J (1994) The biological degradation of cellulose. FEMS Microbiol Rev 13:25–58. https://doi.org/10.1111/j.1574-6976.1994.tb00033.x

Beltz L, Neira D, Axtell C et al (2001) Immunotoxicity of explosives-contaminated soil before and after bioremediation. Arch Environ Contam Toxicol 40:311–317. https://doi.org/10.1007/s002440010177

Bhat M, Bhat S (1997) Cellulose degrading enzymes and their potential industrial applications. Biotechnol Adv 15:583–620. https://doi.org/10.1016/s0734-9750(97)00006-2

Blanco A, Díaz P, Zueco J, Parascandola P, Pastor F (1999) A multidomain xylanase from a Bacillus sp. with a region homologous to thermostabilizing domains of thermophilic enzymes. Microbiology 145:2163–2170. https://doi.org/10.1099/13500872-145-8-2163

Boerjan W, Ralph J, Baucher M (2003) Lignin biosynthesis. Annu Rev Plant Biol 54:519–546. https://doi.org/10.1146/annurev.arplant.54.031902.134938

Brown R (1996) The biosynthesis of cellulose. J Macromol Sci A 33:1345–1373. https://doi.org/10.1080/10601329608014912

Bugg T, Ahmad M, Hardiman E, Rahmanpour R (2011) Pathways for degradation of lignin in bacteria and fungi. Nat Prod Rep 28:1883–1896. https://doi.org/10.1039/c1np00042j

Carvalheiro F, Duarte LC, Gírio FG (2008) Hemicellulose biorefineries: a review on biomass pretreatments. J Sci Ind Res 67(2008):849–864

Chandra R, Bharagava RN (2013) Bacterial degradation of synthetic and Kraft lignin by axenic and mixed culture and their metabolic products. J Environ Biol 34:991–999

Chandra R, Raj A, Purohit H, Kapley A (2007) Characterisation and optimisation of three potential aerobic bacterial strains for Kraft lignin degradation from pulp paper waste. Chemosphere 67:839–846. https://doi.org/10.1016/j.chemosphere.2006.10.011

Chang V, Holtzapple M (2000) Fundamental factors affecting biomass enzymatic reactivity. Appl Biochem Biotechnol 84-86:5–38. https://doi.org/10.1385/abab:84-86:1-9:5

de Oliveira PL, Duarte MCT, Ponezi AN, Durrant LR (2009) Purification and partial characterization of manganese peroxidase from Bacillus pumilus and Paenibacillus sp. Braz J Microbiol 40 (4):818–826

Dedeyan B, Klonowska A, Tagger S, Tron T, Iacazio G, Gil G, Le Petit J (2000) Biochemical and molecular characterization of a laccase from Marasmius quercophilus. Appl Environ Microbiol 66:925–929. https://doi.org/10.1128/aem.66.3.925-929.2000

Dixon RA, Chen F, Guo D, Parvathi K (2001) The biosynthesis of monolignols: a "metabolic grid", or independent pathways to guaiacyl and syringyl units? Phytochemistry 57(7):1069–1084. https://doi.org/10.1016/s0031-9422(01)00092-9

Eriksson K, Wood T (1985) Biodegradation of cellulose. In: Biosynthesis and biodegradation of wood components, pp 469–503. https://doi.org/10.1016/b978-0-12-347880-1.50021-0

Greetham D (2014) Phenotype microarray technology and its application in industrial biotechnology. Biotechnol Lett 36:1153–1160. https://doi.org/10.1007/s10529-014-1481-x

Hakkila P (1989) Utilization of residual forest biomass. In: Springer series in wood science. Springer, Berlin. https://doi.org/10.1007/978-3-642-74072-5

Hatakka A (1994) Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. FEMS Microbiol Rev 13(2–3):125–135

Heinzelman P, Komor R, Kanaan A, Romero P, Yu X, Mohler S, Snow C, Arnold F (2010) Efficient screening of fungal cellobiohydrolase class I enzymes for thermostabilizing sequence blocks by SCHEMA structure-guided recombination. Protein Eng Des Sel 23:871–880. https://doi.org/10.1093/protein/gzq063

Hendriks A, Zeeman G (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 100:10–18. https://doi.org/10.1016/j.biortech.2008.05.027

Hess M, Sczyrba A, Egan R, Kim T, Chokhawala H, Schroth G, Luo S, Clark D, Chen F, Zhang T, Mackie R, Pennacchio L, Tringe S, Visel A, Woyke T, Wang Z, Rubin E (2011) Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. Science 331:463–467. https://doi.org/10.1126/science.1200387

Heux S, Meynial-Salles I, O'Donohue MJ, Dumon C (2015) White biotechnology: state of the art strategies for the development of biocatalysts for biorefining. Biotechnol Adv 33(8):1653–1670

Hofrichter M (2002) Review: lignin conversion by manganese peroxidase (MnP). Enzyme Microb Technol 30:454–466. https://doi.org/10.1016/s0141-0229(01)00528-2

Hon D (1994) Cellulose: a random walk along its historical path. Cellul 1:1–25. https://doi.org/10.1007/bf00818796

Howard R, Abotsi E, Jansen V, Howard S (2003) Lignocellulose biotechnology: issues of bioconversion and enzyme production. Afr J Biotechnol 2:602–619. https://doi.org/10.5897/ajb2003.000-1115

Huber G, Iborra S, Corma A (2006) Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering. Chem Rev 106:4044–4098. https://doi.org/10.1021/cr068360d

Jeffries TW (1994) Biodegradation of lignin and hemicelluloses. In: Ratledge C (ed) Biochemistry of microbial degradation. Springer, Dordrecht, pp 233–277

Jenkinson D, Adams D, Wild A (1991) Model estimates of CO2 emissions from soil in response to global warming. Nature 351:304–306. https://doi.org/10.1038/351304a0

Kirk TK, Cullen D (1998) Enzymology and molecular genetics of wood degradation by white-rot fungi. In: Young RA, Akhtar M (eds) Friendly technologies for the pulp and paper industry. Wiley, Hoboken, NJ, pp 273–308

Kondo A, Ishii J, Hara K, Hasunuma T, Matsuda F (2013) Development of microbial cell factories for bio-refinery through synthetic bioengineering. J Biotechnol 163:204–216. https://doi.org/10.1016/j.jbiotec.2012.05.021

Kumar R, Singh S, Singh O (2008) Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives. J Ind Microbiol Biotechnol 35:377–391. https://doi.org/10.1007/s10295-008-0327-8

Leschine SB (1995) Cellulose degradation in anaerobic environments. Annu Rev Microbiol 49:399–426. https://doi.org/10.1146/annurev.mi.49.100195.002151

Lin L, Thomson J (1991) An analysis of the extracellular xylanases and cellulases of Butyrivibrio fibrisolvensH17c. FEMS Microbiol Lett 84:197–204. https://doi.org/10.1111/j.1574-6968.1991.tb04596.x

Lundqvist J, Jacobs A, Palm M, Zacchi G, Dahlman O, Stålbrand H (2003) Characterization of galactoglucomannan extracted from spruce (Picea abies) by heat-fractionation at different conditions. Carbohydr Polym 51:203–211. https://doi.org/10.1016/s0144-8617(02)00111-x

Mah RA (1981) The methanogenic bacteria, their ecology and physiology. In: Hollaender A, Rabson R, Rogers P, Pietro AS, Valentine R, Wolfe R (eds) Trends in the biology of fermentations for fuels and chemicals. Basic life sciences. Springer, Boston, MA. https://doi.org/10.1007/978-1-4684-3980-9_21

Mariscal R, Maireles-Torres P, Ojeda M, Sádaba I, López Granados M (2016) Furfural: a renewable and versatile platform molecule for the synthesis of chemicals and fuels. Energ Environ Sci 9:1144–1189. https://doi.org/10.1039/c5ee02666k

Mester T, Tien M (2000) Oxidation mechanism of ligninolytic enzymes involved in the degradation of environmental pollutants. Int Biodeter Biodegr 46:51–59. https://doi.org/10.1016/s0964-8305(00)00071-8

Moura J, Bonine C, de Oliveira Fernandes Viana J, Dornelas M, Mazzafera P (2010) Abiotic and biotic stresses and changes in the lignin content and composition in plants. J Integr Plant Biol 52:360–376. https://doi.org/10.1111/j.1744-7909.2010.00892.x

O'sullivan AC (1997) Cellulose: the structure slowly unravels. Cellul 4:173–207. https://doi.org/10.1023/A:1018431705579

Prasad S, Sheetal K, Venkatramanan V, Kumar S, Kannojia S (2019) Sustainable energy: challenges and perspectives. In: Sustainable green technologies for environmental management, pp 175–197. https://doi.org/10.1007/978-981-13-2772-8_9

Prasad S, Venkatramanan V, Singh A (2021) Renewable energy for a low-carbon future: policy perspectives. In: Venkatramanan V, Shah S, Prasad R (eds) Sustainable bioeconomy. Springer, Singapore. https://doi.org/10.1007/978-981-15-7321-7_12

Raj A, Chandra R, Reddy M, Purohit H, Kapley A (2007) Biodegradation of Kraft lignin by a newly isolated bacterial strain, Aneurinibacillus aneurinilyticus from the sludge of a pulp paper mill. World J Microbiol Biotechnol 23:793–799. https://doi.org/10.1007/s11274-006-9299-x

Saha B (2003) Hemicellulose bioconversion. J Ind Microbiol Biotechnol 30:279–291. https://doi.org/10.1007/s10295-003-0049-x

Samuels AL, Rensing KH, Douglas CJ, Mansfield SD, Dharmawardhana DP, Ellis BE (2002) Cellular machinery of wood production: differentiation of secondary xylem in Pinus contorta var. latifolia. Planta 216(1):72–82. https://doi.org/10.1007/s00425-002-0884-4

Sánchez C (2009) Lignocellulosic residues: biodegradation and bioconversion by fungi. Biotechnol Adv 27:185–194. https://doi.org/10.1016/j.biotechadv.2008.11.001

Scheller H, Ulvskov P (2010) Hemicelluloses. Annu Rev Plant Biol 61:263–289. https://doi.org/10.1146/annurev-arplant-042809-112315

Shah S, Venkatramanan V (2019) Advances in microbial technology for upscaling sustainable biofuel production. In: New and future developments in microbial biotechnology and bioengineering, pp 69–76. https://doi.org/10.1016/b978-0-444-63504-4.00005-0

Shah S, Venkatramanan V, Prasad R (eds) (2019) Sustainable green technologies for environmental management. Springer Nature, Singapore. https://doi.org/10.1007/978-981-13-2772-8

Takano M, Nishida A, Nakamura M (2001) Screening of wood-rotting fungi for Kraft pulp bleaching by the poly R decolorization test and biobleaching of hardwood Kraft pulp by Phanerochaete crassa WD1694. J Wood Sci 47:63–68. https://doi.org/10.1007/bf00776647

Tuomela M, Vikman M, Hatakka A, Itävaara M (2000) Biodegradation of lignin in a compost environment: a review. Bioresour Technol 72(2):169–183. https://doi.org/10.1016/S0960-8524(99)00104-2

Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W (2010) Lignin biosynthesis and structure. Plant Physiol 153:895–905. https://doi.org/10.1104/pp.110.155119

Venkatramanan V, Shah S, Prasad R (eds) (2020) Global climate change and environmental policy: agriculture perspectives. Springer Nature, Singapore. https://doi.org/10.1007/978-981-13-9570-3

Venkatramanan V, Shah S, Prasad R (eds) (2021a) Exploring synergies and trade-offs between climate change and the sustainable development goals. Springer, Singapore. https://doi.org/10.1007/978-981-15-7301-9

Venkatramanan V, Shah S, Prasad R (eds) (2021b) Sustainable bioeconomy: pathways to sustainable development goals. Springer Nature, Singapore. https://doi.org/10.1007/978-981-15-7321-7

Vogels GD (1979) The global cycle of methane. Antonie Van Leeuwenhoek 45:347–352. https://doi.org/10.1007/BF00443274

Wilson CA, Wood TM (1992) The anaerobic fungus *Neocallimastix frontalis:* isolation and properties of a cellulosome-type enzyme fraction with the capacity to solubilize hydrogen-bond-ordered cellulose. Appl Microbiol Biotechnol 37:125–129. https://doi.org/10.1007/BF00174216

Wolin M, Miller T (1987) Bioconversion of organic carbon to CH4and CO2. Geomicrobiol J 5:239–259. https://doi.org/10.1080/01490458709385972

Wood T (1985) Properties of cellulolytic enzyme systems. Biochem Soc Trans 13:407–410. https://doi.org/10.1042/bst0130407

Wyman C, Decker S, Himmel M, Brady J, Skopec C, Viikari L (2004) Hydrolysis of cellulose and hemicellulose. In: Dumitriu S (ed) Polysaccharides: structural diversity and functional versatility, 2nd edn. CRC, Boca Raton, FL. https://doi.org/10.1201/9781420030822.ch43

Yang SH (2008) Plant fiber chemistry. China Light Industry Press, Beijing

Zhang J, Choi Y, Yoo C, Kim T, Brown R, Shanks B (2015) Cellulose–hemicellulose and cellulose–lignin interactions during fast pyrolysis. ACS Sustain Chem Eng 3:293–301. https://doi.org/10.1021/sc500664h

Zimbardi F, Viggiano D, Nanna F, Demichele M, Cuna D, Cardinale G (1999) Steam explosion of straw in batch and continuous systems. Appl Biochem Biotechnol 77:117–126. https://doi.org/10.1385/abab:77:1-3:117

Biorefinery: Potential and Prospects for Utilisation of Biogenic Waste

14

Shachi Shah, V. Venkatramanan, and Ram Prasad

Abstract

The biorefinery is considered as a sustainable way of converting different feedstocks into energy-rich products, chemicals and value-added products through a well-established conversion technology. The biorefinery concept emerged with the purpose of efficient utilisation of biomass, waste biovalorization and at the same time to minimise the environmental impacts of waste management. Biorefinery technology enables the production of biomass-derived bioenergy, biofuels and development of circular bioeconomy. The bio-based economy has the potential to mitigate climate change and to achieve sustainable development goals. Biorefining technology refers to the conversion of biomass through processes such as pre-treatment, conversion and processing of products. Biorefinery technology enables thermochemical or biochemical conversion of biomass into bioenergy, biofuels and value-added products. Biorefineries generate low-volume but high-value products and high-volume but low-value products. The biorefineries are classified based on the feedstocks used, conversion processes, platforms or key intermediate products and target products. The feedstocks utilised in the biorefinery are diverse and the sustainability of the biorefinery system depends on (a) availability and characteristics of feedstocks, (b) environmental impacts of feedstock production, (c) amenability and suitability of feedstock for bioconversion and (d) feedstock bioconversion and its contribution to greenhouse gases intensity and energy

S. Shah (✉) · V. Venkatramanan
School of Interdisciplinary and Transdisciplinary Studies, Indira Gandhi National Open University, New Delhi, Delhi, India
e-mail: sshah@ignou.ac.in

R. Prasad
Department of Botany, School of Life Sciences, Mahatma Gandhi Central University, Motihari, Bihar, India

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_14

balance. The feedstocks include “lignocellulosic biomass”, “food wastes”, “algal biomass”, “municipal solid waste”, etc. Based on the feedstocks used, the biorefineries are called as lignocellulosic biorefinery, waste biorefinery, algal biorefinery, etc. However, lignocellulosic waste biorefineries are well studied for its potential and prospects. It gained prominence on account of (a) availability of lignocellulosic waste feedstocks; (b) sustainability of the feedstocks; and (c) diverse value-added chemicals generated from the bioconversion of lignocellulosic waste biomass. The success of biorefineries and development of bio-based industries requires positive intervention from the government in the form of proactive bioeconomy policy.

Keywords

Biorefinery · Biogenic wastes · Bioeconomy · Biomass · Food waste

14.1 Introduction

The biorefinery is considered as a sustainable way of converting different feedstocks into energy-rich products, chemicals and value-added products through a well-established conversion technology. Further, an important challenge for the humanity is to replace or substitute the use of non-renewable fossil fuels with renewable energy sources so that the energy demand and the demand for bioproducts are met sustainably and pragmatically (Shah et al. 2019). The biorefinery concept emerged with a noble purpose of efficient utilisation of biomass, waste biovalorization and at the same time to minimise the environmental impacts of waste management (Venkatramanan et al. 2021a). Biorefinery technology enables the production of biomass-derived bioenergy (Prasad et al. 2019a) and the production of biofuels (Prasad et al. 2019b). The bio-based economy has immense potential to mitigate climate change (Venkatramanan et al. 2020) and to achieve sustainable development goals (Venkatramanan et al. 2021a) warranting a sustainable biofuel policy (Prasad et al. 2020). A growing interest in circular bioeconomy and the need to achieve different dimensions of sustainability, namely environmental, economic and social dimensions calls for a technological innovation like biorefinery (Ubando et al. 2020). Further, the biorefinery concept endeavour to achieve both “energy goal” and “economic goal”. The former aims to replace fossil fuel-based energy sources. The latter economic goal reflects the promotion of bioeconomy and sustainable bio-based industries (Liguori and Faraco 2016). Of late, the biogenic waste biorefinery has received importance on account of its potential to substitute the fossil fuel-based refineries and also for its potential to produce value-added products and chemicals.

14.2 Biorefinery Concept

The concept of biorefinery is similar to petro-refineries which produce a series of products from petroleum or fossil fuels. Nevertheless, biorefineries utilise the biomass as feedstocks. So, the process of biorefining involves the sustainable conversion or bioconversion of biomass into a group of valuable products, energy and biofuels. The biorefining process is defined as "*sustainable processing of biomass into a spectrum of marketable products (food, feed, chemicals and materials) and energy (fuels, power and/or heat)*" (IEA 2009). The plant biomass is generally composed of cellulose, hemicellulose, lignin and other substances. Biorefinery technology utilises different conversion processes to transform/convert biomass into different intermediary compounds and value-added products (Kamm and Kamm 2007). Green biorefineries are defined as "*complex systems of the sustainable, environment- and resource-friendly technologies for the comprehensive (holistic) utilisation and the exploitation of biological raw materials in the form of green and residue biomass from a targeted sustainable regional land utilisation*" (Kamm and Kamm 2007). It can be considered that the biorefineries are equivalent to petroleum refineries. The difference being the renewable feedstocks that are used in the biorefineries. Cherubini et al. (2009) classified the biorefineries based on certain key characteristics, namely "*(a) the used platforms or key intermediate products and processes; (b) the targeted products; (c) the used feedstocks; and (d) the used processes"*.

The biorefinery concept was promoted to efficiently produce value-added products from diverse feedstocks (Ubando et al. 2020), namely "lignocellulosic biomass" (Özdenkçi et al. 2017; Sawatdeenarunat et al. 2018), "food wastes" (Bastidas-Oyanedel and Schmidt 2018; Dahiya et al. 2018; Esteban and Ladero 2018), "microbial-treated wastes", "algal biomass" (Bastiaens et al. 2017; De Bhowmick et al. 2019) and "manures" (Chen et al. 2005). Of late, enzymatic technologies and biotechnology have been dovetailed with biorefinery to produce biofuels (Singh et al. 2019).

14.3 Types of Biorefineries

The biomass feedstocks for the biorefineries are diverse. However, the major sources are agricultural waste, municipal solid waste, industrial waste materials, etc. The agricultural biomass can be either lignocellulosic waste materials such as residues and corn stover, or green biomass such as grasses and clovers. Other sources of biomass are food wastes, sugarcane bagasse and molasses (sugar industry), spent grains (breweries), etc. (Fava et al. 2015; Vea et al. 2018).

14.3.1 Lignocellulosic Biorefinery

Lignocellulosic waste biorefineries are well studied for its potential and prospects. It gained prominence on account of (a) availability of lignocellulosic waste feedstocks; (b) sustainability of the feedstocks; (c) diverse value-added chemicals generated from the bioconversion of lignocellulosic waste biomass (Piccolo and Bezzo 2009). The lignocellulosic waste biomass is composed of cellulose, hemicellulose and lignin. However, the ratio of these vital constituents greatly differs from the source materials (Qian 2014; Isikgor and Becer 2015). They are the widely preferred alternative to the first-generation biomass feedstocks. The first-generation feedstocks face competition from the food crops and land-use changes. However, second-generation biomass feedstocks have no such issue. It is reported that the lignocellulose waste biomass is widely generated on the global landscape. It is reported that while global lignocellulosic biomass production is about 1.3 billion tons per annum, only a meagre 3% of lignocellulosic materials are used for generation of bioenergy, biochemicals and other co-products (Baruah et al. 2018). The source of lignocellulosic waste biomass includes but not limited to corn stover, coconut husk, sugarcane bagasse, crop residues, paddy straw, sorghum stalks, wheat straw and barley straw (Zhang 2008). Studies recommend the use of lignocellulosic biorefineries for the sustainable production of biofuels and value-added products (De Bhowmick et al. 2018). Further, process integration (integration of different bioconversion processes) and recycle and reuse of lignocellulosic wastes are indeed pathways to achieve sustainable development goals in particular energy security and circular bioeconomy (Venkatramanan et al. 2021a, b).

14.3.2 Algal Biorefinery

The algal biomass is also considered as a valuable feedstock for the biorefineries. Algal biomass is grouped into macroalgae and microalgae (Chew et al. 2017; Torres et al. 2019). Algal biomass is construed as the third-generation biorefinery and it is best known for its benefits, namely (a) higher productivity; (b) lower land requirement. Microalgae are the photosynthetic organisms that are capable of synthesising their food from solar energy and the microalgae are rich in valuable global biological substances (Leu and Boussiba 2014). Further, the microalgae are cultivated in reactor systems such as photobioreactor which essentially occupy lesser land due to the vertical designing and other improvements in the technology (Ubando et al. 2016). Further, the algal biorefinery enables the utilisation of microalgae to produce a series of microalgal based bioproducts (López Barreiro et al. 2014; Chew et al. 2017). Macroalgae are another group of organisms which thrive mostly off the shore and “coastal shorelines” (Lehahn et al. 2016). These macroalgae are known as seaweeds. The macroalgae biorefinery, of late, is gaining prominence on account of its feedstocks and also its potential to produce value-added products and biofuels (Jiang et al. 2016; Ingle et al. 2018).

14.3.3 Waste Biorefinery

Waste biorefineries are another group of biorefineries, which utilise waste materials as feedstocks. The waste materials include biogenic waste materials and also non-edible biomass (Venkata Mohan 2014). The waste biorefinery is a technology that aims at waste valorization and through sustainable conversion processes, the waste materials are transformed into value-added products. As with other types of biorefineries, waste biorefineries are also promoted to produce valuable products such as biofuels, bioproducts and biochemicals. The waste management strategy of reuse and recycle greatly exhibited in the case of waste biorefineries. Further, the technologies and conversion pathways applied in waste biorefineries are robust. It must be noted that to augment the efficiency and effectiveness of waste conversion, there is a need to characterise the wastes and accordingly develop the biorefinery plants (Chandak et al. 2015; Skaggs et al. 2018). Studies on the applicability of biorefinery concept on waste materials, namely lignocellulosic waste (Serrano et al. 2017; Singh et al. 2019), food waste (Bastidas-Oyanedel and Schmidt 2018) and municipal solid waste (Barampouti et al. 2019) have been undertaken to understand the sustainability and economic dimensions of waste biorefineries. Nizami et al. (2017) reviewed the waste biorefinery production process in the context of developing countries and also the potential role of waste biorefinery towards circular bioeconomy. Further, the generation of an enormous amount of waste calls for waste biorefinery approach which works within the broad ambit of circular bioeconomy. The circular bioeconomy in addition to incorporating sustainable waste management strategy addresses the socio-economic and environmental concerns of waste management (Minelgaitė and Liobikienė 2019). Further, the organic solid waste biorefineries too utilise the organic waste generated from the sectors such as agriculture, urban system and industries. Organic solid waste biorefineries are also encouraging the development of bio-based industries and circular bioeconomy. Nevertheless, the constraints in the domain of technology, strategy and market influence the sustainable functioning of the biorefinery systems. Also, the factors like the degree of contamination in organic solid waste, fluctuations in the availability of waste biomass, marketability of the bioproducts and residue management are key aspects that demand intensive study.

14.4 Biorefinery Feedstocks

The feedstocks utilised in the biorefinery are diverse and the sustainability of the biorefinery system depends on (a) availability and characteristics of feedstocks, (b) environmental impacts of feedstock production, (c) amenability and suitability of feedstock for bioconversion and (d) feedstock bioconversion and its contribution to greenhouse gases intensity and energy balance. The feedstocks are grouped into first-generation, second-generation and third-generation feedstocks based on the characteristics of feedstocks. First-generation feedstocks include mainly "*sugar crops (sugar cane, sugar beet, sweet sorghum), starchy crops (cassava, corn,*

sorghum, sweet potato), oilseed crops (coconut, oil palm, jatropha) and waste feedstock (citrus peels)" (Shah and Venkatramanan 2019). Nevertheless, since the first-generation feedstocks include agricultural food crops, there is a conflict between the use of crops either for fuel or food. Further, in the recent past, widespread criticism surfaced on providing incentives to grow oil and energy crops at the cost of food grain crops. Issues such as national food security, agricultural land use policy, the rising cost of energy resources and increasing energy demand led to the search for alternative feedstocks. The characteristics that were looked for in the alternative feedstocks are the availability of non-food crop plant biomass and its amenability to bioconversion, etc. The second-generation feedstocks are non-food crop feedstocks. They are mainly lignocellulosic waste biomass. Such feedstocks are known to grow on marginal lands and have high biomass productivity. For instance, plants like eucalyptus, alfalfa, green grasses (switch grasses), Napier and poplar are found growing on marginal lands and they are good candidates for second-generation feedstocks. Further, the second-generation feedstocks include agricultural crop residues and agro-industrial waste as well. The agricultural crop residues include paddy straw, rice bran, wheat straw, wheat bran, sawdust, forest thinning, sugarcane bagasse, etc. On the other hand, the agro-industrial wastes include apple pomace, oil cake, orange peel, spent coffee grounds, etc. (Hassan et al. 2019). Few examples of forestry residues are treetops, needles, branches, etc. The second-generation feedstocks do not compete with food crops and hence there is no conflict of interest between the second-generation feedstocks and food crops. Further, lignocellulosic waste biomass is the most prevalent biomass in the global landscape. Lignocellulosic biomass is made up of cellulose, hemicellulose and lignin. However, there is a demand for innovative technology and sustainable production process to convert the lignocellulosic waste biomass into energy and value-added products (Shah and Venkatramanan 2019; Prasad et al. 2019a, b).

The third-generation feedstocks particularly algal biomass is of recent development. Algae is being promoted as non-food, marine biomass, capable of producing valuable products through the conversion process. Algae include both macroalgae and microalgae. Macroalgae are commonly called seaweeds. They include species such as *Ulva lactuca, Gracilaria vermiculophylla and Saccharina latissima*. It is reported that green algae are being under intensive study for biofuel production (Kawai and Murata 2016; Hassan et al. 2019). The cellulose content is reportedly low in the seaweeds (Rocca et al. 2015; Hassan et al. 2019). On the other hand, microalgae are found to be rich in lipid, and hence it is a potential candidate (feedstock) for biodiesel production. Nevertheless, the cost involved in biodiesel production from microalgae is relatively higher as compared to the second-generation feedstocks. Further, the biodiesel production capacity from the microalgae is relatively low. But the metabolic significance of the microalgae is a point to be noted. Examples of microalgae include but not limited to *Schizochytrium* sp., *Botryococcus braunii, Nitzschia, Hantzschia* and *Neochloris oleoabundans.* The microalgae are potential feedstocks to produce bioenergy, biochemicals and high value-added products. Further, tapping the potential of microalgae through circular bioeconomy approach is not only a sustainable environmental and economic option

but also a strategy to improve resource recovery from microalgae (Venkata Mohan et al. 2020). Algal biomass feedstocks have certain advantages such as the cultivation of algae in different environments. Algae is reported to be grown in photobioreactors, open ponds and closed-loop system (Shah and Venkatramanan 2019). As stated earlier, the triglycerides synthesised by the algal biomass can be transformed into biodiesel. Further, the algae can be genetically modified to produce valuable products including biofuel and other value-added chemicals.

14.5 Biorefinery Technology

Biorefining technology refers to the conversion of biomass through processes such as pre-treatment, conversion and processing of products. In other words, it includes upstream, midstream and downstream processing steps. Based on the feedstock and conversion process employed in a biorefinery, varied products are produced (Cherubini et al. 2009; De Buck et al. 2020). The conversion processes employed in the biorefinery can be grouped into thermochemical and biochemical conversion processes. The biochemical conversion processes employ microorganisms and enzymes for the conversion of biomass into valuable products. Further, the technologies such as pyrolysis, fermentation, gasification, anaerobic digestion (AD) and incineration have been employed in the conversion of biomass such as sugarcane bagasse, grasses, corn stover, paddy straw, municipal solid waste and industrial wastes (Naik et al. 2010). Nevertheless, it is reported that the biorefinery technology based on the type and availability of feedstock produce specific fuels. In other words, in the integrated waste biorefinery, multiple/mixed technologies are adopted to convert multiple feedstocks into bioenergy, biofuels and chemicals, etc. (Posada et al. 2013). In effect, the conversion processes employed in the biorefineries include thermochemical processes (*"pyrolysis, incineration, carbonisation, gasification, hydrothermal liquefaction"*), physico-chemical processes (*"transesterification and microbial mediation"*) and biochemical processes (*"aerobic and anaerobic digestion, microbial electrochemical technology and enzymes"*) (Ubando et al. 2020).

14.6 Conclusion

Biorefinery technology enables thermochemical or biochemical conversion of biomass into bioenergy, biofuels and value-added products. Biorefinery concept and technology are the need of the hour. It is important to address the challenge of waste management, energy demand, climate change and energy security. Sustainable energy is the best alternative available presently to replace fossil fuels. Biorefineries generate low-volume but high-value products and high-volume but low-value products. The economic success of biorefinery system depends on the production of low-volume but high-value products such as chemicals which has huge market potential. The assessment including life cycle assessment and sustainability

assessment revealed that biorefineries have potential (a) to utilise a diverse group of feedstocks, (b) to minimise greenhouse gas emissions, (c) to produce high-value products, (d) to recycle and reuse wastes and (e) to support the circular bioeconomy. Nevertheless, popularisation of biorefineries is checkmated by the challenges, namely financial barriers, technical barriers, etc. The success of biorefineries and development of bio-based industries requires positive intervention from the government in the form of proactive policy.

References

Barampouti E, Mai S, Malamis D, Moustakas K, Loizidou M (2019) Liquid biofuels from the organic fraction of municipal solid waste: a review. Renew Sustain Energy Rev 110:298–314. https://doi.org/10.1016/j.rser.2019.04.005

Baruah J, Nath B, Sharma R, Kumar S, Deka R, Baruah D, Kalita E (2018) Recent trends in the pretreatment of lignocellulosic biomass for value-added products. Front Energy Res 6. https://doi.org/10.3389/fenrg.2018.00141

Bastiaens L, Van Roy S, Thomassen G, Elst K (2017) Biorefinery of algae. In: Microalgae-based biofuels and bioproducts, pp 327–345. https://doi.org/10.1016/b978-0-08-101023-5.00014-5

Bastidas-Oyanedel J, Schmidt J (2018) Increasing profits in food waste biorefinery—a techno-economic analysis. Energies 11:1551. https://doi.org/10.3390/en11061551

Chandak S, Chari K, Memon M (2015) Converting waste agricultural biomass into energy: experiences and lessons learnt from a capacity building and technology demonstration project in India. J Jpn Inst Energy 94:1129–1147. https://doi.org/10.3775/jie.94.1129

Chen S, Wen Z, Liao W, Liu C, Kincaid R, Harrison J, Elliott D, Brown M, Stevens D (2005) Studies into using manure in a biorefinery concept. Appl Biochem Biotechnol 124:0999–1016. https://doi.org/10.1385/abab:124:1-3:0999

Cherubini F, Jungmeier G, Wellisch M, Willke T, Skiadas I, Van Ree R, de Jong E (2009) Toward a common classification approach for biorefinery systems. Biofuels Bioprod Biorefin 3:534–546. https://doi.org/10.1002/bbb.172

Chew K, Yap J, Show P, Suan N, Juan J, Ling T, Lee D, Chang J (2017) Microalgae biorefinery: high value products perspectives. Bioresour Technol 229:53–62. https://doi.org/10.1016/j.biortech.2017.01.006

Dahiya S, Kumar A, Shanthi Sravan J, Chatterjee S, Sarkar O, Mohan S (2018) Food waste biorefinery: sustainable strategy for circular bioeconomy. Bioresour Technol 248:2–12. https://doi.org/10.1016/j.biortech.2017.07.176

De Bhowmick G, Sarmah A, Sen R (2018) Lignocellulosic biorefinery as a model for sustainable development of biofuels and value-added products. Bioresour Technol 247:1144–1154. https://doi.org/10.1016/j.biortech.2017.09.163

De Bhowmick G, Sarmah A, Sen R (2019) Zero-waste algal biorefinery for bioenergy and biochar: a green leap towards achieving energy and environmental sustainability. Sci Total Environ 650:2467–2482. https://doi.org/10.1016/j.scitotenv.2018.10.002

De Buck V, Polanska M, Van Impe J (2020) Modeling biowaste biorefineries: a review. Front Sustain Food Syst. https://doi.org/10.3389/fsufs.2020.00011

Esteban J, Ladero M (2018) Food waste as a source of value-added chemicals and materials: a biorefinery perspective. Int J Food Sci Technol 53:1095–1108. https://doi.org/10.1111/ijfs.13726

Fava F, Totaro G, Diels L, Reis M, Duarte J, Carioca O, Poggi-Varaldo H, Ferreira B (2015) Biowaste biorefinery in Europe: opportunities and research & development needs. N Biotechnol 32:100–108. https://doi.org/10.1016/j.nbt.2013.11.003

Hassan S, Williams G, Jaiswal A (2019) Moving towards the second generation of lignocellulosic biorefineries in the EU: drivers, challenges, and opportunities. Renew Sustain Energy Rev 101:590–599. https://doi.org/10.1016/j.rser.2018.11.041

IEA (2009) IEA Bioenergy-Task 42. Biorefineries: adding value to the sustainable utilisation of biomass. IEA Bioenergy. https://www.ieabioenergy.com/wp-content/uploads/2013/10/Task-42-Booklet1.pdf

Ingle K, Vitkin E, Robin A, Yakhini Z, Mishori D, Golberg A (2018) Macroalgae biorefinery from Kappaphycus alvarezii: conversion modeling and performance prediction for India and Philippines as examples. Bioenergy Res 11:22–32. https://doi.org/10.1007/s12155-017-9874-z

Isikgor F, Becer C (2015) Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. Polym Chem 6:4497–4559. https://doi.org/10.1039/c5py00263j

Jiang R, Ingle K, Golberg A (2016) Macroalgae (seaweed) for liquid transportation biofuel production: what is next? Algal Res 14:48–57. https://doi.org/10.1016/j.algal.2016.01.001

Kamm B, Kamm M (2007) Biorefineries—multi product processes. Adv Biochem Eng Biotechnol 105:175–204. https://doi.org/10.1007/10_2006_040

Kawai S, Murata K (2016) Biofuel production based on carbohydrates from both Brown and red macroalgae: recent developments in key biotechnologies. Int J Mol Sci 17:145. https://doi.org/10.3390/ijms17020145

Lehahn Y, Ingle K, Golberg A (2016) Global potential of offshore and shallow waters macroalgal biorefineries to provide for food, chemicals and energy: feasibility and sustainability. Algal Res 17:150–160. https://doi.org/10.1016/j.algal.2016.03.031

Leu S, Boussiba S (2014) Advances in the production of high-value products by microalgae. Ind Biotechnol 10:169–183. https://doi.org/10.1089/ind.2013.0039

Liguori R, Faraco V (2016) Biological processes for advancing lignocellulosic waste biorefinery by advocating circular economy. Bioresour Technol 215:13–20. https://doi.org/10.1016/j.biortech.2016.04.054

López Barreiro D, Samorì C, Terranella G, Hornung U, Kruse A, Prins W (2014) Assessing microalgae biorefinery routes for the production of biofuels via hydrothermal liquefaction. Bioresour Technol 174:256–265. https://doi.org/10.1016/j.biortech.2014.10.031

Minelgaitė A, Liobikienė G (2019) Waste problem in European Union and its influence on waste management behaviours. Sci Total Environ 667:86–93. https://doi.org/10.1016/j.scitotenv.2019.02.313

Naik S, Goud V, Rout P, Dalai A (2010) Production of first and second generation biofuels: a comprehensive review. Renew Sustain Energy Rev 14:578–597. https://doi.org/10.1016/j.rser.2009.10.003

Nizami A, Rehan M, Waqas M, Naqvi M, Ouda O, Shahzad K, Miandad R, Khan M, Syamsiro M, Ismail I, Pant D (2017) Waste biorefineries: enabling circular economies in developing countries. Bioresour Technol 241:1101–1117. https://doi.org/10.1016/j.biortech.2017.05.097

Özdenkçi K, De Blasio C, Muddassar H, Melin K, Oinas P, Koskinen J, Sarwar G, Järvinen M (2017) A novel biorefinery integration concept for lignocellulosic biomass. Energ Conver Manage 149:974–987. https://doi.org/10.1016/j.enconman.2017.04.034

Piccolo C, Bezzo F (2009) A techno-economic comparison between two technologies for bioethanol production from lignocellulose. Biomass Bioenergy 33:478–491. https://doi.org/10.1016/j.biombioe.2008.08.008

Posada J, Patel A, Roes A, Blok K, Faaij A, Patel M (2013) Potential of bioethanol as a chemical building block for biorefineries: preliminary sustainability assessment of 12 bioethanol-based products. Bioresour Technol 135:490–499. https://doi.org/10.1016/j.biortech.2012.09.058

Prasad S, Sheetal K, Venkatramanan V, Kumar S, Kannojia S (2019a) Sustainable energy: challenges and perspectives. In: Sustainable Green technologies for environmental management, pp 175–197. https://doi.org/10.1007/978-981-13-2772-8_9

Prasad S, Venkatramanan V, Kumar S, Sheetal K (2019b) Biofuels: a clean technology for environment management. In: Sustainable Green technologies for environmental management, pp 219–240. https://doi.org/10.1007/978-981-13-2772-8_11

Prasad S, Kumar S, Sheetal K, Venkatramanan V (2020) Global climate change and biofuels policy: Indian perspectives. In: Global climate change and environmental policy, pp 207–226. https://doi.org/10.1007/978-981-13-9570-3_6

Qian E (2014) Pretreatment and saccharification of lignocellulosic biomass. In: Research approaches to sustainable biomass systems, pp 181–204. https://doi.org/10.1016/b978-0-12-404609-2.00007-6

Rocca S, Agostini A, Giuntoli J, Marelli L (2015) Biofuels from algae: technology options, energy balance and GHG emissions. JRC science for policy report. https://publications.jrc.ec.europa.eu/repository/bitstream/JRC98760/algae_biofuels_report_21122015.pdf. Accessed 10 Sept 2020

Sawatdeenarunat C, Nam H, Adhikari S, Sung S, Khanal S (2018) Decentralized biorefinery for lignocellulosic biomass: integrating anaerobic digestion with thermochemical conversion. Bioresour Technol 250:140–147. https://doi.org/10.1016/j.biortech.2017.11.020

Serrano A, Fermoso F, Alonso-Fariñas B, Rodríguez-Gutierrez G, Fernandez-Bolaños J, Borja R (2017) Olive mill solid waste biorefinery: high-temperature thermal pre-treatment for phenol recovery and biomethanization. J Clean Prod 148:314–323. https://doi.org/10.1016/j.jclepro.2017.01.152

Shah S, Venkatramanan V (2019) Advances in microbial Technology for Upscaling Sustainable Biofuel Production. New and future developments in microbial. Biotechnol Bioeng:69–76. https://doi.org/10.1016/b978-0-444-63504-4.00005-0

Shah S, Venkatramanan V, Prasad R (eds) (2019) Sustainable green technologies for environmental management. Springer Nature, Singapore. https://doi.org/10.1007/978-981-13-2772-8

Singh A, Rodríguez Jasso R, Gonzalez-Gloria K, Rosales M, Belmares Cerda R, Aguilar C, Singhania R, Ruiz H (2019) The enzyme biorefinery platform for advanced biofuels production. Bioresour Technol Rep 7:100257. https://doi.org/10.1016/j.biteb.2019.100257

Skaggs R, Coleman A, Seiple T, Milbrandt A (2018) Waste-to-Energy biofuel production potential for selected feedstocks in the conterminous United States. Renew Sust Energ Rev 82:2640–2651. https://doi.org/10.1016/j.rser.2017.09.107

Torres M, Kraan S, Domínguez H (2019) Seaweed biorefinery. Rev Environ Sci Biotechnol 18:335–388. https://doi.org/10.1007/s11157-019-09496-y

Ubando A, Cuello J, El-Halwagi M, Culaba A, Promentilla M, Tan R (2016) Application of stochastic analytic hierarchy process for evaluating algal cultivation systems for sustainable biofuel production. Clean Techn Environ Policy 18:1281–1294. https://doi.org/10.1007/s10098-015-1073-z

Ubando A, Felix C, Chen W (2020) Biorefineries in circular bioeconomy: a comprehensive review. Bioresour Technol 299:122585. https://doi.org/10.1016/j.biortech.2019.122585

Vea E, Romeo D, Thomsen M (2018) Biowaste valorisation in a future circular bioeconomy. Proc CIRP 69:591–596. https://doi.org/10.1016/j.procir.2017.11.062

Venkata Mohan S (2014) Reorienting waste remediation towards harnessing bioenergy. In: Industrial wastewater treatment, recycling and reuse, pp 235–281. https://doi.org/10.1016/b978-0-08-099968-5.00006-4

Venkata Mohan S, Hemalatha M, Chakraborty D, Chatterjee S, Ranadheer P, Kona R (2020) Algal biorefinery models with self-sustainable closed loop approach: trends and prospective for blue-bioeconomy. Bioresour Technol 295:122128. https://doi.org/10.1016/j.biortech.2019.122128

Venkatramanan V, Shah S, Prasad R (eds) (2020) Global climate change and environmental policy: agriculture perspectives. Springer Nature, Singapore. https://doi.org/10.1007/978-981-13-9570-3

Venkatramanan V, Shah S, Prasad R (eds) (2021a) Sustainable bioeconomy: pathways to sustainable development goals. Springer Nature, Singapore. https://doi.org/10.1007/978-981-15-7321-7

Venkatramanan V, Shah S, Rai AK, Prasad R (2021b) Nexus between crop residue burning, bioeconomy and sustainable development goals over North-western India. Front Energy Res 8:614212. https://doi.org/10.3389/fenrg.2020.614212

Zhang Y (2008) Reviving the carbohydrate economy via multi-product lignocellulose biorefineries. J Ind Microbiol Biotechnol 35:367–375. https://doi.org/10.1007/s10295-007-0293-6

Life Cycle Assessment of Lignocellulosic Waste Biorefinery

15

V. Venkatramanan, Shachi Shah, Ram Prasad, and Mrinalini Shah

Abstract

The twenty-first century is witnessing fossil fuel depletion, increase in the atmospheric concentration of greenhouse gases, industrialization, urbanization and global climate change. There is a growing need to switch over to renewable energy resources and move towards circular bioeconomy. Sustainable bioeconomy has been promoted to replace fossil fuels and to produce bioenergy, chemicals and high value-added products. Biorefineries play a pivotal role in circular bioeconomy. Adoption of biorefineries is a win-win proposition both from the perspective of energy security and waste management. "*Biorefining is defined as the sustainable synergetic processing of biomass into a spectrum of marketable food and feed ingredients, products (chemicals, materials) and energy (fuels, power, heat)*". Biorefinery system endeavours to maximize the production of useful products from the biomass. Biorefineries adopt technologies which aim to process the biomass into diverse building blocks. The building blocks are further processed to generate biochemicals and biofuels. The biorefineries are classified based on key features such as (a) feedstocks used in the biorefinery, (b) conversion processes, (c) platform or intermediary products and (d) targeted products. The feedstocks including its characteristics, availability and biodegradability is one of the pertinent factors deciding the sustainability of

V. Venkatramanan (✉) · S. Shah
School of Interdisciplinary and Transdisciplinary Studies, Indira Gandhi National Open University, New Delhi, Delhi, India
e-mail: vvramanan@ignou.ac.in

R. Prasad
Department of Botany, School of Life Sciences, Mahatma Gandhi Central University, Motihari, Bihar, India

M. Shah
Institute of Management Technology, Ghaziabad, Uttar Pradesh, India

S. Shah et al. (eds.), *Bio-valorization of Waste*, Environmental and Microbial Biotechnology, https://doi.org/10.1007/978-981-15-9696-4_15

biorefinery system. The debate between food and fuel has led to the search for second-generation biorefineries, which thrives on non-food biomass. The second-generation biorefineries utilize feedstocks such as residual biomass, lignocellulosic biomass and waste streams. The alternative biomass resources have huge potential for energy generation and can minimize fossil fuel use. Lignocellulose is the most abundant source of unutilised biomass. The positive attributes of lignocellulose biomass are year-round availability of biomass, renewability, sustainability, and amenability to conversion. Nevertheless, lignocellulosic waste biomass requires pretreatment for augmenting the efficiency of the conversion process. Several pretreatment strategies and methods such as physical, chemical and biological methods are adopted to enable lignin deconstruction. The pretreated lignocellulosic biomass through thermochemical conversion (combustion, gasification, hydrothermal processing, liquefaction, pyrolysis) and biochemical conversion are converted into bioenergy, biofuels, speciality chemicals and value-added products. Nevertheless, it is important to assess the impacts of biorefinery on the environment from the perspective of feedstocks, product generation and economic returns. The sustainability of the biorefineries is assessed through the life cycle assessment methodology. Life cycle assessment of biorefineries gains currency on account of (a) technological advancement, (b) bioconversion of diverse feedstocks into value-added products, (c) evaluation of the environmental performance of the biorefineries and (d) validating the sustainable conversion processes. As per ISO 14040, LCA involves four important components, namely goal, scope and functional unit; inventory analysis; impact assessment and interpretation. It has been observed that LCA of lignocellulosic biorefineries is greatly influenced by the methodological attributes, namely the "functional unit", "system boundaries", "allocation methods", LCA approach, etc. LCA studies on lignocellulosic biorefineries reveal that the accuracy and reliability of LCA study are influenced by factors, not limited to data inadequacy, certain assumptions in LCA study and site-specific or local conditions. Though there are challenges to LCA of lignocellulosic waste biorefinery, importance must be placed on the sustainable production of value-added products, efficient utilization of resources, biovalorization and energy efficiency of the biorefinery system. The future research can be directed towards (a) sustainable biorefineries; (b) waste valorization; (c) upscaling the production of value-added products; (d) optimisation of bioconversion processes; (e) sustainable design configuration of the biorefinery; (f) role of biorefineries in the circular economy and (g) contribution of biorefineries in climate change mitigation.

Keywords

Life cycle assessment (LCA) · Biorefinery · Lignocellulosic Biorefinery · Feedstocks · Biomass

15.1 Introduction

The twenty-first century is witnessing fossil fuel depletion, increase in the atmospheric concentration of greenhouse gases, industrialization, urbanization and global climate change (Venkatramanan et al. 2020, 2021a). The key issues in the domain of energy sector include switching over to renewable energy resources (Prasad et al. 2021), use of bio-based feedstocks, waste valorization and bioenergy generation (Venkatramanan et al. 2021b). The need to reduce greenhouse gases emissions, to increase the dependence on sustainable energy resources (Prasad et al. 2019a, 2020) and to upscale the production of biofuel (Prasad et al. 2019b; Shah and Venkatramanan 2019) demands the development of circular bioeconomy (Venkatramanan et al. 2021b). Sustainable bioeconomy has been promoted to replace fossil fuels and to produce bioenergy, chemicals and high value-added products (Palmeros Parada et al. 2016; Venkatramanan et al. 2021b). The alternative biomass resources particularly the lignocellulosic feedstocks, agro-wastes, and food wastes have huge potential for energy generation and can minimize fossil fuel use. The utilization of lignocellulosic waste for the production of value-added products downplays the concerns about fossil fuel use and also the consequences of the population growth (Bello et al. 2018). Further, across the world, countries have initiated steps to tap the potential of bioeconomy. Use of alternative bio-based resources through biorefinery concept and related technology influence environmental sustainability (Shah et al. 2019).

LCA study is prominently adopted in the sustainability assessment of the biorefineries. The biorefineries in the recent past are multifunctional and multi-product based and also use a diverse group of feedstocks. In effect, the environmental profile of the biorefineries is greatly influenced by the feedstocks used, the bioconversion processes adopted and also the system design of the biorefineries. In this context, the role of LCA study in biorefineries is significant. As per ISO 14040, LCA involves four important components, namely goal, scope and functional unit; inventory analysis; impact assessment; and interpretation. LCA of lignocellulosic biorefineries is greatly influenced by the methodological attributes, namely the "functional unit", "system boundaries", "allocation methods", LCA approach, etc. The accuracy and also the reliability of LCA study are influenced by factors, not limited to data inadequacy, certain assumptions in LCA study and site-specific or local conditions. The uncertainty in the environmental profile of lignocellulosic waste biorefinery calls for sensitivity analysis. The sensitivity analysis enables to quantify the influence of an input (Bezergianni and Chrysikou 2020). The challenges to LCA of lignocellulosic waste biorefinery demand LCA study to be systematic, comprehensive and well-designed. Nevertheless, in the LCA study of lignocellulosic waste biorefineries, importance must be given on the sustainable production of value-added products, efficient utilization of resources, biovalorization and energy efficiency of the biorefinery system.

15.2 Lignocellulosic Waste Biomass

"Lignocellulose is the most abundant source of unutilised biomass" (Menon and Rao 2012). Lignocellulosic biomass which includes agricultural waste, agro-industrial wastes and energy crops, due to positive features like year-round availability of biomass, renewability, sustainability and amenability to conversion are gaining significance in the era of global change (Bilal and Iqbal 2020). Important constituents of lignocellulosic biomass are cellulose (40 to 50%), hemicellulose (25 to 30%) and lignin (15 to 20%). However, the rate of cellulose, hemicellulose and lignin varies significantly based on the feedstock (Table 15.1) (De Buck et al. 2020). Cellulose is the prime component of the cell wall and they are made up of glucose units linked through β (1 → 4) glycosidic linkages. In other words, the cellulose is composed of cellobiose chains. The cellobiose is a disaccharide, which is formed by the condensation of a pair of glucose molecules. The cellulose chains through hydrogen bonds form microfibrils. These cellulose microfibrils are attached by hemicellulose and polymers (pectin) (Fig. 15.1) (Menon and Rao 2012). So, hemicellulose enables interlinking of cellulose fibres and also interlinking of cellulose and lignin. Hemicellulose is composed of C5 (xylose) and C6 (glucose, galactose, mannose). The composition of hemicellulose varies with the feedstocks. The hemicellulose in hardwood is mostly xylans. In the case of softwoods, the hemicellulose is made of glucomannans (De Buck et al. 2020). Lignin is a "polyphenolic polymer". It is made of paracoumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Lignin is a polymerization product of paracoumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Nevertheless, the ratio of these alcohol components varies with layers of the cell wall, tissues and plant parts. Lignin due to tightly linked aromatic polymer is resistant to the hydrolytic process.

15.3 A Primer on Biorefinery

The International Energy Agency (IEA) Bioenergy Task 42 defines "*biorefining as the sustainable synergetic processing of biomass into a spectrum of marketable food & feed ingredients, products (chemicals, materials) and energy (fuels, power, heat)*" (IEA 2014). Biorefinery system includes "*upstream (biomass production, transportation, pretreatment), midstream (biomass conversion to the targeted products) and downstream (product distribution) processing of bio-based feedstocks*" (Bezergianni and Chrysikou 2020). Biorefinery system endeavours to maximize the production of useful products from the biomass. Biorefineries adopt technologies which aim to process the biomass into diverse building blocks. The building blocks are further processed to generate biochemicals and biofuels (Fig. 15.2). Cherubini et al. (2009) attempted to classify or group the biorefineries based on key features such as (a) feedstocks used in the biorefinery, (b) conversion processes, (c) platform or intermediary products and (d) targeted products.

Table 15.1 Composition of representative lignocellulosic feedstocks

Feedstocks	Carbohydrate composition (% dry wt)			References
	Cellulose	Hemicellulose	Lignin	
Barley hull	34	36	19	Kim et al. (2008)
Barley straw	36–43	24–33	6.3–9.8	Garda-Aparicio et al. (2006) and Rowell (1992)
Bamboo	49–50	18–20	23	Alves et al. (2010)
Corn cob	32.3–45.6	39.8	6.7–13.9	Cao et al. (1997) and McKendry (2002)
Corn stover	35.1–39.5	20.7–24.6	11.0–19.1	Mosier et al. (2005)
Cotton	85–95	5–15	0	Kadolph and Langford (1998)
Cotton stalk	31	11	30	Rubio et al. (1998)
Douglas fir	35–48	20–22	15–21	Schell et al. (1999)
Eucalyptus	45–51	11–18	29	Pereira (1988) and Alves et al. (2010)
Hardwood stems	40–55	24–40	18–25	Howard et al. (2003) and Malherbe and Cloete (2002)
Rice straw	29.2–34.7	23–25.9	17–19	Brylev et al. (2001) and Prasad et al. (2007)
Rice husk	28.7–35.6	11.96–29.3	15.4–20	Allen et al. (2001) and Abbas and Ansumali (2010)
Wheat straw	35–39	22–30	12–16	Prasad et al. (2007)
Wheat bran	10.5–14.8	35.5–39.2	8.3–12.5	Miron et al. (2001)
Grasses	25–40	25–50	10–30	Stewart et al. (1997)
Newspaper	40–55	24–39	18–30	Howard et al. (2003)
Sugarcane bagasse	25–45	28–32	15–25	Alves et al. (2010) and Singh et al. (2009)
Sugarcane tops	35	32	14	Jeon et al. (2010)
Pine	42–49	13–25	23–29	Pereira (1988)
Poplar wood	45–51	25–28	10–21	Torget and Hsu (1994)
Olive tree biomass	25.2	15.8	19.1	Cara et al. (2008)
Jute fibres	45–53	18–21	21–26	Mosihuzzaman et al. (1982)
Switchgrass	35–40	25–30	15–20	Howard et al. (2003)
Grasses	25–40	25–50	10–30	Howard et al. (2003) and Malherbe and Cloete (2002)
Winter rye	29–30	22–26	16.1	Petersson et al. (2007)
Oilseed rape	27.3	20.5	14.2	Petersson et al. (2007)
Softwood stem	45–50	24–40	18–25	Howard et al. (2003) and Malherbe and Cloete (2002)
Oat straw	31–35	20–26	10–15	Rowell (1992)
Nut shells	25–30	22–28	30–40	Sinner et al. (1979)
Sorghum straw	32–35	24–27	15–21	Herrera et al. (2003) and Vázquez et al. (2007)

(continued)

Table 15.1 (continued)

Feedstocks	Carbohydrate composition (% dry wt)			References
	Cellulose	Hemicellulose	Lignin	
Tamarind kernel powder	10–15	55–65	–	Menon et al. (2010)
Water hyacinth	18.2–22.1	48.7–50.1	3.5–5.4	Nigam (2002) and Aswathy et al. (2010)

Source: With permission from Menon and Rao (2012)

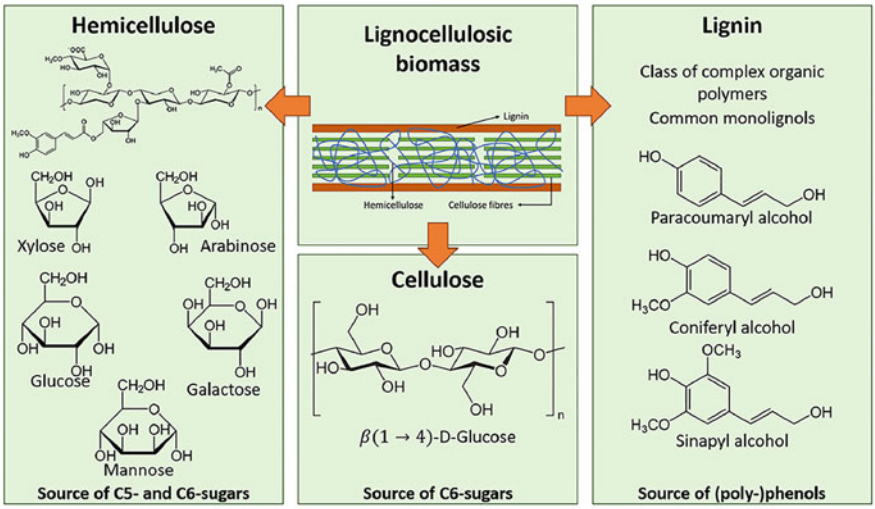


Fig. 15.1 Lignocellulosic biomass. (Source: Isikgor and Becer 2015; De Buck et al. 2020. "Modelling Biowaste Biorefineries: A review" by De Buck et al. 2020 is licensed under CC BY. Accessed at https://www.frontiersin.org/articles/10.3389/fsufs.2020.00011/full)

15.3.1 Biorefinery Generations and Associated Feedstocks

First-generation biorefineries utilize feedstocks such as food crops to produce biofuels and other value-added products (De Buck et al. 2020). Nevertheless, the sustainability of first-generation biorefineries is challenged by the debate on food versus fuel. It must be noted that crops such as corn, rapeseed, etc. contribute immensely to the production of bioethanol. For long-term sustainability and increasing food demand, the focus has been shifted towards other feedstocks that are rich in carbohydrates and available in plenty. Among the non-food crop-based feedstocks, lignocellulosic waste, municipal solid waste, agricultural wastes, food wastes, etc. are potential candidates. The second-generation biorefinery utilizes feedstocks such as residual biomass, lignocellulosic biomass and waste streams (De Buck et al. 2020). The waste normally used as feedstocks in the second-generation biorefineries include agricultural farm wastes/residues, forestry wastes, industrial wastes,

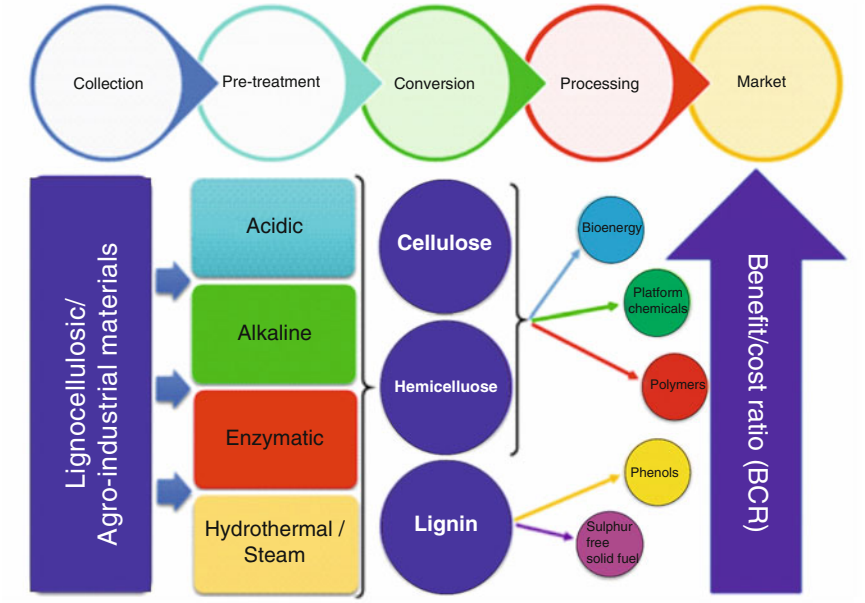


Fig. 15.2 Concept of biorefinery. (Source: With permission from Arevalo-Gallegos et al. 2017)

municipal solid wastes, kitchen wastes, etc. Biowaste refineries are prevalent widely and possess sustainability features as compared to the first-generation biorefineries. The third-generation biorefineries utilize feedstocks such as algal biomass (Bezergianni and Chrysikou 2020). These algal biorefineries are still under development and require research and development (De Buck et al. 2020).

15.3.2 Conversion Platforms

The conversion processes employed in biorefineries are significant, as they influence (a) economic and environmental feasibility of the biorefinery system, (b) process carbon efficiency and (c) environmental impacts like greenhouse gas emissions, eutrophication, acidification, etc. To better understand the conversion processes, Cherubini et al. (2009) grouped the conversion platforms into categories such as biochemical, thermochemical and hybrid processes (De Buck et al. 2020). The thermochemical conversion processes generally employ chemical processes such as pyrolysis and gasification for the conversion of feedstocks into valuable products. On the other hand, the biochemical processes use the action of microorganisms and enzymes for the bioconversion of feedstocks. In the case of hybrid processes, both the thermochemical and biological processes are employed in the conversion of biological feedstocks (De Buck et al. 2020).

15.3.3 Lignocellulosic Biorefinery

Sustainable bioeconomy provides a gateway to checkmate the global challenges including depletion of fossil fuels and climate change. "Bioeconomy involves the production and sustainable use of biological resources to further growth of the sustainable economy through generation of information, knowledge, bioproducts, ecosystem services and innovative processes" (Venkatramanan et al. 2021b). Bioeconomy has been encouraged as a strategy to replace fossil fuels and to produce bioenergy, chemicals and value-added products (Palmeros Parada et al. 2016). In this context, the concept of biorefineries is gaining currency. Lignocellulosic waste biorefineries are prominent due to the key characteristics of lignocellulosic waste feedstocks. The lignocellulosic feedstocks are known for its "sustainability, bio-renewability, availability round the year, recyclability" (Bilal and Iqbal 2020).

Lignocellulosic waste biomass requires pretreatment for augmenting the efficiency of the conversion process. Several pretreatment strategies and methods such as physical, chemical, and biological methods are adopted to enable lignin deconstruction (Fig. 15.3) (Menon and Rao 2012; Galbe and Wallberg 2019; De Buck et al. 2020; Bilal and Iqbal 2020). Through physical pretreatment, the lignocellulosic biomass is treated using methods like grinding, milling, irradiation and extrusion. The basic aims of physical pretreatment are size reduction, improving

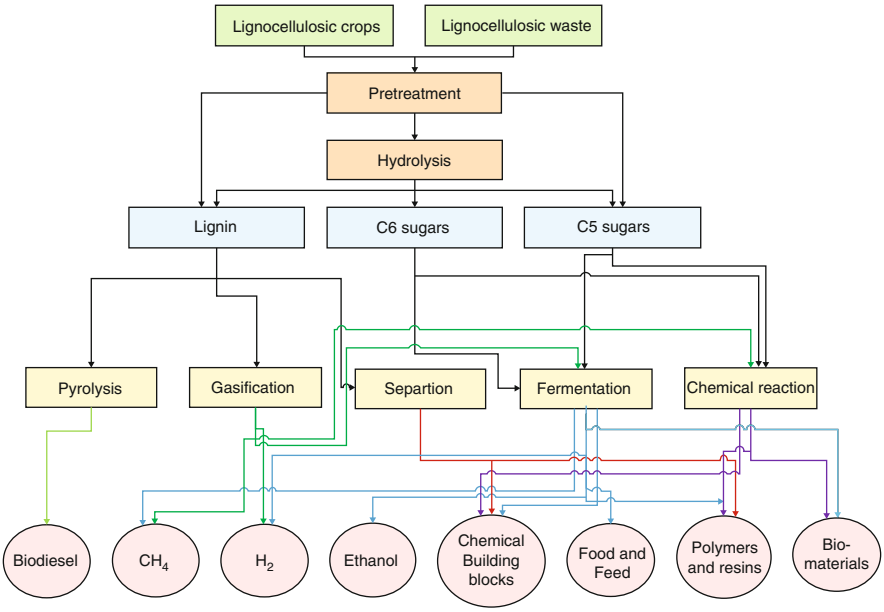


Fig. 15.3 A schematic representation of lignocellulosic biorefinery. (Source: Galbe and Wallberg 2019; "Pre-treatment for biorefineries: a review of common methods for efficient utilisation of lignocellulosic materials" by Galbe and Wallberg 2019 is licensed under CC BY. Accessed at https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-019-1634-1)

enzymatic hydrolysis process, biodegradation of lignocellulosic waste and reducing crystallinity. Nevertheless, the physical pretreatment methods are energy-intensive and cost-intensive (Menon and Rao 2012; Galbe and Wallberg 2019; De Buck et al. 2020).

The physico-chemical treatment process integrates both physical and chemical process to increase the efficiency of the pretreatment process and enable degradation of lignocellulosic biomass. In this category, the common methods employed are steam explosion, ammonia fibre explosion (AFEX), ammonia recycle percolation (ARP), microwave-chemical pretreatment, liquid hot water pretreatment, etc. (Menon and Rao 2012). In case of steam explosion, the lignocellulosic waste biomass is treated with saturated steam (160–260 °C) (0.69–4.83 MPa) for a few minutes and subsequently, the pressure is reduced leading to explosive decompression. The water in the biomass explodes during the process of explosive decompression. The steam explosion pretreatment aims at hemicellulose hydrolysis and lignin degradation. In the case of liquid hot water treatment, the biomass is treated (cooked) in hot water at high pressure. This pretreatment process increases the "cellulose digestibility" and "sugar extraction". Concerning ammonia fibre explosion, the biomass is treated with liquid ammonia (1–2 kg of ammonia/kg of dry mass) at high temperature (90 °C) for about 30 min. This process enables the degradation of cellulose and hemicellulose and aids in augmenting the fermentation rate (Menon and Rao 2012; Galbe and Wallberg 2019; De Buck et al. 2020).

The chemical pretreatment methods are studied among the pretreatment methods. They include acid pretreatment, alkaline pretreatment, green solvents, etc. The basic purpose of chemical pretreatment methods is to improve the degradation of cellulose and to remove the lignin. In the case of acid pretreatment, acids (dilute or concentrated sulphuric acid/hydrochloric acid/phosphoric acid/nitric acid) are used to enable degradation of lignocellulosic waste. As regards the alkali pretreatment, bases such as sodium hydroxide and lime are used to treat the lignin-rich waste biomass (Menon and Rao 2012; Galbe and Wallberg 2019). Alkali pretreatment results in "*structural alteration of lignin, cellulose swelling, partial decrystallization of cellulose*" (Menon and Rao 2012).

Biological pretreatment methods employ the microorganisms and enzyme products for the treatment of lignocellulosic biomass. Interestingly, many microorganisms including fungi and bacteria are reported to degrade and to modify the chemical composition of lignocellulosic biomass. The rot fungi (basidiomycetes) are best known for the degradation of lignin. Particularly, the white-rot fungi like *Phanerochaete chrysosporium* are best known for lignin degradation. Nevertheless, the biological pretreatment process is time-consuming and demands controlled growth conditions for microbial activity (Menon and Rao 2012; Galbe and Wallberg 2019; De Buck et al. 2020).

The pretreated lignocellulosic biomass through thermochemical conversion (combustion, gasification, hydrothermal processing, liquefaction, pyrolysis) and biochemical conversion are converted into bioenergy, biofuels, speciality chemicals and value-added products. The products of pretreatment of lignocellulosic biomass are cellulose (C6), hemicellulose (C5/C6) and lignin. These intermediary compounds are transformed into biofuels and value-added chemicals (Fig. 15.4)

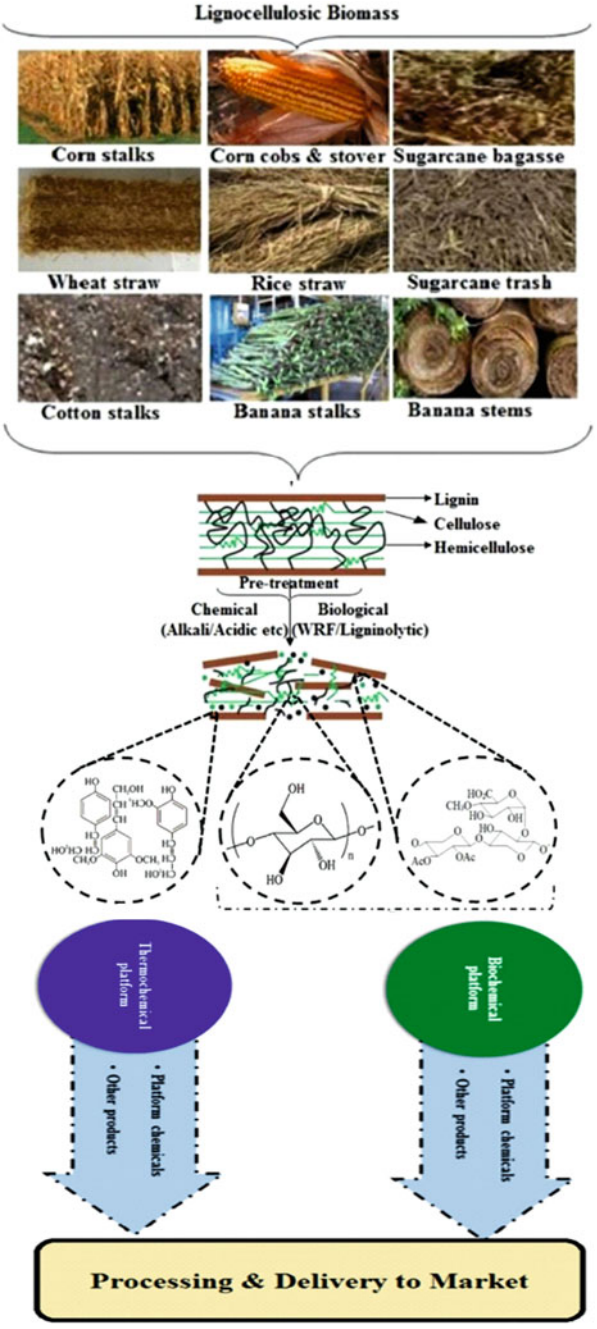


Fig. 15.4 Lignocellulosic waste bioconversion into platform chemicals. (Source: With permission from Arevalo-Gallegos et al. 2017)

Table 15.2 Value-added chemicals potentially derived from lignocellulosic biomass

Biomass	Constituents	Polymers	
Lignocellulosic biomass	Cellulose	Levulinic acid	Succinic acid, THF, MTHF, 1,4 butanediol, NMP, lactones
		Ethanol	Acrylic acid, acetaldehyde
		Lactic acid	2,3-pentanedione, Pyruvic acid
		3-hydroxypropionic acid	3-methyl THF, 3-methyl pyrrolidone
		Itaconic acid	2, methyl-1,4-butane diamine Itaconic diamide
		Glutamic acid Glucuronic acid Succinic acid	2-pyrrolidones, 1,4-butanediol, tetrahydrofuran
	Hemicellulose	Xylitol	
		Ethanol, butanol, 2,3-butanediol	
		Ferulic acid	Vanillin, vanillic acid, Protocatechuic acid
		Lactic acid	
		Furfural	
		Chitosan	
		Xylooligosaccharides	
	Lignin	Syngas	
		Syngas products	Methanol/dimethyl Ether, ethanol, mixed Liquid fuels
		Hydrocarbons	Cyclohexanes, higher Alkylates
		Phenols	Cresols, eugenol, Coniferols, syringols
		Oxidized products	Vanillin, Vanillic acid, DMSO, aldehydes, quinones, aromatic and aliphatic acids
		Macromolecules	Carbon fibres, Activated carbon, Polymer alloys, Polyelectrolytes, Substituted lignins, Thermosets, Composites, wood Preservatives, Nutraceuticals/drugs, Adhesives and resins

Source: With permission from Menon and Rao (2012)

(Table 15.2) (Menon and Rao 2012; Arevalo-Gallegos et al. 2017; Galbe and Wallberg 2019; De Buck et al. 2020; Bilal and Iqbal 2020).

15.4 Life Cycle Assessment

Growing population demands more food, feed and energy and consequently, there is a dire need to optimize the production of biomass and value-added products from the biomass. Generation of energy and high value-added chemicals and products from biomass provides a fillip to the bio-based economy and reduces the dependence on fossil fuels (Venkatramanan et al. 2021b). In this regard, technological developments like biorefineries are highly significant as they have the potential to use diverse feedstocks ranging from the food crops, non-food crops to lignocellulosic wastes, municipal solid wastes and food wastes (Parajuli et al. 2017). Nevertheless, the impact of biorefinery on the environment need to be assessed from the perspective of feedstocks, product generation and economic returns. The sustainability of the biorefineries is assessed through the life cycle assessment methodology. Life cycle assessment of biorefineries gains currency on account of (a) technological advancement, (b) bioconversion of diverse feedstocks into value-added products, (c) evaluation of the environmental performance of the biorefineries and (d) validating the sustainable conversion processes (Bezergianni and Chrysikou 2020).

Nevertheless, sustainable assessment of a biorefinery system should involve more than identification and quantification of environmental impacts. Multi-product biorefineries and integrated biorefineries need an assessment on eco-efficiency. Several studies have noted the significance of assessment of lignocellulosic biorefineries both from the environmental and economic perspectives. The LCA methodology and the eco-efficiency concept enable a comprehensive assessment of biorefinery sustainability. Further, extending the horizon of LCA methodology to incorporate the social dimensions through social life cycle assessments adds immense value and credibility to the assessment methodology. To gauge the social sustainability of the biorefineries, socio-economic indicators are widely used (Palmeros Parada et al. 2016). The integration of sustainability principles in the design of biorefineries is critical for the advancement of bioeconomy.

Life cycle assessment is a comprehensive and intensive approach that endeavours to assess the environmental impacts of products in its production process (Pant et al. 2011). An intensive assessment of the biorefinery system also aids in reducing or minimizing the negative impacts. Life cycle assessment methodology intents to figure out the environmental impacts related to a production process. Assessment of the production process through LCA methodology reveals the process subsystems that greatly influence the environmental consequence of a system. In other words, the LCA study of a biorefinery system provides a valuable output in terms of identification of "process hotspots" in the process value chain. Further, optimization of the process hotspots in the lignocellulosic biorefineries entails the optimization of the pretreatment process, technologies and production of value-added products (Bello et al. 2018).

Life cycle assessment of biorefineries reveals the "environmental profile of the biorefineries", "feedstock optimization" and "process configuration" (Bezergianni and Chrysikou 2020). Life cycle assessment studies can be grouped into attributional

and consequential LCA study based on the processes that are included in the system boundary. The attributional LCA study identifies and quantifies the environmental impact of a product/system through a time-tested process. The attributional LCA study provides inputs regarding the hotspots in the production process. On the other hand, the consequential LCA study reflects on the potential impacts emanating from the future decisions that have significant influences on the study systems (Bezergianni and Chrysikou 2020).

15.4.1 Purpose of LCA in Biorefineries

Studies on LCA of biorefinery system (Uihlein and Schebek 2009; Bernstad Saraiva 2017; Julio et al. 2017; Van Hung et al. 2020; Bezergianni and Chrysikou 2020) throws light on the following purpose of undertaking LCA study.

(a) To optimize and efficiently use the feedstocks in a biorefinery system.
(b) To identify efficient conversion and recycling paths.
(c) To identify sustainable biorefinery system.
(d) To produce specific bioproducts (value-added products and chemicals) from the diverse group of feedstocks.
(e) To improve and upscale the production processes and generation of value-added products.
(f) To identify the negative impacts kindred with the biorefinery process.
(g) To identify the hotspots in the production process or the life cycle of biorefinery.
(h) To identify a sustainable pathway for feedstock conversion from the perspectives of technology, value-addition and eco-efficiency.
(i) To develop sound decision support tool and consequently to perform strategic planning and policymaking.

15.4.2 LCA Framework

As per ISO 14040, LCA involves four important components (Fig. 15.5). They are as follows (Van Hung et al. 2020):

- The goal, scope and functional unit.
- Inventory analysis.
- Impact assessment.
- Interpretation.

The first and foremost step in LCA is goal setting. The goal relates to the motivation and purpose of the LCA study. The goal also states the target audience and also the potential application of LCA study. In the LCA study on biorefinery producing bioenergy, bioethanol and value-added chemicals from switchgrass as feedstock,

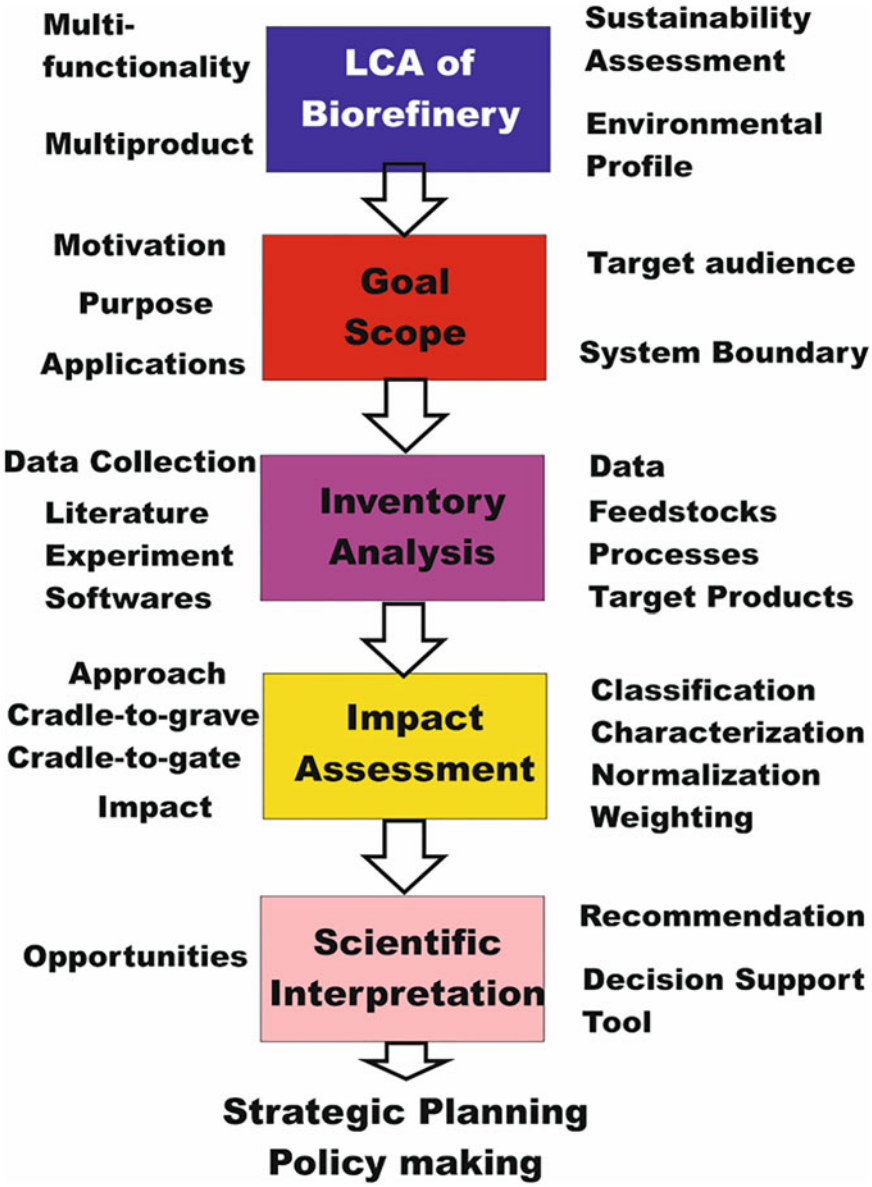


Fig. 15.5 LCA methodology and framework

Cherubini and Jungmeier (2010) stated the goal as a comparative analysis of fossil fuel-based system with the biorefinery system. The goal of the LCA study draws the broad contour of opportunities and scope of LCA study. The LCA study on biorefineries incorporates assessments related to greenhouse gas emissions, waste

management, bioenergy, value-added chemicals and bioproducts (Gnansounou 2017). The goal of the LCA study defines the functional unit. The functional unit reflects the function of the system under study and also the targeted value-added products. Generally, in the LCA study, the functional unit can be the "mobility indicator" such as kilometre or energy unit (megajoules). Energy unit is adopted as a functional unit in case of comparative analysis of biorefineries adopting different pathways to produce bioethanol. In effect, approaches adopted to define the functional unit in case of integrated biorefineries include either biomass input or the targeted product. The goal and scope definition including the system boundaries and, functional unit decides the methodology of the LCA study (Bezergianni and Chrysikou 2020).

In the recent past, biorefineries are looked upon as a bio-based technology with an intent to produce multiple products. The multifunctionality of the integrated biorefineries calls for allocating the environmental impacts of the biorefineries among various outputs as well (IEA 2019). The multi-products generated from the biorefinery possess varied attributes and also diverse applications. Under such circumstances, the LCA of multi-product biorefinery is complicated. Similarly, in the case of multifunctional biorefineries wherein a single activity may have multiple functions. For instance, the lignocellulosic biorefineries involve in addition to waste management, generation of energy and high value-added products. In this case, as well, there is a need to allocate the burdens or environmental impacts between the production processes (Bezergianni and Chrysikou 2020). In the LCA of multi-product biorefinery, it is a great challenge to allocate the inputs and outputs between different products. However, ISO 14044 does not recommend allocation. Due to the multifunctionality of the integrated biorefineries, there is a need for a fool-proof allocation procedure. The procedures such as economic allocation, gross energy allocation and mass allocation are adopted to allocate the resource input and releases among the products of the biorefinery system. Allocation in the LCA of biorefineries can be applied either through system expansion or by partitioning method. In the former case, the functional unit is suitably reframed to incorporate the functions of all the co-products. In the latter case, the environmental impacts are allocated among the products based on their "mass, volume or energy content" or economic features like the market price of the products, etc. (Bezergianni and Chrysikou 2020). However, it must be noted that the methods of allocation should be implemented aptly.

Life cycle inventory analysis is a very significant step in the LCA study. Based on the goal of the LCA study, the LCA inventory analysis includes an intensive collection of data about the feedstocks or inputs, production processes and targeted value-added products. Data inadequacy is a challenge in the LCA study. In this regard, software such as SimaPro and GEMIS envisages simplification of the environmental assessment of biorefineries (Bezergianni and Chrysikou 2020). In the life cycle analysis, the system boundaries are specified. The system boundary states the biomass production, bioconversion process into value-added products and energy supply system. The system boundaries in case of LCA of biorefineries depend on the feedstocks (Bernstad Saraiva 2017). As regards the feedstocks for

biorefineries, it can be either a dedicated biomass feedstock or lignocellulosic residues as in the case of lignocellulosic waste biorefineries. LCA of biorefineries involving dedicated biomass as feedstock, factor in the environmental impacts due to the inputs and also land-use changes. On the other hand, LCA of lignocellulosic biorefineries which uses lignocellulosic wastes as feedstock, allocate zero environmental burdens to the feedstock.

As regards the LCA approach, IEA (2019) recommends generally cradle-to-grave life cycle approach. Nevertheless, due to data inadequacy, a cradle-to-gate approach is also followed. While the cradle-to-gate approach involves life cycle of the study system until the production stage, the cradle-to-grave life cycle approach involves life cycle of the system including reuse and recycle of the products (Bezergianni and Chrysikou 2020). For instance, in the case of LCA of rice-based biorefinery, the cradle-to-grave life cycle approach incorporates the environmental impacts emanating from the rice cultivation stage to the ultimate consumption of the products by the consumers. In the case of cradle-to-gate approach, the LCA of rice-based biorefinery incorporates the environmental impacts from paddy cultivation, crop residue collection, transportation and final processing at the biorefinery plant (Sreekumar et al. 2020). In both the approaches, the consumption of resources or inputs and the emissions are quantified (Bezergianni and Chrysikou 2020).

The life cycle impact assessment follows the life cycle inventory analysis. In other words, the output of the life cycle inventory analysis forms an input to the life cycle impact assessment. The life cycle impact assessment involves steps such as classification, characterization normalization and weighting. The environmental impact categories considered in LCA study are “global warming”, “abiotic and biotic resource depletion”, “acidification”, “stratospheric ozone depletion”, “eutrophication”, “photochemical oxidation” and “human toxicity” (Gnansounou 2017; Bezergianni and Chrysikou 2020). Studies observe that the impact categories considered in the LCA of biorefineries should not be limited to the greenhouse gas emissions and energy balances (Finkbeiner 2009). Studies by Uihlein and Schebek (2009) on LCA of lignocellulosic biorefineries categorically included the impact categories like fossil fuel use, land use and human toxicity. As stated by Bezergianni and Chrysikou (2020), the LCA of biorefineries include impact categories such as “greenhouse gas emissions”, “acidification potential”, “ozone-depleting potential” and “photochemical ozone creation potential”. However, it must be noted that a comprehensive inclusion of impact categories provides a detailed environmental profile of the lignocellulose biorefinery.

Scientific interpretation follows the life cycle impact assessment. Scientific interpretation aids to figure out the opportunities and provides scope for improvement in the lignocellulosic waste biorefineries.

In the LCA study, for assessing the environmental profile of lignocellulosic biorefinery, there is a need for a reference system for comparative analysis. Since, most often, the main product of biorefinery is the biofuel, the reference system will be a fossil fuel-based refinery system. In such a case, it will be also beneficial to assess the sustainability of switching over from fossil fuel-based system to biorefinery (Bezergianni and Chrysikou 2020).

LCA of lignocellulosic biorefineries is greatly influenced by the methodological attributes, namely the "functional unit", "system boundaries", "allocation methods", LCA approach, etc. The accuracy and also the reliability of LCA study are influenced by factors, not limited to data inadequacy, certain assumptions in LCA study and site-specific or local conditions. Uncertainties exist in the environmental assessment of lignocellulosic biorefineries, perhaps due to the methodological aspects of LCA study. The uncertainty in the environmental profile of lignocellulosic waste biorefinery calls for sensitivity analysis. Through sensitivity analysis, the variables that cause significant environmental impacts can be identified (Julio et al. 2017). In other words, the sensitivity analysis enables to quantify the influence of an input (Bezergianni and Chrysikou 2020).

15.4.3 Challenges

The LCA of the lignocellulosic waste biorefinery is indeed a complicated process. It involves detailed inputs from (a) types of lignocellulosic waste feedstocks; (b) amount of lignocellulose waste available; (c) characteristic features of lignocellulosic waste feedstocks; (d) bioconversion processes; (e) energy intensity; (f) co-products/value-added products, etc. The LCA methodology adopted in the study of lignocellulosic waste biorefinery endeavours to identify and quantify the environmental impacts. In the process, in addition to eliciting the environmental impacts of the lignocellulosic waste biorefinery, the LCA study figures out the hotspots in the production process/bioconversion process. Further, the challenges to LCA of lignocellulosic waste biorefineries are data inadequacy, the rigidity of the system boundary, diverse co-products generation, local environmental conditions, etc. The challenges to LCA of lignocellulosic waste biorefinery demand LCA study to be systematic, comprehensive and well-designed. Nevertheless, in the LCA study of lignocellulosic waste biorefineries, importance must be given on the sustainable production of value-added products, efficient utilization of resources, biovalorization and energy efficiency of the biorefinery system.

15.5 Conclusion

The biorefinery system is being promoted to replace a fossil fuel-based energy use. Further, adoption of biorefineries greatly aids in the utilization of a diverse group of renewable feedstocks, waste valorization and development of sustainable bioeconomy and circular economy. The biorefineries through the "thermochemical processes" and "biochemical processes" convert the biomass into bioenergy, chemicals and value-added products. The future research can be directed towards (a) sustainable biorefineries; (b) waste valorization; (c) upscaling the production of value-added products; (d) optimization of bioconversion processes; (e) sustainable design configuration of the biorefinery; (f) role of biorefineries in the circular economy and (g) contribution of biorefineries in climate change mitigation.

References

Abbas A, Ansumali S (2010) Global potential of rice husk as a renewable feedstock for ethanol biofuel production. Bioenergy Res 3:328–334

Allen SG, Schulman D, Lichwa J, Antal MJ, Laser M, Lynd LR (2001) A comparison between hot liquid water and steam fractionation of corn fiber. Ind Eng Chem Res 40:2934–2941

Alves FF, Bose SK, Francis RC, Colodette JL, Iakovlev M, Heiningen AV (2010) Carbohydrate composition of eucalyptus, bagasse and bamboo by a combination of methods. Carbohydr Polym 82:1097–1101

Arevalo-Gallegos A, Ahmad Z, Asgher M, Parra-Saldivar R, Iqbal H (2017) Lignocellulose: a sustainable material to produce value-added products with a zero-waste approach—a review. Int J Biol Macromol 99:308–318. Accessed 9 Sep 2020. https://doi.org/10.1016/j.ijbiomac.2017.02.097

Aswathy US, Sukumaran RK, Devi GL, Rajasree KP, Singhania RR, Pandey A (2010) Bio-ethanol from water hyacinth biomass: an evaluation of enzymatic saccharification strategy. Bioresour Technol 101:925–930

Bello S, Ríos C, Feijoo G, Moreira M (2018) Comparative evaluation of lignocellulosic biorefinery scenarios under a life-cycle assessment approach. Biofuels Bioprod Biorefin 12:1047–1064. https://doi.org/10.1002/bbb.1921

Bernstad Saraiva A (2017) System boundary setting in life cycle assessment of biorefineries: a review. Int J Environ Sci Technol 14:435–452. https://doi.org/10.1007/s13762-016-1138-5

Bezergianni S, Chrysikou LP (2020) Application of life-cycle assessment in biorefineries. Waste Biorefin 17:455–480. https://doi.org/10.1016/B978-0-12-818228-4.00017-4

Bilal M, Iqbal H (2020) Recent advancements in the life cycle analysis of lignocellulosic biomass. Curr Sustain Renew Energy Rep 7:100–107. https://doi.org/10.1007/s40518-020-00153-5

Brylev AN, Adylov DK, Tukhtaeva GG, Dinova NAK, Abidova LD, Rakhimov DA (2001) Polysaccharides of rice straw. Chem Nat Compd 37:569–570

Cao N, Xia Y, Gong CS, Tsao GT (1997) Production of 2,3-butanediol from pretreated corn cob by Klebsiella oxytoca in the presence of a fungal cellulase. Appl Biochem Biotechnol 63–65:129–139

Cara C, Ruiz E, Oliva JM, Sáez F, Castro E (2008) Conversion of olive tree biomass into fermentable sugars by dilute acid pretreatment and enzymatic saccharification. Bioresour Technol 99:1869–1876

Cherubini F, Jungmeier G (2010) LCA of a biorefinery concept producing bioethanol, bioenergy, and chemicals from switchgrass. Int J Life Cycle Assess 15:53. https://doi.org/10.1007/s11367-009-0124-2

Cherubini F, Jungmeier G, Wellisch M, Willke T, Skiadas I, Van Ree R, de Jong E (2009) Toward a common classification approach for biorefinery systems. Biofuels Bioprod Biorefin 3:534–546. https://doi.org/10.1002/bbb.172

De Buck V, Polanska M, Van Impe J (2020) Modeling biowaste biorefineries: a review. Front Sustain Food Syst 4:11. https://doi.org/10.3389/fsufs.2020.00011

Finkbeiner M (2009) Carbon footprinting—opportunities and threats. Int J Life Cycle Assess 14:91–94. https://doi.org/10.1007/s11367-009-0064-x

Galbe M, Wallberg O (2019) Pretreatment for biorefineries: a review of common methods for efficient utilisation of lignocellulosic materials. Biotechnol Biofuels 12:294. https://doi.org/10.1186/s13068-019-1634-1. https://biotechnologyforbiofuels.biomedcentral.com/articles/10.1186/s13068-019-1634-1

Garda-Aparicio MAP, Ballesteros I, Gonzalez A, Oliva JWM, Ballesteros M, Negro MAJ (2006) Effect of inhibitors released during steam-explosion pretreatment of barley straw on enzymatic hydrolysis. Appl Biochem Biotechnol 129:278–288

Gnansounou E (2017) Fundamentals of life cycle assessment and specificity of biorefineries. In: Life-cycle assessment of biorefineries, pp 41–75. https://doi.org/10.1016/b978-0-444-63585-3.00002-4

Herrera A, Téllez-Luis SJ, Ramírez JA, Vázquez M (2003) Production of xylose from sorghum strow using hydrochloric acid. J Cereal Sci 37:267e74

Howard RL, Abotsi E, Rensburg EL, Howard S (2003) Lignocellulose biotechnology: issues of bioconversion and enzyme production. Afr J Biotechnol 2:602e19

IEA (2014) IEA bioenergy—Task 42 biorefining: sustainable and synergetic processing of biomass into marketable food & feed ingredients, chemicals, materials and energy (fuels, power, heat). https://www.ieabioenergy.com/wp-content/uploads/2014/09/IEA-Bioenergy-Task42-Biorefining-Brochure-SEP2014_LR.pdf. Accessed 10 Sept 2020)

IEA (2019) IEA bioenergy—Task 42 biorefining: technical, economic and environmental assessment of biorefinery concepts: developing a practical approach for characterisation. https://www.ieabioenergy.com/wp-content/uploads/2019/07/TEE_assessment_report_final_20190704-1.pdf. Accessed 10 Sept 2020

Isikgor F, Becer C (2015) Lignocellulosic biomass: a sustainable platform for production of bio-based chemicals and polymers. Polym Chem 6:4497–4559. https://doi.org/10.1039/C5PY00263J

Jeon YJ, Xun Z, Rogers PL (2010) Comparative evaluations of cellulosic raw materials for second generation bioethanol production. Lett Appl Microbiol 51:518–524

Julio R, Albet J, Vialle C, Vaca-Garcia C, Sablayrolles C (2017) Sustainable design of biorefinery processes: existing practices and new methodology. Biofuels Bioprod Biorefin 11:373–395. https://doi.org/10.1002/bbb.1749

Kadolph SJ, Langford AL (1998) Textiles, 8th edn. Prentice Hall, Upper Saddle River, NJ

Kim TH, Taylor F, Hicks KB (2008) Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment. Bioresour Technol 99:5694–5702

Malherbe S, Cloete TE (2002) Lignocellulose biodegradation: fundamentals and applications: a review. Environ Sci Biol Technol 1:105–114

McKendry P (2002) Energy production from biomass (part 1): overview of biomass. Bioresour Technol 83:37–43

Menon V, Rao M (2012) Trends in bioconversion of lignocellulose: biofuels, platform chemicals & biorefinery concept. Prog Energy Combust Sci 38:522–550. https://doi.org/10.1016/j.pecs.2012.02.002

Menon V, Prakash G, Rao M (2010) Enzymatic hydrolysis and ethanol production using xyloglucanase and Debaromyces hansenii from tamarind kernel powder: galactoxyloglucan predominant hemicellulose. J Biotechnol 148:233–239

Miron J, Yosef E, Ben-Ghedalia D (2001) Composition and in vitro digestibility of monosaccharide constituents of selected byproduct feeds. J Agric Food Chem 49:2322–2326

Mosier NS, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M et al (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96:673–686

Mosihuzzaman M, Theander O, Aman P (1982) Comparative study of carbohydrates in the two major species of jute (Corchorus capsularis and Corchorus olitorius). J Sci Food Agr 33:1207–1212

Nigam JN (2002) Bioconversion of water-hyacinth (Eichhornia crassipes)hemicellulose acid hydrolysate to motor fuel ethanol by xylose-fermenting yeast. J Biotechnol 97:107–116

Palmeros Parada M, Osseweijer P, Posada Duque J (2016) Sustainable biorefineries, an analysis of practices for incorporating sustainability in biorefinery design. Ind Crop Prod 106:105. Accessed 4 Sep 2020. https://doi.org/10.1016/j.indcrop.2016.08.052

Pant D, Singh A, Van Bogaert G, Gallego YA, Diels L, Vanbroekhoven K (2011) An introduction to the life cycle assessment (LCA) of bioelectrochemical systems (BES) for sustainable energy and product generation: relevance and key aspects. Renew Sustain Energy Rev 15:1305–1313. https://doi.org/10.1016/j.rser.2010.10.005

Parajuli R, Knudsen MT, Birkved M, Djomo SN, Corona A, Dalgaard T (2017) Environmental impacts of producing bioethanol and biobased lactic acid from standalone and integrated

biorefineries using a consequential and an attributional life cycle assessment approach. Sci Total Environ 598:497. https://doi.org/10.1016/j.scitotenv.2017.04.087

Pereira H (1988) Variability in the chemical composition of plantation eucalyptus. Wood Fiber Sci 20:82–90

Petersson A, Thomsen MH, Hauggaard-Nielsen H, Thomsen A- B. (2007) Potential bioethanol and biogas production using lignocellulosic biomass from winter rye, oilseed rape and faba bean. Biomass Bioenergy 31:812–819

Prasad S, Singh A, Joshi HC (2007) Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resour Conserv Recycl 50:1–39

Prasad S, Sheetal K, Venkatramanan V, Kumar S, Kannojia S (2019a) Sustainable energy: challenges and perspectives. In: Sustainable Green technologies for environmental management, pp 175–197. https://doi.org/10.1007/978-981-13-2772-8_9

Prasad S, Venkatramanan V, Kumar S, Sheetal K (2019b) Biofuels: a clean technology for environment management. In: Sustainable Green technologies for environmental management, pp 219–240. https://doi.org/10.1007/978-981-13-2772-8_11

Prasad S, Kumar S, Sheetal K, Venkatramanan V (2020) Global climate change and biofuels policy: Indian perspectives. In: Global climate change and environmental policy, pp 207–226. https://doi.org/10.1007/978-981-13-9570-3_6

Prasad S, Venkatramanan V, Singh A (2021) Renewable energy for a low-carbon future: policy perspectives. In: Venkatramanan V, Shah S, Prasad R (eds) Sustainable bioeconomy. Springer, Singapore. https://doi.org/10.1007/978-981-15-7321-7_12

Rowell MR (1992) Emerging technologies for material and chemicals from biomass. In: Proceedings of symposium. American Chemical Society, Washington, DC, pp 26–31

Rubio M, Tortosa JF, Quesada J, Gomez D (1998) Fractionation of lignocellulosics: solubilization of corn stalk hemicelluloses by autohydrolysis in aqueous medium. Biomass Bioenergy 15:483–491

Schell DJ, Ruth MF, Tucker MP (1999) Modeling the enzymatic hydrolysis of dilute acid pretreated Douglas fir. Appl Biochem Biotechnol 77:67–81

Shah S, Venkatramanan V (2019) Advances in microbial technology for upscaling sustainable biofuel production. In: New and future developments in microbial biotechnology and bioengineering, pp 69–76. https://doi.org/10.1016/b978-0-444-63504-4.00005-0

Shah S, Venkatramanan V, Prasad R (eds) (2019) Sustainable green technologies for environmental management. Springer Nature, Singapore. https://doi.org/10.1007/978-981-13-2772-8

Singh R, Varma AJ, Laxman RS, Rao M (2009) Hydrolysis of cellulose derived from steam exploded bagasse by *Penicillium cellulases*: comparison with commercial cellulase. Bioresour Technol 100:6679–6681

Sinner M, Puls J, Dietrichs H (1979) Carbohydrate composition of nut shells and some other agricultural residues. Starch 31:267–269

Sreekumar A, Shastri Y, Wadekar P, Patil M, Lali A (2020) Life cycle assessment of ethanol production in a rice-straw-based biorefinery in India. Clean Technol Environ Policy 22:409. https://doi.org/10.1007/s10098-019-01791-0

Stewart D, Azzini A, Hall A, Morrison I (1997) Sisal fibres and their constituent noncellulosic polymers. Ind Crop Prod 6:17–26

Torget R, Hsu TA (1994) Two temperature dilute-acid prehydrolysis of hardwood xylan using a percolation process. Appl Biochem Biotechnol 45:5–22

Uihlein A, Schebek L (2009) Environmental impacts of a lignocellulose feedstock biorefinery system: an assessment. Biomass Bioenergy 33:793–802. https://doi.org/10.1016/j.biombioe.2008.12.001

Van Hung N, Migo M, Quilloy R, Chivenge P, Gummert M (2020) Life cycle assessment applied in rice production and residue management. In: Sustainable rice straw management, pp 161–174. https://doi.org/10.1007/978-3-030-32373-8_10

Vázquez M, Oliva M, Téllez-Luis SJ, Ramírez JA (2007) Hydrolysis of sorghum straw using phosphoric acid: evaluation of furfural production. Bioresour Technol 98:3053e60

Venkatramanan V, Shah S, Prasad R (eds) (2020) Global climate change and environmental policy: agriculture perspectives. Springer Nature, Singapore. https://doi.org/10.1007/978-981-13-9570-3

Venkatramanan V, Shah S, Prasad R (eds) (2021a) Exploring synergies and trade-offs between climate change and the sustainable development goals. Springer, Singapore. https://doi.org/10.1007/978-981-15-7301-9

Venkatramanan V, Shah S, Prasad R (eds) (2021b) Sustainable bioeconomy: pathways to sustainable development goals. Springer Nature, Singapore. https://doi.org/10.1007/978-981-15-7321-7