Fungal Biology

Manish Srivastava
Neha Srivastava
Pramod W. Ramteke
Pradeep Kumar Mishra *Editors*

Approaches to Enhance Industrial Production of Fungal Cellulases

Springer

Fungal Biology

Series Editors

Vijai Kumar Gupta
Department of Chemistry and Biotechnology
Tallinn University of Technology
Akadeemia tee, Tallinn, Estonia

Maria G. Tuohy
School of Natural Sciences
National University of Ireland Galway
Galway, Ireland

Fungal biology has an integral role to play in the development of the biotechnology and biomedical sectors. It has become a subject of increasing importance as new fungi and their associated biomolecules are identified. The interaction between fungi and their environment is central to many natural processes that occur in the biosphere. The hosts and habitats of these eukaryotic microorganisms are very diverse; fungi are present in every ecosystem on Earth. The fungal kingdom is equally diverse, consisting of seven different known phyla. Yet detailed knowledge is limited to relatively few species. The relationship between fungi and humans has been characterized by the juxtaposed viewpoints of fungi as infectious agents of much dread and their exploitation as highly versatile systems for a range of economically important biotechnological applications. Understanding the biology of different fungi in diverse ecosystems as well as their interactions with living and non-living is essential to underpin effective and innovative technological developments. This series will provide a detailed compendium of methods and information used to investigate different aspects of mycology, including fungal biology and biochemistry, genetics, phylogenetics, genomics, proteomics, molecular enzymology, and biotechnological applications in a manner that reflects the many recent developments of relevance to researchers and scientists investigating the Kingdom Fungi. Rapid screening techniques based on screening specific regions in the DNA of fungi have been used in species comparison and identification, and are now being extended across fungal phyla. The majorities of fungi are multicellular eukaryotic systems and therefore may be excellent model systems by which to answer fundamental biological questions. A greater understanding of the cell biology of these versatile eukaryotes will underpin efforts to engineer certain fungal species to provide novel cell factories for production of proteins for pharmaceutical applications. Renewed interest in all aspects of the biology and biotechnology of fungi may also enable the development of "one pot" microbial cell factories to meet consumer energy needs in the 21st century. To realize this potential and to truly understand the diversity and biology of these eukaryotes, continued development of scientific tools and techniques is essential. As a professional reference, this series will be very helpful to all people who work with fungi and should be useful both to academic institutions and research teams, as well as to teachers, and graduate and postgraduate students with its information on the continuous developments in fungal biology with the publication of each volume.

More information about this series at http://www.springer.com/series/11224

Manish Srivastava • Neha Srivastava
Pramod W. Ramteke • Pradeep Kumar Mishra
Editors

Approaches to Enhance Industrial Production of Fungal Cellulases

Springer

Editors
Manish Srivastava
Department of Physics and Astrophysics
University of Delhi
Delhi, Delhi, India

Pramod W. Ramteke
Department of Biological Sciences
Sam Higginbottom University
of Agriculture
Technology and Sciences (SHUATS)
Allahabad, Uttar Pradesh, India

Neha Srivastava
Department of Chemical Engineering
and Technology
Indian Institute of Technology (Banaras
Hindu University) Varanasi
Varanasi, Uttar Pradesh, India

Pradeep Kumar Mishra
Department of Chemical Engineering
and Technology
Indian Institute of Technology (Banaras
Hindu University) Varanasi
Varanasi, Uttar Pradesh, India

ISSN 2198-7777 ISSN 2198-7785 (electronic)
Fungal Biology
ISBN 978-3-030-14725-9 ISBN 978-3-030-14726-6 (eBook)
https://doi.org/10.1007/978-3-030-14726-6

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

Renewable energy alternatives are urgently needed owing to serious environmental issues such as increasing pollution, global warming and crises of fossil fuels. Thus far, technologies related to renewable energy are noneconomic and have difficulty with their commercialization. Bioenergy generation using cellulosic waste biomasses is a renewable, eco-friendly and potentially economically viable process and thus gaining attention worldwide. In spite of having a number of advantages over fossil fuels, production process of biomass to bioenergy is far from its practical implementation and hence could not be commercialized at global scale. Enzymatic hydrolysis of biomass and downstream processing are the major hurdles which make the process of biomass to biofuels economically unviable. Application of biotechnology and bioprocess engineering is advancing to resolve numerous issues associated with the existing bottlenecks of the process to make it cost-effective.

Publication of *Approaches to Enhance Industrial Production of Fungal Cellulases* book is a commendable step in this area. I am glad to read this book as a researcher interested in the area of biomass to biofuels conversion. This book comprises of 10 chapters presenting various feasible approaches to improve the cellulase enzymes production and efficiency at industrial level. Production of cellulase using low-cost cellulosic substrate *via* low-cost technology at large scale is the major focus of this book. This book also attempts to fill the current gap of unavailability of feasible technology to produce cellulase for sustainable biofuels production using cellulosic biomass at commercial scale. In my opinion, this book will be treated as a milestone in the renewable energy area consisting of valuable information for the scientists, researchers, teachers, students and industries who are interested in biomass-based biofuels production process.

I congratulate to Dr. Manish Srivastava [DU, Delhi], Dr. Neha Srivastava [IIT (BHU), Varanasi], Prof. (Dr.) Pradeep Kumar Mishra [IIT (BHU), Varanasi] and Prof. (Dr.) Pramod W. Ramteke [SHUATS, Allahabad] for *Approaches to Enhance*

Industrial Production of Fungal Cellulases book. The in-depth efforts made by the editors will help reduce the gap and fulfill the need of industries, scientists, teachers, researchers and students. I appreciate the efforts by the editors of this book.

Virginia Commonwealth University
Richmond, VA, USA

Ram B. Gupta

Preface

Lignocellulosic biomass is the most abandoned waste being generated from the agricultural sector and is regarded as a potential source for green energy production. Due to rich source of cellulose and other organic components, it is significant of value addition transformation for commercial implementation of waste to wealth concept. Nevertheless, sustainable and economic transformations of these waste biomasses into biofuels are prime issues for commercial viability of the process. Cellulosic structure, high production cost, nonefficiency and unavailability of productive process are some major rollbacks which have to be overcome for making biomass waste to biofuels production process more economical and sustainable.

Co-culture or mixed microbial culture concept for fermentation, genetic engineering, thermophilic/thermotolerant microbes, bioprocess parameters designing, and implementation of nanotechnology are effective and growing tools in this area for improving biomass to biofuels production process.

The aim of this volume, "Approaches to Enhance Industrial Production of Fungal Cellulases," is to keep the readers informed about the latest opportunity and practical viability in biofuels production technology using different and new viable approaches. This volume consists of 11 different chapters contributed by the author(s) having in-depth experience in teaching and research in a broad area of bioprocess technology and biofuels production. An introduction of biomass to biofuels production process with limited technology and sustainable application opportunity has been given in Chaps. 1 and 2 while fermentation technologies availability with microorganisms for process is discussed in Chaps. 3, 4, and 5. A detailed description about feedstocks and their potential is explored in Chaps. 5, 6, and 7. Immobilization strategies to improve enzyme stability are discussed in Chaps. 8 and 9. Moreover, Chaps. 10 and 11 explore about the genetic engineering strategies to improve biomass to biofuels production process using microbes and protein engineering to improve stability and productivity of the process. Further, this book is targeted toward exploration of various sustainable, viable, and economical approaches which can help to improve biofuels production from cellulosic waste at practical ground. Vast discussion about existing loophole in the process and

estimation of recent advancement enhance the quality of this book and make it suitable for the students, scholars, and eminent scientists working in this area.

We express our deep indebtedness to all the contributed authors, people involved directly or indirectly for sustained guidance, invaluable prolonged discussion, criticism, and all that went to contribute substantially in the completion of this task.

We also extend our thanks to the Director, IIT (BHU) Varanasi, and Head, Department of Chemical Engineering and Technology, IIT (BHU) Varanasi (U.P., India).

Delhi, Delhi, India — Manish Srivastava
Varanasi, Uttar Pradesh, India — Neha Srivastava
Allahabad, Uttar Pradesh, India — Pramod W. Ramteke
Varanasi, Uttar Pradesh, India — Pradeep Kumar Mishra

Acknowledgments

We thankfully acknowledge all the authors, academicians, and scientists for their contributions that have enriched this volume. From the core of heart, a deep sense of gratitude to our parents is also expressed, whose blessings have always encouraged us to pursue academic activities profoundly. While compiling this work, it is possible that some mistakes might have snuck in text unintentionally and for these we owe undiluted responsibility. We are thankful to the Department of Chemical Engineering and Technology, IIT (BHU), Varanasi, for the support during the compilation of this work. Editor M.S. also acknowledges the Department of Science and Technology (DST) for DST-INSPIRE Faculty [IFA-13-MS-02] award.

Contents

About the Editors

Manish Srivastava is working as DST INSPIRE faculty in the Department of Physics and Astrophysics, University of Delhi, India. He has published 41 research articles in peer-reviewed journals, authored several book chapters and filed 1 patent. He worked as a postdoctorate fellow in the Department of BIN Fusion Technology, Chonbuk National University, from August 2012 to August 2013. He was an assistant professor in the Department of Physics, DIT School of Engineering, Greater Noida, from July 2011 to July 2012. He received his PhD in Physics from the Motilal Nehru National Institute of Technology (MNNIT), Allahabad, India, in 2011. Presently, he is working on the synthesis of graphene-based metal oxide hybrids and their applications as catalysts. His area of interest is synthesis of nanostructured materials and their applications as catalyst for development of electrode materials in energy storage, biosensors and biofuels production.

Field of Expertise: Synthesis of nanomaterials and their application as catalyst for development of electrode materials in energy storage, biosensors and biofuels productions

Neha Srivastava is currently working as postdoctorate fellow in the Department of Chemical Engineering and Technology, IIT (Banaras Hindu University) Varanasi, India. She has published 26 research articles in the peer-reviewed journals and has 3 patents. She has completed her PhD from the Department of Molecular and Cellular Engineering, SHUATS, India, in 2016 in the area of bioenergy. She has received six Young Scientist Awards. Presently, she is working on biofuels production (cellulase enzymes, production and enhancement, biohydrogen production from waste biomass, bioethanol production).

Field of Expertise: Biofuels production, microbial bioprocessing and enzyme technologies

Pramod W. Ramteke is presently dean of Postgraduate Studies and head of Departments of Biological Sciences and Genetics & Plant Breeding at Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Allahabad, India.

He is elected fellow of the National Academy of Agricultural Sciences, National Academy of Biological Sciences, The Linnean Society of London, Academy of Microbial Sciences India (AMI), The Biotech Research Society of India, Society for Plant Protection Sciences, Mycological Society of India, Uttar Pradesh Academy of Agricultural Sciences, Maharashtra Academy of Sciences, Association of Biotechnology and Pharmacy, Society of Biological Sciences and Rural Development and The Academy of Environmental Biology.

He is recipient of J.C. Bose Gold Medal, SCON Memorial Award for Excellence in Science, Dr. J. C. Edward Medal, Prof. K. S. Bilgrami Memorial Award, Er. V. S. Chauhan Gold Medal, Biotechnology Overseas Associateship and International Fellowship by Biotechnology and Biological Sciences Research Council, UK. He is member of editorial board of several journals. He has been a visiting scientist to Hacettepe University, Turkey; Institute of Food Research, UK; the University of Liège, Belgium; the Korea Institute of Science and Technology, South Korea; the University of Szeged, Hungary; and Institute of Hydrobiology, Czech Republic.

In addition to 3 patents, 3 books and over 150 research papers are to his credit. He has implemented 7 major projects from DBT, DST, CSIR, UPCAR and CST, UP, and supervised 21 PhDs and more than 40 PG thesis/projects. He was a member of 18th Indian Scientific Expedition to Antarctica (1998–1999).

Field of Expertise: Biofuels production, plant biology, microbial technology

Pradeep Kumar Mishra is currently professor and head of the Department of Chemical Engineering & Technology, Indian Institute of Technology (BHU), Varanasi, India. He obtained his PhD in Chemical Engineering from Indian Institute of Technology (Banaras Hindu University) in 1995. He has authored/co-authored over 60 technical papers published in reputed national/international journals and supervised more than 20 doctoral students. He has received several awards and honours and has five patents. He is fellow of the Institution of Engineers of India. He has received several awards and honours at national/international levels. He has also made significant contribution towards development of entrepreneurship ecosystem in Eastern part of the country. He is coordinator of Technology Business Incubator at the Institute and member executive committee of NIESBUD, Ministry of Skill Development, Government of India.

Field of Expertise: Biofuels production, microbial bioprocessing and enzyme technologies

Chapter 1
Fungal Cellulases: New Avenues in Biofuel Production

Spriha Raven, Chitra Srivastava, Himanshi Kaushik, Veralu Hesuh, and Archana Tiwari

1.1 Introduction

Cellulase actions are required for the microbial hydrolysis of insoluble cellulose, and it changes the complex polymer into a simple one. Fungi are one of the big decomposers of cellulose among all the microorganisms, and it is the reason for 80% of the breakdown of cellulose. Generally in forest, fungi have remained the reason for cellulose degradation. Members of fungi like *Ascomycota*, *Basidiomycota*, and *Deuteromycota* are important for the breakdown of cellulose. But in industry, the most preferred are the aerobic fungal cellulases because of their good characteristics like they are extracellular and adaptive in different conditions and during growth its secretions are in large quantity (Gil et al. 2009). *Aspergillus*, *Alternaria*, *Penicillium*, *Trichoderma*, etc. are species which represent the cellulolytic fungi.

Fungal cellulases are quite simpler than the bacterial cellulases. It contains two domains which are connected at the N-terminal of the catalytic domain by a short polylinker region. The two domains are catalytic domain and cellulose-binding domain. There are various applications of fungal cellulases such as in the food industry, beer and wine production, textile, paper, and agriculture. They are also used in the making of detergents for better cleaning action; they don't damage the fabric and remove dirt properly. It is also utilized in the fermentation process for the beer and wine production. It provides improved filtration rate and also gives wine stability.

In the fruit industry, it is utilized for the clarification mostly by removing pulp of juices and for improving texture and quality. Now in the textile industry, it is used

S. Raven · C. Srivastava · H. Kaushik · A. Tiwari (✉)
Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, India

V. Hesuh
Amity Institute of Horticulture Studies and Research, Amity University, Noida, Uttar Pradesh, India

M. Srivastava et al. (eds.), *Approaches to Enhance Industrial Production of Fungal Cellulases*, Fungal Biology,
https://doi.org/10.1007/978-3-030-14726-6_1

for improving fabric quality and for softening of fabrics, and it also removes extra dye from fabrics. In the pulp and paper industry, the mechanical pumping processes like refining and grinding make the pulp with fines and stiffness, but the biochemical pulping with the help of cellulases causes energy savings up to 20–40%. Cellulases with hemicellulases help in improving drainage in the paper mills.

Cellulase with xylanase is used for drinking or removing ink from paper to recycle it (Gil et al. 2009). *T. reesei* is the most commonly studied aerobic fungi; it has the capacity to hydrolyze the native cellulose. Submerged fermentation is the common way to the large scale of cellulase production enzyme (Smitha et al. 2013). The soluble substrate is spread in the free-flowing liquid, but in the liquid medium, fungi like *Aspergillus* and *Penicillium* lead to the cellulase production in large amount. In submerged fermentation, the controlling and monitoring process becomes easy. There are several factors which are influencing the activity of cellulase like pH, medium composition, temperature, inducer, etc. Fungi use different substrates in the cellulase production, like *A. heteromorphus* use wheat straw as its substrate and *A. niger* use maize straw as its substrate for production. Other substrates which can be used include banana peels, sawdust, grass, rice straw, sugarcane bagasse, etc. Purification is also a very necessary step. It includes different methods like precipitation, dialysis, and column purification. In the global market, cellulase enzymes own the third place in the enzyme industry. Enzymes like cellulase are favorable because they are nonpolluting that means safe for the environment, they are specific, and substrates are cheap making elevated applications of fungal cellulases.

1.2 Classification, Production, and Mode of Action

Cellulase is classified into different types like endocellulase which cleaves the internal bonds. Another one is exocellulase which cleave at the end of the chain from endocellulase which gives rise to tetrasaccharides or disaccharides. Cellobiases hydrolyze the exocellulase product and form it in the monosaccharides. There are two types of cellulase: progressive and nonprogressive. The progressive one binds with the polysaccharide, and the nonprogressive will bind once and then forms interaction with another polysaccharide. Cellulase action is also known as the synergistic. Due to the enhancement of population globally, industrialization has also elevated to meet the requirement of people without destroying the environment products from natural resources. Products like fossil fuel are getting exhausted, so we need the renewable energy resources, and fungal cellulases are a good option.

Cellulase is produced by different microorganisms which include both fungi and bacteria. The conversion of cellulose into useful products, which is sustainable and not harmful to the environment, is the key function of cellulases (Kuhad et al. 2011). Fungi which are employed for the production of cellulase are *A. niger*, *A. nidulans*, *A. oryzae*, *H. grisea*, *Penicillium brasilianum*, etc. Some species of brown and white rot fungi are also utilized for cellulase production.

The methods included for the making of cellulase are solid-state fermentation and submerged fermentation. One of the important reasons behind environment pollution is the waste from industries and agriculture, and if we convert this waste into the useful product, then the problem of pollution can be solved. Agriculture waste includes wheat bran, rice bran, and the husk from the green and black gram. And these products can be utilized for the substrate for production of fungal cellulase. In solid-state fermentation, there is the cultivation of enzymes on a solid surface without the presence of microorganisms. And in the submerged fermentation, production of cellulose takes place in free-flowing liquid. Ease in monitoring and downstream processing make this process more attractive. The disadvantages of the submerged fermentation are the long fermentation time and low production rate.

Solid-state fermentation is on a solid surface; there is no free water. The solid support is also the source of nutrients. It not only helps in giving the value-added products but also enables recycling. The production rate is high and the cost is also low. With the help of these methods, fungal cellulase can be produced. After production, it includes purification which gives us the improved product. Purification includes precipitation, dialysis, etc. There are so many applications for the fungal cellulase like in paper industry, food, textile, alcoholic beverages, etc. Fungal cellulase products are renewable and cause less pollution to the environment. It is cost-effective and also helps in using industrial and agriculture waste. In the paper industry, we use it for the removal of ink from waste and old paper. Biomechanical methods are also there, but with the cellulase, it's more effective and also improves the physical properties like its mechanical strength. It also reduces the human labor (Arja 2007). With the aid of gene technology, immunology identification of enzymes can be done, and we can also know about their structure and properties. Cellulase has much application due to its action. In paper and pulp industry, it acts by improving the physical condition of the sheets.

The waste papers across the world are used as raw materials for the pulp and paper industry (Cui et al. 2015). We are recycling the waste paper as it reduces the solid waste from our environment and also minimizes the deforestation. With all these qualities, it adds one more quality in paper industry, which is the solving process of the drainage system, it dissolves the clogged fiber residues. Cellulase also helps in making of the tissue paper, cardboard, and different kinds of sanitary paper which are easily biodegradable and don't harm the environment. In the textile industry also, its mode of action leads to the improvement in the softness and brightness of the fabric. Enzymes like cellulase also help in biostoning of denim jeans (Cortez et al. 2001). It also removes the extra dye from the fibers.

In the food industry, cellulase is used in the clarification of fruit juices by removing pulp, purees, and in cooking oils. Cellulase with other degrading enzymes helps in enhancing the taste of the fruits by decreasing its bitterness. Cellulase is also used in generating wine and beer; it enhances the production and stability with the help of degrading enzymes which degrade the cell wall during malting and fermentation process. It is used in fermented foods which also helps in the enhancement of the nutritional quality of different products, and it also improves the digestibility. Not only in the food, textile, and paper industry, cellulase also plays a big role in the

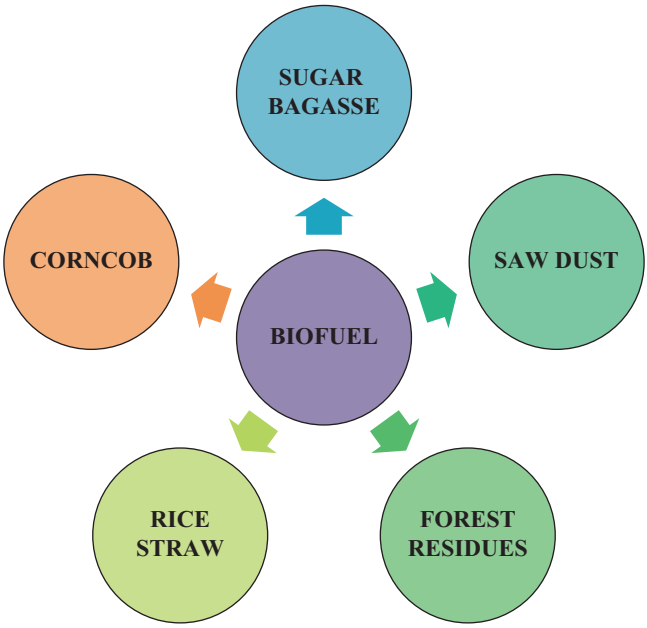

Fig. 1.1 Substrates used for the production of biofuel

production of bioethanol. Due to growth in the utilization of fossil fuels, it has caused environmental pollution and also reduced its reserves. So to overcome this problem, cellulase helps in the bioethanol production; this is renewable and is also not harmful to the environment (Rosegrant et al. 2006). It is estimated that by the year 2020, fossil fuel consumption will be replaced by 20%, which is a good sign for the environment. Cellulase helps in the cellulosic resource conversion into glucose, which leads to the bioethanol production, and this glucose and other simple fermented sugars can be used as the substrate. Other raw materials which can be used as a substrate are straws of rice, wheat, corn stover, and sugarcane bagasse. Figure 1.1 shows various substrates used in biofuel generation. Filamentous fungi with these raw materials produce the bioethanol. A lot of research is going on in this field to produce more and more stuff which is renewable, is cost-effective, and also doesn't harm the environment. Products from fungal cellulase also give the higher productivity and improve the quality of the product.

1.3 Cellulase as Biofuels

Cellulase has great applications in the biofuel industry (Ahmed and Bibi 2018). There are certain fungi, such as *Aspergillus niger*, *A. nidulans*, *A. oryzae*, *Fusarium solani*, *Penicillium brasilianum*, etc., having cellulolytic power. The important

constituents are endoglucanase, exoglucanase, and ß-glucosidase that convert lignocellulosic biomass into fermentable sugars. Cellulolytic microbes develop an interactive kinship in association with non-cellulolytic species in cellulosic wastes; association directs to the whole debasement of cellulose. Enormous consumption of fossil fuels causes CO_2 emission; thus alternative origins are being searched for the welfare of mankind. Biofuels produced from fungal cellulase have huge potential and are renewable in nature. The various stages involved in the generation of biofuels are mentioned below.

1.3.1 Bioethanol

The interest of biofuel generation was initiated in the 1980s, and it has also been implemented in the many countries because of the catastrophic utilization of fossil fuels that cause environmental destruction. Bioethanol is also regarded as ethyl alcohol/C_2H_5OH/EtOH. It basically benefits the transportation department. In comparison to fossil fuels, bioethanol is less destructive, is readily perishable, and brings about little air-borne wastes. Fungi play an essential part in the generation of bioethanol by the fermentation of sugars to ethanol. Developing third-generation biofuel is basically obtained from microorganisms such as fungi, bacteria, etc. Bioethanol generation from different feedstocks is abundant in sucrose with different yeast forms, and fermentation status is provided in order to give different ethanol concentration. The main focus of this review is to enhance the generation of fungal cellulase so that it may benefit the mankind (Azhara et al. 2017).

1.3.1.1 Stages/Processing Route

Two different advances are the biochemical and thermochemical transition for bioethanol generation. It resolves into fragments of cellulose and hemicellulose. Hydrolysis of polysaccharides leads to the production of sugars and subsequently forms bioethanol. The thermochemical route requires an enormous level of heat that forms a synthesis gas involving CO, H_2, and CO_2 that converts into a variety of alcohols and further separated via distillation. The biochemical route involves biological pretreatment and physical pretreatment. And the physical pretreatment basically enhances the cellulose handiness to cellulases and hemicellulases to generate sugars so that further it may be transformed into bioethanol and be purified via distillation (Achinas et al. 2016). The processing route of bioethanol has been depicted in Fig. 1.2.

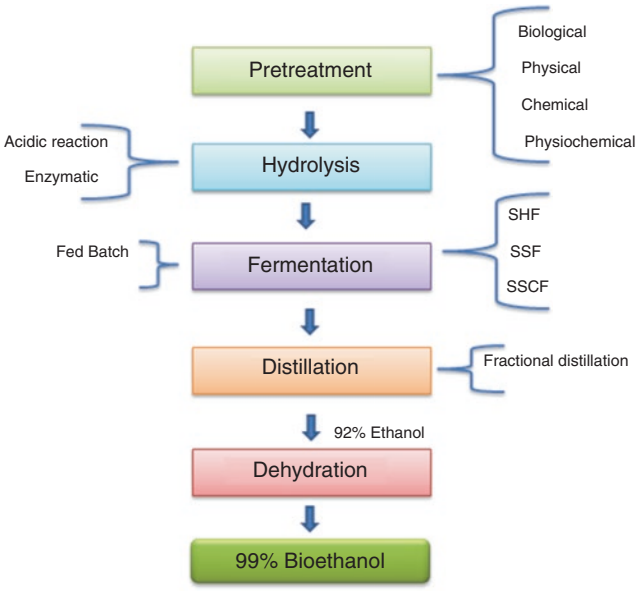

Fig. 1.2 Processing route for bioethanol production

Pretreatment

Pretreatment makes the hydrolysis gentler and has the greatest role in the operations. Downstream processing and hydrolysis handling can be revised by efficient pretreatment. The elemental strategies embrace physical and thermochemical operations that destroy the disaffected materials and facilitate the polysaccharide to endure the chemical reaction with larger potency and lower-energy usage. Operations involved in pretreatments are biological, physical, chemical, and physiochemical. Dissimilar fungal species are involved in biological operations. Furan derivatives and various inorganic compounds (iron, nickel) are formed that are toxic in nature and cause inhibition of cell growth, thus reducing ethanol productivity (Achinas et al. 2016). And the detoxification process is followed that enhances pretreatment performance.

Hydrolysis

Hydrolysis has the greatest connection with pretreatment. Meanwhile, in this phase, cellulose and hemicellulose are hydrolyzed into simple compounds that further convert fermentable sugars into ethanol. There are two types of hydrolysis: enzymatic and acidic reactions. And the acidic reaction has two forms: dilute and concentrated reactions. Generally the hydrolysis used is dilute acid hydrolysis. This process,

called acid hydrolysis of lignocellulosic biomass, is led by a two organized method as the breakdown of pentose sugar more promptly correlated to hexose sugars.

Hydrolysis of hemicellulosic biomass in the initial level adopting hydrolysis, i.e., dilute acid, although hydrolysis of cellulose in the next level practicing concentrated acid. The concentrated acid operations generate more sugar improvement (90%) in the lower span of time. The deprivation of acid hydrolysis is the adversity of operating acid reestablishment and recovering operation that boosts the generation expenditure.

Enzymes are required to hydrolyze the feedstock by enzymatic hydrolysis for the formation of fermentable sugars. There are three classes of enzymes that help in the degradation of cellulose such as endo-ß-1,4-glucanases, cellobiohydrolases, and ß-glucosidases. Cellulose gets debased into diminishing sugars beneath gentle response conditions at pH of 4.8–5.0 and a temperature of 45–50 °C. Additionally, it does not create an erosion issue within the reactors, which can lead to large sugar production. It has been explained that under unfavorable condition of lignin on cellulases performs in such a way that it gets broken into two components such as ammonium and N-based components (Sewalt et al. 1997). It has been stated that the enzymatic method can be achieved in a concurrent approach with the co-fermentation process to deliver ethanol from woody biomass (Spindler et al. 1991).

Fermentation

The three types that are generally utilized in the generation of bioethanol involve processes such as separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and simultaneous saccharification and co-fermentation (SSCF). The SHF is the conventional strategy for bioethanol generation (Zabed et al. 2014).

There are numerous advantages of the batch system involving full sterilization: no need for labor expertise, simple to handle the feedstocks, easily controlled, and flexible to different product particularization. However, the abundance is less and requires deeper and more labor costs. Fed-batch fermentation is a mixture of two operations such as batch and continuous operations that include substrate addition without hampering the medium. It has comparatively higher potency, dissolved oxygen is greater in the medium, and it has lesser time for fermentation and diminishing the harmful contents of the medium ingredients in comparison to different types of fermentation. However, ethanol strength in fed-batch is restrained by feed rate and cell mass level (Azhara et al. 2017). The fed-batch strategy is by and large practiced beneficially in nonuniform SSF framework by enumerating continually a pretreated substrate to accomplish more sugar and ethanol concentration. Continous mode is performed by persistent addition of substrates and culture medium followed by nutrient addition into a bioreactor carrying active microorganisms. Moreover, contamination is more than any other modes of fermentation (Azhara et al. 2017).

The ability of yeasts for the production of ethanol from continuous operation is reducing due to higher generation time. The final output is ethanol and water from fermentation process that needs to be separated through the process of distillation. Fractional distillation is a method with the help of which ethanol is isolated depending on different volatilities. Heat is given to the distillation column, and the distillate (bioethanol) is accumulated on the top of the column, as the boiling point is less, i.e., 78.3 °C, though the boiling point of water is 100 °C. Moreover, 92% of ethanol concentrate is obtained, and in addition, dehydration is required to achieve pure ethanol (Achinas et al. 2016).

1.3.1.2 Factors Affecting Bioethanol Manufacturing

Few factors have been mentioned below that affect the bioethanol generation, such as temperature, PH, agitation rate, and fermentation time, shown in Fig. 1.3 (Ragauskas et al. 2006).

Temperature

Temperature afflicts a great role in the growth rate of the microorganism. The stress factor of microorganism is developed when the temperature is elevated. 20 °C and 35 °C is the temperature range for fermentation. Near 30 °C is an optimum temperature for the free cells of *S. cerevisiae* although cells which are immobilized have marginally greater optimum temperature due to its capability to transfer heat into the cells from the particle surface. There are certain enzymes that performs microbial activity, and enzymes responsible for fermentation method are more prone to high temperature, thus leading to denaturation of its tertiary structure and hence

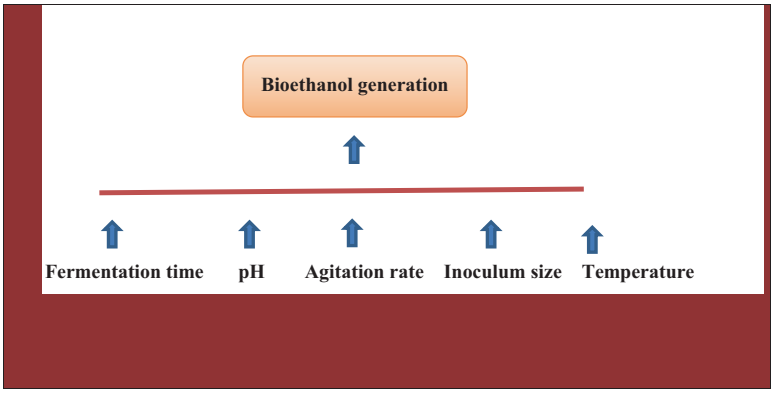

Fig. 1.3 Factors affecting for bioethanol production

stops activation of enzymes. Therefore, temperature is controlled all over the fermentation process (Azhara et al. 2017).

Inoculum

Inoculum concentration doesn't accord eloquent result on the final alcohol concentration; however, it alters the alcohol efficiency and consumption rate of sugar. Moreover, the lower the inoculum size, the lesser will be the rate of productivity in ethanol fermentation (Azhara et al. 2017).

Agitation Rate

It boosts the quantity of sugar utilization and lessens the blockage of alcohol on cells. The excess rate of agitation isn't applicable to sleek alcohol generation because it creates a restriction to the metabolic actions of the cells.

Fermentation Time

Fermentation time alters the microorganism growth. Lower fermentation time causes ineffective fermentation due to insufficient microorganism growth. On the opposite hand, longer fermentation time accords noxious result on growth of microbes, notably in batch operation because of the high ethanol concentration within the fermented broth. The entire fermentation is often accomplished at diminished temperature by utilizing high fermentation time that leads to lowest fermentation alcohol production (Azhara et al. 2017).

1.3.1.3 Bioethanol-Based Economy

The recent scenarios in developing countries show that energy usage is anticipated to rise more than 50% by 2025 (Tong et al. 2010). Petroleum oil is considered as the primary resource of energy, but in the United States and in a few parts of the world,

Table 1.1 Different liquid fuels

Name of liquid fuel	Percentage of ethanol blends with gasoline		Application
E10	10% ethanol	90% gasoline	Runs in all standard automobiles, without modification
E15	15% ethanol	85% gasoline	Runs in most US automobiles
E85	85% ethanol	15% gasoline	Runs in flex-fuel vehicles or FFVs

it is being exhausted rapidly. The days aren't faraway when we will be close to the "peak oil," the condition at which oil requirement will surpass oil supply. Ultimately, petroleum reserves will be completely exhausted. This upcoming scenario acts as a physically powerful impulse to look for substitute resources to meet up the ever-growing stipulates for energy.

Liquid fuels like fuel ethanol contain ethanol as its major constituents (approximately 99%). Poisonous additives are added to liquid ethanol with the goal that it can't be utilized as an alternative to refreshment liquor. It can likewise be utilized as an additive alternate in other liquid fuels. For example, E10 gasoline is a mixture of 10% ethanol, and E85 is with 85% ethanol (Table 1.1).

Moreover, for transport fuel (other than petroleum) which are based on minerals like hydrogen, bioethanol and biodiesel are considered as suitable substitutes and contribute to approximately 2 percent of the aggregate of total transport fuel (IEA 2011). A petrol substitute processed from the fermentation of starch-based crops is bioethanol and is also known as grain. The isotopic composition of carbon atoms of bioethanol and synthetic (petroleum based) is the only distinguishable point, as both have the same chemical composition (C_2H_5OH).

Globally, bioethanol is the major alternative fuel to petroleum and, thus, is also most widely utilized (mainly in America). In Brazil, most of the automobiles are being fueled by bioethanol, which are generated from sugar, as most of the people majorly invest in sugarcane to ethanol fuel industries. However, in South Africa, bioethanol contributes to liquid fuel as a resource around the 1930s and till late 1960s, but ensuring abundant and cheap crude oil (Cartwright 2007).

In comparison to bioethanol, cellulosic ethanol is derived from cellulose having glucose, a 6-carbon sugar molecule, as a raw material. For gasoline, cellulosic ethanol served as an alternate option which comes from waste paper and energy crops like switchgrass and poplar. At the same time, it is chemically identical to ethanol made from food crops such as sugarcane and corn. The major problem for the world is its production as unlike starch from grains, cellulose is tough in its structural properties which can't be easily broken down into glucose sugar.

1.3.1.4 Recent Status of Bioethanol Production

Bioethanol generation from lignocellulose at a wide range has not yet been determined as a cost-effective alternative. One has to follow a line of investigation to target a next-generation bioethanol, which is based on cellulose because it has a great capacity to be enhanced (Achinas et al. 2016).

A broad kind of industry issues develops within the totally special steps of processing bioethanol – from pretreatment to the last water-ethanol mixture separation. Though the linkage between science and applied technology is vital to spot the void and gap of the research field, and through worldwide analysis, conditions are often determined (Russo and Ladisch 2008).

However, with a specific end goal to decrease the price of bioethanol generation, it is important to clear the vital mechanical advances. Numerous organizations are hereby generating enzymes to hoist the scope of utilizations and the conduct of the enzymatic cellulose hydrolysis and hemicellulose.

The hydrolysis might need the appliance of microorganisms such as fungi, yeast, bacteria, or enzymes. The preference of microorganisms and enzymes must be created in terms of stability and conversion rate. However, the utilization of enzymes and microorganisms elevates the generation value of lignocellulosic alcohol and leads to the production of biofuels.

Further discovery must be coordinated in this region to hoist the transformation effected that lessen the price of microorganisms and enzymes that lead to profitable lignocellulosic-based ethanol generation plants (Achinas et al. 2016).

The flight was controlled by a mix of 75% air turbine fuel and 25% bio-jet fuel. A blend of oil from Jatropha seeds and avionics turbine fuel pushed the India's first-ever bio-jet fuel-controlled trip on 27 August 2018 among Dehradun (city in the northern region) and Delhi (capital). A mix of 25% of bio stream fuel and 75% of flight turbine fuel (ATF) was conveyed in one of the two motors of the plane, while the other motor conveyed just ATF. Universal norms allow a mix rate of up to 50% biofuel with ATF.

1.4 Advantages of Fungal Cellulase

- Cellulase includes the third incredible advanced catalysts, i.e., 15% after amylase, as amylase contributes almost 25% and protease contributes almost 18%, on around the world market. Enzymatic breakdown of cellulosic biomass offers an appealing choice, for the case, bioethanol (Yinbo et al. 2006), natural acids (Shen and Xia 2006; Zhang et al. 2007), free sugars, anti-infective operators, and animal feeds (Cao et al. 2014).
- Cellulases have a wide range of applications in completely different businesses including cleaning materials, paper and pulp industry, organics and bioethanol; a few of them are discussed below.
- Fungal cellulase can convert biomass utilized as feedstock and can without much of a stretch be changed over into biosugars, for example, glucose and xylose. Glucose and xylose are boundless substrates, which allow the making of a wide combination of cutting-edge biofuels, for example, green gas, green diesel, and bio-jet fuel that is more sustainable synthetic substances, bioplastics, cellulosic ethanol, and so forth.
- Cellulosic ethanol can be delivered by utilizing an assortment of biomass feedstocks including reused paper; urban waste paper redirected from municipal solid waste (MSW); horticultural squanders like sugarcane bagasse and corn stover; vitality crops like poplar, willow, and switchgrass; wood waste; and waste streams from mash and paper factories.

- Cellulosic ethanol produced using biomass will deliver littler measures of ozone-depleting substances than corn ethanol, and far not as much as the fuel it will supplant. This nursery decrease potential can be assessed by Life Cycle Analysis (LCA), utilized by various gatherings to assess the net vitality and carbon adjustments of ethanol from corn and cellulosic biomass.
  Cellulases are mostly utilized in paper industry for the pulping and deinking of squander papers.
- Usage of cellulase during pulping updates the essentialness efficiency of the method, and besides, it enhances the physical properties, for case, between fiber holding and mechanical quality of the final paper thing (Chen et al. 2012). In addition, it gives better strategies, compelling the utilization of hazardous manufactured substances. In the course of deinking, ink connected to the plane of reused filaments of cellulose was discharged by breakdown of enzymes, prompting the detaching of individual packages or strands (Kuhad et al. 1997).
- In material industry, cellulases act as bio-cleaners of cotton fabrics. For biostoning of denim pants to grant faded appearance, celullases are again used. In the course of biostoning procedure, cellulases help in the hydrolysis process for fiber projections from their surface, and it discharges the indigo color coupled to fibers, bringing about the faded appearance to the pants.
- Cellulases are produced to have potential applications in nourishment and feed handling ventures also. It is an indispensable piece of the squashing chemical complex such as pectinase, cellulase, and xylanase, which are utilized for the withdrawal and illumination of fruits and vegetable juices, nectars, oils, and purees (Rai et al. 2007; Ajayi et al. 2015). Cellulase-helped extraction of flavonoids from blooms and seeds improved the production and diminished the extraction time and warmth harm, when contrasted with the traditional corrosive/antacid/natural dissolvable/warm extraction techniques (Cao et al. 2014).

The manifold misuse and use of petroleum products have unwittingly decreased its characteristic holds and caused extreme natural contamination by means of the arrival of greenhouse and harmful gases. Thus, the world economy has centered around biofuels, particularly bioethanol from sustainable sources, that is depended upon to supplant 20% of the petroleum item utilization by 2020 (Rosegrant et al. 2006; Msangi et al. 2007). The foremost viably researched utilization of cellulase is biofuel generation, particularly bioethanol. Cellulases viably alter over the cellulosic boundless resources into glucose and other fundamental fermentable sugars that can be utilized as substrates for the era of bioethanol. Lignocelluloses are involved in a multistep procedure for production of bioethanol. In its pretreatment lignocellulosic biomass are involved either mechanically or enzymatically to expel lignin and hemicellulose parts, trailed by the treatment with cellulase to discharge fermentable sugars (pentoses and hexoses). At this point cellulosic deposition is seen by the activity of enzyme hydrolase, which is utilized for microbial maturation to deliver cellulosic ethanol (Zhang et al. 2002). Agribusiness buildup like straw of wheat, corn, rice, sugarcane bagasse, wheat grain, corn stover, and so forth are effectively utilized as crude substrates for the generation of bioethanol, utilizing

cellulase created by different filamentous organisms including *Aspergillus*, *Trichoderma*, and *Penicillium* (Zhou et al. 2008; Binod et al. 2010; Chen and Qiu 2010; Li et al. 2011).

1.5 Industrial Application

Cellulase plays a critical part for the breakdown of cellulose. Various fungi perform cellulolytic abilities that lead them to produce a whole cellulase system. There is one alternative source which has been found in the biofuel generation that has less effect on environmental imbalance. Cellulase is considered as the third important enzyme that is beneficial to the human needs. In spite of the fact that the generation rate and low yield of cellulase obstruct its utilization within the businesses, still fungal cellulase has the incredible upgrade within the future era which is still a progressing handle (Sajith et al. 2016). Cellulase production has contributed to the development of various industrial operations such as biofuel industries, textile industries, paper industries, agriculture, biochemical industry, etc., shown in Table 1.2 and Fig. 1.3.

1.5.1 Function of Cellulase in Several Industries

1.5.1.1 Biofuels

Of all the microbial cellulose present, fungi are a major source of cellulase that has been used in industries. The focus has been moved now for biofuels due to the limitations and the harmful effects of fossil fuels on living beings (Ahmed and Bibi 2018). At present the most prominent lignocellulosic materials for biofuels are sugar bagasse, corncob, rice straw, switchgrass, sawdust, and forest residues. Agricultural waste/lignocellulosic material transformation into functional biofuel requires multiple steps. Pretreatment is the first step that is done by the mechanical, chemical/biological processes, and secondly by the polymer breakdown to give fermentable sugars such as pentoses and hexoses. Then for microbial fermentation, hydrolyzed cellulosic residue is utilized to produce ethanol.

Table 1.2 Application of cellulase from different industries

Food	Clarification of juices, improves the quality of fruits, improves the texture and color, controls the bitterness of fruits	Sajith et al. (2016)
Pulp and paper industry	Help in deinking of waste paper, improve the brightness of fiber, improve the drainage in the paper mills	Sajith et al. (2016)
Textile	Jeans biostoning, for softening of garments, removes the extra dye from fabrics, improves the quality of the clothes	Kuhad et al. (2011)
Fermented products	Wine and beer production, improve the rate of filtration and also improve the wine stability	Kuhad et al. (2011)

1.5.1.2 Textile Industry

In the textile industry, cellulase enzymes are widely used for different purposes. It is mostly used in surface modification of cotton fabrics and biostoning of denims (Kuhad et al. 2011). Biostoning of denim jeans with cellulose-based treatment over the old-style stone washing has resulted in several advantages such as less damage of fibers, less work intensive, and environment-friendly. The breaking down of tiny protrusions of the fiber removes the woolliness of the surface, thus making the surface of the cotton cloth sleeker and more glazed in looks. Cellulases are utilized in various applications such as detergents, laundry, and household soaps for refining fabric softness and brightness. *T. viride*, *T. harzianum*, *T. reesei*, and *A. niger* are the various fungi which are used to produce mild alkaline and thermotolerant cellulases for this purpose.

1.5.1.3 Pulp and Paper Industry

The function of cellulase in the pulp industry has expanded drastically in the few decades from 320 to 395 million tons (Ahmed and Bibi 2018). In pulp and paper mill, the main usage of cellulases is in pulping and deinking of papers, that is, removing ink from waste paper. Utilization of cellulases proves to lessen the energy use and increase the physical properties and mechanical strength of the concluding paper product. Furthermore, it contributes to an environmentally beginning process, reducing the use of hazardous chemicals. Cellulases are also utilized for waste reason in paper process. Cellulose helps in making biodegradable paper which disturbs the environment.

1.5.1.4 Agriculture Industries

Cellulases help the improvement of crops and also help in controlling diseases. Cellulases can damage the cell wall of the plant pathogen that helps in managing of disease. Various cellulolytic fungi are noted to play a major role in farming by enhancing sprouting of seed, faster development of the plant, and flowering, enhancing root system, and improving crop production. Conventionally using a straw is a crucial method to enrich soil quality. Applications of fungi such as *Aspergillus*, *Chaetomium*, and *Trichoderma* and actinomycetes on straw for accelerating the decomposition of straw have shown a positive outcome.

1.5.1.5 Animal Feed Industries

Cellulose has the ability to increase the properties of animal feed; that's why it has drawn attention in this field also (Kuhad et al. 2011). The enzyme can increase the nutritional value of the product and can also remove the anti-nutritional stuff from it and gives certain digestive enzymes such as protease, amylases, and glucanases.

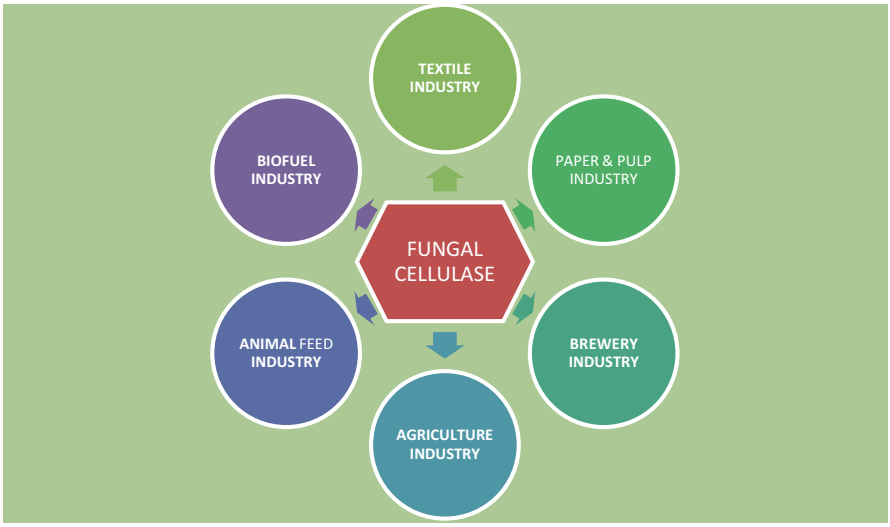

Fig. 1.4 Application of fungal cellulase

β-Glucanases and xylanases play an important application in the feed of omnivores and some herbivores animals to breaking down on starch polysaccharides such as β-glucans and arabinoxylans. These enzymes can also trigger an increment in weight of chickens and piglets by making a difference in digestion and absorption of nourished materials.

1.5.1.6 Other Applications

Another important application is in laundry and detergent industry with lipase and proteases being the major enzymatic constituent. Recently an inventive method of using alkaline cellulases, proteases, and lipase has shown a betterment of color intensity and dirt elimination from the clothes.

Cellulase is also utilized in wine and brewery industry. Fragrance of wine can be intensified by β-glucosidases through hydrolyzed glycosylated precursors into their aglycones and glucose (Ahmed and Bibi 2018) (Fig. 1.4).

1.6 Current Status

Products which are made from fungal cellulase are renewable and not harmful to the environment; that's why researches are going on in this field to make more products from it. Well, it has a lot of applications in paper, food, and textile industry. It is also

used in the bioethanol production and in clarification of fruit juices, purees, cooking oils, etc. Products which are made from it are renewable, reduce human labor, and increase the production rate and also the quality of the product. But more research work is required in this field which is in process. The main goals of industries are low economic rate and sustainable process, and using products made from fungal cellulase can fit into that.

Raw materials which we use are mostly waste products like wheat bran, rice bran, wheat husk, etc. (Sajith et al. 2016), which makes the process eco- and environment-friendly. Most places in this world are moving toward the utilization of fungal cellulase products. Only the biofuel production has reached 105 billion liters by 2010. Cellulase is used in various parts of the world in the food industry; it is also used in laundry detergents as a fabric softener and in the fermentation of wine and beer. So from this, it is quite clear that the use of cellulase in different products is beneficial and supportive and more products in the environment ought to be accessible for sustainable improvement.

1.7 Conclusion

Fungal cellulases hold immense opportunities in diverse industries, and further investigations will trigger the fungal biorefinery approach. There are many facts to be unraveled to synchronize the cellulose-mediated renewable and eco-friendly bioethanol on commercial scale. Exploring the diversity, function, and mode of action of fungal cellulose is essential for their magnificent contribution in the biofuel industry. The multifaceted applications of fungal cellulases stay to be unraveled, which can suitably address different challenges toward worldwide renewable maintainable economy for distant better tomorrow.

References

Achinas S, Jan G, Euverink W (2016) Consolidated briefing of biochemical ethanol production from lignocellulosic biomass. Electron J Biotechnol 23:44–53

Ahmed A, Bibi A (2018) Fungal cellulase; production and applications: minireview. Int J Health Life-Sci 4(1):19–36

Ajayi A, Peter-Albert C, Adedeji O (2015) Modification of cell wall degrading enzymes from soursop (*Annona muricata*) fruit deterioration for improved commercial development of clarified soursop juice (a review). Med Aromat Plants 4:2167–2412

Arja M-O (2007) Cellulases in the textile industry. In: Industrial enzymes. Springer, Dordrecht, pp 51–63

Azhara HMS, Abdullaa R, Jamboa SA, Marbawia H, Gansaua JA, Faika AAM Rodrigues KF (2017) Yeasts in sustainable bioethanol production: a review. Biochem Biophys Rep 10:52–61

Binod P, Sindhu R, Singhania RR, Vikram S, Devi L (2010) Bioethanol production from rice straw: an overview. Bioresour Technol 101:4767–4774

Cao Y, Zhang H, Xiong P (2014) Application of cellulase-assisted method to extract flavonoids from plants. Agric Sci Technol 15:729

Cartwright A (2007) Biofuels trade and sustainable development: an analysis of South African Bioethanol International Institute for Environment and Development, working document

Chen H, Qiu W (2010) Key technologies for bioethanol production from lignocellulose. Biotechnol Adv 28:556–562

Chen Y, Wan J, Zhang X, Ma Y, Wang Y (2012) Effect of beating on recycled properties of unbleached eucalyptus cellulose fiber. Carbohydr Polym 87:730–736

Cortez JM, Ellis J, Bioshop DP (2001) Cellulase finishing of woven, cotton fabrics in jet and which machines. J Biotechnol 89:239–245

Cui L, Meddeb-Mouihi F, Laframboise F, Beauregard M (2015) Effects of commercial cellulases and refining on kraft pulp properties: correlations between treatment impacts and enzymatic activity components. Carbohydr Polym 115:193–199

Gil N, Gil C, Amaral ME, Costa AP (2009) Use of enzymes to improve the refining of a bleached Eucalyptus globules kraft pulp. Biochem Eng J 46:89–95

IEA (2011) Technology roadmap: biofuels for transport. Organisation for Economic Cooperation and Development, International Energy Agency, Paris

Kuhad RC, Singh A, Eriksson KE (1997) Microorganisms and enzymes involved in the degradation of plant fiber cell walls. Adv Biochem Eng Biotechnol 57:45–125

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

Li DC, Li AN, Papageorgiou AC (2011) Cellulases from thermophilic fungi: recent insights and biotechnological potential. Enzym Res. https://doi.org/10.4061/2011/308730

Msangi S, Sulser T, Rosegrant M, Valmonte-Santos R (2007) Global scenarios for biofuels: impacts and implications for food security and water use. In: Paper presented at the 10th annual conference on global economic analysis. Purdue University, Indiana

Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, Frederick WJ Jr, Hallett JP, Leak DJ, Liotta CL, Mielenz JR, Murphy R, Templer R, Tschaplinski T (2006) The path forward for biofuels and biomaterials. Science 311:484–489

Rai P, Majumdar G, Gupta SD, De S (2007) Effect of various pretreatment methods on permeate flux and quality during ultrafiltration of mosambi juice. J Food Eng 78:561–568

Rosegrant MW, Msangi S, Sulser T, Valmonte-Santos R (2006) Biofuels and the global food balance. In: Bioenergy and agriculture: promises and challenges. International Food Policy Research Institute (IFPRI), Washington, DC

Russo L, Ladisch M (2008) Gaps in the research of 2nd generation transportation biofuels. Final report of task 41, project 2. IEA Bioenergy (2):1

Sajith S, Priji P, Sreedevi S, Benjamin S (2016) An overview on fungal cellulases with an industrial perspective. J Nutr Food Sci 6(1). https://doi.org/10.4172/2155-9600.1000461

Sewalt VJH, Glasser WG, Beauchemin KA (1997) Lignin impact on fiber degradation. Reversal of inhibition of enzymatic hydrolysis by chemical modification of lignin and by additives. J Agric Food Chem 45:1823–1828. https://doi.org/10.1021/jf9608074

Shen X, Xia L (2006) Lactic acid production from cellulosic material by synergetic hydrolysis and fermentation. Appl Biochem Biotechnol 133:251–262

Smitha R, Jisha V, Sajith S, Benjamin S (2013) Dual production of amulase and d-endotoxin by *Bacillus thuringiensis subsp. Kurstakidurng* biphasic fermentation. Microbiology 82:794–800

Spindler DD, Wyman CE, Grohmann K (1991) The simultaneous saccharification and fermentation of pretreated woody crops to ethanol. Appl Biochem Biotechnol 28/29:773–786

Tong Z, Pullammanappallil P, Teixeira AA (2010) How ethanol is made from cellulosic biomass. Energy Bulletin, University of Florida, 1–4

Yinbo Q, Zhu M, Liu K, Bao X, Lin J (2006) Studies on cellulosic ethanol production for sustainable supply of liquid fuel in China. Biotechnol J 1:1235–1240

Zabed H, Faruq G, Sahu JN et al (2014) Bioethanol production from fermentable sugar juice. Sci World J 2014:1–11

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

Zhang ZY, Jin B, Kelly JM (2007) Production of lactic acid from renewable materials by Rhizopus fungi. Biochem Eng J 35:251–263

Zhou J, Wang YH, Chu J, Zhuang YP, Zhang SL, Yin P (2008) Identification and purification of the main components of cellulases from a mutant strain of *Trichoderma viride* T 100-14. Bioresour Technol 99:6826–6833

Chapter 2
An Insight into Fungal Cellulases and Their Industrial Applications

Kavitha Sampathkumar, Valarmathi Kumar, Selvaraju Sivamani, and Nallusamy Sivakumar

2.1 Introduction

Cellulases are not a single enzyme but are a family of three groups of enzymes, exoglucanase, endoglucanase, and glucosidase (Adhyaru et al. 2015). Exoglucanase or glucan cellobiohydrolase (CBH) or avicelase attacks the ends of the cellulose chain and produces the disaccharide, cellobiose, as the resultant product. Endoglucanase or glucan glucanohydrolase or carboxymethylcellulase (CMCase or EG) acts on the inner part of cellulosic molecules that produces oligosaccharides. Glucosidase or cellobiase specifically attacks cellobiose and produces glucose (Adsul et al. 2007). Figure 2.1 depicts the mechanism of cellulolytic action. Cellulases have a wide range of applications in agriculture, biotransformation and fermentation, detergents and laundry, the pulp and paper industry, textiles, and the food industry (Adsul et al. 2009). Figure 2.2 represents the industrial applications of cellulases. Structurally, fungal cellulases are simpler than bacterial cellulases. Fungal cellulases have two domains: the catalytic domain and the cellulose-binding molecule (Ahmed et al. 2009).

The factors that influence the production of cellulases are the type of organism (fungi or bacteria or actinomycetes), the fermentation method (submerged or solid), the constituents of the production medium (carbon source, nitrogen source, and trace elements), and the process parameters (substrate concentration, pH,

K. Sampathkumar · V. Kumar
Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, India

S. Sivamani (✉)
Chemical Engineering Section, Engineering Department, Salalah College of Technology, Salalah, Oman

N. Sivakumar
Department of Biology, College of Science, Sultan Qaboos University, Muscat, Oman

M. Srivastava et al. (eds.), *Approaches to Enhance Industrial Production of Fungal Cellulases*, Fungal Biology,
https://doi.org/10.1007/978-3-030-14726-6_2

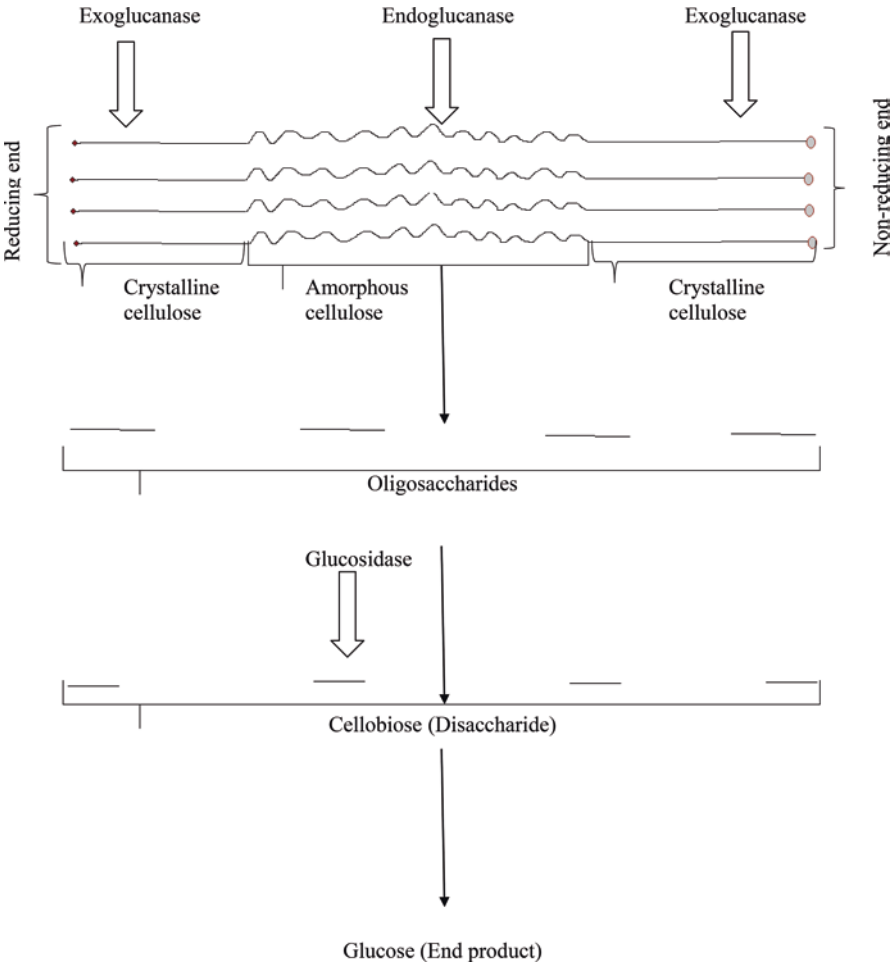

Fig. 2.1 Mechanism of cellulases action

temperature, time, inoculum size, and stirring rate) (Amir et al. 2011). Solid state fermentation is a process used in the production of fuels, food, pharmaceutical, and industrial products using microorganisms in a controlled environment. It is used as an alternative to submerged fermentation. Fermentation in the solid state takes place in the absence of free water. The advantages of solid state fermentation include a simpler process that requires less energy; produces higher volumetric productivity, similar to the natural environment of certain mushrooms; and makes purification easier than submerged fermentation (Anand et al. 2008).

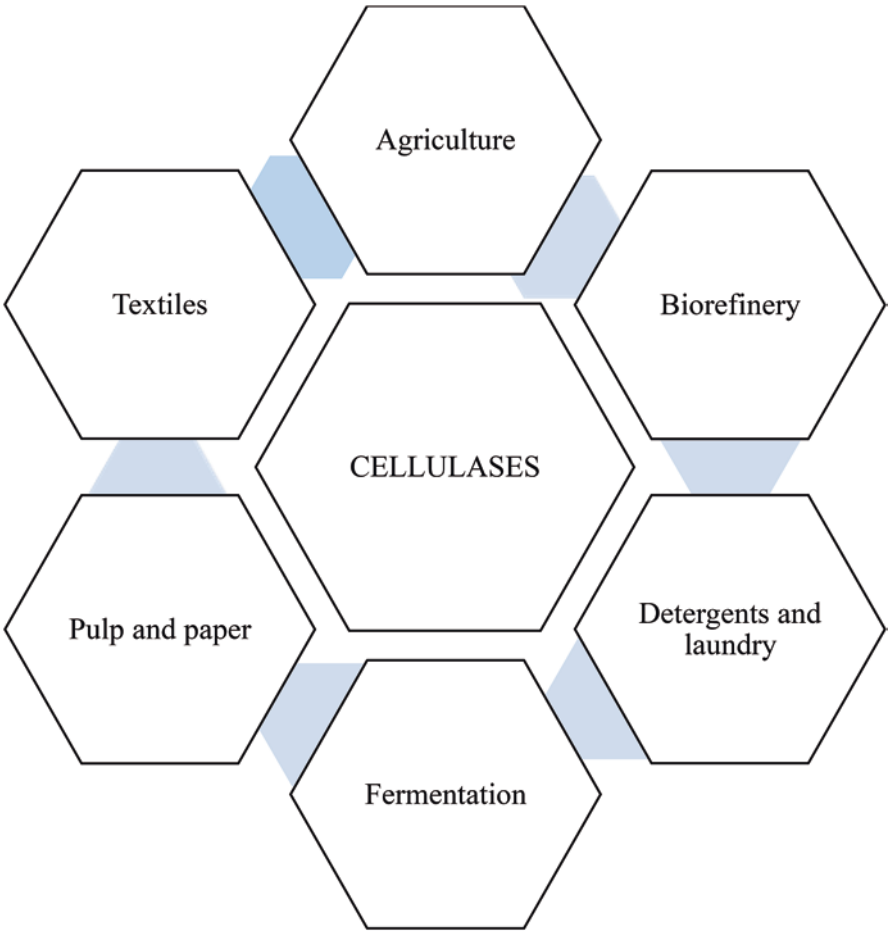

Fig. 2.2 Industrial applications of cellulases

2.2 Fungal Cellulases

Although it is known that bacteria, actinomycetes, and fungi produce cellulases, fungi play a major role. Of the fungi, the genera *Aspergillus*, *Trichoderma*, and *Penicillium* are predominant in the production of cellulases. *Aspergillus niger* and *Trichoderma reesei* were the most common microorganisms that produce cellulases (Anish et al. 2007). *Aspergillus* has more activity with respect to endoglucanase or CMCase (C_x) than exoglucanase or avicelase (C_1) and glucosidase or cellobiase, whereas *Trichoderma* has more significant activity of endoglucanase and exoglucanase than glucosidase. *Penicillium* produces more endoglucanase and glucosidase (Anita et al. 2009).

2.2.1 *Cellulases from* Aspergillus

Baba et al. have characterized *Aspergillus aculeatus* β-glucosidase 1 (AaBGL1), which promotes hydrolysis of cellulose by the *Trichoderma* cellulases system (Baba et al. 2015). Current research has also compared certain properties with a commercially available *A. niger* orthologue (AnBGL) to elucidate the benefits of recombinant AaBGL1 (rAaBGL1) for a synergistic effect on *Trichoderma* enzymes. Steady-state kinetic studies revealed that rAaBGL1 exhibited high catalytic efficiency for β protein-linked glucooligosaccharides. Milala et al. evaluated cellulases activity in *Aspergillus candidus* with rice husks, millet straw, guinea corn stalks, and sawdust as substrates (Milala et al. 2009). The substrates were pretreated with 5% NaOH and autoclaved. Fermentation studies showed that husks of rice, millet straw, and guinea corn stalks exhibited maximum cellulases activity of 7.50, 6.88, and 5.84 IU, respectively.

Schmidt et al. tested whether ochratoxin A (OTA) production of *Aspergillus niger* and *A. carbonarius* was related to a particular genotype and the identification of marker sequences with diagnostic value identifying *A. carbonarius* concerning the production of OTA in food and feed materials (Schmidt et al. 2004). The ability of isolates to produce OTA was tested by thin-layer chromatography (TLC). Strains were genetically characterized by AFLP fingerprints and compared with each other and with reference strains. Gomathi et al. explained the potential for CMCase production with the selective species *Aspergillus flavus* (Gomathi et al. 2012). The expression of CMCase in *A. flavus* was evaluated under different processing conditions using submerged fermentation (SmF) on various agricultural by-products. *A. flavus* produced high levels of CMCase under optimized culture conditions on the third day of incubation at optimal pH 6.0, at a temperature of 30 °C, and at a graft size of 4% in Czapek Dox using wheat bran as a substrate for SmF.

Immanuel et al. studied the production capacity of cellulases enzymes of *Aspergillus niger* and *A. fumigatus* against lignocellulose waste at pH 5–9 and at temperature of 20–50 °C (Immanuel et al. 2007). Enzyme production was analyzed separately with dinitrosalicylic acid (DNS) and filter paper (FPA). In the FPA method, *A. fumigatus* (0.292 IU/mL) and pH 6 of *A. niger* (0.262 IU/mL) resulted in a high level of enzyme production when coconut waste and sawdust were used as substrates, respectively. Similarly, with varying temperatures, both organisms achieved a high level of enzyme production at 40 °C with both substrates. Tao et al. purified endoglucanase (EG) from *Aspergillus glaucus* XC9 developed on 0.3% sugarcane bagasse as a carbon source from the culture filtrate using ammonium sulfate, a fast-flowing DEAE-Sepharose column and a Sephadex G-100 column, with a purge fold of 21.5% and a recovery of 22.3% (Tao et al. 2010).

Anita et al. studied the production of *Aspergillus heteromorphus* cellulases by submerged fermentation using wheat straw as a substrate (Anita et al. 2009). Process parameters such as pH, temperature, and time have been optimized for saccharification. The maximum reducing sugars were produced on the fifth day at pH 5 and 30 °C. Under optimal conditions, the activities of the filter paper and the CMCase

were, respectively, 3.2 IU/mL and 83 IU/mL. Herculano et al. studied the separation and purification of *Aspergillus japonicus* URM5620 cellulases in aqueous two-phase systems (ATPS) (Herculano et al. 2012). A factorial model (2^4) was used to determine the influence of the molarity of polyethylene glycol (PEG) (1000 to 8000 g/mol), its concentration (20.0–24.0% (w/w)), sodium citrate concentration (15–20% (w/w)), and pH (6.0–8.0) on the differential distribution and purification of the cellulolytic complex consisting of β-glucosidase (βG), endoglucanase (CMCase), and total cellulases (FPase). This process ensures an efficient and attractive increase in the purification factor.

Koseki et al. expressed the recombinant AkCel61, the wild-type enzyme (rAkCel61), and a truncated enzyme consisting of the catalytic domain (rAkCel61ACBM) in *Pichia pastoris* and analyzed their biochemical properties (Koseki et al. 2008). The purified rAkCel61 and rAkCel61ACBM migrated on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and their apparent molecular weights were 81 kDa and 34 kDa, respectively. The rAkCel61 protein bound to crystalline cellulose but not to arabinoxylan. The rAkCel61 and rAkCel61ACBM proteins produced small amounts of oligosaccharides from soluble carboxymethylcellulose. However, they showed no detectable activity against microcrystalline cellulose, arabinoxylan, and pectin. Lockington et al. characterized two genes encoding *A. nidulans* exo-cellulases and a gene encoding an endo-cellulases that is complementary to the endo-cellulases coding gene, for example, *eglA* (Lockington et al. 2002). The 5′ putative regulatory regions of all genes contain potential binding sites for the global carbon and nitrogen regulatory proteins, CreA and AreA. The 5′ *eglA* and *eglB* sequences contain potential consensus of XlnR-binding sites involved in induction in *A. niger*, but none of the 5′ sequence contains an exact copy of the AceII DNA binding consensus sequence involved in induction of *Trichoderma reesei*. Therefore, it is likely that they can be induced by different regulatory proteins specific to a pathway.

Coral et al. prepared a CMCase from a wild *Aspergillus niger* Z10 strain (Coral et al. 2002). Analyses of the enzyme preparation by SDS-PAGE revealed two protein bands with cellulolytic activity. The molecular weight of these bands has been estimated at about 83 kDa and 50 kDa. The optimum temperature of the enzyme was observed at about 40 °C. It was found that the activity of the enzyme had a broad pH range between 3 and 9 and that 41.2% of the initial activity was preserved after heat treatment at 90 °C for 15 minutes. Immanuel et al. also investigated the ability to produce cellulases enzymes from *Aspergillus niger* and *A. fumigatus* using lignocellulose wastes at pH 5–9 and at temperature of 20–50 °C (Immanuel et al. 2007).

Hui et al. studied the direct microbial conversion of wheat straw to lipid via a cellulolytic fungus of *Aspergillus oryzae* A-4 in solid state fermentation (SSF) (Hui et al. 2010). *A. oryzae* A-4 gave a lipid of 36.6 mg/g dry substrate (DS) and a cellulases activity of 1.82 FPU/g DS, with 25.25% of the holocellulose use in the substrates was detected. e Silva et al. studied the production of cellulolytic enzymes by the fungus *Aspergillus phoenicis* (e Silva et al. 2009). Grape waste from the wine industry has been selected as a growth substrate between various agro-industrial by-products. A centralized design was carried out with the quantity of grape and

peptone waste as independent variables. The fungus was cultured in a submerged fermentation at 120 °C and 120 rpm, and the activities of total cellulases, endoglucanases, and β-glucosidases were measured. The optimal production of the three cellulolytic activities was observed at values close to the central point. *A. phoenicis* has the potential to produce cellulases using grape waste as a growth substrate.

Gao et al. studied the production of extracellular cellulases by the thermoacidophilic fungus *Aspergillus terreus* M11 on lignocellulosic materials in solid state fermentation (SSF) (Gao et al. 2008). The results showed that the high-level cellulases activities of 581, 243, and 128 U/g of carbon source were obtained for endoglucanase, FPase, and β-glucosidase at 45 °C, pH 3, and 80% of moisture with corn straw and 0.8% yeast extract as sources of carbon and nitrogen. Adhyaru et al. investigated xylanase and cellulases activity using *A. tubingensis* FDHN1 and agricultural residues such as foundry waste, sugarcane bagasse, wood shavings, wheat straw, corn straw, peanut shell rice, and barley straw by solid state fermentation (Adhyaru et al. 2015). Wood chips showed maximum cellulases activity of 2.81 U/g with *A. tubingensis*.

In addition to the literature mentioned above, *A. ellipticus* (Hu et al. 2011), *A. flavus* (Obruca et al. 2012; Ojumu et al. 2003), *A. heteromorphus* (Singh et al. 2009a; b), *A. japonicas* (Herculano et al. 2011), *A. niger* (Mrudula and Murugammal 2011; Omojasola and Jilani 2008), *A. oryzae* (Kotaka et al. 2008), *A. terreus* (Narra et al. 2012), *A. tubingensis* (Decker et al. 2001), and other *Aspergillus* species were also found to produce cellulases.

2.2.2 *Cellulases from* Trichoderma

Kovacs et al. improved the cellulases production process and developed more efficient enzymes for lignocellulose degradation to reduce the costs of the enzymes required for the biomass to bioethanol process (Kovacs et al. 2009). Lignocellulolytic enzymatic complexes were produced by the mutant *Trichoderma atroviride* TUB F-1663 on three different pretreated lignocellulosic substrates, namely, fir, wheat straw, and sugarcane bagasse. The filter paper activities of the enzymes produced on the three materials were very similar, while β-glucosidase and hemicellulases activities were more dependent on the nature of the substrate. Ahmed et al. produced and partially purified cellulases complex using *T. harzianum* with carbon sources such as glucose, carboxymethylcellulose (CMC), corncobs, birch xylan, and wheat bran (Ahmed et al. 2009). Between cellulases complexes, exoglucanase showed more activity than endoglucanase and glucosidase. *T. harzianum* showed maximum cellulases activity with 1% CMC at 120 °C and pH 5.5 for 120 hours. Under optimal conditions, the enzymes were partially purified by ammonium sulfate precipitation and then by chromatography on Sephadex G-200 and Sephadex G-50 gel. Specific activities were found to be 49.22, 0.63, and 0.35 U/mg, respectively.

Omojasola et al. used sweet orange scrap as a substrate for cellulases production (Omojasola and Jilani 2008). The skin, the fruit wall, and the pulp were treated with

alkali and steam. Next, the pretreated materials were hydrolyzed by cellulolytic enzymes. The cellulases activities of *Trichoderma longi*, *Aspergillus niger*, and *Saccharomyces cerevisiae* were expressed in terms of reducing sugar concentrations and were found to be 3.86, 2.94, and 2.30 mg/mL, respectively. Leghlimi et al. isolated the native cellulolytic fungus *Trichoderma longibrachiatum* (GHL) from the soil near an Algerian hot spring and used it for the production of cellulases by submerged fermentation on Mandel's medium with Avicel cellulose (1%) as single source of carbon (Leghlimi et al. 2013). The endoglucanase and filter paper activities of the wild-type *Trichoderma* strain were compared to hypercellulolytically mutated *Trichoderma reesei* Rut C-30 in shake flask cultures at 35 °C. After 7 fermentation days, *T. longibrachiatum* has activities equivalent to *T. reesei* (10.61 IU/mL endoglucanase (CMCase) and 2.04 IU/mL filter paper activity (FPA)). On the other hand, the β-glucosidase activity of *Trichoderma* GHL was twice as great as that of *T. reesei*.

Boer et al. tested the heterologous expression of *T. reesei* cellobiohydrolase Cel7A in methylotrophic yeast *Pichia pastoris*, both under the *P. pastoris* alcohol oxidase (AOX1) promoter and glyceraldehyde-3-phosphate dehydrogenase (GAP) in a fermenter (Boer et al. 2000). The production of Cel7A with the AOX1 promoter gave a better yield. The k_{cat} and K_m values for the purified protein on soluble substrates are comparable to the values found for native *Trichoderma* Cel7A. The optimum pH measured also closely resembles that of purified *T. reesei* Cel7A. Circular dichroism (CD) measurements indicate that the formation of disulfide bridges is an important step in the correct folding of Cel7A. Van Wyk and Mohulatsi treated different waste materials with the enzyme *Trichoderma viride* cellulases, which convert their cellulosic component into fermentable sugars (Van Wyk and Mohulatsi 2003). All the materials exhibited different susceptibilities for cellulases as well as for the production of non-similar sugar release cartridges as increasing amounts of paper were treated with a solid enzyme concentration. A general decrease in hydrolytic efficiency was observed when sugar concentrations were increased during the biodegradation of all wastes.

In addition to the above scientific literature, *T. atroviride* (Kovács et al. 2008; Kovács et al. 2009), *T. harzianum* (da Silva Delabona et al. 2012; El-Katatny et al. 2001; Maeda et al. 2011), *T. reesei* (Kovács et al. 2009; Krishna et al. 2000; Lee and Koo 2001; Rocky-Salimi and Hamidi-Esfahani 2010; Singhania et al. 2006; Turner et al. 2003), and other *Trichoderma* species have also proved to produce cellulases.

2.2.3 *Cellulases from* Penicillium

Adsul et al. improved the strain of *Penicillium janthinellum* by mutation with ethyl methyl sulfonate for 24 hours and then by UV irradiation for 3 minutes (EMS-UV8) (Adsul et al. 2007). Subsequent mutation and selection led to the isolation of two promising mutants, one selected on the basis of Avicel hydrolysis (EU1) and the other based on the hydrolysis of Walseth cellulose in the presence of 2-deoxy-D-glucose (EU2D-21). All of these mutants produced twice as much FPase and

CMCase activity as the parental strain. Enzymatic preparation derived from Avicel hydrolyzed mutant EU1 to a greater extent. Adsul et al. produced high levels of CMCase and glucosidase from one of the *P. janthinellum* (EU2D-21) mutants on wheat bran (4 g) and wheat bran (3 g) with steam-exploded cane bagasse (2 g) as substrates by fermentation in the solid state (Adsul et al. 2009). The stability of the cellulases prepared from one of the mutants (EMS-UV8) was studied in one of the ionic liquids, 1-butyl-3-methylimidazolium chloride ([bmim] Cl), and revealed that all the enzymes exhibited significant activity at a concentration of 20% ionic liquid.

Belghith et al. studied the thermal stability of *Penicillium occitanis* cellulases (Po16) by spray drying and the effect of additives (Belghith et al. 2001a). The results showed that the CMCase activity assures a good stability at 50 °C, even after 60 hours of incubation. In addition, β-glucosidase activity was more sensitive and showed a 50% loss and reacted to total cellulases activity (FPU). The addition of hydrophilic agents such as ethylene glycol and polyethylene glycol (PEG6000) increased the enzyme activity. The effect of PEG and maltodextrin, another agent reducing the activity of water, was then tested during spray drying of Pol6 cellulases. The presence of 1% PEG provided the best recovery but had a negative effect on the stability of the enzyme, whereas 1% maltodextrin had a negative effect on the recovery of the enzyme but a positive effect on the recovery of the enzyme and its stability. Belghith et al. cultured the mutant *Penicillium occitanis* (Po16), which separated a large amount of cellulases into a fermenter using local paper pulp as an inducing substrate (Belghith et al. 2001b). High extracellular cellulases activity was obtained after batch treatment: 23 IU/mL filter paper, 21 IU/mL CMCase activity (endoglucanase units), and 25 mg/mL protein. This cellulases preparation was applied in a biodegradation process on an industrial scale. The abrasive effect of *P. occitanis* cellulases was very uniform and with comparable efficiency to that obtained commercially.

Camassola and Dillon treated bagasse with sugarcane containing the white rot fungus *Pleurotus sajor-caju* PS 2001 and were then used for the production of cellulases and xylanases by the fungus *Penicillium echinulatum* for saccharification (Camassola and Dillon 2009). Despite the environmental benefits offered by this type of pretreatment, the enzymatic activity obtained with the pretreated sugarcane bagasse (PSCB) was lower than that of the control treatments. Although the enzymatic activities of the culture with PSCB are inferior to those of cultures made with untreated sugarcane bagasse, it should be noted that the production of enzymes from the cellulases and hemicellulases complex after the production of mushrooms is another way to add value to this agricultural residue.

Camassola and Dillon studied the production of cellulases and xylanases from *Penicillium echinulatum* 9A02S1 by solid state fermentation (SSF) with different mass ratios of sugarcane bagasse (SCB) and wheat bran (WB) (Camassola and Dillon 2010). The largest FPase obtained was 45.82 ± 1.88 U/g DS in a culture containing 6 SCB/4 WB on the third day. The most important β-glucosidase activities were 40.13 ± 5.10 U/g DS obtained on the third day for culture at 0 SCB/10 WB. For endoglucanase, the highest activity was 290.47 ± 43.57 U/g DSF for culture 6 SCB/4 WB on the fourth day of culture.

Camassola and Dillon evaluated the production of cellulases and hemicellulases by *Penicillium echinulatum* 9A02S1 of cellulases and hemicellulases with different concentrations of pretreated cane bagasse (PSCB) and wheat bran (WB) (Camassola and Dillon 2007). The highest activities of FPase, β-glucosidase, and endoglucanases were measured at 32.89 ± 1.90, 58.95 ± 2.58, and 282.36 ± 1.23 U/g DS. The inclusion of inexpensive sources in lignocellulosic enzyme production media would help reduce the cost of producing enzyme complexes capable of hydrolyzing lignocellulose residues for the formation of fermented syrups, thereby contributing to the economic production of bioethanol. Camassola et al. characterized *Penicillium echinulatum* cellulases for their FPase and β-glucosidase activities (Camassola et al. 2004). Both activities showed maximum values between pH 4 and 5. The activities were slightly higher in citrate buffer than in acetate buffer with the same pH. The thermal stability of both activities was good at 55 °C. FPase was significantly reduced at higher temperature.

Penicillium brasilianum (Jørgensen and Olsson 2006; Jørgensen et al. 2003; Jung et al. 2015; Krogh et al. 2010; Panagiotou et al. 2006), *P. citrinum* (Dutta et al. 2008; Ng et al. 2010), *P. echinulatum* (Dillon et al. 2011; Martins et al. 2008; Sehnem et al. 2006), *P. funiculosum* (de Castro et al. 2010), *P. janthinellum* (Singhania et al. 2014), *P. purpurogenum* (Davies et al. 2000; Lee and Koo 2001), and other *Penicillium* species also produce cellulases.

2.2.4 Cellulases from Other Genera

In addition to *Aspergillus*, *Trichoderma*, and *Penicillium*, the following organisms have also been considered to produce cellulases:

Amir et al. optimized the pH (3–9), the time (1–7 days), and the temperature (25–40 °C) for maximal enzymatic activity with *Alternaria alternata* with the corncob as a source of carbon by fermentation in the solid state (Amir et al. 2011). *A. alternata* exhibited a maximum cellulases activity of 31.24 μg/mL with 5 g corn at 35 °C and a pH of 6 for 96 hours. Anand et al. produced more cellulolytic enzymes, namely, C_1 and C_x in vitro, virulent isolates of *Colletotrichum capsici*, and *Alternaria alternata* and that the activity of these enzymes increased with increasing age of culture (Anand et al. 2008). Anish et al. used an alkali-stable endoglucanase from the alkalothermophilic society *Thermomonospora* sp. (T-EC) for denim biofinishing (Anish et al. 2007). The current study has shown that the use of acidic and neutral cellulases causes staining back of the indigo dye on the tissue. T-EG is effective at removing hair with negligible weight loss and soft tissue. Higher abrasion activity with lower background staining was a preferred feature for denim biofinishing presented by T-EG. The enzyme was also effective under non-swab conditions, which is an added advantage for use in the textile industry. An enzymatic finishing mechanism of the cotton fabric is presented based on the unique properties of T-EG.

Baba et al. cloned two cDNAs homologous to the rce1 gene of *Rhizopus oryzae*, called mce1 and mce2 cDNAs, from *Mucor circinelloides*, a member of the *Zygomycota* subdivision (Baba et al. 2005). The mcel cDNA encoded an endoglucanase (family 45 glycoside hydrolase) with a carbohydrate-binding module (CBM), called mce1, and the mce2 cDNA encoded the same endoglucanase with two replicate tandem CBMs, called mce2. The specific activity of CMCase of mce2 was almost identical to that of mce1, whereas the specific activity of avicelase of mce2 was twice as high as that of mce1. In addition, mce2, of which two tandem CBMs would be more effective for the degradation of crystalline cellulose than CBM, was excreted only in an early culture phase in which crystalline cellulose was abundant.

Baldrian and Gabriel studied the activities of cellulolytic (endo-1,4-L-glucanase, exo-1,4-L-glucanase, 1,4-L-glucosidase), hemicellulolytic (endo-1,4-L-xylanase, 1,4-L-xylosidase, and 1,4-L-mannosidase), and ligninolytic (Mn-peroxidase and laccase) during growth of *Pleurotus ostreatus* on wheat straw in the presence and absence of cadmium (Baldrian and Gabriel 2003). The activities of endo-1,4-L-glucanase, 1,4-L-glucosidase, and laccase were increased in the presence of cadmium. Boisset et al. examined the digestion of bacterial cellulosic tapes with mixtures of ternary enzymes consisting of recombinant cellulases (two cellobiohydrolases, Cel6A and Cel7A, and the endoglucanase Cel45A) from *Humicola insolens* over a wide range of mixture compositions (Boisset et al. 2001). The degree of digestion was followed by saccharification analysis and transmission electron microscopy (TEM) observations. It has been found that the addition of very small amounts of Cel45A induces a dramatic increase in the saccharification of the substrate with Cel7A or the mixture of Cel6A and Cel7A. But only moderate saccharification resulted from mixing Cel45A and Cel6A.

Bhatti et al. produced β-glucosidase from *Fusarium solani* with agricultural waste using solid state fermentation (SSF) (Bhatti et al. 2013). The optimal β-glucosidase activity of 3206 U/g DS was obtained with a rice husk at pH 5, a 60% moisture content, 65 °C, and a 72-hour fermentation period with the supplemented medium in lactose. Then, the enzyme was partially purified with ammonium sulfate precipitation to give a specific activity of 97.5 U/mg. It was observed that β-glucosidase was thermally stable at 65 °C. β-Glucosidase was subjected to kinetic studies. The K_m and V_{max} values were 1 mM and 55.6 μmol/min, respectively. Mg^{2+} ions increased enzymatic activity. These characteristics suggest that β-glucosidase isolated from *F. solani* can be used in various applications such as textile, paper, biofuel, starch, animal feed, and fruit industries.

Agaricus arvensis (Jeya et al. 2010b), *Alternaria alternate* (Eshel et al. 2002), *Brassica napus* (Mølhøj et al. 2001), *Chaetomium thermophilum* (Li et al. 2003), *Clostridium cellulolyticum* (Desvaux 2005; Guedon et al. 2002; Higashide et al. 2011), *Colletotrichum capsici* (Anand et al. 2008), *Coniophora puteana* (Kajisa et al. 2009; Kajisa et al. 2004), *Coriolopsis caperata* (Deswal et al. 2014), *Fomitopsis palustris* (Deswal et al. 2011; Shimokawa et al. 2008), *Fusarium solani* (Obruca et al. 2012), *Fusarium oxysporum* (Panagiotou et al. 2005; Panagiotou et al. 2003; Ramanathan et al. 2010), *Fusarium chlamydosporum* (Qin et al. 2010), *Gloeophyllum trabeum* (Cohen et al. 2005; Deswal et al. 2014; Niemenmaa et al. 2008), *Humicola*

insolens (Davies et al. 2000; Mariyam 2011), *Humicola grisea* (Nascimento et al. 2010; Takashima et al. 2007), *Kluyveromyces marxianus* (Ballesteros et al. 2004; Pessani et al. 2011; Suryawati et al. 2009; Tomás-Pejó et al. 2009), *Melanocarpus albomyces* (Haakana et al. 2004; Hirvonen and Papageorgiou 2003; Miettinen-Oinonen et al. 2004; Parkkinen et al. 2008; Szijártó et al. 2008), *Mucor circinelloides* (Saha 2004), *Mucor indicus* (Karimi et al. 2006), *Neurospora crassa* (Dogaris et al. 2009; Phillips et al. 2011; Tian et al. 2009), *Paecilomyces inflatus* (Kluczek-Turpeinen et al. 2005), *Phanerochaete chrysosporium* (Martinez et al. 2004; Shi et al. 2008), *Phlebia gigantea* (Niranjane et al. 2007), *Piptoporus betulinus* (Valášková and Baldrian 2006), *Pleurotus ostreatus* (Obodai et al. 2003; Reddy et al. 2003; Taniguchi et al. 2005; Valášková and Baldrian 2006), *Pleurotus florida* (Deswal et al. 2014), *Pleurotus sajor-caju* (Reddy et al. 2003), *Poria placenta* (Highley et al. 2007; Niemenmaa et al. 2008), *Rhizopus oryzae* (Karimi et al. 2006; Murashima et al. 2002; Park et al. 2004), *Saccharomyces cerevisiae* (Den Haan et al. 2007; Karimi et al. 2006; Omojasola and Jilani 2008), *Sporotrichum thermophile* (Dimarogona et al. 2012; Kaur and Satyanarayana 2004), *Thermoascus aurantiacus* (Kalogeris et al. 2003), *Trametes hirsuta* (Jeya et al. 2009), *Trametes versicolor* (Valášková and Baldrian 2006), and other fungi are also reported to produce cellulases.

2.3 Conclusion

Cellulases and cellulolytic microorganisms constitute one of the most important groups of enzymes for industrial applications, as emphasized in this review. They exhibit good catalytic properties which make them versatile. Based on the progress in recent research on fungal cellulases, their applications have been broadened to reduce the costs. There are still many limitations to overcome. One of the problems is the practical feasibility of commercial cellulases. Their complex nature and the downstream processing stages result in the low yield of cellulases. The utilization of residues from agro-industrial residues as substrates can improve their applications. Also, various microorganisms used to produce fungal cellulases, mechanism of cellulolytic action, cheap substrates used for enzyme production, and the industrial applications were emphasized.

References

Adhyaru DN, Bhatt NS, Modi HA (2015) Optimization of upstream and downstream process parameters for cellulases-poor-thermo-solvent-stable xylanase production and extraction by Aspergillus tubingensis FDHN1. Bioresour Bioprocess 2(1):3

Adsul MG, Bastawde KB, Varma AJ, Gokhale DV (2007) Strain improvement of Penicillium janthinellum NCIM 1171 for increased cellulases production. Bioresour Technol 98(7):1467–1473

Adsul MG, Terwadkar AP, Varma AJ, Gokhale D (2009) Cellulases from Penicillium janthinellum mutants: solid-state production and their stability in ionic liquids. Bioresources 4(4):1670–1681

Ahmed S, Bashir A, Saleem H, Saadia M, Jamil A (2009) Production and purification of cellulose-degrading enzymes from a filamentous fungus Trichoderma harzianum. Pak J Bot 41(3):1411–1419

Amir IJAZ, Anwar Z, Zafar Y, Iqbal H, Aish M, Muhammad I, Sajid M (2011) Optimization of cellulases enzyme production from corn cobs using *Alternaria alternata* by solid state fermentation. J Cell Mol Biol 9(2):51–56

Anand T, Bhaskaran R, Karthikeyan TG, Rajesh M, Senthilraja G (2008) Production of cell wall degrading enzymes and toxins by Colletotrichum capsici and *Alternaria alternata* causing fruit rot of chillies. J Plant Protect Res 48(4):437–451

Anish R, Rahman MS, Rao M (2007) Application of cellulases from an alkalothermophilic Thermomonospora sp. in biopolishing of denims. Biotechnol Bioeng 96(1):48–56

Anita S, Namita S, Bishnoi NR (2009) Production of cellulases by Aspergillus heteromorphus from wheat straw under submerged fermentation. Int J Env Sci Eng 1(1):23–26

Baba Y, Shimonaka A, Koga J, Kubota H, Kono T (2005) Alternative splicing produces two endoglucanases with one or two carbohydrate-binding modules in Mucor circinelloides. J Bacteriol 187(9):3045–3051

Baba Y, Sumitani JI, Tani S, Kawaguchi T (2015) Characterization of Aspergillus aculeatus β-glucosidase 1 accelerating cellulose hydrolysis with Trichoderma cellulases system. AMB Express 5(1):3

Baldrian P, Gabriel J (2003) Lignocellulose degradation by Pleurotus ostreatus in the presence of cadmium. FEMS Microbiol Lett 220(2):235–240

Ballesteros M, Oliva JM, Negro MJ, Manzanares P, Ballesteros I (2004) Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with Kluyveromyces marxianus CECT 10875. Process Biochem 39(12):1843–1848

Belghith H, Chaabouni SE, Gargouri A (2001a) Stabilization of Penicillium occitanis cellulases by spray drying in presence of Maltodextrin. Enzym Microb Technol 28(2–3):253–258

Belghith H, Ellouz-Chaabouni S, Gargouri A (2001b) Biostoning of denims by Penicillium occitanis (Pol6) cellulases. J Biotechnol 89(2–3):257–262

Bhatti HN, Batool S, Afzal N (2013) Production and characterization of a novel (beta)-glucosidase from *Fusarium solani*. Int J Agric Biol 15(1):140–144

Boer H, Teeri TT, Koivula A (2000) Characterization of Trichoderma reesei cellobiohydrolase Cel7A secreted from Pichia pastoris using two different promoters. Biotechnol Bioeng 69(5):486–494

Boisset C, Pétrequin C, Chanzy H, Henrissat B, Schülein M (2001) Optimized mixtures of recombinant Humicola insolens cellulases for the biodegradation of crystalline cellulose. Biotechnol Bioeng 72(3):339–345

Camassola M, Dillon AJP (2007) Production of cellulases and hemicellulases by Penicillium echinulatum grown on pretreated sugarcane bagasse and wheat bran in solid-state fermentation. J Appl Microbiol 103(6):2196–2204

Camassola M, Dillon AJ (2009) Biological pretreatment of sugarcane bagasse for the production of cellulases and xylanases by Penicillium echinulatum. Ind Crop Prod 29(2–3):642–647

Camassola M, Dillon AJ (2010) Cellulases and xylanases production by Penicillium echinulatum grown on sugarcane bagasse in solid-state fermentation. Appl Biochem Biotechnol 162(7):1889–1900

Camassola M, De Bittencourt LR, Shenem NT, Andreaus J, Dillon AJP (2004) Characterization of the cellulases complex of Penicillium echinulatum. Biocatal Biotransformation 22(5–6):391–396

Cohen R, Suzuki MR, Hammel KE (2005) Processive endoglucanase active in crystalline cellulose hydrolysis by the brown rot basidiomycete Gloeophyllum trabeum. Appl Environ Microbiol 71(5):2412–2417

Coral G, Arikan B, Ünaldi MN, Güvenmez H (2002) Some properties of crude carboxymethyl cellulases of Aspergillus niger Z10 wild-type strain. Turk J Biol 26(4):209–213

da Silva Delabona P, Farinas CS, da Silva MR, Azzoni SF, da Cruz Pradella JG (2012) Use of a new Trichoderma harzianum strain isolated from the Amazon rainforest with pretreated sugarcane bagasse for on-site cellulases production. Bioresour Technol 107:517–521

de Castro AM, de Carvalho MLDA, Leite SGF, Pereira N (2010) Cellulases from Penicillium funiculosum: production, properties and application to cellulose hydrolysis. J Ind Microbiol Biotechnol 37(2):151–158

Davies GJ, Brzozowski AM, Dauter M, Varrot A, Schülein M (2000) Structure and function of Humicola insolens family 6 cellulases: structure of the endoglucanase, Cel6B, at 1.6 Å resolution. Biochem J 348(1):201–207

Decker C, Visser J, Schreier P (2001) β-glucosidase multiplicity from Aspergillus tubingensis CBS 643.92: purification and characterization of four β-glucosidases and their differentiation with respect to substrate specificity, glucose inhibition and acid tolerance. Appl Microbiol Biotechnol 55(2):157–163

Den Haan R, Rose SH, Lynd LR, van Zyl WH (2007) Hydrolysis and fermentation of amorphous cellulose by recombinant *Saccharomyces cerevisiae*. Metab Eng 9(1):87–94

Desvaux M (2005) Clostridium cellulolyticum: model organism of mesophilic cellulolytic clostridia. FEMS Microbiol Rev 29(4):741–764

Deswal D, Khasa YP, Kuhad RC (2011) Optimization of cellulases production by a brown rot fungus Fomitopsis sp. RCK2010 under solid state fermentation. Bioresour Technol 102(10):6065–6072

Deswal D, Gupta R, Nandal P, Kuhad RC (2014) Fungal pretreatment improves amenability of lignocellulosic material for its saccharification to sugars. Carbohydr Polym 99:264–269

Dillon AJP, Bettio M, Pozzan FG, Andrighetti T, Camassola M (2011) A new Penicillium echinulatum strain with faster cellulases secretion obtained using hydrogen peroxide mutagenesis and screening with 2-deoxyglucose. J Appl Microbiol 111(1):48–53

Dimarogona M, Topakas E, Olsson L, Christakopoulos P (2012) Lignin boosts the cellulases performance of a GH-61 enzyme from Sporotrichum thermophile. Bioresour Technol 110:480–487

Dogaris I, Vakontios G, Kalogeris E, Mamma D, Kekos D (2009) Induction of cellulases and hemicellulases from Neurospora crassa under solid-state cultivation for bioconversion of sorghum bagasse into ethanol. Ind Crop Prod 29(2–3):404–411

Dutta T, Sahoo R, Sengupta R, Ray SS, Bhattacharjee A, Ghosh S (2008) Novel cellulases from an extremophilic filamentous fungi Penicillium citrinum: production and characterization. J Ind Microbiol Biotechnol 35(4):275–282

e Silva LAD, Lopes FC, Silveira ST, Brandelli A (2009) Production of cellulolytic enzymes by Aspergillus phoenicis in grape waste using response surface methodology. Appl Biochem Biotechnol 152(2):295–305

El-Katatny M, Gudelj M, Robra KH, Elnaghy M, Gübitz G (2001) Characterization of a chitinase and an endo-β-1, 3-glucanase from Trichoderma harzianum Rifai T24 involved in control of the phytopathogen Sclerotium rolfsii. Appl Microbiol Biotechnol 56(1–2):137–143

Eshel D, Lichter A, Dinoor A, Prusky D (2002) Characterization of *Alternaria alternata* glucanase genes expressed during infection of resistant and susceptible persimmon fruits. Mol Plant Pathol 3(5):347–358

Gao J, Weng H, Zhu D, Yuan M, Guan F, Xi Y (2008) Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal Aspergillus terreus M11 under solid-state cultivation of corn stover. Bioresour Technol 99(16):7623–7629

Gomathi D, Muthulakshmi C, Kumar DG, Ravikumar G, Kalaiselvi M, Uma C (2012) Submerged fermentation of wheat bran by Aspergillus flavus for production and characterization of carboxy methyl cellulases. Asian Pac J Trop Biomed 2(1):S67–S73

Guedon E, Desvaux M, Petitdemange H (2002) Improvement of cellulolytic properties of Clostridium cellulolyticum by metabolic engineering. Appl Environ Microbiol 68(1):53–58

Haakana H, Miettinen-Oinonen A, Joutsjoki V, Mäntylä A, Suominen P, Vehmaanperä J (2004) Cloning of cellulases genes from Melanocarpus albomyces and their efficient expression in Trichoderma reesei. Enzym Microb Technol 34(2):159–167

Herculano PN, Porto TS, Moreira KA, Pinto GA, Souza-Motta CM, Porto ALF (2011) Cellulases production by Aspergillus japonicus URM5620 using waste from castor bean (*Ricinus communis* L.) under solid-state fermentation. Appl Biochem Biotechnol 165(3–4):1057–1067

Herculano PN, Porto TS, Maciel MH, Moreira KA, Souza-Motta CM, Porto AL (2012) Partitioning and purification of the cellulolytic complex produced by Aspergillus japonicus URM5620 using PEG–citrate in an aqueous two-phase system. Fluid Phase Equilib 335:8–13

Higashide W, Li Y, Yang Y, Liao JC (2011) Metabolic engineering of Clostridium cellulolyticum for isobutanol production from cellulose. Appl Environ Microbiol 77:2727

Highley TL, Wolter KE, Evans FJ (2007) Polysaccharide-degrading complex produced in wood and in liquid media by the brown-rot fungus Poria placenta. Wood Fiber Sci 13(4):265–274

Hirvonen M, Papageorgiou AC (2003) Crystal structure of a family 45 endoglucanase from Melanocarpus albomyces: mechanistic implications based on the free and cellobiose-bound forms. J Mol Biol 329(3):403–410

Hu HL, Van den Brink J, Gruben BS, Wösten HAB, Gu JD, De Vries RP (2011) Improved enzyme production by co-cultivation of Aspergillus niger and Aspergillus oryzae and with other fungi. Int Biodeter Biodegr 65(1):248–252

Hui L, Wan C, Hai-Tao D, Xue-Jiao C, Qi-Fa Z, Yu-Hua Z (2010) Direct microbial conversion of wheat straw into lipid by a cellulolytic fungus of Aspergillus oryzae A-4 in solid-state fermentation. Bioresour Technol 101(19):7556–7562

Immanuel G, Bhagavath CMA, Raj PI, Esakkiraj P, Palavesam A (2007) Production and partial purification of cellulases by Aspergillus niger and *A. fumigatus* fermented in coir waste and sawdust. Internet J Microbiol 3(1):1–20

Jeya M, Zhang YW, Kim IW, Lee JK (2009) Enhanced saccharification of alkali-treated rice straw by cellulases from Trametes hirsuta and statistical optimization of hydrolysis conditions by RSM. Bioresour Technol 100(21):5155–5161

Jeya M, Moon HJ, Kim SH, Lee JK (2010b) Conversion of woody biomass into fermentable sugars by cellulases from Agaricus arvensis. Bioresour Technol 101(22):8742–8749

Jørgensen H, Olsson L (2006) Production of cellulases by Penicillium brasilianum IBT 20888—Effect of substrate on hydrolytic performance. Enzym Microb Technol 38(3–4):381–390

Jørgensen H, Eriksson T, Börjesson J, Tjerneld F, Olsson L (2003) Purification and characterization of five cellulases and one xylanase from Penicillium brasilianum IBT 20888. Enzym Microb Technol 32(7):851–861

Jung DU, Yoo HY, Kim SB, Lee JH, Park C, Kim SW (2015) Optimization of medium composition for enhanced cellulases production by mutant Penicillium brasilianum KUEB15 using statistical method. J Ind Eng Chem 25:145–150

Kajisa T, Yoshida M, Igarashi K, Katayama A, Nishino T, Samejima M (2004) Characterization and molecular cloning of cellobiose dehydrogenase from the brown-rot fungus Coniophora puteana. J Biosci Bioeng 98(1):57–63

Kajisa T, Igarashi K, Samejima M (2009) The genes encoding glycoside hydrolase family 6 and 7 cellulases from the brown-rot fungus Coniophora puteana. J Wood Sci 55(5):376

Kalogeris E, Christakopoulos P, Katapodis P, Alexiou A, Vlachou S, Kekos D, Macris BJ (2003) Production and characterization of cellulolytic enzymes from the thermophilic fungus Thermoascus aurantiacus under solid state cultivation of agricultural wastes. Process Biochem 38(7):1099–1104

Karimi K, Emtiazi G, Taherzadeh MJ (2006) Ethanol production from dilute-acid pretreated rice straw by simultaneous saccharification and fermentation with Mucor indicus, Rhizopus oryzae, and *Saccharomyces cerevisiae*. Enzym Microb Technol 40(1):138–144

Kaur G, Satyanarayana T (2004) Production of extracellular pectinolytic, cellulolytic and xylanoytic enzymes by thermophilic mould Sporotrichum thermophile Apinis in solid state fermentation. Indian J Biotechnol 3(4):552–557

Kluczek-Turpeinen B, Maijala P, Tuomela M, Hofrichter M, Hatakka A (2005) Endoglucanase activity of compost-dwelling fungus Paecilomyces inflatus is stimulated by humic acids and other low molecular mass aromatics. World J Microbiol Biotechnol 21(8–9):1603

Koseki T, Mese Y, Fushinobu S, Masaki K, Fujii T, Ito K et al (2008) Biochemical characterization of a glycoside hydrolase family 61 endoglucanase from Aspergillus kawachii. Appl Microbiol Biotechnol 77(6):1279

Kotaka A, Bando H, Kaya M, Kato-Murai M, Kuroda K, Sahara H et al (2008) Direct ethanol production from barley β-glucan by sake yeast displaying Aspergillus oryzae β-glucosidase and endoglucanase. J Biosci Bioeng 105(6):622–627

Kovács K, Megyeri L, Szakacs G, Kubicek CP, Galbe M, Zacchi G (2008) Trichoderma atroviride mutants with enhanced production of cellulases and β-glucosidase on pretreated willow. Enzym Microb Technol 43(1):48–55

Kovacs K, Macrelli S, Szakacs G, Zacchi G (2009) Enzymatic hydrolysis of steam-pretreated lignocellulosic materials with Trichoderma atroviride enzymes produced in-house. Biotechnol Biofuels 2(1):14

Kovács K, Szakacs G, Zacchi G (2009) Comparative enzymatic hydrolysis of pretreated spruce by supernatants, whole fermentation broths and washed mycelia of Trichoderma reesei and Trichoderma atroviride. Bioresour Technol 100(3):1350–1357

Krishna SH, Rao KS, Babu JS, Reddy DS (2000) Studies on the production and application of cellulases from Trichoderma reesei QM-9414. Bioprocess Eng 22(5):467–470

Krogh KB, Harris PV, Olsen CL, Johansen KS, Hojer-Pedersen J, Borjesson J, Olsson L (2010) Characterization and kinetic analysis of a thermostable GH3 β-glucosidase from Penicillium brasilianum. Appl Microbiol Biotechnol 86(1):143–154

Lee SM, Koo YM (2001) Pilot-scale production of cellulases using Trichoderma reesei Rut C-30 fed-batch mode. J Microbiol Biotechnol 11(2):229–233

Leghlimi H, Meraihi Z, Boukhalfa-Lezzar H, Copinet E, Duchiron F (2013) Production and characterization of cellulolytic activities produced by Trichoderma longibrachiatum (GHL). Afr J Biotechnol 12(5):465–475

Li DC, Lu M, Li YL, Lu J (2003) Purification and characterization of an endocellulases from the thermophilic fungus Chaetomium thermophilum CT2. Enzym Microb Technol 33(7):932–937

Lockington RA, Rodbourn L, Barnett S, Carter CJ, Kelly JM (2002) Regulation by carbon and nitrogen sources of a family of cellulases in Aspergillus nidulans. Fungal Genet Biol 37(2):190–196

Maeda RN, Serpa VI, Rocha VAL, Mesquita RAA, Santa Anna LMM, De Castro AM et al (2011) Enzymatic hydrolysis of pretreated sugarcane bagasse using Penicillium funiculosum and Trichoderma harzianum cellulases. Process Biochem 46(5):1196–1201

Mariyam I (2011) Multistep mutagenesis for the over-expression of cellulases in Humicola insolens. Pak J Bot 43(1):669–677

Martinez D, Larrondo LF, Putnam N, Gelpke MDS, Huang K, Chapman J et al (2004) Genome sequence of the lignocellulose degrading fungus Phanerochaete chrysosporium strain RP78. Nat Biotechnol 22(6):695

Martins LF, Kolling D, Camassola M, Dillon AJP, Ramos LP (2008) Comparison of Penicillium echinulatum and Trichoderma reesei cellulases in relation to their activity against various cellulosic substrates. Bioresour Technol 99(5):1417–1424

Miettinen-Oinonen A, Londesborough J, Joutsjoki V, Lantto R, Vehmaanperä J, Biotec PL (2004) Three cellulases from Melanocarpus albomyces for textile treatment at neutral pH. Enzym Microb Technol 34(3–4):332–341

Milala MA, Shehu BB, Zanna H, Omosioda VO (2009) Degradation of agro-waste by cellulases from Aspergillus candidus. Asian J Biotechnol 1(2):51–56

Mølhøj M, Ulvskov P, Dal Degan F (2001) Characterization of a functional soluble form of a *Brassica napus* membrane-anchored endo-1, 4-β-glucanase heterologously expressed in Pichia pastoris. Plant Physiol 127(2):674–684

Mrudula S, Murugammal R (2011) Production of cellulases by Aspergillus niger under submerged and solid state fermentation using coir waste as a substrate. Braz J Microbiol 42(3):1119–1127

Murashima K, Nishimura T, Nakamura Y, Koga J, Moriya T, Sumida N et al (2002) Purification and characterization of new endo-1, 4-β-D-glucanases from Rhizopus oryzae. Enzym Microb Technol 30(3):319–326

Narra M, Dixit G, Divecha J, Madamwar D, Shah AR (2012) Production of cellulases by solid state fermentation with Aspergillus terreus and enzymatic hydrolysis of mild alkali-treated rice straw. Bioresour Technol 121:355–361

Nascimento CV, Souza FHM, Masui DC, Leone FA, Peralta RM, Jorge JA, Furriel RPM (2010) Purification and biochemical properties of a glucose-stimulated β-D-glucosidase produced by Humicola grisea var. thermoidea grown on sugarcane bagasse. J Microbiol 48(1):53–62

Ng IS, Li CW, Chan SP, Chir JL, Chen PT, Tong CG et al (2010) High-level production of a thermoacidophilic β-glucosidase from Penicillium citrinum YS40-5 by solid-state fermentation with rice bran. Bioresour Technol 101(4):1310–1317

Niemenmaa O, Uusi-Rauva A, Hatakka A (2008) Demethoxylation of [O 14 CH 3]-labelled lignin model compounds by the brown-rot fungi Gloeophyllum trabeum and Poria (Postia) placenta. Biodegradation 19(4):555

Niranjane AP, Madhou P, Stevenson TW (2007) The effect of carbohydrate carbon sources on the production of cellulases by Phlebia gigantea. Enzym Microb Technol 40(6):1464–1468

Obodai M, Cleland-Okine J, Vowotor KA (2003) Comparative study on the growth and yield of Pleurotus ostreatus mushroom on different lignocellulosic by-products. J Ind Microbiol Biotechnol 30(3):146–149

Obruca S, Marova I, Matouskova P, Haronikova A, Lichnova A (2012) Production of lignocellulose-degrading enzymes employing *Fusarium solani* F-552. Folia Microbiol 57(3):221–227

Ojumu TV, Solomon BO, Betiku E, Layokun SK, Amigun B (2003) Cellulases production by Aspergillus flavus Linn isolate NSPR 101 fermented in sawdust, bagasse and corncob. Afr J Biotechnol 2(6):150–152

Omojasola PF, Jilani OP (2008) Cellulases production by Trichoderma longi, Aspergillus niger and *Saccharomyces cerevisiae* cultured on waste materials from orange. Pak J Biol Sci 11(20):2382–2388

Panagiotou G, Kekos D, Macris BJ, Christakopoulos P (2003) Production of cellulolytic and xylanolytic enzymes by Fusarium oxysporum grown on corn stover in solid state fermentation. Ind Crop Prod 18(1):37–45

Panagiotou G, Christakopoulos P, Olsson L (2005) Simultaneous saccharification and fermentation of cellulose by Fusarium oxysporum F3—growth characteristics and metabolite profiling. Enzym Microb Technol 36(5–6):693–699

Panagiotou G, Granouillet P, Olsson L (2006) Production and partial characterization of arabinoxylan-degrading enzymes by Penicillium brasilianum under solid-state fermentation. Appl Microbiol Biotechnol 72(6):1117–1124

Park EY, Anh PN, Okuda N (2004) Bioconversion of waste office paper to L (+)-lactic acid by the filamentous fungus Rhizopus oryzae. Bioresour Technol 93(1):77–83

Parkkinen T, Koivula A, Vehmaanperä J, Rouvinen J (2008) Crystal structures of Melanocarpus albomyces cellobiohydrolase Cel7B in complex with cello-oligomers show high flexibility in the substrate binding. Protein Sci 17(8):1383–1394

Pessani NK, Atiyeh HK, Wilkins MR, Bellmer DD, Banat IM (2011) Simultaneous saccharification and fermentation of Kanlow switchgrass by thermotolerant Kluyveromyces marxianus IMB3: the effect of enzyme loading, temperature and higher solid loadings. Bioresour Technol 102(22):10618–10624

Phillips CM, Beeson WT IV, Cate JH, Marletta MA (2011) Cellobiose dehydrogenase and a copper-dependent polysaccharide monooxygenase potentiate cellulose degradation by Neurospora crassa. ACS Chem Biol 6(12):1399–1406

Qin Y, He H, Li N, Ling M, Liang Z (2010) Isolation and characterization of a thermostable cellulases-producing Fusarium chlamydosporum. World J Microbiol Biotechnol 26(11):1991–1997

Ramanathan G, Banupriya S, Abirami D (2010) Production and optimization of cellulases from Fusarium oxysporum by submerged fermentation. J Sci Ind Res 69(6):454–459

Reddy GV, Babu PR, 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(10):1457–1462

Rocky-Salimi K, Hamidi-Esfahani Z (2010) Evaluation of the effect of particle size, aeration rate and harvest time on the production of cellulases by Trichoderma reesei QM9414 using response surface methodology. Food Bioprod Process 88(1):61–66

Saha BC (2004) Production, purification and properties of endoglucanase from a newly isolated strain of Mucor circinelloides. Process Biochem 39(12):1871–1876

Schmidt H, Taniwaki MH, Vogel RF, Niessen L (2004) Utilization of AFLP markers for PCR-based identification of Aspergillus carbonarius and indication of its presence in green coffee samples. J Appl Microbiol *97*(5):899–909

Sehnem NT, de Bittencourt LR, Camassola M, Dillon AJ (2006) Cellulases production by Penicillium echinulatum on lactose. Appl Microbiol Biotechnol 72(1):163–167

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

Shimokawa T, Shibuya H, Nojiri M, Yoshida S, Ishihara M (2008) Purification, molecular cloning, and enzymatic properties of a family 12 endoglucanase (EG-II) from Fomitopsis palustris: role of EG-II in larch holocellulose hydrolysis. Appl Environ Microbiol 74(18):5857–5861

Singh A, Singh N, Bishnoi NR (2009a) Production of cellulases by Aspergillus heteromorphus from wheat straw under submerged fermentation. Int J Environ Sci Eng 1:1

Singh R, Kumar R, Bishnoi K, Bishnoi NR (2009b) Optimization of synergistic parameters for thermostable cellulases activity of Aspergillus heteromorphus using response surface methodology. Biochem Eng J 48(1):28–35

Singhania RR, Sukumaran RK, Pillai A, Prema P, Szakacs G, Pandey A (2006) Solid-state fermentation of lignocellulosic substrates for cellulases production by Trichoderma reesei NRRL 11460. Indian J Biotechnol 5(3):332–336

Singhania RR, Saini JK, Saini R, Adsul M, Mathur A, Gupta R, Tuli DK (2014) Bioethanol production from wheat straw via enzymatic route employing Penicillium janthinellum cellulases. Bioresour Technol 169:490–495

Suryawati L, Wilkins MR, Bellmer DD, Huhnke RL, Maness NO, Banat IM (2009) Effect of hydrothermolysis process conditions on pretreated switchgrass composition and ethanol yield by SSF with Kluyveromyces marxianus IMB4. Process Biochem 44(5):540–545

Szijártó N, Siika-aho M, Tenkanen M, Alapuranen M, Vehmaanperä J, Réczey K, Viikari L (2008) Hydrolysis of amorphous and crystalline cellulose by heterologously produced cellulases of Melanocarpus albomyces. J Biotechnol 136(3–4):140–147

Takashima S, Ohno M, Hidaka M, Nakamura A, Masaki H, Uozumi T (2007) Correlation between cellulose binding and activity of cellulose-binding domain mutants of Humicola grisea cellobiohydrolase 1. FEBS Lett 581(30):5891–5896

Taniguchi M, Suzuki H, Watanabe D, Sakai K, Hoshino K, Tanaka T (2005) Evaluation of pretreatment with Pleurotus ostreatus for enzymatic hydrolysis of rice straw. J Biosci Bioeng 100(6):637–643

Tao YM, Zhu XZ, Huang JZ, Ma SJ, Wu XB, Long MN, Chen QX (2010) Purification and properties of endoglucanase from a sugarcane bagasse hydrolyzing strain, Aspergillus glaucus XC9. J Agric Food Chem 58(10):6126–6130

Tian C, Beeson WT, Iavarone AT, Sun J, Marletta MA, Cate JH, Glass NL (2009) Systems analysis of plant cell wall degradation by the model filamentous fungus Neurospora crassa. *Proc Nat Acad Sci* 106:22157–22162

Tomás-Pejó E, García-Aparicio M, Negro MJ, Oliva JM, Ballesteros M (2009) Effect of different cellulases dosages on cell viability and ethanol production by Kluyveromyces marxianus in SSF processes. Bioresour Technol 100(2):890–895

Turner MB, Spear SK, Huddleston JG, Holbrey JD, Rogers RD (2003) Ionic liquid salt-induced inactivation and unfolding of cellulases from Trichoderma reesei. Green Chem 5(4):443–447

Valášková V, Baldrian P (2006) Estimation of bound and free fractions of lignocellulose-degrading enzymes of wood-rotting fungi Pleurotus ostreatus, Trametes versicolor and Piptoporus betulinus. Res Microbiol 157(2):119–124

Van Wyk JPH, Mohulatsi M (2003) Biodegradation of wastepaper by cellulases from Trichoderma viride. Bioresour Technol 86(1):21–23

Chapter 3
Comparative Study of Cellulase Production Using Submerged and Solid-State Fermentation

Prabhakara Rao Dasari, Pramod W. Ramteke, Satyam Kesri, and Prasada Rao Kongala

3.1 Introduction

Cellulases have been studied for most of the twentieth century. The cost of cellulase production may be brought down by using cheap lignocellulosic substrates and by using cost-effective fermentation strategies like solid-state fermentation. Cellulases have wide mechanical and economical applications. The applications of cellulases are present in nourishment, material, creature nourish, fuel, paper, chemical businesses and mash industry, squander administration, protoplast generation, medical/pharmaceutical industry, hereditary designing, contamination treatment of pectinases are in juice preparing, handling of alcoholic refreshments, vegetable oil extraction and an assortment of application in nourishment businesses (Sukumaran et al. 2009).

The Submerged Fermentation is one type of fermentation method, which is utilized for the industrial enzyme production because of it's for process and separation. It utilizes the liquid substances such as molasses and broth. The biomolecules are secreted into fermentation broth, and then, it's easy to purify it. This process is highly suitable for microbes, e.g., bacteria and fungus. In this fermentation, yield differs for each substrate, so it is important to select the correct substrate and conditions for fermentation to be optimized (Limayem and Ricke 2012; Du et al. 2012). Cellulase production is carried out in aerobic aseptic culture. The growth medium contains salts, nutrients, surfactant, and inducers which are required for the fungus to survive and grow in medium. The important metal ions which are required for

P. R. Dasari · S. Kesri · P. R. Kongala
Department of Chemistry, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad, Department of Biological Sciences, Sam Higginbottom University of Agriculture Technology & Sciences, Prayagraj, Uttar Pradesh, India

P. W. Ramteke (✉)
Department of Biological Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Allahabad, Uttar Pradesh, India

M. Srivastava et al. (eds.), *Approaches to Enhance Industrial Production of Fungal Cellulases*, Fungal Biology,
https://doi.org/10.1007/978-3-030-14726-6_3

cellulose production are iron, cobalt, copper, magnesium, calcium, potassium, manganese, and ammonium. The microbial contamination is avoided using antibiotics and by sterilization of equipment before inoculation. The progress in cellulose production was averaged doubling every 2 years due to the strain improvement and optimization of fermentation process (Jabasingh and Nachiyar 2011; Graminha et al. 2008; Romero-Gómez et al. 2000).

Solid-state fermentation has emerged as a potential technology for the production of microbial cellulase. SSF refers to the process where microbial growth and product formation occur on the surface of solid materials. This process involves the absence (or near absence) of "free" water; however, the moisture is absorbed to the solid substrate to support growth and microbial metabolism (Da Silva et al. 2012; Coradi et al. 2013; Ang et al. 2013; Pirota et al. 2014). The use of solid-state fermentation to upgrade the nutritive value of agricultural by-products and for enzyme production has been increased due to the higher quantity of residues produced in several countries, representing a potential solution to feeding animals in developing countries. The advantage of using SSF to achieve both goals is the low-tech fermentation system required plus the possibility of having it carried out on farms (Robinson and Nigam 2003; Robinson et al. 2001; Hafiz Muhammad Nasir et al. 2011). The fermentation methods such as SSF and SmF processes are attractive methods to produce cellulase. In this context, several groups of researchers examined the production of microbial cellulases in SmF and SSF processes. These SSF and SmF cultivation with sugarcane bagasse as substrate for cellulase production (Agrawal et al. 2013; Florencio et al. 2015; Ray 2011).

3.2 Cellulase Production by Submerged Fermentation

Cellulase alludes to a bunch of hydrolytic proteins competent of hydrolyzing cellulose to glucose. The cellulolytic proteins are created by a huge number of the microorganisms counting parasites and microbes. Generally in microorganisms, chemicals are either cell-bound cellular. The capacity to deliver additional cellular cellulolytic proteins is far-reaching in organisms, and these protein frameworks have been broadly considered (Enari 1983). Due to effective potential and many more extensive applications of cellulases, it is essential to consider a few viewpoints related to their generation. The thought of utilizing cheaper crude materials for pectinase and cellulase generation is a vital parameter in valuable mechanical improvement. There have been a few ponders distributed as of late concerning the cellulase generation exclusively from distinctive cheaper crude materials beneath submerged and solid-state aging (Debing et al. 2006; Acharya et al. 2008; Datta et al. 1989; Palaniyappan et al. 2009). Be that as it may, the generation of cellulase by the same living being beneath the same maturation conditions is required to be examined. Subsequently ponder and bargains showed that the optimization of aging conditions utilizing diverse carbon sources with regard to ideal abdicate of cellulase (Kumar et al. 2011; Grajek 1987).

Cellulases are a combination of hydrolytic chemical channels paperase, carboxymethyl cellulase, and glucosidase and are capable of discharging sugars within the

bioconversion of the lignocellulosic biomass into an assortment of esteem-included items. The cellulase produced by *Aspergillus niger* on person lignocellulosic substrates in both submerged and also Solid State Fermentations. Rice bran backed most extreme chemical yields taken after by wheat bran in both fermentations. Among different combinations with rice bran tried, combination of rice bran and wheat bran served the best combination for production of cellulolytic enzymes (Reddy et al. 2015).

Cellulose is the foremost copiously accessible biomass on Earth. Cellulose is the most important material of photosynthesis in earthly situations, and most copious renewable bio-source created in biosphere. Cellulose is commonly corrupted by a protein cellulase. Cellulases are utilized within the nourishment, material, clothing, heating, brewing, mash and paper businesses from biomass and hereditary building (Emert and Katzen 1980; Lonsane and Ramakrishna 1989; Ragauskas et al. 2006; Bhat 2000; Kirk et al. 2002; Padmavathi et al. 2012). Cellulase is an important extracellular microbial protein, which hydrolyzes cellulose. The foremost reasonable sources of biomass utilized for the generation of squeezing within the natural product juice industry and other manufacturing plants through protein bioconversion, which demonstrates to have a tall mechanical esteem. An extraordinary number of microorganisms, generally parasites, are able to debase cellulose for their development and delivered a total set of cellulose for the hydrolysis of cellulose to dissolvable sugar (Shobana and Maheswari 2013; Coughlan 1985; Pandey 1992; Hong et al. 2001).

In the present days context the bioconversions of cellulose materials are major problem to investigate the advancement of a large-scale transformation prepare useful to mankind. Reduce the deficiencies of nourishment and creature bolsters, fathom present day squander transfer issue and lessen man's reliance on fossil powers by giving a helpful and capable of being renewed source of vitality within the frame of glucose. The different range of cellulose producing microorganism. A few of creators have been confined and distinguished over a long time, and they still proceed to develop quickly. One of the foremost broadly examined organisms is *Trichoderma reesei*, which changes over local as well as inferred cellulose to glucose. The cellulose producing microorganisms such as *Trichoderma* sp, *Humicola* sp, *Penicillium* sp, and *Aspergillus* sp. In this examination, a cellulase creating strain of *Trichoderma viride*, confined from metropolitan solid squander, was subjected to optimization of media and development parameters for cellulase generation (Coughlan 1985; Pandey 1992; Hong et al. 2001). The Lignocellulolytic biomass, is a rich source of carbon for microbial chemical generation; and lignocellulosic biomass, such as corn stover, wheat bran, corn cob, exceptionally plenteous, cheap and effectively accessible. Different rural microbial and substrate by-product societies have been effectively utilized within the solid-state maturation for cellulase generation (Yang et al. 2006; Isaac and Abu-Tahon 2015; Sun and Cheng 2002).

Cellulase can proficiently corrupt the complex polysaccharide components of lignocellulosic into monomeric sugars, which could be a key preparation in biomass bioconversion. The cellulase generation, be that as it may, remains a major deterrent that ruins scale-up of biotransformation of lignocellulose. The procedure of cellulase generation would be of extraordinary esteem for the productive utilization of lignocellulose and the decrease of cellulase taken a toll (Yang et al. 2006; Isaac and Abu-Tahon 2015; Klein-Marcuschamer et al. 2012; Lee et al. 2007). All inclusive

the evaluated amount of the squanders era was 12 billion tones in the year 2002 of which 11 billion tones were mechanical squanders and 1.6 billion tones were civil solid squanders. Around 90 billion tons of solid squanders are anticipated to be created every year by the year 2025. Yearly, Asia alone produces 4.4 billion tons of solid squanders, and metropolitan solid squander comprises 790 million tons of which approximately 48 million tons are produced in India. Informal transfer of squander causes an unfavorable effect on all the components of environment and human well-being. Agrarian squander is additionally caused by natural contamination (Gautam et al. 2010; Lawal and Ugheoke 2010).

Some of agro-industrial squanders are renewable shape of assets created circular to over the world. Wheat, rice bran, corn husks, sugar cane bagasse, corn cobs, citrus, mango peel, etc. are vital squanders of nourishment businesses. Around 63% of cellulase substances (Pandey et al. 2000), corn husks speak to an alluring substrate for the cellulase generation. The micro-organisms for bioconversion and bio-waste into esteem included items has been highlighted within the later decades, particularly in chemical preparations (Ahuja et al. 2004; Jalak et al. 2012). Among such chemicals cellulases are picking up notoriety in this respect. These proteins are for the most part considered to comprise of three chemical bunches for the cellulose hydrolysis into the glucose monomers, specifically endoglucanases, exoglucanases, and cellobioses. Collaboration between proteins is critical for hydrolysis handle. Cellulases have a variety of applications in numerous distinctive businesses such as nourishment, brewery, mash, wine, paper, material, cleanser, and horticulture (Nema et al. 2015; Karmakar and Ray 2011; Bhat 2000; Ming et al. 2008).

Cellulose is the cheapest and the foremost inexhaustible renewable assets on soil and it is a successful way for moo carbon and economic advancement to create fuel, nourish and chemical items by aging with agrarian squanders. The cellulose plays a vital part in a few zones counting utilize of renewable assets (Gutierrez-Correa et al. 1999; Sukumaran et al. 2009), advancement of bioenergy (Lever et al. 2010; Kuhad et al. 2010) and security of environment (Zhiyou et al. 2004; Singhania et al. 2010). There are two ways for the generation of cellulose. One is solid-state aging, and another is submerged maturation. Both of these two strategies have focal points; solid-state maturation is much better in utilizing reactor and chemical generation. Hence, mechanical generation of cellulose is more as often as possible done by the strategy of solid-state maturation (Liu et al. 2012; Pandey 2003). Solid-state submerged aging holds huge potential for generation of proteins and other things. Agro-industrial buildups or squanders are by and large considered as reasonable substrates for the generation of proteins particularly cellulases in SSF and SmF handle. Among these fermentation methods, the SmF and SSF have been broadly utilized for the generation of proteins and to get it physiological angles of the blend of proteins (Patil and Dayanand 2006). These two Submerged Fermentation (SmF) and Solid State Fermentation (SSF) were uses shown in Fig. 3.1 and it was collected from (https://www.biofueljournal.com/article_53201.html).

3.3 Cellulase Production by Solid-State Fermentation

Solid-state fermentation utilizes solid substrates like bagasse, bran, and paper pulp. The main advantage of using these substrates is nutrient-rich waste materials can be easily recycled as substrates. In this fermentation technique, these substrates are utilized very slowly and steadily, so the similar substrate can be used for long fermentation periods. Hence, this technique supports controlled release of some nutrients. SSF is best suited for fermentation techniques involving the fungi and microorganisms that require very less moisture content. However, it can't be utilized in fermentation processes and involving many organisms that require high water activity and bacteria (Subramaniyam and Vimala 2012; Babu and Satyanarayana 1996).

Solid-state maturation utilizes solid substrates, like bran, bagasse, and paper mash. The most advantage of utilizing these substrates is that nutrient-rich squander materials can be effectively reused as substrates. In this aging strategy, the substrates are utilized exceptionally gradually and relentlessly, so the similar substrate can be utilized for long aging periods. Bolsters were controlled discharge of supplements. SSF is best suited for aging procedures including organisms and microorganisms that require less dampness substance. In any case, it cannot be utilized in aging forms including life forms that require water movement, such as microbes (Babu and Satyanarayana 1996). The framework has been refined in see of diverse param-

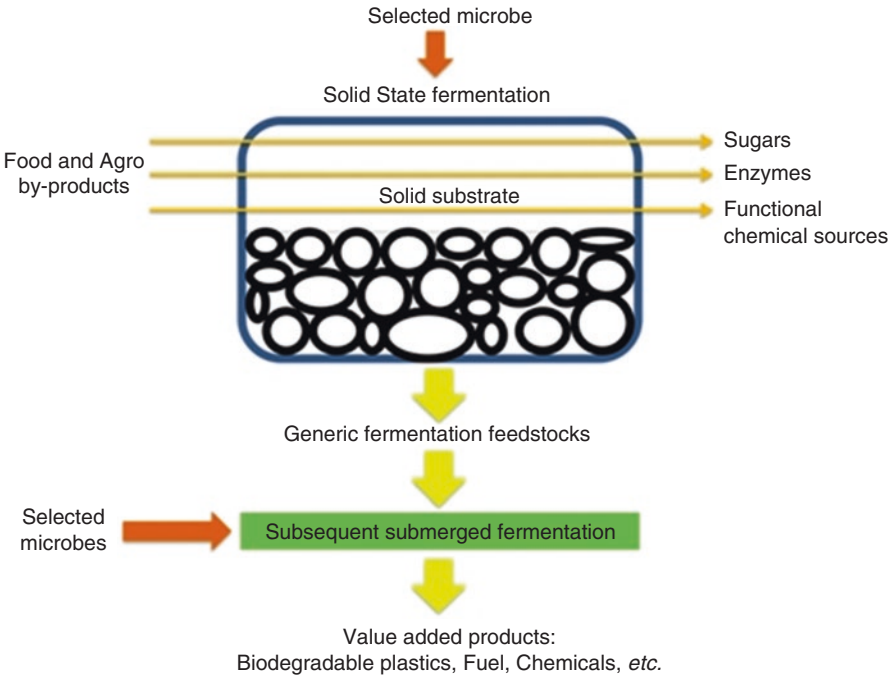

Fig. 3.1 Submerged fermentation (SmF) and solid-state fermentation (SSF)

eters, for case, the substrates utilized, environmental parameters and the life shapes utilized for development. The bioactive blends were watched to be the made in higher sums in SSF, in spite of the fact that diverse blends have been removed utilizing SmF. Development has been requested into SSF and SmF for the foremost portion in light of the kind of substrate utilized in the midst of maturing (Demain 1999; Basha et al. 2009).

Solid-state advancement, a plausibility for submerged developing for compound creation, was seen to be more exceptional, which can be performed beneath compelled budgetary and work necessities. Agro-industrial residuals have conceivably been utilized as substrate in SSF for protein creation and additionally other optional metabolites. In this perspective, among agro industries particularly oil palm industry, will be at remarkable favoured point of see. In these expansion, different examination hones cantered on SSF, which had incite a wide collection of utilizations at ask approximately office scale additionally at pilot and mechanical scale (Gupte and Madamwar 1997; Kotwal et al. 1998; Sekar and Balaraman 1998; Lee et al. 2011; Durand and Chereau 1988; Xue et al. 1992; Pandey 1991).

The bioreactor gives a control condition to enhancement and advancement of the microorganism that did characteristic responses (Pandey 1991). Of course, the choice of a fitting kind of bioreactor is besides noteworthy since bioreactor is the "heart" of the improvement strategy. Other than that, there are different sorts of SSF bioreactors, and each one of them is one of a kind (Mitchell et al. 2006). The cellulase compound utilizing *Aspergillus niger* USM AI 1 made on sugar adhere bagasse and palm portion cake (PKC). The effect of SSF method for compound creation have been asked almost. These included degree of substrate, sogginess of substance, discussion of course rate and time, blending rate, and blending control. In the interim, relative examinations with *Trichoderma reesei* have been done (Kotwal et al. 1998; Sekar and Balaraman 1998; Mitchell et al. 2006; Cunha et al. 2012). Dynamic solid-state and submerged headway with sugarcane bagasse as substrate for cellulase creation by *Aspergillus niger* A12 was overviewed by surveying endoglucanase action. A whimsical pre-culture with a covered up irresistible headway orchestrate beneath solid-state change was trailed by altering submerged improvement by including the fluid culture medium to the mycelium made on solid substrate. For examination, control tests were driven utilizing typical submerged enhancement (Cen and Xia 1999).

Solid-state developing presents different purposes of eagerness counting tall volumetric efficiency and unassumingly tall centralization of the proteins made. However, it would be consolidate a lower capital meander and lower working taken a toll. Another significant component of SSF is that it utilizes heterogeneous delayed consequences of agribusiness (for the most part agrarian stores) and side effects of agro-based organizations (Raimbault 1998). In solid-state development of cellulase age, cellulosic substrate goes approximately as both the carbon source and as an inducer for cellulase creation. The two microorganisms and advancements can utilize cellulose as a fundamental carbon source. Most diminutive life shapes, in any case, are unequipped for demolishing crystalline cellulose since their cellulase frameworks are inadequate. The cellulolytic impetuses made by one or two

of improvements for the most part join each one of the three sorts of manufactured compounds, so are to a extraordinary degree solid within the scarification of boundless pretreated lignocellulosic materials. Parasitic strains that make cellulases for the most part included *Trichoderma*, *Aspergillus*, *Penicillium*, and *Fusarium* genera. *Trichoderma reesei* (*T. reesei*) is a thermostatic widely utilized living being for the production of cellulolytic manufactured concoctions and has been broadly analyzed (Stockton et al. 1991; Liu and Tzeng 1998).

Some of variables are responsible for obliging the advancement of microorganisms. Working conditions like temperature, pH, and suddenness of substance are principal for microbial advancement and reasonable cellulolytic impulse framework creation within the middle of solid-state improvement. The strategy requests overhaul of essential parameters that impact microbial progression and thing plan. As regularly conceivable movement of different parameters could be a testing and dull errand. Reaction surface thinking (RSM) can be utilized to assess the criticalness of a few of components particularly when collaborations exist among components and are bewildering to select (Brijwani et al. 2010; Ishida et al. 2006). The strategy can be exhausted a sensible time scale. The illustrate reasonableness of solid state developing structure, for occurrence, a inactive plate bioreactor, utilizing blended social orders of *T. reesei* and *A. oryzae* with soybean edges and wheat grain as solid media beneath idealize handle conditions within the making of adjusted and irrelevant effort cellulolytic protein frameworks that can profitably hydrolyses lignocellulosic biomass for bioethanol and bioenergy (Ruth et al. 1999).

The genuine methodologies utilized as a portion of era of cellulolytic compounds by parasites are solid-state maturing and submerged culture of the microorganisms. Be that because it may, it is uncommonly exorbitant to provide cellulolytic catalysts by submerged development (Gupte and Madamwar 1997; Moo-Young et al. 1983). The centrality of solid-state maturing over submerged maturing can't be demonized. The production of cellulolytic compounds through SSF is moo and abdicate is tall. However, essentialness used is moo, it is simple to do and a small squander thing is delivered. Other than, cellulolytic chemicals made by SSF are more amassed within the way of life filtrate. The cellulolytic compounds produced by *P. ostreatus* and *P. pulmonarius* utilizing corn cob, saw build-up and rice wheat as substrates utilizing solid state development (Ekundayo et al. 2017; Raimbault 1988; Assamoi et al. 2008).

Solid-state maturing is for the foremost portion utilized for ordinary food planning and for parasitic proteins era. The solid maturing is known for a long time of protein, on a very basic level for gluco-amylase creation derivate from the ordinary Koji prepare portrayed by Takamine in 1914 as the Rotten Bran Prepare utilizing Aspergillus oryzae created on mix of wheat grain and rice. It was the foremost advanced handle for compound era by solid substrate development. Directly, many methods are delineated within the composing to convey parasitic catalysts (Neagu et al. 2012). Solid state development has been expanded re-established excitement from researchers as of late and is routinely utilized for the creation of proteins since of traditionalist and practical inclinations, for illustration, straightforwardness, moo capital costs for equipment and working, tall volumetric benefit, bring down space

necessities, and less requesting downstream planning (Wen et al. 2005; Singh et al. 2009). Cellulases contain a complex of proteins locked in with the characteristic debasement of cellulose, genuine polysaccharide of the plant cells. These enzymatic complexes can be alter over cellulose to the oligosaccharides and glucose. The microorganisms, for illustration, living beings and organisms are basic producers of cellulases. Substrate costs speak to a critical portion of the costs of cellulase era, and the utilization of impoverished biomass resources as substrates can decrease cellulase costs (Shweta 2015; Hesseltine 1972).

Successful utilization of cellulosic fabric as a limitless carbon source depends on the progression of fiscally achievable preparation developments for the era of cellulose. This catalyst has distinctive mechanical applications and is directly considered as noteworthy in gathering of advanced protein. The benefits of cellulase through solid state development, when differentiated with submerged maturing, the sorts of cellulolytic microorganisms and unmistakable agro-modern wastes utilized for cellulose creation. The biotechnological portion of cellulase investigation and their future prospects are moreover inspected (Hesseltine 1977).

Solid-state maturations are those methods in which all or parts of the substrate are in a solid state. They are more ordinary than liquid maturations since they approach the conditions beneath which most microorganisms create in nature. The social occasions of microorganisms, for illustration, some minuscule life forms and yeasts, create in liquids, a significant number of distinctive microorganisms gotten to be added to solid substrates. The genuine get-togethers of microorganisms utilized as a portion of maturing are infinitesimal living beings, actinomycetes, yeasts, and parasites. Solid-state development offers different focuses of intrigue for the era of mass engineered compounds and proteins (Hesseltine 1987; Roussos et al. 1994; Nampoothiri and Pandey 1996). Some of places has been an extending number of reports on the utilization of solid state development shapes for the creation of different microbial things (Pandey 1996; Pandey et al. 1999; Vandenberghe et al. 2000). This can be for the most part on the grounds that solid-state shapes have brought down essentialness of necessities and made significantly less wastewater and biological concerns since exchange is of solid wastes. The SSF progression of microorganisms in a moo water-condition on a non-dissolvable fabric that showings both as physical offer assistance and wellspring of supplements; besides it isn't critical to connect the portion of offer assistance and substrate however or maybe mirror the states of moo water activity and tall oxygen transference by utilizing a nutritiously dormant fabric sprinkled with a supplement course of action (Ghildyal et al. 1985; Hui et al. 2010).

Some chemicals have been gotten from the submerged aging since ease of dealing with and more noteworthy control of natural variables such as temperature and pH. In any case, solid state aging procedure can move forward abdicate and decreases the taken a toll of chemical generation. Filamentous organisms are foremost commonly utilized microorganisms in the SSF since they are capable to develop on solid materials with moo water substance (Biswas et al. 1990; Singhania et al. 2010). There are a few reports portraying utilization of agro-mechanical buildups for the generation of cellulose such as rice straw, wheat straw, and wheat bran as substrates (Goyal et al. 2008; Zeng and Chen 2009; Szendefy et al. 2006).

The SSF points incorporate predominant efficiency, basic method; moo capital speculation, moo vitality prerequisite and less water yield, way better item recuperation and need of froth construct up and detailed to be most fitting handle for creating nations (Mrudula and Murugammal 2011; Shweta 2015; Acharya et al. 2010).

The warm conductivity of the solid medium utilized in SSF confines the expulsion of abundant warm created by microbial digestion system, and thus SSF requires air circulation. The increment in temperature in bioreactors may lead to denaturation of thermolabile proteins. Besides, SSF forms lead to improvement of distinctive slopes (dampness, temperature, substrate concentration, and others) along the bioreactor, which may negatively impact the method. The most common disadvantage of SSF is subsequently the scale-up of the method, basically because of warm exchange and culture homogeneity issues. The pointed towards the improvement of bioreactors for SSF frameworks; be that as it may, the accessible data has not demonstrated any perfect bioreactor however. They are investigated issues related to cellulase prepare innovation, and a extraordinary bargain more work should be done some time recently it's down to earth applications are realized (Lever et al. 2010; Roussos 1989; Sadhu and Maiti 2013).

Cellulases were customarily created utilizing the submerged aging, in which the microorganisms are developed in a watery environment containing supplements. Contrastingly, solid-state maturation may be a handle whereby an insoluble substrate is aged with adequate dampness but within the nonattendance of free-flowing water. The moo dampness substance implies that maturation can as it were be carried out by a constrained number of microorganisms, basically yeasts, parasites, and a few microbes. The past decade has taken note a reestablished interest in SSC incompletely, due to the realization that numerous microorganisms, at the side GMOs, may create their items more successfully by SSF. Cellulases created in solid-state culture appear momentous soundness toward temperature, pH, metal particles, etc. The optimization of SSF might be advance progress. The large generation financial matters and besides can make it an alluring procedure for cellulase generation (Fadel 2000; Souza and Magalhaes 2010). The comparison of submerged and solid-state fermentation reported for pectinase production from *Aspergillus niger* by dividing the yield obtained from SSF and SmF in culture broth. The cellulase production by *Aspergillus niger* in SSF and SmF using different substrate especially cellulose. When comparing between the SmF and SSF, production of total cellulase by SSF was higher than that of SmF (Mrudula and Murugammal 2011; Farinas 2018). A tenfold diminishment within the generation fetched when SSF is utilized for generation. Table 3.1 lists a few parametric comparisons between SmF and SSF for cellulose generation. The cellulase generation by the filamentous parasites in SmF and SSF was examined broadly (Farinas 2018; Shweta 2015). Be that as it may, there's no report on the comparison of cellulase generation by SmF and SSF conditions. The living organisms was disconnected and illustrated for their moved forward proficiency in SmF and SSF are for generation of the cellulase utilizing agro-industrial squander as crude fabric. The impact of different parameters was assessed beneath two various fermentation conditions. The comparison of the cellulase generation by *Aspergillus niger* in SmF and SSF frameworks (Mrudula and Murugammal 2011; Vintila et al. 2009; Durand et al. 1996).

3.4 Summary

Comparative study of cellulase production by utilized submerge and solid-state fermentation. The best cellulase activities were achieved by submerged and solid-state fermentation techniques. In the presence of solid-state medium obtained cellulase than submerged process. The cellulase obtained by SSF. In the literature there is a relatively large amount of information on the production of cellulases by submerged fermentation and but there is information on the production of cellulases in SSF. This paper reports the results of research on the production of cellulases by the submerged and solid-state fermentations. The biological parameters applicable to SSF and SmF. Thus, the perspective of SSF would gain in prevailing significance in the industrial production of cellulose, compared with SmF, it is more effective in several aspects including lower energy and sterility demands as well as higher stability of

Table 3.1 Comparisons between the SSF and SmF for cellulose production (Shweta 2015)

Parameter	SSF	SmF
Aseptic conditions	Aseptic conditions can be curtailed under water-limited conditions of fermentation	Required to avoid contamination under high moisture conditions
Cellulosic substrate	Cellulosic substrate	Cellulosic substrate
Downstream processing	Air-dried fermented solids can be directly used as source of enzyme eliminating the need of expenses on downstream processing	Requires downstream processing
Effluent generation	Virtually, no effluent generation	Large volumes of effluents discarded
Energy consumption	Low	High
Moisture requirements	Carried out in the absence of free-flowing water	Large volumes of water needed
Productivity	100–300 g/L, 2–3 times higher enzyme production as well as protein rate also enhanced titers of the product in the medium	30–80 g/L
Process parameters	Generally operated under static conditions	Generally involves mixing, aeration, control, and monitoring of temperature, pH, dissolved oxygen, and gas flow rates and therefore profitable in terms of its higher degree of process control and monitoring
Scale-up	Scale-up is problematic; new design equipments are needed	Easy scale-up and industrial equipments are available
Substrate utilization	The amenability of SSF technique to utilize 20–30% of the substrate makes it more promising	A maximum utilization of only 5% in SmF process

products and several of microorganisms and especially the d of mixed cultures. In this present study indicated that the various agro-industrial residues studied, cellulose production in SmF as well as SSF. SSF showed for highest production, possibilities of the effective utilization of the cellulose for the value of addition through biotechnological. Such processes would not only help in reducing the cost of production but also pave the way in effective solid-state fermentation management.

3.5 Conclusion

The comparative study between from the production of cellulose by using submerge and solid state fermentation. The result of present study clearly indicates that the potential can successfully produce the production of cellulase. These methods are useful in the production of cellulose and reducing disposal problem; therefore it can be effectively utilized by a potential strain like microbes, plants, etc. for production of cellulase which is commercially important. Solid medium was used in the solid-state fermentation and restricts the removal of excess heat by microbial metabolism. The increase in temperature in bioreactors may lead to denaturation of thermolabile proteins. The SSF processes lead to development of different gradients (moisture, temperature, substrate concentration, and others) along the bioreactor, which may negatively influence the process. The main drawbacks of SSF process are mainly because of heat transfer and culture homogeneity problems. The conclusion has shown that the results obtained in this study indicated that among the several agro-industrial residues studied, wheat bran was a suitable substrate for the protease synthesis in SmF as well as SSF. SSF showed superiority for cellulose production and also revealed the possibilities of effective utilization of wheat bran and agro-industrial residues for the value of addition through the biotechnology. Such processes would not only help in reducing cost of production but also cover the way in effective solid-state management. Fermentation, with its wide array of application and immense benefits, has proved to be a main contender to fill this void. However, due to the variations of fermentation techniques, a lot of work still needs to be done in the terms of comparison of these techniques. The exploration was carried out to identify the sustainable substrates and processes to maintain productivity and quality. These methods can help for the increasing reduction and production of these compounds.

References

Acharya PB, Acharya DK, Modi HA (2008) Optimization for cellulase production by Aspergillus Niger using saw dust as substrate. Afr J Biotechnol 7:4147–4152

Acharya BK, Mohana S, Jog R, Divecha J, Madamwar D (2010) Utilization of anaerobically treated distillery spent wash for production of cellulases under solid-state fermentation. J Environ Manag 91:2019–2027

Agrawal R, Satlewal A, Verma AK (2013) Production of an extracellular cellobiase in solid state fermentation. J Microbiol Biotechnol Food Sci 2:2339–2350

Ahuja SK, Ferreira GM, Moreira AR (2004) Utilization of enzymes for environmental applications. Crit Rev Biotechnol 24(2–3):125–154

Ang SK, Shaza EM, Adibah Y, Suraini AA, Madihah MS (2013) Production of cellulases and xylanase by Aspergillus fumigatus SK1 using untreated oil palm trunk through solid state fermentation. Process Biochem 48:1293–1302

Assamoi AA, Jacqueline D, Delvigne F, Lognay G, Thonart P (2008) Solid-state fermentation of xylanase from *Penicillium canescens 10-10c* in a multi-layer-packed bed reactor. Appl Biochem Biotechnol 145:87–97

Babu KR, Satyanarayana T (1996) Production of bacterial enzymes by solid state fermentation. J Sci Ind Res 55:464–467

Basha NS, Rekha R, Komala M, Ruby S (2009) Production of extracellular anti-leukaemic enzyme lasparaginase from marine actinomycetes by solid state and submerged fermentation: purification and characterisation. Trop J Pharm Res 8(4):353–360

Bhat MK (2000) Cellulases and related enzymes in biotechnology. Biotechnol Adv 18(5):355–383

Biswas SR, Jana SC, Mishra AK, Nanda G (1990) Production, purification and characterization of xylanase from a hyperxylanotic mutant of *Aspergillus ochraceus*. Biotechnol Bioeng 35:244–251

Brijwani K, Oberoi HS, Vadlani PV (2010) Production of a cellulolytic enzyme system in mixed-culture solid-state fermentation of soybean hulls supplemented with wheat bran. Process Biochem 45(1):120–128

Cen P, Xia L (1999) Production of cellulase in solid state fermentation. In: Scheper T (ed) Recent progress in bioconversion of lignocellulosics, Advances in biochemical engineering/biotechnology, vol 65. Springer, Berlin, p 69

Coradi GV, Da Visitação VL, De Lima EA, Saito LYT, Palmieri DA, Takita MA et al (2013) Comparing submerged and solid-state fermentation of agro-industrial residues for the production and characterization of lipase by Trichoderma harzianum. Ann Microbiol 63:533–540

Coughlan MP (1985) Cellulase production, properties and applications. Biochem Soc Trans 13:405–406

Cunha FM, Esperanca MN, Zangirolami TC, Badino AC, Farinas CS (2012) Sequential solid-state and submerged cultivation of Aspergillus Niger on sugarcane bagasse for the production of cellulase. Bioresour Technol 112:270–274

Da Silva DP, Pirota RDB, Codima CA, Tremacoldi CR, Rodrigues A, Farinas CS (2012) Using Amazon forest fungi and agricultural residues as a strategy to produce cellulolytic enzymes. Biomass Bioenergy 37:243–250

Datta M, Patel S, Parikh H (1989) Solid state fermentation for cellulases and β-glucosidase production by Aspergillus Niger. J Ferment Bioeng 67:424–426

Debing J, Peijun L, Stagnitti F, Xianzhe X, Li L (2006) Pectinase production by solid fermentation from Aspergillus Niger by a new prescription experiment. Ecotox Environ Safe 64:244–250

Demain AL (1999) Pharmaceutically active secondary metabolites of microorganisms. Appl Microbiol Biotechnol 52:455–463

Du R, Su R, Li X, Tantai X, Liu Z, Yang J (2012) Controlled adsorption of cellulase onto pretreated corncob by pH adjustment. Cellulose 19:371–380

Durand A, Chereau D (1988) A new pilot reactor for solid-state fermentation: application to the protein enrichment of sugar beet pulp. Biotechnol Bioeng 31(5):476–486

Durand R, Renaud J, Maratray SA, Diez M (1996) INRA-Dijon reactors for solid state fermentation: designs and applications. J Sci Ind Res 55(5–6):317–332

Ekundayo FO, Ekundayo EA, Ayodele BB (2017) Comparative studies on glucanases and beta-glucosidase activities of Pleurotus ostreatus and P. Pulmonarius in solid state fermentation. Mycosphere 8(8):1201–1209

Emert GH, Katzen R (1980) Gulf's cellulose-to-ethanol process. CHEMTECH 10(10):610–615

Enari TM (1983) Microbial cellulases. In: Forgaty WF (ed) Microbial enzymes and biotechnology, vol I. Applied Sciences Publishers, London, pp 83–223

Fadel M (2000) Production physiology of cellulases and β-glucosidase enzymes of *Aspergillus niger* grown under solid state fermentation conditions. Online Biol Sci 1:401–411

Farinas CS (2018) Solid-state fermentation for the on-site production of cellulolytic enzymes and their use in the saccharification of lignocellulosic biomass. In: Current developments in biotechnology and bioengineering. Elsevier, Amsterdam, pp 169–183

Florencio C, Cunha FM, Badino AC, Farinas CS (2015) Validation of a novel sequential cultivation method for the production of enzymatic cocktails from Trichoderma strains. Appl Biochem Biotechnol 175:1389–1402

Gautam SP, Bundela PS, Pandey AK, Awasthi MK, Sarsaiya S (2010) Optimization of the medium for the production of cellulase by the Trichoderma viride using submerged fermentation. Int J Environ Sci 1(4):656

Ghildyal NP, Lonsane BK, Sreekantiah KR, Sreenivasa Murthy V (1985) Economics of submerged and solid state fermentation for the production of amyloglucosidase. J Food SciTechnol 22:171–176

Goyal M, Kalra KL, Sareen VK, Soni G (2008) Xylanase production with xylan rich lignocelulasic waste by a local soil isolate of *Trichoderma viride*. Braz J Microbiol 39:535–541

Grajek W (1987) Comparative studies on the production of cellulases by thermophilic fungi in submerged and solid-state fermentation. Appl Microbiol Biotechnol 26(2):126–129

Graminha EB, Gonçalves AZ, Pirota RD, Balsalobre MA, Da Silva R, Gomes E (2008) Enzyme production by solid-state fermentation: application to animal nutrition. Anim Feed Sci Technol 144(1–2):1–22

Gupte A, Madamwar D (1997) Solid state fermentation of lignocellulosic waste for cellulase and β-glucosidase production by cocultivation of *Aspergillus ellipticus* and *Aspergillus fumigatus*. Biotechnol Prog 13(2):166–169

Gutierrez-Correa M, Portal L, Moreno P, Tengerdy RP (1999) Mixed culture solid substrate fermentation of Trichoderma reesei with Aspergillus Niger on sugar cane bagasse. Bioresour Technol 68(2):173–178

Hafiz, Muhammad Nasir I, Ishtiaq A, Muhammad Anjum Z, Muhammad I (2011) Purification and characterization of the kinetic parameters of cellulase produced from wheat straw by Trichoderma viride under SSF and its detergent compatibility. Adv Biosci Biotechnol 2(3): 149–156

Hesseltine CW (1972) Solid state fermentations. Biotechnol Bioeng 14(5):17–32

Hesseltine CW (1977) Solid state fermentation-part 1. Process Biochem 12:24–27

Hesseltine CW (1987) Solid state fermentation—an overview. Int Biodeterior 23(2):79–89

Hong J, Tamaki H, Akiba S et al (2001) Cloning of a gene encoding a highly stable endo-b-1,4-glucanase from *Aspergillus niger* and its expression in yeast. J Biosci Bioeng 92:434–441

Hui L, Wan C, Hai-tao D, Xue-jiao C, Qi-fa Z, Yu-hua Z (2010) Direct microbial conversion of wheat straw into lipid by a cellulolytic fungus of *Aspergillus oryzae* A-4 in solid-state fermentation. Bioresour Technol 101:7556–7562

Isaac GS, Abu-Tahon MA (2015) Enhanced alkaline cellulases production by the thermohalophilic Aspergillus terreus AUMC 10138 mutated by physical and chemical mutagens using corn stover as substrate. Braz J Microbiol 46(4):1269–1277

Ishida N, Saitoh S, Ohnishi T, Tokuhiro K et al (2006) Metabolic engineering of Saccharomyces cerevisiae for efficient production of pure L-(+)-lactic acid. Appl Biochem Biotechnol 131:795–807

Iqbal, Hafiz Muhammad Nasir, et al. "Purification and characterization of the kinetic parameters of cellulase produced from wheat straw by Trichoderma viride under SSF and its detergent compatibility." Advances in Bioscience and Biotechnology 2.03 (2011): 149–156.

Jabasingh SA, Nachiyar CV (2011) Utilization of pretreated bagasse for the sustainable bioproduction of cellulase by *Aspergillus nidulans* MTCC344 using response surface methodology. Ind Crop Prod 34:1564–1571

Jalak J, Kurasin M, Teugjas H, Valjama P (2012) Endo-exo synergism in cellulose hydrolysis revisited. J Biol Chem 287(34):28802–28815

Karmakar M, Ray R (2011) Current trends in research and application of microbial cellulases. Res J Microbiol 6:41–53

Kirk O, Borchert TV, Fuglsang CC (2002) Industrial enzyme applications. Curr Opin Biotechnol 13(4):345–351

Klein-Marcuschamer D, Oleskowicz-Popiel P, Simmons BA, Blanch HW (2012) The challenge of enzyme cost in the production of lignocellulosic biofuels. Biotechnol Bioeng 109(4):1083–1087

Kotwal SM, Gote MM, Sainkar SR, Khan MI, Khire JM (1998) Production of α-galactosidase by thermophilic fungus Humicola sp. in solid-state fermentation and its application in soyamilk hydrolysis. Process Biochem 33(3):337–343

Kuhad RC, Mehta G, Gupta R, Sharma KK (2010) Fed batch enzymatic saccharification of newspaper cellulosics improves the sugar content in the hydrolysates and eventually the ethanol fermentation by Saccharomyces cerevisiae. Biomass Bioenergy 7:1–6

Kumar S, Sharma HK, Sarkar BC (2011) Effect of substrate and fermentation conditions on pectinase and cellulase production by Aspergillus Niger NCIM 548 in submerged (SmF) and solid state fermentation (SSF). Food Sci Biotechnol 20(5):1289

Lawal SA, Ugheoke BI (2010) Investigation of alpha-cellulose content of agro-waste products as alternatives for paper production. AU J Technol 13:258–260

Lee YB, Lee B, Jo K, Lee N, Chang C, Lee Y, Lee J (2007) Purification and characterization of cellulase by *Bacillus amyoliquefaciens* DL3, utilizing rice hull. Bioresour Technol 98(2):288–297

Lee CK, Darah I, Ibrahim CO (2011) Production and optimization of cellulase enzyme using Aspergillus niger USM AI 1 and comparison with Trichoderma reesei via solid state fermentation system. *Biotechnol Res Int* 2011:1

Lever M, Ho G, Cord-Ruwisch R (2010) Ethanol from lignocellulose using crude unprocessed cellulose from solid-state fermentation. Bioresour Technol 101(18):7083–7087

Limayem A, Ricke SC (2012) Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects. Prog Energy Combust Sci 38:449–467

Liu BL, Tzeng YM (1998) Optimization of growth medium for the production of spores from Bacillus thuringiensis using response surface methodology. Bioprocess Biosyst Eng 18:413–418

Liu J, Xue D, He K, Yao S (2012) Cellulase production in solid-state fermentation by marine Aspergillus sp. ZJUBE-1 and its enzymological properties. Adv Sci Lett 16(1):381–386

Lonsane BK, Ramakrishna M (1989) Microbial enzymes in food processing industry: present status and future prospects in India. Indian Food Industry 8(4):15–31

Ming C, Jing Z, Liming X (2008) Enzymatic hydrolysis of maize straw polysaccharides for the production of reducing sugars. Carbohydr Polym 71(3):411–415

Mitchell DA, Berovic M, Krieger N (2006) Introduction to solid state fermentation bioreactors. In: Mitchell DA, Krieger N, Berovi M (eds) Solid state bioreactors: fundamentals of design and operation. Springer, Berlin, pp 33–44

Moo-Young M, Moriera AR, Tengerdy RP (1983) Principles of solid state fermentation. In: Smith JE, Berry DR, Kristiansen B (eds) The filamentous fungi, Fungal biotechnology, vol 4. Edward Arnold Publishers, London, pp 117–144

Mrudula S, Murugammal R (2011) Production of cellulase by Aspergillus Niger under submerged and solid state fermentation using coir waste as a substrate. Braz J Microbiol 42(3):1119–1127

Nampoothiri KM, Pandey A (1996) Solid-state fermentation for L-glutamic acid production using Brevibacterium sp. Biotechnol Letts 18(2):199–204

Neagu DA, DESTAIN J, THONART P, SOCACIU C (2012) Trichoderma reesei cellulase produced by submerged versus solid state fermentations. Bull UASVM Agric 69:2

Nema N, Alamir L, Mohammad M (2015) Production of cellulase from *Bacillus cereus* by submerged fermentation using corn husks as substrates. Int Food Res J 22(5):1831–1836.

Padmavathi T, Nandy V, Agarwal P (2012) Optimization of the medium for the production of cellulases by Aspergillus terreus and Mucor plumbeus. Eur J Exp Biol 2(4):1161–1170

Palaniyappan M, Vijayagopa V, Viswanathan R, Viruthagiri T (2009) Screening of natural substrates and optimization of operating variables on the production of pectinase by submerged fermentation using Aspergillus niger MTCC 281. Afr J Biotechnol 8:682–686

Pandey A (1991) Aspects of fermenter design for solid-state fermentations. Process Biochem 26(6):355–361

Pandey A (1992) Recent development in solid state fermentation. Process Biochem 27(2):109–117
Pandey A (1996) Recent developments in solid-state fermentation. Process Biochem 27:109–117
Pandey A (2003) Sold-state fermentation. Biochem Eng J 13:81–84
Pandey A, Selvakumar P, Soccol CR, Nigam P (1999) Solid-state fermentation for the production of industrial enzymes. Curr Sci 77:149–162
Pandey A, Soccol CR, Nigam P, Soccol VT (2000) Biotechnological potential of agro- industrial residues: sugarcane bagasse. Bioresour Technol 74(1):69–80
Patil SR, Dayanand A (2006) Exploration of regional agrowastes for the production of pectinase by *Aspergillus niger*. Food Technol Biotech 44:289–292
Pirota RD, Delabona PS, Farinas CS (2014) Simplification of the biomass to ethanol conversion process by using the whole medium of filamentous fungi cultivated under solid-state fermentation. Bioenergy Res 7:744–752
Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, Mielenz JR (2006) The path forward for biofuels and biomaterials. Science 311(5760):484–489
Raimbault M (1988) Enzymes production by solid state fermentation. In: Solid state fermentation in bioconversion of agro-industrial raw materials, vol 5. ORSTOM Centre Montpellier, Montpellier
Raimbault M (1998) General and microbiological aspects of solid substrate fermentation. Electron J Biotechnol 27:498–503
Ray RC (2011) Solid-state fermentation for production of microbial cellulase: an overview. In: Cellulase: types and action, mechanism, and uses. Nova Science Publishers Inc, New York
Reddy GPK, Narasimha G, Kumar KD, Ramanjaneyulu G, Ramya A, Kumari BS, Reddy BR (2015) Cellulase production by Aspergillus Niger on different natural lignocellulosic substrates. Int J Curr Microbiol App Sci 4(4):835–845
Robinson T, Nigam P (2003) Bioreactor design for protein enrichment of agricultural residues by solid state fermentation. Biochem Eng J 13:197–203
Robinson T, Singh D, Nigam P (2001) Solid-state fermentation: a promising microbial technology for secondary metabolite production. Appl Microbiol Biotechnol 55:284–289
Romero-Gómez SJ, Augur C, Viniegra-González G (2000) Invertase production by Aspergillus Niger in submerged and solid-state fermentation. Biotechnol Lett 22(15):1255–1258
Roussos S (1989) Sugarcane used in solid state fermentation for cellulases production. *Solid State Ferment*:139–150
Roussos S, Hannibal L, Aquiahuatl MA, Trejo M, Marakatis S (1994) Caffeine degradation by Penicillium verrucosum in solid fermentation of coffee pulp: critical effect of additional inorganic and organic nitrogen sources. J Food Sci Technol 31(4):316–319
Ruth MF, Howard JA, Nikolov Z, Hooker BS et al (1999) Preliminary economic analysis of agricultural production for use in cellulose hydrolysis. In: Abstracts from the 21st symposium on biotechnology for fuels and chemicals, Fort Collins, Colorado, Mays, Abstract, 3–49
Sadhu S, Maiti TK (2013) Cellulase production by bacteria: a review. Brit Microbial Res J 3(3): 235–258
Sekar C, Balaraman K (1998) Optimization studies on the production of cyclosporin A by solid state fermentation. Bioprocess Eng 18(4):293–296
Shobana P, Maheswari NU (2013) Production of cellulase from Aspergillus fumigatus under submerged and solid state fermentation using agricultural waste. Int J Adv Pharm Biol Chem 2(4):595–599
Shweta A (2015) Solid state fermentation for cellulase production. Biotechnol Res 1(1):108–112
Singh A, Singh N, Bishnoi NR (2009) Production of cellulases by Aspergillus heteromorphus from wheat straw under submerged fermentation. Int J Environ Sci Eng 1:1
Singhania RR, Sukumaran RK, Patel AK, Larroche C, Pandey A (2010) Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. Enzym Microb Technol 46(7):541–549
Souza PM, Magalhaes PO (2010) Application of microbial - amylase in industry-a review. Braz J Microbiol 41:850–861

Stockton BC, Mitchell DJ, Grohmann K, Himmel ME (1991) Optimum b-D glucosidase supplementation of cellulase for efficient conversion of cellulose to glucose. Biotechnol Lett 13:57–62

Subramaniyam R, Vimala R (2012) Solid state and submerged fermentation for the production of bioactive substances: a comparative study. Int J Sci Nat 3(3):480–486

Sukumaran RK, Singhania RR, Mathew GM, Pandey A (2009) Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bioethanol production. Renew Energy 34(2):421–424

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

Szendefy J, Szakacs G, Christopher L (2006) Potential of solid-state fermentation enzymes of *Aspergillus oryzae* in biobleaching of paper pulp. Enzym Microb Tech 39:1354–1360

Vandenberghe LP, Soccol CR, Pandey A, Lebeault JM (2000) Solid-state fermentation for the synthesis of citric acid by Aspergillus Niger. Bioresour Technol 74(2):175–178

Vintila T, Dragomirescu M, Jurcoane S, Vintila D, Caprita R, Maniu M (2009) Production of cellulase by submerged and solid-state cultures and yeasts selection for conversion of lignocellulose to ethanol. Rom Biotechnol Lett 14(2):4275–4281

Wen Z, Liao W, Chen S (2005) Production of cellulase/β-glucosidase by the mixed fungi culture Trichoderma reesei and Aspergillus phoenicis on dairy manure. Process Biochem 40:3087–3094

Xue M, Liu D, Zhang H, Qi H, Lei Z (1992) A pilot process of solid state fermentation from sugar beet pulp for the production of microbial protein. J Ferment Bioeng 73(3):203–205

Yang SQ, Yan QJ, Jiang ZQ et al (2006) High-level of xylanase production by the thermophilic *Paecilomyces thermophila* J18 on wheat straw in solid-state fermentation. Bioresour Technol 97:1794–1800

Zeng W, Chen HZ (2009) Air pressure pulsation solid state fermentation of feruloyl esterase by *Aspergillus niger*. Bioresour Technol 100:1371–1375

Zhiyou W, Liao W, Shulin C (2004) Hydrolysis of animal manure lignocellulosics for reducing sugar production. Bioresour Technol 91(1):31–39

Chapter 4
Microorganisms for Cellulase Production: Availability, Diversity, and Efficiency

Mayara C. S. de Barcelos, Carlos H. Carvalho e Silva, Cintia L. Ramos, and Gustavo Molina

4.1 Introduction

Cellulose, hemicellulose, and lignin are the main constituents of lignocellulosic biomass, the most plentiful organic matter of the planet, in which cellulose represents 30–50% of organic matter total weight (Bilal et al. 2018). In addition, the discovery of the relationship between degradation of lignocellulosic biomass and biofuel production has made the search for more cost-effective and less aggressive processes, besides considerably more sustainable (Chander et al. 2016; Jiang et al. 2017).

In this scenario, enzymes, biological catalysts which possess high substrate specificity and are able to perform the degradation of organic matter, achieve high yields and low environmental impact (Jegannathan and Nielsen 2013; Sarrouh 2012).

Regarding lignocellulosic biomass, this degradation occurs through the action of cellulases. Cellulases are popularly known as the enzymes responsible for catalyzing depolymerization reactions of cellulose, which occurs through the hydrolysis of the glycosidic bonds that bind sugars, separating them into fermentable units (Behera and Ray 2016).

Cellulase is a broad term used to characterize cellulolytic enzymes capable of cleaving linkages that form the polysaccharide chain of cellulose (Chander et al. 2016). According to the Carbohydrate-Active Enzyme Database (CAZy), these enzymes belong to the group of glycosidic hydrolases, which means that they act by hydrolyzing glycosidic bonds between carbohydrates or between a carbohydrate and a non-carbohydrate in a chain with two or more constituents (Panchapakesan and Shankar 2016).

M. C. S. de Barcelos · C. H. Carvalho e Silva · G. Molina (✉)
Laboratory of Food Biotechnology, Institute of Science and Technology, Food Engineering, UFVJM, Diamantina, Minas Gerais, Brazil

C. L. Ramos
Department of Basic Sciences, UFVJM, Diamantina, Minas Gerais, Brazil

M. Srivastava et al. (eds.), *Approaches to Enhance Industrial Production of Fungal Cellulases*, Fungal Biology,
https://doi.org/10.1007/978-3-030-14726-6_4

More specifically, cellulases act on the cleavage of β-1,4-d-glucan bonds in the cellulose structure, thus providing the release of compounds such as glucose, cellobiose, and cello-oligosaccharides (Srivastava et al. 2017). This enzyme complex consisted of the enzymes endo-1,4-β-D-glucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) (Behera and Ray 2016; Singhania et al. 2016) is the most elucidated by researchers, in which each of the enzymes have a specific mechanism of action.

The action of this enzymatic complex can be described as a series of sequence reactions that complement each other until the release of the monomeric fermentable sugar at the end (Srivastava et al. 2017). The enzymatic degradation of the cellulose is divided into two stages of hydrolysis: in the former, the endo-1,4-β-D-glucanase cleaves β-1,4-d-glucan bonds, favoring the appearance of reducing and nonreducing groups in the polymer chain. Then, exoglucanases produce cello-oligosaccharides and also cellobiose, acting in the reducing and nonreducing groups that are now abundant (Srivastava et al. 2014; Do Vale et al. 2014). In the second stage, which occurs in a completely liquid medium, the β-glucosidases cleave the cellobiose aiming the release of fermentable monomers that can later be applied in different ways (Srivastava et al. 2017).

The paper and cellulose industry has considerable influence on the global economy, being one of the largest economic sectors in a wide range of countries. This isolated fact shows the great use of cellulose in industrial processes that certainly require relatively expensive treatment processes, having, also, environmental impacts. In this approach, enzymatic technology emerges as an alternative to these processes, in which cellulases have reached extremely significant size in industrial routines (Singh et al. 2016).

Cellulases are also extensively used in the food, veterinary, pharmaceutical, textile, and general cleaning industries, among others, establishing itself as the third largest market for industrial enzymes worldwide (Yoon et al. 2014; Chander et al. 2016; Singhania et al. 2016). Such enzyme was an important part of the prominent fashion revolution in the 1970s, where textile industries began to use it as the main agent in the manufacture of jeans with lighter color and softer texture, since at that time these products were considered hard and consequently uncomfortable (Tolan and Foody 1999).

This was only the initial kickoff in the discovery of this important industrial enzyme which, shortly thereafter, gained prominence in other sectors of the industry until then considered consolidated with its well-known chemical techniques and already of common use in the industrial routine (Sarrouh 2012).

The presence of cellulases in detergents and laundry products helps to remove the famous “lint” that appear on the clothes after some time of use and are able to soften the texture of the tissues. In this way, the use of cationic softeners, which cause environmental problems when discarded, has drastically reduced (Olsen and Falholt 1998). In the veterinary industry, this compound is able to potentiate the digestive tract of animals facilitating the absorption of nutrients from feed and food supplements that are dense and quite viscous and therefore difficult to digest (Tolan and Foody 1999).

In addition to these applications, another industrial sector has been developing in stride and has further increased the weight of cellulases in the global market of enzymes: the development of biofuels derived from lignocellulosic biomass. Classified as second-generation biofuels, these biofuels came as an alternative to the use of nonrenewable organic materials, being a clean and sustainable production route for the world fuel market (Ferreira et al. 2014; Jiang et al. 2017). In this way, the industrial demand for cellulases presents growth potential proportional to the technological advance in this sector (Srivastava et al. 2017).

The production of ethanol through fermentative processes involving sugarcane or corn, for example, has harmful impacts to the final value of the product, making it less advantageous to the consuming public when compared to fossil fuels. Therefore, the use of lignocellulosic biomass treated with suitable enzymes can make the production of biofuels head in the global market (Toogood and Scrutton 2018).

Despite the great potential of application and the numerous operational advantages, the use of enzymes in the biofuel industry faces a significant number of obstacles: the structural complexity of the lignocellulosic biomass makes the process of cleavage of its molecules in smaller units extremely difficult, drastically decreasing the number of enzymes capable of degrading this material (Yamamoto and Tamaru 2016).

Another factor that contributes to further limit the advance of this market is the fact that the efficiency of the ethanol production process, the substance of interest, from sugar and starch is extremely superior when compared to the production through lignocellulosic biomass (Bilal et al. 2018). Thus, the degradation process of this biomass requires the use of a large number of enzymes with different properties and routes of action, making studies of identification and characterization of these molecules necessary before considering a possible industrial application area.

It is observed, therefore, that the use of cellulases in industrial applications has already proven extremely effective in several areas, being this enzyme a faithful ally in the production of quality products with lower environmental impacts. However, there are still several barriers to be broken so that this class of enzymes is also used in the large production scale of biofuels, among other processes of enzymatic catalysis starting from complex substrates and difficult to cleave.

In this approach, the main goal of this chapter is to offer an in-depth outline on the identification of new strains capable of producing cellulases and overview the fermentative parameters, process optimization, and genetic engineering for process development, highlighting how microorganisms can be efficient tools for the production of cellulases with realistic potential of industrial applicability.

4.2 Identification of New Strains with Potential for Cellulose Production

The conversion of lignocellulosic biomass by cellulolytic enzymes is an essential step for a great variety of applications such as paper recycling, cotton processing, biofuel production, laundry, food processing, pharmaceutical applications, and

others (Kuhad et al. 2011; Gaurav et al. 2017). Thus, the search and identification of novel strains producing these enzymes have been a constant subject of many studies. Several reports have been published of microorganisms producing cellulosic enzymes, including mainly fungi and bacteria, isolated from different environments around the world. Cellulases are inducible enzymes, and to find novel organisms producing these hydrolytic enzymes is essential to discover different route and conditions for cellulolytic biomass hydrolysis.

It is known that lignocellulosic biomass hydrolysis depends on the synergistic action of three main groups of enzymes: β-glucosidases, endoglucanases, and exo-β-glucanases. In general, fungi are the most studied and employed strains due to their high ability to secrete large amounts of cellulases to the medium which facilitates the enzyme purification (Maki et al. 2009). One of the most extensively studied and industrially applied cellulase is produced by *Trichoderma reesei*, which converts native and derived cellulose to glucose. Other fungal species of *Trichoderma*, *Humicula*, *Penicillium*, and *Aspergillus* genera in addition to bacterial species of *Bacillus*, *Pseudomonas*, *Cellulomonas*, *Streptomyces*, and *Actinomucor* have also been studied and described as cellulase producers (Kuhad et al. 2011; Wilson 2011; Behera et al. 2017).

Although the fungi enzymes have been successfully produced and employed in a diversity of industrial applications, some bioprocess applications require enzymes which are able to act in extreme conditions, such as extreme temperatures, salinity, wide range of pH, and presence of heavy metal (Acharya and Chaudhary 2012). In general, these characteristics are not commonly provided by the available commercial enzymes. Some authors defend the idea that bacteria are good candidates to be applied for cellulase production. These microorganisms show high growth rate in association to enzyme secretion, in addition to their ability to survive in stressful conditions imposed by industrial bioconversion processing. Further, bacteria are less affected by the bioconversion products (feedback inhibition) (Kadarmoidheen et al. 2012). Therefore, several bacterial genera of cellulase producers have been widely reported such as *Clostridium*, *Cellulomonas*, *Ruminococcus*, *Pseudomonas*, *Bacillus*, and *Streptomyces* (Pennacchio et al. 2018; Hussain et al. 2017; Yuan et al. 2015; Kojima et al. 2013; Huang et al. 2012; Sheng et al. 2012).

Nowadays, studies have focused their efforts to find novel strains producing cellulase in a wide range of extreme conditions such as Antarctic soils and the halophilic Algerian arid and semiarid wetland ecosystems (Cong et al. 2017; Silva et al. 2017; Menasria et al. 2018). The researchers have suggested that the extreme ecosystems are an important reservoir of novel strains able to produce hydrolytic enzymes with optimum activity at extreme range of salinity, temperature, and pH. From rhizosphere of *Colobanthus quitensis* in Antarctic, the bacterial species *Rhodococcus* sp. and *Rhizobium* sp. were isolated and showed cellulolytic activity at 4 °C, 12 °C, and 25 °C, an important characteristic to be applied in industrial processes employing low temperatures (Silva et al. 2017). Another study conducted in Antarctic Pole isolated a fungi *Aspergillus sydowii* MS-19 which showed to synthesize low-temperature lignocellulolytic degrading enzymes. The transcriptome

analysis obtained 17 genes involved in cellulase degradation (eight cellobiohydrolase and nine endo-1,3-β-glucanase) (Cong et al. 2017). In regard to halophilic environments, 24 extreme halophilic archaea were isolated and showed extracellular cellulase activity at high salt concentration (20% w/v), demonstrating important biotechnological potential (Menasria et al. 2018).

Other environments have also been studied to isolate cellulolytic microorganisms. A well-known cellulolytic environment is the rumen where the animal digestion is assisted by the complex anaerobic microbiota hydrolyzing the plant cell wall. It is estimated a population around 1010 bacteria per ml in the rumen; however, even if the main carbon source in the rumen is cellulose, only 10% of the bacteria are cellulolytic (Russell et al. 2009). Fungi and protozoa are also cellulolytic microorganisms found in animal rumen, but studies have shown that bacteria are the major cellulolytic group. A new strain of *Escherichia coli* ZH-4 able to degrade cellulose was isolated from the Inner Mongolia bovine rumen. The strain showed potential for biotechnological application due to their ethanol and hydrogen production using cellulose and corn straw as substrate (Pang et al. 2017). Potential cellulolytic bacterial strain (e.g., *Arthrobacter* sp. HPG166) was also found in the gut of insect larvae (Huang et al. 2012, 2015; Sheng et al. 2012). According to Joynson et al. (2017), the herbivore gut microbiome is an important reservoir of lignocellulolytic enzymes due to its ability to degrade plant cell wall, as observed by metagenomic study of black slug *Arion ater* gut microbiome.

Wastes, plants, composts, and soils have also been often exploited as cellulose-degrading environments. Recycled paper sludge (Heinz et al. 2017), mangrove (Behera et al. 2017), soil, ward poultry (Hussain et al. 2017), the *Eucalyptus camaldulensis* and *Populus nigra* plants (Pennacchio et al. 2018), compost, sugarcane bagasse, and animal wastes (Mohamed et al. 2017) are some examples of studied source of potential novel cellulolytic strains. From these sources, the bacterial strains *Bacillus amyloliquefaciens* SA5, *B. subtilis* BTN7A, *B. megaterium* BMS4, *Anoxybacillus flavithermus* BTN7B, *Streptomyces flavogriseus* AE64X, and *Streptomyces flavogriseus* AE63X and the fungus *Trichoderma virens* in addition to the yeast *Trichosporon laibachii* MG270406-1A14 were isolated and described as novel cellulolytic strains with potential to be used in a diversity of biotechnological applications (Hussain et al. 2017; Pennacchio et al. 2018; Zeng et al. 2016; Giese et al. 2017).

Although the studies and strains secreting cellulolytic enzymes have increased substantially lately, it is estimated that only a small percentage (1–2%) of the microorganisms present in the environment can be cultured and then remained unknown. Uncultured methodologies such as the metagenomic tools, based on total nucleic acids extracted from the samples, have been employed to obtain a better knowledge of phylogeny identification and functional capability of microbiota in order to increase this estimative. This is an important strategy to search for novel enzymes since the genes can be easily identified. However, the microbial strains with potential biotechnological applications still limited to the culturable isolates.

4.3 Microorganisms as Tools for Efficient Cellulase Production

4.3.1 Fermentative Process Parameters

The cellulase production by microorganisms is reported for different species, such as fungi, bacteria, and actinomycetes, but only a few are able to produce significant amounts, where fungi are highlighted, especially due to the excretion of the enzyme (Amaeze et al. 2015; Bansal et al. 2012; Juturu and Wu 2014). Normally, these microorganisms come from environmental sources such as hot water springs, compost, sewage, soil samples obtained from forest and nature reserves, animal manure, and bovine rumen (Juturu and Wu 2014).

There are no general process parameters, such as fermentation type, carbon source, pH, temperature, and time, suitable for all cellulase producer microorganisms that will guaranty high yields and efficiency, but the understanding of such parameters (Fig. 4.1) and how they affect the production of the enzymes can improve the bioprocess (Table 4.1).

Submerged and solid fermentation systems are currently used for cellulase production. However, solid systems offer advantages concerning energy and cost saving, since it requires lower capital and energy and simpler fermentation medium and achieves high productivity, even though rigorous control of process parameters is not required (Bansal et al. 2012; Behera and Ray 2016). Besides, less wastewater is produced, and the use of lignocellulosic waste as substrates is possible (Cunha et al. 2012). Although more dilute products are generated at submerged fermentation, the availability of well-established bioreactor monitoring and control techniques facilitates the process (Cunha et al. 2012).

For solid-state fermentation (SSF), the particle size, chemical composition, cost, moisture content, and availability of the substrate are essential, providing all components necessary for adequate microorganism growth and enzyme production (Behera and Ray 2016; Idris et al. 2017). The pretreatment of some

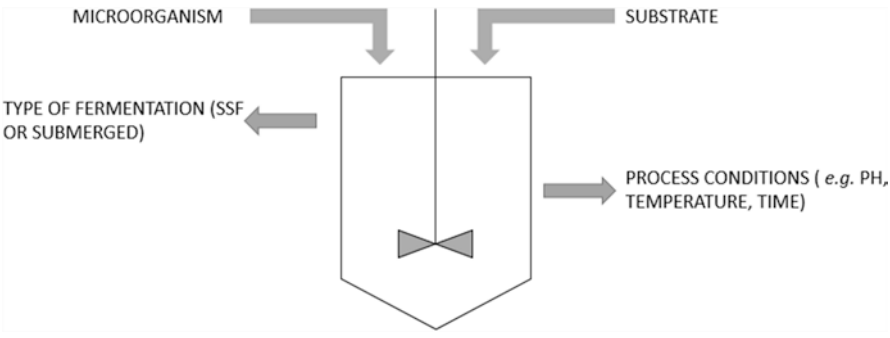

Fig. 4.1 Factors that affect microbial cellulase production

Table 4.1 Production of cellulases from several biocatalysts and process parameters

Microorganism	Process	Carbon source	Time (days)	pH	Temperature (°C)	Cellulase activity/ productivity	Reference
Fungi							
Aspergillus niger	Shake flask	Pineapple peel	5	4	45	0.385 mg/mL	Amaeze et al. (2015)
	SSF	Wheat bran	4	6.5	30	296.0 U/gds	Bansal et al. (2012)
	SSF and shake flask	Glucose followed by sugarcane bagasse	4	6	32	57 IU/L.h	Cunha et al. (2012)
	SSF	Banana peel	5	–	30	41.1 U/mg	Mandal and Ghosh, (2018)
Aspergillus fumigatus	SSF	Corn stover	4	4	50	526.3 U/gds	Liu et al. (2011)
Saccharomyces cerevisiae	Shake flask	Pineapple peel	3	5	45	0.330 mg/mL	Amaeze et al. (2015)
Trichoderma reesei	Shake flask	Lactose	5	4.8	–	10 FPU/g h	Callow et al. (2016)
	Shake flask	Soybean hulls	5	5	–	23.5 g/l	Ellilä et al. (2017)
	Fed-batch fermentation	Glucose followed by lactose	4	4.8	28	2.11 times higher than batch fermentation	Fang and Xia (2015)
	SSF	Wheat bran	9	4.8	30	298.35 IU/gds	Idris et al. (2017)
Trichoderma harzianum	SSF and shake flask	Glycerol followed by sugarcane bagasse	4	5	29	2.27 FPU/mL	Delabona et al. (2016)
	Bubble column reactor	Domestic wastewater supplement with microcrystalline cellulose	5	–	28	64.6 U/L.h	Libardi et al. (2017)
Mixed culture of *Aspergillus niger* and *Trichoderma reseei*	SSF	Rice straw and wheat bran	4	–	30	24.17 IU/gds	Dhillon et al. (2011)

(continued)

Table 4.1 (continued)

Microorganism	Process	Carbon source	Time (days)	pH	Temperature (°C)	Cellulase activity/ productivity	Reference
Mixed cultures of *Trichoderma reesei* and *Aspergillus niger* or *Aspergillus phoenicis*	Shake flask	Lactose	3	5.5	28	1.6-fold when compared to single cultures	Gutiérrez-Correa and Villena (2012)
Trichosporon laibachii	Shake flask	Avicel and cellobiose	5	6	30	0.3 U/mL	Giese et al. (2017)
Bacteria							
Bacillus licheniformis	Shake flask	Wheat bran	3	5	37	Sevenfold when compared to synthetic medium	Gupta et al. (2015)
Bacillus sp. strain SMIA-2	Shake flask	Sugarcane bagasse and corn steep liquor	5	7.5	50	0.83 U/mL	Ladeira et al. (2015)
Paenibacillus terrae	Shake flask	Wheat bran	2.5	8	28	2.08 U/mL	Liang et al. (2014)

substrates before use in SSF processes is a suitable alternative, facilitating the microbial growth (Bansal et al. 2012).

Alkali-treated substrates are reported to achieve higher productivities of cellulose than acid-treated. Acid-treated substrates have the hemicellulose component solubilized, which produces hydroxymethylfurfural, furfural, monomers, and other volatile products. These products, along with lignin, affect negatively the microbial growth, while in alkali-treated substrates, lignin is released causing modification and solubilization of the crystalline state of cellulose (Bansal et al. 2012).

At submerged fermentation, the use of surfactants and specific shear rates are used to morphology control, which facilitates enzyme separation improving downstream process (Callow et al. 2016).

To evaluate the influence of submerged and solid-state fermentation at cellulase production, a sequential cultivation of *Aspergillus niger* was conducted using sugarcane bagasse. The strain was grown at SSF followed by submerged fermentation, having control experiments with only submerged fermentation using glucose as only carbon source. A productivity of 57 IU/L/h was achieved, representing a threefold improvement. The sequential cultivation had the advantage to improve the assimilation of the substrate and fungal growth morphology, which develop at a dispersed filamentous form, enhancing cell-substrate interaction (Cunha et al. 2012).

The carbon source used possess influence at the microbial morphology and total protein secretion (Delabona et al. 2016). Carbon sources reported as cellulase inducers are lactose (Callow et al. 2016), glycerol (Delabona et al. 2016), pure cellulose (Ellilä et al. 2017), microcrystalline cellulose (Libardi et al. 2017), and wheat bran (Liang et al. 2014), meanwhile glucose leads to a strong repression of cellulase gene transcription in fungi due to catabolism repression (Delabona et al. 2016). The use of two different carbon sources is an alternative to increase cellulase productivity, where the first carbon source is used for microbial biomass growth and the second a cellulase inducer for enzyme secretion (Cunha et al. 2012; Delabona et al. 2016; Fang and Xia 2015). Concerning the nitrogen source, it is observed satisfactory results when inorganic sources are used, such as ammonium compounds (Mandal and Ghosh 2018; Sethi and Gupta 2014).

Optimum incubation temperature for cellulase production is suggested to vary according to the strain used. For fungi, high temperatures (above 30 °C) can alter the membrane composition which stimulates protein catabolism leading to cell death (Bansal et al. 2012). However, cellulase production is reported for *Aspergillus niger* and *Saccharomyces cerevisiae* at 45 °C (Amaeze et al. 2015) and *Aspergillus fumigatus* at 50 °C (Liu et al. 2011).

The pH control of cellulase production is considered the major challenge of this bioprocess, since the synthesis and secretion of different cellulase components require different optimal pH ranges, which vary with the strain used (Li et al. 2013). The changes in pH observed during microbial growth also affects the enzyme stability at the medium (Sethi and Gupta 2014). Values ranging from 4 to 6.5 can be observed at cellulase production by fungi and varying from 5 to 8 when produced by bacteria (Table 4.1).

The use of different by-products as substrates for cellulase production is one strategy for a cost-effective bioprocess, with already proven efficiency for pineapple and orange peels (Amaeze et al. 2015), kitchen wastes (Bansal et al. 2012), glycerol (Delabona et al. 2016), sugarcane bagasse (Cunha et al. 2012; Delabona et al. 2016), soybean hulls (Ellilä et al. 2017), wheat bran (Dhillon et al. 2011; Gupta et al. 2015; Idris et al. 2017), and domestic wastewater (Libardi et al. 2017), among others. Other alternative is the use of mixed culture of microorganisms to obtain higher yields when compared to single cultures, such as *Aspergillus niger* and *Trichoderma reseei* or *Aspergillus phoenicis* and *Trichoderma reseei* (Dhillon et al. 2011; Gutiérrez-Correa and Villena 2012).

The comparison between producer-cellulase strains is a difficult task due to the differences in fermentation conditions, media composition, and raw materials employed, besides the use of different methods and assays for cellulase activity determination, which do not allow a direct comparison of cellulase yields (Kuhad et al. 2016).

4.3.2 Optimization Process for Enhanced Cellulose Production

Optimization of process parameters is a critical factor for economical viable bioprocess, indispensable when maximum production is desirable (Sirajunnisa et al. 2016). The optimization of cellulase production is driven by the increased industrial demand for sustainable raw materials and, consequently, cellulose degradation, aiming enhancement at enzyme activity and productivity (Baz et al. 2016).

The cellulase production is a multivariable process, including media composition and external factors, which directly influence its activity and productivity (Sangwan et al. 2015; De Sousa et al. 2018). Besides, both medium composition and fermentation strategy must be optimized to obtain high productivity and activity and lower costs (Han et al. 2017).

Nowadays, cellulase is commercially produced by filamentous fungi, such as *Aspergillus niger* and *Trichoderma reesei*, using submerged fermentation (Baz et al. 2016); however its use is limited due to high production costs (De Sousa et al. 2018).

In order to expand industrial options and reduce costs, the optimization and modelling of several variables, determining the optimum process conditions, are being studied, for which statistical approaches are commonly used, such as response surface methodology (RSM), one factor at a time, Plackett-Burman, full factorial design, and central composite design (CCD), as it can be seen in Table 4.2.

Another strategy for enhanced cellulase production is the induced mutagenesis using UV irradiation with exposure time and distance of 220 seconds and 9 cm. This method was tested using *Aspergillus niger*, in which a twofold increase at total cellulase activity was observed when compared to the wild strain (4.159 IU/ml) (Jafari et al. 2017).

Table 4.2 Optimum conditions for enhanced production of cellulase by different microorganisms

Microorganism	Optimization methodology	Optimum conditions for cellulase production	Cellulase activity/ productivity	Reference
Fungi				
Aspergillus tubingensis IMMIS2	RSM	Using 40 mesh size substrate, 8 g substrate, 80% moisture, 5 mL inoculum, 0.5 g urea, 0.1 g KCl, 0.1 g $CaCl_2$, and 0.06 g $MgSO_4$	112 μg/mL/min	Imran et al. (2017)
Penicillium oxalicum RE-10	CCD and RSM	Use of fed batch fermentation, with no influence of carbon and nitrogen source	158.38 U/L/h	Han et al. (2017)
Trichoderma sp. CMIAT 041	Full factorial design	Growth media with 25.0 g/L of cellulose, 20.0 g/L of crude glycerol, 0.6 g/L of yeast extract, and 1.5 g/L of ammonium sulfate	89.35 FPU/L	De Sousa et al. (2018)
Trichoderma sp. CMIAT 054	Full factorial design	Growth media with 25.0 g/L of cellulose, 10.0 g/L of crude glycerol, 1.4 g/L of yeast extract, and 3.5 g/L of ammonium sulfate	138.48 FPU/L	De Sousa et al. (2018)
Trichoderma atroviride	RSM	Solid state fermentation during 5.5 days, 32.5 °C, pH 5.5, and spore suspension of 1.75 mL	90.43 IU/gds	Sangwan et al. (2015)
Trichoderma viride	RSM	Growth media volume of 30%, 1.5 g/L peptone, and 0.4 g/L of surfactant	1.066–2.99 IU/mL (64% increase)	Baz et al. (2016)
Pycnoporus sanguineus	CCD	70% (v/v) palm oil mill effluent concentration, 350 rpm agitation speed, and 1.0 vvm aeration rate	16.073 IU/mL	Teoh et al. (2017)
Bacteria				
Bacillus stratosphericus	RSM	Growth media with esculin (1.9 g/L), K_2HPO_4 (0. 5 g/ L) and $MgSO_4$ (0.3 g/L)	3340 IU	Dutta et al. (2017)
Bacillus subtilis K-18	CCD and RSM	Media with 2% substrate concentration, 2% inoculum size, 1% yeast extract, pH 5.0, incubation temperature of 50 °C for 24 h of fermentation period	3.50 ± 0.11 IU/ml	Irfan et al. (2017)

(continued)

Table 4.2 (continued)

Microorganism	Optimization methodology	Optimum conditions for cellulase production	Cellulase activity/ productivity	Reference
Halobacillus sp. QLS 31	Plackett-Burman	Temperature of 30 °C, fermentation time of 2 days, pH 9, CMC concentration (1%), inoculum size (1%), yeast extract concentration (0.1%), ammonium sulfate ($(NH_3)_2SO_4$) concentration (0.1%), sodium chloride (NaCl) concentration (20%), and metal inducers: $ZnSO_4$ (0.1%) and Ca/Mg ratio (0.01%)	23.19–175.47 U/mg (7.5-fold increase)	Korany et al. (2017)
Achromobacter xylosoxidans	RSM	Solid state fermentation at 40 °C, pH 5, moisture of 60%, and incubation time of 6 hours	107.7–512.98 U/gds (3.7-fold increase)	Hareesh et al. (2016)
Bacillus licheniformis NCIM 5556	RSM	Growth media with carboxy methyl cellulose, 19.21 g/L, $CaCl_2$-$6H_2O$, 25.06 mg/L, Tween-20, 2.96 mL/L and temperature of 43.35 °C	42.99 IU/mL (threefold increase)	Shajahan et al. (2017)

Optimization tools will support several process advances, to maximize production, process concentration, and overall yield, with clear economic potential.

4.4 Conclusion

The use of cellulases in industrial applications has already been proven to be extremely effective in several areas, this enzyme being a faithful ally in the production of quality products with lower environmental impacts. However, there are still several barriers to be broken so that this class of enzymes is also used in the large production scale of biofuels, among other processes of enzymatic catalysis starting from complex substrates and difficult to cleave. The cellulase production by microorganisms is reported for different species, such as fungi, bacteria, and actinomycetes, but only a few are able to produce significant amounts, where fungi are highlighted, especially due to the excretion of the enzyme. Several approaches have been used to support and maximize their production, such as bioprospecting and screening of new biocatalysts, optimization process, and genetic engineering, among others. The use of genetic engineering still focus on higher tolerance of microorganisms against adverse conditions but little on how different genetic techniques can improve cellulase production. In this scenario, further studies are needed. The advances in this area should make the obtaining of these biocatalysts in the quantity and quality sufficient for the industrial scale, opening several precedents for its application in commercial products.

Acknowledgments Authors acknowledge CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico – Process number: 460897/2014-4), Fapemig (Fundação de Amparo à Pesquisa do Estado de Minas Gerais – Process number: CAG-APQ-01056-17), and ICT/UFVJM.

References

Acharya S, Chaudhary A (2012) Optimization of fermentation conditions for cellulases production by *Bacillus licheniformis* MVS1 and *Bacillus* sp. MVS3 isolated from Indian hot spring. Braz Arch Biol Technol 55:497–503

Amaeze NJ, Okoliegbe IN, Francis ME (2015) Cellulase production by Aspergillus niger and Saccharomyces cerevisiae using fruit wastes as substrates. Int J Appl Microbiol Biotechnol Res 3:36–44

Bansal N, Tewari R, Soni R, Soni SK (2012) Production of cellulases from Aspergillus niger NS-2 in solid state fermentation on agricultural and kitchen waste residues. Waste Manag 32:1341–1346. https://doi.org/10.1016/j.wasman.2012.03.006

Baz AE, Shetaia Y, Eldin H, ElMekawy A (2016) Optimization of cellulase production by Trichoderma viride using response surface methodology. Current Biotechnol 5. https://doi.org/10.2174/2211550105666160115213402

Behera SS, Ray RC (2016) Solid state fermentation for production of microbial cellulases: recent advances and improvement strategies. Int J Biol Macromol 86:656–669. https://doi.org/10.1016/j.ijbiomac.2015.10.090

Behera BC, Sethi BK, Mishra RR, Dutta SK, Thatoi HN (2017) Microbial cellulases – diversity & biotechnology with reference to mangrove environment: a review. J Genet Eng Biotechnol 15:197–210

Bilal M, Iqbal MN, Hu H, Wang W, Zhang X (2018) Metabolic engineering and enzyme-mediated processing: a biotechnological venture towards biofuel production – a review. Renew Sustain Energy Rev 82:436–447. https://doi.org/10.1016/j.rser.2017.09.070

Callow NV, Ray CS, Kelbly MA, Ju LK (2016) Nutrient control for stationary phase cellulase production in Trichoderma reesei Rut C-30. Enzym Microb Technol 82:8–14. https://doi.org/10.1016/j.enzmictec.2015.08.012

Chander R, Deswal D, Sharma S, Bhattacharya A, Kumar K, Kaur A et al (2016) Revisiting cellulase production and redefining current strategies based on major challenges. Renew Sust Energ Rev 55:249–272. https://doi.org/10.1016/j.rser.2015.10.132

Cong B, Wang N, Liu S, Liu F, Yin X, Shen J (2017) Isolation, characterization and transcriptome analysis of a novel Antarctic *Aspergillus sydowii* strain MS-19 as a potential lignocellulosic enzyme source. BMC Microbiol 17:129

Cunha FM, Esperança MN, Zangirolami TC, Badino AC, Farinas CS (2012) Sequential solid-state and submerged cultivation of Aspergillus niger on sugarcane bagasse for the production of cellulase. Bioresour Technol 112:270–274. https://doi.org/10.1016/j.biortech.2012.02.082

De Sousa KA, da Faheina Junior GS, de Azevedo DCS, Pinto GAS (2018) Optimization of cellulase production by Trichoderma strains using crude glycerol as a primary carbon source with a 24 full factorial design. Waste Biomass Valoriz 9(3):357–367. https://doi.org/10.1007/s12649-016-9806-8

Delabona P d S, Lima DJ, Robl D, Rabelo SC, Farinas CS, da Cruz Pradella JG (2016) Enhanced cellulase production by Trichoderma harzianum by cultivation on glycerol followed by induction on cellulosic substrates. J Ind Microbiol Biotechnol 43:617–626. https://doi.org/10.1007/s10295-016-1744-8

Dhillon GS, Oberoi HS, Kaur S, Bansal S, Brar SK (2011) Value-addition of agricultural wastes for augmented cellulase and xylanase production through solid-state tray fermentation

employing mixed-culture of fungi. Ind Crop Prod 34:1160–1167. https://doi.org/10.1016/j.indcrop.2011.04.001

Do Vale LHF, Filho EXF, Miller RNG, Ricart CAO, De Sousa MV (2014) Cellulase systems in Trichoderma: an overview. Biotechnol Biol Trichoderma. https://doi.org/10.1016/B978-0-444-59576-8.00016-3

Dutta SG, Shaik AB, Ganesh Kumar C, Kamal A (2017) Statistical optimization of production conditions of β-glucosidase from Bacillus stratosphericus strain SG9. 3 Biotech 7. https://doi.org/10.1007/s13205-017-0866-7

Ellilä S, Fonseca L, Uchima C, Cota J, Goldman GH, Saloheimo M et al (2017) Development of a low-cost cellulase production process using Trichoderma reesei for Brazilian biorefineries. Biotechnol Biofuels 10:1–17. https://doi.org/10.1186/s13068-017-0717-0

Fang H, Xia L (2015) Cellulase production by recombinant Trichoderma reesei and its application in enzymatic hydrolysis of agricultural residues. Fuel 143:211–216. https://doi.org/10.1016/j.fuel.2014.11.056

Ferreira NL, Margeot A, Blanquet S, Berrin JG (2014) Use of cellulases from Trichoderma reesei in the twenty-first century—part I: current industrial uses and future applications in the production of second ethanol generation. Biotechnol Biol Trichoderma 1. https://doi.org/10.1016/B978-0-444-59576-8.00017-5

Gaurav N, Sivasankari S, Kiran GS, Ninawe A, Selvin J (2017) Utilization of bioresources for sustainable biofuels: a review. Renew Sust Energ Rev 73:205–214

Giese EC, Dussán KJ, Pierozzi M, Chandel AK, Pagnocca FC, Silva SS (2017) Cellulase production by *Trichosporon laibachii*. Orbital Electron J Chem 9:271–278. https://doi.org/10.17807/orbital.v9i4.1024

Gupta M, Sharma M, Singh S, Gupta P, Bajaj BK (2015) Enhanced production of cellulase from Bacillus licheniformis k-3 with potential for saccharification of rice straw. Energy Technol 3:216–224. https://doi.org/10.1002/ente.201402137

Gutiérrez-Correa M, Villena GK (2012) Batch and repeated batch cellulase production by mixed cultures of Trichoderma reesei and Aspergillus niger or Aspergillus phoenicis. J Microbiol Biotechnol Res 2:929–935

Han X, Song W, Liu G, Li Z, Yang P, Qu Y (2017) Improving cellulase productivity of Penicillium oxalicum RE-10 by repeated fed-batch fermentation strategy. Bioresour Technol 227:155–163. https://doi.org/10.1016/j.biortech.2016.11.079

Hareesh ES, Faisal PA, Benjamin S (2016) Optimization of parameters for the production of cellulase from Achromobacter xylosoxidans BSS4 by solid-state fermentation. Electron J Biol 12:443–448

Heinz KGH, Zanoni PRS, Oliveira RR, Medina-Silva R, Simão TLL, Trindade FJ, Pereira LM, Tavares LBB, Giongo A (2017) Recycled paper sludge microbial community as a potential source of cellulase and xylanase enzymes. Waste Biomass Valor 8:1907–1917

Huang SW, Sheng P, Zhang HY (2012) Isolation and identification of cellulolytic bacteria from the gut of *Holotrichia parallela* larvae (Coleoptera: Scarabaeidae). Int J Mol Sci 13:2563–2577

Huang S, Deng G, Yang Y, Wu Z, Wu L (2015) Optimization of endoglucanase production from a novel bacterial isolate, *Arthrobacter* sp. HPG166 and characterization of its properties. Braz Arch Biol Technol 58:692–701

Hussain AA, Abdel-Salam MS, Abo-Ghalia HH, Hegazy WK, Hafez SS (2017) Optimization and molecular identification of novel cellulose degrading bacteria isolated from Egyptian environment. J Genet Eng Biotechnol 15:77–85

Idris ASO, Pandey A, Rao SS, Sukumaran RK (2017) Cellulase production through solid-state tray fermentation, and its use for bioethanol from sorghum stover. Bioresour Technol 242:265–271. https://doi.org/10.1016/j.biortech.2017.03.092

Imran M, Anwar Z, Irshad M, Javid A, Hussain A, Ali S (2017) Optimization of cellulase production from a novel strain of Aspergillus Tubingensis IMMIS2 through response surface methodology. Biocatal Agric Biotechnol 12:191–198. https://doi.org/10.1016/j.bcab.2017.10.005

Irfan M, Mushtaq Q, Tabssum F, Shakir HA, Qazi JI (2017) Carboxymethyl cellulase production optimization from newly isolated thermophilic Bacillus subtilis K-18 for saccharification using response surface methodology. AMB Express 7. https://doi.org/10.1186/s13568-017-0331-3

Jafari N, Jafarizadeh-Malmiri H, Hamzeh-Mivehroud M, Adibpour M (2017) Optimization of UV irradiation mutation conditions for cellulase production by mutant fungal strains of Aspergillus Niger through solid state fermentation. Green Process Synth 6:333–340. https://doi.org/10.1515/gps-2016-0145

Jegannathan KR, Nielsen PH (2013) Environmental assessment of enzyme use in industrial production-a literature review. J Clean Prod 42:228–240. https://doi.org/10.1016/j.jclepro.2012.11.005

Jiang Y, Xin F, Lu J, Dong W, Zhang W, Zhang M et al (2017) State of the art review of biofuels production from lignocellulose by thermophilic bacteria. Elsevier Ltd, New York. https://doi.org/10.1016/j.biortech.2017.05.142

Joynson R, Pritchard L, Osemwekha E, Ferry N (2017) Metagenomic analysis of the gut microbiome of the common black slug *Arion ater* in search of novel lignocellulose degrading enzymes. Front Microbiol 8:2181

Juturu V, Wu JC (2014) Microbial cellulases: engineering, production and applications. Renew Sust Energ Rev 33:188–203. https://doi.org/10.1016/j.rser.2014.01.077

Kadarmoidheen M, Saranraj P, Stella D (2012) Effects of cellulolytic fungi on the degradation of cellulosic agricultural wastes. Int J Appl Microbiol Sci 1:13–23

Kojima M, Okamoto K, Yanase H (2013) Direct ethanol production from cellulosic materials by *Zymobacter palmae* carrying *Cellulomonas* endoglucanase and *Ruminococcus* β-glucosidase genes. Appl Microbiol Biotechnol 97:5137–5147

Korany AH, Ali AE, Essam TM, Megahed SA (2017) Optimization of cellulase production by Halobacillus sp. QLS 31 isolated from Lake Qarun, Egypt. Appl Biochem Biotechnol 183:189–199. https://doi.org/10.1007/s12010-017-2438-z

Kuhad RC, Gupta R, Singh A (2011) Microbial cellulases and their industrial applications. Enzym Res 2011:ID280696

Kuhad RC, Deswal D, Sharma S, Bhattacharya A, Jain KK, Kaur A et al (2016) Revisiting cellulase production and redefining current strategies based on major challenges. Renew Sust Energ Rev 55:249–272. https://doi.org/10.1016/j.rser.2015.10.132

Ladeira SA, Cruz E, Delatorre AB, Barbosa JB, Martins MLL (2015) Cellulase production by thermophilic Bacillus sp. SMIA-2 and its detergent compatibility. Electron J Biotechnol 18:110–115. https://doi.org/10.1016/j.ejbt.2014.12.008

Li C, Yang Z, He Can Zhang R, Zhang D, Chen S, Ma L (2013) Effect of pH on cellulase production and morphology of Trichoderma reesei and the application in cellulosic material hydrolysis. J Biotechnol 168:470–477. https://doi.org/10.1016/j.jbiotec.2013.10.003

Liang Y-L, Zhang Z, Wu M, Wu Y, Feng J-X (2014) Isolation, Screening, and Identification of Cellulolytic Bacteria from Natural Reserves in the Subtropical Region of China and Optimization of Cellulase Production by *Paenibacillus terrae* ME27-1. Biomed Res Int 2014:1–13. https://doi.org/10.1155/2014/512497

Libardi N, Soccol CR, Góes-Neto A, de OJ, Vandenberghe LP de S (2017) Domestic wastewater as substrate for cellulase production by Trichoderma harzianum. Process Biochem 57:190–199. https://doi.org/10.1016/j.procbio.2017.03.006

Liu D, Zhang R, Yang X, Wu H, Xu D, Tang Z et al (2011) Thermostable cellulase production of Aspergillus fumigatus Z5 under solid-state fermentation and its application in degradation of agricultural wastes. Int Biodeterior Biodegrad 65:717–725. https://doi.org/10.1016/j.ibiod.2011.04.005

Maki M, Leung KT, Qin W (2009) The prospects of cellulose-producing bacteria for the bioconversion of lignocellulosic biomass. Int J Biol Sci 5:500–516

Mandal M, Ghosh U (2018) Value addition to horticultural solid waste by applying it in biosynthesis of industrially important enzyme: cellulase. In: Ghosh SK (ed) Utilization and management of bioresources. Springer Nature, Singapore, pp 279–289. https://doi.org/10.1007/978-981-10-5349-8

Menasria T, Aguilera M, Hocine H, Benammar L, Ayachi A, Bachir AS, Dekak A, Monteoliva-Sánchez M (2018) Diversity and bioprospecting of extremely halophilic archaea isolated from Algerian arid and semi-arid wetland ecosystems for halophilic-active hydrolytic enzymes. Microbiol Res 207:289–298

Mohamed AH, Youseif SH, El-Mageed FHA, Heikal NZ, Moussa TAA, Saleh SA (2017) Production of cellulase, exoglucanase and xylanase by different microorganisms cultivated on agricultural wastes. Res J Pharm Biol Chem Sci 8:435–452

Olsen HS, Falholt P (1998) The role of enzymes in modern detergency. J Surfactant Deterg 1:555–567. https://doi.org/10.1007/s11743-998-0058-7

Panchapakesan A, Shankar N (2016) Fungal cellulases: an overview. In: Gupta VK (ed) New and future developments in microbial biotechnology and bioengineering. Elsevier B.V., Amsterdam, pp 9–18

Pang J, Liu Z-Y, Hao M, Zhang Y-F, Qi Q-S (2017) An isolated cellulolytic *Escherichia coli* from bovine rumen produces ethanol and hydrogen from corn straw. Biotechnol Biofuels 10(165):165

Pennacchio A, Ventorino V, Cimini D, Pepe O, Schiraldi C, Inverso M, Faraco V (2018) Isolation of new cellulase and xylanase producing strains and application to lignocellulosic biomasses hydrolysis and succinic acid production. Bioresour Technol 259:325–333

Russell JB, Muck RE, Weimer PJ (2009) Quantitative analysis of cellulose degradation and growth of cellulolytic bacteria in the rumen. FEMS Microbiol Ecol 67:183–197

Sangwan P, Mor V, Dharnkhar R, Sukhani S (2015) Optimization of process paramerts for cellulase and xylanase production using rice husk. Int J Pharm Biol Sci 6:755–762

Sarrouh B (2012) Up-to-date insight on industrial enzymes applications and global market. J Bioprocess Biotech s1. https://doi.org/10.4172/2155-9821.S4-002

Sethi S, Gupta S (2014) Optimization of cultural parameters for cellulase enzyme production from fungi. Biolife Int Q J Biol Life Sci 2:989–996

Shajahan S, Moorthy IG, Sivakumar N, Selvakumar G (2017) Statistical modeling and optimization of cellulase production by Bacillus licheniformis NCIM 5556 isolated from the hot spring, Maharashtra, India. J King Saud Univ Sci 29:302–310. https://doi.org/10.1016/j.jksus.2016.08.001

Sheng P, Huang SW, Wang Q, Wang AL, Zhang HY (2012) Isolation, screening and optimization of the fermentation conditions of high cellulolytic bacteria from the hindgut of *Holotrichia parallela* larvae (Coleoptera: Scarabaeidae). Appl Biochem Biotechnol 167:270–284

Silva AC, Rachid CTCC, Jesus HE, Rosado AS, Peixoto RS (2017) Predicting the biotechnological potential of bacteria isolated from Antarctic soils, including the rhizosphere of vascular plants. Polar Biol 40:1393–1407

Singh S, Singh VK, Aamir M, Dubey MK, Patel JS, Upadhyay RS, Gupta VK (2016) Cellulase in pulp and paper industry. In: Gupta VK (ed) New and future developments in microbial biotechnology and bioengineering, 1st edn. Elsevier B.V., Amsterdam, pp 153–163

Singhania RR, Adsul M, Pandey A, Patel AK (2016) Cellulases. In: Current developments in biotechnology and bioengineering: production, isolation and purification of industrial products. Elsevier, Amsterdam, pp 73–101. https://doi.org/10.1016/B978-0-444-63662-1.00004-X

Sirajunnisa AR, Vijayagopal V, Sivaprakash B, Viruthagiri T, Surendhiran D (2016) Optimization, kinetics and antioxidant activity of exopolysaccharide produced from rhizosphere isolate, *Pseudomonas fluorescens* CrN6. Carbohydr Polym 135:35–43. https://doi.org/10.1016/j.carbpol.2015.08.080

Srivastava N, Rawat R, Oberoi H (2014) Application of thermostable cellulase in bioethanol production from lignocellulosic waste. In: Kumar S, Sarma A, Tyagi S, Yadav Y (eds) Recent advances in bioenergy research. Sardar Swaran Singh National Institute of Renewable Energy, Kapurthala, pp 121–129

Srivastava N, Srivastava M, Mishra PK, Gupta VK, Molina G (2017) Applications of fungal cellulases in biofuel production: advances and limitations. Renew Sust Energ Rev:0–1 v.82. https://doi.org/10.1016/j.rser.2017.08.074

Teoh YP, Don MM, Fadzilah K (2017) Optimization of cellulase production by Pycnoporus sanguineus in 5 L stirred tank bioreactor and enhanced fermentation by employing external loop. Chiang Mai J Sci 44:774–787

Tolan JS, Foody B (1999) Cellulase from Submerged Fermentation. Adv Biochem Eng Biotechnol 65:41–67. https://doi.org/10.1007/3-540-49194-5_3

Toogood HS, Scrutton NS (2018) Retooling microorganisms for the fermentative production of alcohols. Curr Opin Biotechnol 50:1–10. https://doi.org/10.1016/j.copbio.2017.08.010

Wilson DB (2011) Microbial diversity of cellulose hydrolysis. Curr Opin Microbiol 14:259–263

Yamamoto K, Tamaru Y (2016) Important roles of the cellulosome on degradation of plant biomass. In: Gupta VK (ed) New and future developments in microbial biotechnology and bioengineering, 1st edn. Elsevier B.V., pp 3–8

Yoon LW, Ang TN, Ngoh GC, Chua ASM (2014) Fungal solid-state fermentation and various methods of enhancement in cellulase production. Biomass Bioenergy 67:319–338. https://doi.org/10.1016/j.biombioe.2014.05.013

Yuan SF, Wu TH, Lee HL, Hsieh HY, Lin WL, Yang B, Chang C-K, Li Q, Gao J, Huang C-H, Ho M-C, Guo R-T, Liang P-H (2015) Biochemical characterization and structural analysis of a bifunctional cellulase/xylanase from *Clostridium thermocellum*. J Biol Chem 290:5739–5748

Zeng R, Yin X-Y, Ruan T, Hu Q, Hou Y-L, Zuo Z-Y, Huang H, Yang Z-H (2016) A novel cellulase produced by a newly isolated *Trichoderma virens*. Bioengineering 3:13

Chapter 5
Role of Solid-State Fermentation to Improve Cost Economy of Cellulase Production

Sheelendra M. Bhatt and Shilpa Bhat

5.1 Introduction

Cellulase is the third largest industrial enzyme used all around the world because of the growing interest in the demand of the biofuel from the lignocellulosic wastes. Cellulase found its applications in many fields ranging from brewery and wine industries, animal feeding, food and textile, laundry, paper and pulp industries, and biofuel industry as mentioned in Table 5.1. Cellulases are produced by a number of microorganisms including bacteria and fungi. The filamentous fungus *Trichoderma* is well known for its cellulase-producing activity. Cellulases are multienzyme complexes consisting of mainly three different components, endo-1,4-b-D-glucanase (EC 3.2.1.4), exoglucanase/exo-cellobiohydrolase (EC 3.2.1.91), and b-glucosidase (EC 3.2.1.21). All these three parts act synergistically to hydrolyze the cellulose polymer totally into its glucose monomers. Most of the wild strain of *Trichoderma* produces the enzyme with less or no β-glucosidase (Singhania et al. 2010).

Now to improve the economy of cellulase production, rDNA technology has been used to improve the production rate along with specificity and selectivity (Anderson et al. 2005). Therefore enzyme demand is increasing with around 4% rate annually as per the reports of CAGR 2012–2014 (Global Industrial Enzyme Market Report 2013). Now more patent is coming in cellulase improvement as mentioned in Table 5.2.

Cellulase is part of hydrolase (proteases) which captures around 60% of the market share (Sukumaran et al. 2005). The main attempt via rDNA is specificity so that enzyme loading can be increased. North America is the highest user of cellulase, while among developing countries, the Asia Pacific and African countries are the

S. M. Bhatt (✉)
Sant Baba Bhag Singh University, Jalandhar, Punjab, India

S. Bhat
CGC Landran, Mohali, Punjab, India

M. Srivastava et al. (eds.), *Approaches to Enhance Industrial Production of Fungal Cellulases*, Fungal Biology,
https://doi.org/10.1007/978-3-030-14726-6_5

Table 5.1 Applications of cellulases

Type of industry	Enzymes in use	Applications
General purpose	Cellulase	(a) Used for improving the softness and drape of the fabric. For biopolishing of cellulosic fabric, cellulases are used in acidic conditions
		(b) Used for the removal of the indigo dye trapped inside the fibers by combined action of enzymes and mechanical agitation of the fabric
	Pectinase	For bio-scouring of cellulosic fabric, pectinases are used which work better under alkaline conditions
		Pectin and associated hemicelluloses are hydrolyzed from the fabrics and removed in an eco-friendly manner
	Catalase	Used as bleaching agent
Laundry detergents	Alkaline protease	Protein-based stains are degraded into small readily soluble peptides and amino acids and are easily removed during washing process. Hence efficiency of detergents is increased
	Alkaline amylase	To decompose starch-based stains, it is used in automatic dishwashing liquid detergent formulations where starch-based stains are degraded into small, readily soluble compounds and thus can be removed easily during washing process
	Alkaline lipase	Used for decomposition of fatty-based stains like fats, butter, and salad oil

leaders. The leading industry for cellulase production is Novozyme (Global Industrial Enzyme Market Report 2013) followed by DSM and DuPont. The worldwide industrial enzymes market is relied upon to achieve USD 9.63 billion by 2024, as indicated by report of Grand View Research, Inc. (Revenue, USD Million, 2013–2024 (https://www.grandviewresearch.com/press-release/global-industrial-enzymes-market). Novozyme and Genecore both are trying to reduce the cost of production of cellulase, for example, Genecore has developed a mutant strain named as *"Accellerase" 1000* and *"Accellerase" 1500* by genetically modifying strain with high β-glucosidase activity and at lower price. Accellerase produces both cellulase and xylanase together to digest complete cellulose in the form of xylan and glucan (http://www.genencor.com). The main problem lies due to lack of beta-glucosidase which causes accumulation of cellobiose that further causes inhibition of endoglucanase. To cope up with this problem, sometimes an external supply of β-glucosidase is essential for cocktail preparation of enzymes which can be used for biofuel production. Since most of the cellulase acts between pH 4 and 6, most of the hydrolysis experiments use acidic pretreatment.

5.2 SSF Mode of Cellulase Production

Historically, submerged fermentation was used to produce cellulase at large scale. However, it was soon realized that it's very costly due to low cellulase production and overall high operational cost in downstream processing. For example, in

Table 5.2 List of commercial enzymes, their suppliers, and source of production

	Enzyme samples	Supplier	Source
1	Cellubrix (Celluclast)	Novozymes, Denmark	*T. longibrachiatum* and *A. niger*
2	Novozymes 188	Novozymes	*A. niger*
3	Cellulase 2000L	Rhodia-Danisco (Vinay, France)	*T. reesei*, *T. longibrachiatum*
4	Rohament CL	Rohm-AB Enzymes (Finland)	*T. reesei*, *T. longibrachiatum*
5	Viscostar 150L	Dyadic(Jupiter, USA)	*T. reesei*, *T. longibrachiatum*
6	Multifect CL	Genencor Intl. (S. San Francisco, CA)	*T. reesei*
7	Biofeed beta L	Novozymes	*T. reesei*, *T. longibrachiatum*
8	Energex L	Novozymes	*T.reesei*, *T. longibrachiatum*
9	Ultraflo L	Novozymes	*T.reesei*, *T. longibrachiatum*
10	Viscozyme L	Novozymes	*T.reesei*, *T. longibrachiatum*
11	Cellulyve	50L Lyven (Colombelles, France)	*T. reesei*, *T. longibrachiatum*
12	GC 440	Genencor-Danisco (Rochester, USA)	*T. reesei*, *T. longibrachiatum*
13	GC 880	Genencor	*T. reesei*, *T. longibrachiatum*
14	Spezyme CP	Genencor	*T. reesei*, *T. longibrachiatum*
15	GC 220	Genencor	*T. reesei*, *T. longibrachiatum*
16	Accellerase 1500	Genencor	*T. reesei*
17	Cellulase AP30K	Amano Enzyme	*A. niger*
18	Cellulase TRL	Solvay Enzymes (Elkhart, IN)	*T. reesei*, *T. longibrachiatum*
19	Econase CE	Alko-EDC (New York)	*T. reesei*, *T. longibrachiatum*
20	Cellulase TAP106	Amano Enzyme (Troy, VA)	*T. viride*
21	Biocellulase TRI	Quest Intl. (Sarasota, FL)	*T. reesei*, *T. longibrachiatum*
22	Biocellulase A	Quest Intl.	*A. niger*
23	Ultra-low microbial(ULM)	logen (Ottawa, Canada)	*T. reesei*, *T. longibrachiatum*

Source Singhania et al. (2010)

bioethanol production from lignocellulose, cellulase cost is considerable since production cost goes around 20–50% (Himmel et al. 2007).

This was understood since most of the cellulase is produced by the fungus which needs to grow over the solid bed. That's why solid-state fermentation was reported to be more economical as compared to submerged fermentation. Another advantage of solid-state fermentation is that it requires less liquid media, is easy to maintain, minimum cost is required for downstream processing and purification of cellulase enzyme is convenient. The only drawback of SSF is the problem of heat and mass transfer during fermentation which could be solved by using microbes which are thermotolerant. Solid-state fermentation is commonly used to prepare fermented foods like miso, pickles, soya sauce, and other foods in most of the Asian countries (Sukumaran et al. 2005).Therefore, we will discuss about how cellulase is produced and how they can be modified with more focus on SSF mode optimization strategies.

5.3 SmF Versus SSF

Many research reports show a comparison of work in submerged (SmF) versus SSF mode to see which one is more feasible and has high production rate. For details, see Table 5.3 (Deshpande et al. 2009; Gamarra et al. 2010; Gautam et al. 2010; Han et al. 2017; Kannahi and Elangeswari 2015; Radhika et al. 2013).

Various species of *Pleurotus* (viz., *P. florida*, *P. ostreatus*, *and P. sajor-caju*) have been compared for cellulase production in SmF and SSF mode using different substrates like rice husk, paddy straw, rice bran, sawdust, sesame oil cake and wheat bran (Radhika et al. 2013). Based on the work done by different authors, it was reported that cellulase was better produced in SmF, while laccase was produced better with SSF (Gautam et al. 2011).

Some worker uses *Penicillium oxalicum* in SSF mode to investigate commercial cellulase production in shake flask and then scale up to 7-liter vessel. Optimum pH and temperature were pH 5 and 50 °C, respectively. At 50 °C, the produced cellulase retained approximately 50% and 26% of its activity at 48 h and 72 h, respectively. Thus, it was concluded that this strain can be used to produce commercial cellulase (Saini et al. 2015).

In an another study, pineapple peel was used as a substrate for cellulase production under SmF fermentation using *Aspergillus niger* and *Trichoderma viride,* and it was reported that *Aspergillus niger* can be used for large-scale production of cellulase (Kannahi and Elangeswari 2015). In an another study, *Penicillium oxalicum* RE-10 was used for cellulase production under fed-batch fermentation, and optimization was done using Plackett-Burman design (PBD) and central composite design (CCD) (Han et al. 2017). In this work, the author has reported fed-batch fermentation enhances the cellulase production as compared to submerged fermentation and cellulase productivity was increased from 105.75 U/L/h in batch fermentation to 158.38 U/L/h in fed-batch fermentation (Table 5.4).

A decent survey composed over a similar investigation of SmF versus SSF which demonstrates that the economy of cellulase creation lies more with SSF when contrasted with SmF (Srivastava et al. 2018). *Trichoderma reesei* (QM 1914) strain has been also used in submerged fermentation (Neagu et al. 2012) using wheat bran or

Table 5.3 Cellulase enzyme manufacturer and patents' source (Singhania et al. 2010)

Manufacturer	Patents
Novozymes, Bagsvaerd, Denmark	902
Genencor, Copenhagen, Denmark	355
DSM, Delft, the Netherlands	398
AB Enzymes, Feldbergstrasse, Germany	22
ADM, Illinois, USA	2
logen, Ontario Canada	39
Dyadic, Florida, USA	11
Enmex,Tlalnepantla, Mexico	1

sawdust that performed better production at low temperature. Cellulase production with white-rot basidiomycetes fungi in solid-state versus submerged cultivation (Bentil et al. 2018).

The main problem reported during submerged fermentation was high viscosity of the medium and low pH which hampers the growth of fungus *Trichoderma*, and thus it can be resolved by SSF which may prove better for enhanced cellulase production (Chang et al. 2017). Some authors modified parent strain for more glucose consumption so that there should not be oxygen-limiting conditions and thus even in viscous medium cellulase production would not cease. As we have discussed, ameliorating oxygen supply is one of the major challenges encountered in cellulase production in submerged mode. Therefore, modifying *Trichoderma reesei* with bacterial hemoglobin gene has shown to have a profound effect on cellulase production as much as 2.2-fold increase as compared to parent strain (Su et al. 2017).

Table 5.4 Comparison of submerged fermentation (SmF) and solid-state fermentation (SSF)

Parameter	SmF	SSF
1. Cellulosic substrate	Pure cellulose	Utilization of the natural cellulosic wastes as substrates
2. Aseptic conditions	Required to avoid contamination under high-moisture conditions	Aseptic conditions can be curtailed under water-limited conditions of fermentation
3. Substrate utilization	A max utilization of only 5% in SmF process	The amenability of SSF technique to utilize 20–30% of the substrate makes it more promising
4. Moisture requirements	Large volumes of water needed	Carried out in the absence of free flowing water
5. Process parameters	Generally involves mixing, aeration, control, and monitoring of temp, pH, dissolved oxygen, and gas flow rates and therefore profitable in terms of its higher degree of process control and monitoring	Generally operated under static conditions
6. Effluent generation	Large volume of effluents discarded	Virtually no effluent generation
7. Scale-up	Easy scale-up and industrial equipment's are available	Scale-up is problematic, new design equipment's are needed
8. Energy consumption	High	Low
9. Productivity	30–80 g/L	100–300 g/L, 2–3 times higher enzyme production as well as protein rate also enhanced titers of the product in the medium
10. Downstream processing	Requires DSP	Air-dried fermented solids can be directly used as source of enzyme eliminating the need of expenses on DSP

5.4 Microbes and Other Conditions in SSF

Various microbes are used for cellulase production especially various species of fungus like *Trichoderma* and *Aspergillus* using lignocellulosic substrates (Chahal et al. 1996; Mrudula and Murugammal 2011). The fungus is thought to be more beneficial for cellulase production because of the presence of a complete cellulase system, and moreover it secretes enzymes extracellularly as compared to the bacterial system. There are various strains of fungus which lack some important component in enzyme complex such as β-glucosidase, which is not synthesized in *Trichoderma* while endo- and exoglucanases are lacking in *Aspergillus.* Other microbes used in SSF mode are *Penicillium oxalicum* which secrete higher β- glucosidases when grown in cassava residue (Su et al. 2017). It has also an advantage that no inhibition of enzyme is reported due to accumulation of cellobiose. Another advantage was an increase in twofold enzyme secretion without any optimization of media conditions. Similarly with soybean hulls, *Aspergillus* tends to produce high β-glucosidase (Julia et al. 2016). Similarly, high concentration of enzyme is produced in newly designed reactor FERMSOSTAT where *Aspergillus Niger* USM AI 1 was grown on the mixture of sugarcane bagasse and palm kernel cake as substrates at 1:1 (w/w) ratio. As a result, 62.6 U/g of CMCase activity was obtained under optimized conditions. Some modification in SSF mode such as the use of fed-batch SSF also resulted in enhanced results in cellulase enzyme production (Zhu et al. 2006). However, some workers reported that improved enzyme activity was observed at low pH conditions ranging from 3 to 5 (Lee and Koo 2001). In the 50 L reactor, under the fed-batch conditions, high titer of cellulase was recovered using *Trichoderma reesei* (C30) (Hendy et al. 1984). Fed-batch process frequently evacuates impediment of low water and supplement glucose which is a constraint regularly experienced in SSF mode. It has been observed that (Lee and Koo 2001) supply of glucose up to 45 g/L increased cellulase production manifold. This strategy is well suitable for cocktail production of enzymes also (Passos et al. 2018).

5.5 Role of Inducer/Accessory Proteins in Cellulase Production

It has been observed that supply of accessory protein such as LPMO and swollenin helps in swelling of cellulose. LMPO is lytic polysaccharide monooxygenases (Seidl et al. 2008) and was first reported in some insects which is required for an effective deconstruction activity.

Cellulase Secretary Pathway

Groleau et al. demonstrated that some protein aggregates that were larger than 4×10^3 kDa were having a role in cellulase secretion. This protein was called as LMPO. It has been reported that sometimes cellulase is secreted during starvation conditions. Therefore some signaling molecules activated in *Aspergillus nidulans* are responsible for the release of cellulase in the extracellular medium (Brown et al.

2014). In *Clostridium acetobutylicum* ATCC 82, cellulase secretion depends on the specific substrate, for example, glucose and cellobiose inhibited cellulase production, while xylose enhanced cellulase secretion (López-Contreras et al. 2004). Some workers observed the role of farnesol, which was first discovered quorum-sensing molecule, in high secretion of cellulase due to the highly responsive role.

5.6 Cellulase Optimization Strategies

Optimization of cellulase production has been conducted in various studies by (Ellouz Chaabouni et al. 1995; Harendra et al. 2013; Jampala et al. 2017; Muthuvelayudham and Viruthagiri 2007; Prasanna et al. 2016; Sethi et al. 2013). Various microbes were selected such as *Penicillium*, *Trichoderma*, etc. where high endoglucanase activity was reported (Prasanna et al. 2016). Other microbes such as *Aspergillus flavus* NSPR017, *Aspergillus flavus* NSPR016, and *Aspergillus flavus* NSPR019 were used over various agricultural wastes such as orange peel for cellulase production. The author has reported production strategies were optimal with a low-cost system which may be useful for industrial production of cellulase enzyme (Juliet et al. 2013). The various workers have also optimized the media components using CCD (Gunny et al. 2015) and RSM (Muthuvelayudham and Viruthagiri 2010; Saravanan et al. 2013; Thakkar and Saraf 2014). A couple of thermophilic strain likewise has been streamlined utilizing RSM innovation for cellulase generation (Shajahan et al. 2017). Generally, Plackett-Burman design has been used for screening of nutrients and media conditions and then optimization by using RSM selecting more factors with interaction among each factor. The author reported that pH 9 was best for high production of exo-cellulase production from *Bacillus cereus* in submerged conditions (Tabssum et al. 2018). Initial screening of media components using multiple regression analysis shows media factors such as Tween 20, $CaCl_2$, ammonium nitrate, ammonium chloride, and yeast extract affect most of the cellulase production (Shajahan et al. 2017). After optimization, it was revealed that best nitrogen source was yeast extract which boosts growth and cellulase production for *Bacillus subtilis* (Muthuvelayudham and Viruthagiri 2010). Also, sugar produced by microbial cellulase was reported to be 3.85 mg/ml and 2.30 mg/ml after 6 h of incubation at 50 °C, and percent hydrolysis of commercial cellulase and local cellulase was 19.25% and 11.50%, respectively. The low saccharification rate observed in indigenous methods was due to the production of some inhibitors during bioprocessing of cellulose. In recent work (Mohapatra et al. 2018), it has been reported that *Aspergillus fumigatus* produces high FPase and CMCase under optimized conditions using RSM. The total sugar reported was 396.6 and 355.8 (mg/g), and after xylanase addition, it further increased to 478.7 and 483.3 (mg/g). For CMCase, optimum pH reported was 4, while for FPase optimum pH reported was 5. Some authors reported that type of lignocellulose also matters a lot in significant production of cellulase, while the presence of some metal ions such as Mg^{+2} and $Ca + ^{2}$ also plays a significant role in protein synthesis after transcription.

Economical production of cellulase therefore depends on various medium factors and bioprocessing steps involved adapted such as pretreatment, hydrolysis, and fermentation. For example, hydrolysis of the substrate takes around 40% cost of the total production cost in cellulolytic enzyme production. For optimum cellulase production, selection of substrate, microorganisms, other conditions are important parameters (Deshavath et al. 2018), pretreatment of lignocellulosic biomass is also a cost-effective factor for industrial production of cellulase. For example, alkaline pretreatment mostly uses NaOH which may prove helpful in lowering down the cost since it's able to solubilize both hemicellulose and lignin. Sometimes the design of the reactor is also helpful such as stirred tank reactor (STR) which has been successfully employed in increasing enzyme production up to 51.3 FPU/g using sorghum (Deshavath et al. 2018). Recently for economical production of cellulase, stirred tank reactor has been used for alkaline-pretreated agrowaste using *Phanerochaete chrysosporium* NCIM 1106 (Tabssum et al. 2018). One recombinant strain of *Pichia pastoris* is produced where β-glucosidase gene (bgl) was expressed from *Aspergillus niger* (Xia et al. 2018) that resulted in high production of β-glucosidase (129 IU/mL) using corn powder. Some researchers have created a mutant of *Penicillium janthinellum* and checked cellulase production in the small bioreactor in presence of CSL. As a result, production of cellulase was doubled as compared to the normal one (up to 5.44 ± 0.3 FPU/ml at 168 h) (Singh et al. 2018).

Similarly, some gene activator were used recently for producing over an expressive strain of *T. reesei* RUT-C-30 called as Trvib-1. As a result cellulase production reported to increase 200 times (Fig. 5.1) (Zhang et al. 2018). The author has reported that treatment of this strain with corn stover resulted in enhanced sugar production after a 40% improvement in hydrolysis.

Recently some worker reported that some bacterial strain such as *Bacillus subtilis MU S1,* a strain isolated from Eravikulam National Park, resulted in 3.2 times more production of cellulase using CMC after optimization with Plackett-Burman design (Sreena and Sebastian 2018). Optimized cellulase concentration level was 566.66 U/ml at pH 7 and other media components such as yeast extract, CMC, NaCl, $MgSO_4$, and $NaNO_3$.

5.7 Microbial Consortia Applications

Consortia are intuitive groupings of microorganisms, multispecies collections which have been utilized as of late for application in SSF mode for practical cellulase creation and all the while for improved saccharification (Angenent and Wrenn 2008). Use of microbial consortia has also find its applications in expansive scale biogas and biopolymer creation (Kleerebezem and van Loosdrecht 2007). What's more, against monoculture approaches, microbial consortia are more valuable for improved absorption and transformation of lignocellulose biomass (Eiteman et al. 2008; Shao et al. 2009; Szambelan et al. 2004). In efficient production of cellulase,

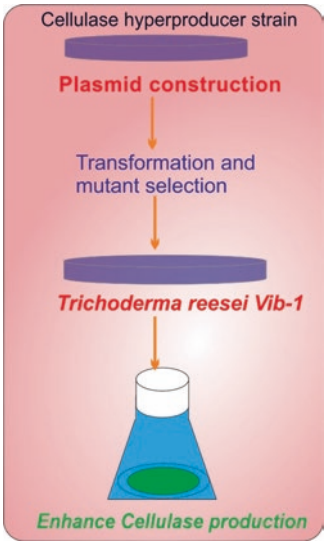

Fig. 5.1 Cellulase hyper-producing strain adapted from (Zhang et al. 2018)

different *Basidiomycetes* species have been accounted for, for example, *Phanerochaete chrysosporium*, *Ceriporiopsis subvermispora*, *Phlebia subserialis*, and *Pleurotus ostreatus*, which likewise has been utilized in delignification of cotton stalks (Kumar et al. 2009); *P. chrysosporium*, *Phanerochaete chrysosporium*, *Phlebia radiata*, *Dichomitus squalens*, *Rigidoporus lignosus*, and *Jungua separabilima* (Shi et al. 2008) have been utilized in delignification of wheat straw (Magnusson et al. 2008). Mostly saprophytic fungi secrete cellulase and xylanase together (Kumar et al. 2012; Zeng et al. 2011). This may result in upgraded saccharification, while a few strains of organisms have been utilized for separation of silica from rice, e.g., *Fusarium* (Bansal et al. 2006; Della et al. 2002; Yu et al. 2010). Moreover, expanded recovery of cellulose has been accounted for from rice straw after consolidated utilization of ammonia and ionic fluid (1-Ethyl-3-methylimidazolium acetate (Emim)Ac) for 82% of the cellulose recovery with 97% of the enzymatic saccharification (Nguyen et al. 2010; hua Li et al. 2010; Binder and Raines 2010). Improved enzymatic recovery of cellulose (30.6%) and 43.3% hemicellulose gotten from rice after microwave helped pretreatment (Ma et al. 2009). Fungal pretreatment isn't just environment-friendly methodology; however cellulose generation additionally is expanded (Sánchez 2009).

Auto hydrolysis and organosolv delignification have been utilized for fractionation of *Eucalyptus globulus* wood (Romaní et al. 2011). As of late, it has been accounted for that building of microbial consortia can complete complex capacities and they may be more powerful to changes in their surroundings than are singular populaces (Brenner et al. 2008). Advancement of symbiotic consortia for lignocellulosic biofuel creation is additionally revealed by (Zuroff and Curtis 2012). Characteristic and designed collaborations might be a promising strategy for community control and regulation. Organism, consortia, and bioprocess configuration must propel as an

inseparable unit with specialized and monetary possibility keeping in mind the end goal to make lignocellulosic biofuels a reality.

5.8 Process Economy of Production and Extraction in SSF Mode

For the process economy of cellulase production, some worker prefer to use consolidated bioprocessing which is a single step and easy to use (Lynd et al. 2008). Also, the major focus was to increase thermo stability and enzyme catalytic efficiency and reuse of enzymes for further lowering down the cost of cellulase production. Some anaerobic bacteria reported possessing cellulosome which is known to degrade cellulose via using carbohydrate-binding modules (CBMs) joined by a flexible linker. It has also been reported that the presence of CBM in cellulosome increases enzyme activity many folds (Byrt et al. 2012). CBM also enables microbes to sustain high temperature and thus high efficiency can be achieved. This activity was reported after site-directed mutagenesis in *Bacillus* (Yin et al. 2011). CBM is thought to play a role in catalytic domain to substrate cellulose and improve the efficiency of the cellulase by enabling digestion within the bulk of the film (Reyes-Ortiz et al. 2013).

For extraction of cellulase, acetate buffer has been employed (Marín et al. 2018) at pH range 4–5. The authors have reported different activities of cellulase in different extraction buffers (Marín et al. 2018) and reported that optimal ratio of solid bed versus buffer should be 1:4 for optimized extraction of beta-endoglucanase using fermented wheat bran and for total extraction of enzyme, the recommended ratio was 1:15. Cellulase activity was measured by FPase activity as per *Ghosh* et al. The authors also compared the static versus dynamic mode of cell separation (centrifugation). It was accounted for that in static mode, enzyme action was more when contrasted with dynamic mode. In theoretical it's fundamental to enhance the extraction condition in SSF mode which relies upon substrate utilized, pretreatment, and pH along with extraction buffer (Pirota et al. 2013). Some author reported that other conditions such as solid to liquid ratio, the type of agitation, the temperature, and the contact time are also an important variable (Castilho et al. 2000). Very few economic analysis have been done for extraction of cellulase (Klein et al. 2016) limited to biofuel production. For that, the author has developed a "Bioeconomics" tool that can simulate total capital cost and cost incurred in total production of lipase enzyme. Based on the work done, the author concluded that for extraction of enzyme, SSF seems to be more costly in terms of total equipment investment since one has to change the equipment in case of SSF as compared to submerged fermentation (SmF). However, in terms of high enzyme production, SSF was most suitable as compared to submerged fermentation. (Klein-Marcuschamer et al. 2012). Here in the experiment, economic analysis was done for calculating cost of enzyme for ethanol production from acid pretreated corn stover. They also focused that overall

half of the total cost of enzyme incurred in equipments via SSF mode and the rest one third of enzyme cost incurred in raw material used.

5.9 Pilot-Scale Production Strategies

Since most recent 10 years, more broad work has been done for pilot-scale production of cellulase (Liming and Xueliang 2004; Juturu and Wu 2014; Ahamed and Vermette 2008; Srivastava et al. 2018). A few authors utilized thermotolerant yeast strain *Kluyveromyces marxianus* CECT 10875 in SSF to deliver ethanol with commercial cellulase after treatment of *Sorghum* sp. bagasse, wheat straw, and *Brassica carinata* residue. A few creators assessed sparing generation systems, and their finding uncovers that when contrasted with SMF, SSF is more prudent and favorable (Srivastava et al. 2018). Steps of pilot-scale production have been shown in Fig. 5.2.

One exceptionally fascinating work has been specified as utilization of coffee husk for cellulase and xylanase generation utilizing microbial consortia (Cerda et al. 2017). According to their tests, just issue confronted was increment in temperature in bioreactor which prompts decrease in cellulase creation for which they have suggested thermotolerant species in bioreactor. The greater part of the worker leans toward SSF mode amid merged handling where microorganisms and substrate are utilized at the same time. An exceptionally intriguing work has been done to enhance pilot-scale generation of cellulase utilizing cane molasses and cellulosic

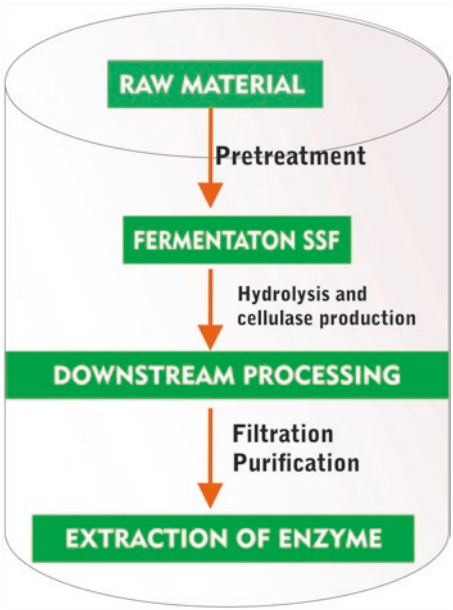

Fig. 5.2 Pilot-scale downstream processing of cellulase

biomass as soybean hull in presence of *Trichoderma reesei* (Ellilï et al. 2017). These authors utilized invertase by including *Aspergillus niger* in the medium for most extreme hydrolysis of cane molasses sugar.

The author detailed that little adjustment in medium can upgrade cellulase generation and can be a decent choice for commercial cellulase preparations. Some novel strategies were adjusted by putting fermentation in semi-simultaneous saccharification and fermentation mode (SSSF) (Gomes et al. 2018). Downstream processing is so far less studied division at pilot scale (Marín, Artola, and Sánchez 2018). For zero waste-based enzyme generation, two-stage enzyme extraction was finished.

5.10 Conclusion

In brief we can conclude that SSF or SmF mode choice depends on the type of substrate used and microorganisms selected. Besides this recombinant DNA, technology has played a vital role in lowering down the cost of cellulase further. Mutant strain is more robust and efficient as compared to wild type in terms of cellulase production. Additional requirements of metals and inducers may boost additional production of enzyme, while SSF mode and batch or fed-batch reactor may further be helpful in improving production of cellulase. Thus overall production of cellulase depends on equipments' cost and medium components (Singhania et al. 2007).

Acknowledgment The author expresses their deep thanks to Professor Pradeep Mishra and Neha Srivastava for providing this opportunity. Also the author declares no conflict of interest lies in the production of article and no fund is involved from anywhere during the writing of article. Tables and figures adapted in articles are properly cited.

References

http://www.genencor.com
https://www.grandviewresearch.com/press-release/global-industrial-enzymes-market
https://www.prweb.com/releases/2013/industrial-enzymes-market/prweb10968094.htm
Ahamed A, Vermette P (2008) Culture-based strategies to enhance cellulase enzyme production from Trichoderma Reesei RUT-C30 in bioreactor culture conditions. Biochem Eng J 40(3):399–407. https://doi.org/10.1016/j.bej.2007.11.030
Anderson WF, Peterson J, Akin DE, Herbert Morrison W (2005) Enzyme pretreatment of grass lignocellulose for potential high-value co-products and an improved fermentable substrate. In: Twenty-sixth symposium on biotechnology for fuels and chemicals. Springer, pp 303–310
Angenent LT, Wrenn BA (2008) Optimising mixed-culture bioprocessing to convert wastes into bioenergy. In: Bioenergy, pp 179–194
Bansal V, Poddar P, Ahmad A, Sastry M (2006) Room-temperature biosynthesis of ferroelectric barium titanate nanoparticles. J Am Chem Soc 128:11958–11963. https://doi.org/10.1021/ja063011m

Bentil JA, Thygesen A, Mensah M, Lange L, Meyer AS (2018) Cellulase production by white-rot basidiomycetous fungi: solid-state versus submerged cultivation. Appl Microbiol Biotechnol 2018. https://doi.org/10.1007/s00253-018-9072-8

Binder JB, Raines RT (2010) Fermentable sugars by chemical hydrolysis of biomass. Proc Natl Acad Sci U S A 107:4516–4521. https://doi.org/10.1073/pnas.0912073107

Brenner K, You L, Arnold FH (2008) Engineering microbial consortia: a new frontier in synthetic biology. Trends Biotechnol 26(9):483–489. https://doi.org/10.1016/j.tibtech.2008.05.004

Brown NA, dos Reis TF, Goinski AB, Savoldi M, Menino J, Almeida MT, Rodrigues F, Goldman GH (2014) The Aspergillus nidulans signalling mucin MsbA regulates starvation responses, adhesion and affects cellulase secretion in response to environmental cues. Mol Microbiol 94(5):1103–1120. https://doi.org/10.1111/mmi.12820

Byrt CS, Cahyanegara R, Grof CPL (2012) Plant carbohydrate binding module enhances activity of hybrid microbial cellulase enzyme. Front Plant Sci 3:254. https://doi.org/10.3389/fpls.2012.00254

Castilho LR, Polato CMS, Baruque EA, Sant'Anna GL Jr, Freire DMG (2000) Economic analysis of lipase production by penicillium restrictum in solid-state and submerged fermentations. Biochem Eng J 4(3):239–247

Cerda A, Mejías L, Gea T, Sánchez A (2017) Cellulase and xylanase production at pilot scale by solid-state fermentation from coffee husk using specialized consortia: the consistency of the process and the microbial communities involved. Bioresour Technol 243:1059–1068. https://doi.org/10.1016/j.biortech.2017.07.076

Chahal PS, Chahal DS, Lê GBB (1996) Production of cellulase in solid-state fermentation with Trichoderma Reesei MCG 80 on wheat straw. Appl Biochem Biotechnol 57–58:433–442. https://doi.org/10.1007/BF02941724

Chang KL, Chen XM, Wang XQ, Han YJ, Potprommanee L, Liu J y, Liao YL, Ning X a, Sun S y, Huang Q (2017) Impact of surfactant type for ionic liquid pretreatment on enhancing delignification of rice straw. Bioresour Technol 227:388–392. https://doi.org/10.1016/j.biortech.2016.11.085

Della VP, Kühn I, Hotza D (2002) Rice husk ash as an alternate source for active silica production. Mater Lett 57:818–821. https://doi.org/10.1016/S0167-577X(02)00879-0

Deshavath NN, Sahoo SK, Panda MM, Mahanta S, Goutham DSN, Goud VV, Dasu VV, Jetty A (2018) The cost-effective stirred tank reactor for cellulase production from alkaline-pretreated agriculture waste biomass. In: Utilization and management of bioresources. Springer, Singapore, pp 25–35

Deshpande P, Nair S, Khedkar S (2009) Water hyacinth as carbon source for the production of cellulase by Trichoderma Reesei. Appl Biochem Biotechnol 158(3):552–560. https://doi.org/10.1007/s12010-008-8476-9

Eiteman MA, Lee SA, Altman E (2008) A co-fermentation strategy to consume sugar mixtures effectively. J Biol Eng 2:3. https://doi.org/10.1186/1754-1611-2-3

Ellilï S, Fonseca L, Uchima C, Cota J, Goldman GH, Saloheimo M, Sacon V, Siika-Aho M (2017) Development of a low-cost cellulase production process using Trichoderma Reesei for Brazilian biorefineries. Biotechnol Biofuels 10:30. https://doi.org/10.1186/s13068-017-0717-0

Ellouz Chaabouni S, Belguith H, Hassairi I, M'Rad K, Ellouz R (1995) Optimization of cellulase production by Penicillium Occitanis. Appl Microbiol Biotechnol 43(2):267–269. https://doi.org/10.1007/BF00172822

Gamarra NN, Villena GK, Gutiérrez-Correa M (2010) Cellulase production by Aspergillus Niger in biofilm, solid-state, and submerged fermentations. Appl Microbiol Biotechnol 87(2):545–551. https://doi.org/10.1007/s00253-010-2540-4

Gautam, S. P., P. S. Bundela, A. K. Pandey, M. K. Awasthi, S. Sarsaiya, and others. 2010. "Optimization of the medium for the production of cellulase by the Trichoderma Viride using submerged fermentation." Int J Environ Sci 1 (4): 656–665

Gautam SP, Bundela PS, Pandey AK, Khan J, Awasthi MK, Sarsaiya S (2011) Optimization for the production of cellulase enzyme from municipal solid waste residue by two novel cellulolytic fungi. Biotechnol Res Int 2011(810425). https://doi.org/10.4061/2011/810425

Gomes A d C, Moysés DN, Santa Anna LMM, de Castro AM (2018) Fed-batch strategies for Saccharification of pilot-scale mild-acid and alkali pretreated sugarcane bagasse: effects of solid loading and surfactant addition. Ind Crop Prod 119:283–289. https://doi.org/10.1016/j.indcrop.2018.04.026

Gunny AAN, Arbain D, Jamal P, Gumba RE (2015) Improvement of halophilic cellulase production from locally isolated fungal strain. Saudi J Biol Sci 22(4):476–483. https://doi.org/10.1016/j.sjbs.2014.11.021

Global Industrial Enzymes Market Report (2013) Edition, 2013, Available at URL: http://www.reportlinker.com/p0848804-summary/Global-Industrial-Enzymes-MarketReport-Edition.html

Han X, Song W, Liu G, Li Z, Yang P, Qu Y (2017) Improving cellulase productivity of Penicillium Oxalicum RE-10 by repeated fed-batch fermentation strategy. Bioresour Technol 227:155–163. https://doi.org/10.1016/j.biortech.2016.11.079

Harendra SK, Kumar BP, Laxmi D (2013) Optimization and production of cellulase from agricultural waste. Res J Agri For Sci 1(7):2320–6063

Hendy NA, Wilke CR, Blanch HW (1984) Enhanced cellulase production in fed-batch culture of Trichoderma Reesei C30. Enzyme Microb Technol 6(2):73–77

Himmel ME, Ding S-Y, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD (2007) Biomass recalcitrance: engineering plants and enzymes for biofuels production. Science 315(5813):804–807

Jampala P, Tadikamalla S, Preethi M, Ramanujam S, Uppuluri KB (2017) Concurrent production of cellulase and xylanase from Trichoderma Reesei NCIM 1186: enhancement of production by desirability-based multi-objective method. 3 Biotech 7(1). https://doi.org/10.1007/s13205-017-0607-y

Julia BM, Belén AM, Georgina B, Beatriz F (2016) Potential use of soybean hulls and waste paper as supports in SSF for cellulase production by Aspergillus Niger. Biocatal Agric Biotechnol 6:1–8. https://doi.org/10.1016/j.bcab.2016.02.003

Juliet AB, Cyrus ET, Oladiti OO (2013) Parameters optimization of cellulase Zymosynthesis by Aspergillus flavus NSPR017 grown on pretreated orange peels. Nat Sci 11(10):80–87

Juturu V, Wu JC (2014) Microbial cellulases: engineering, production and applications. Renew Sustain Energy Rev 33:188–203. https://doi.org/10.1016/j.rser.2014.01.077

Kannahi M, Elangeswari S (2015) Enhanced production of cellulase on different fruit peel under submerged fermentation. Int J Pharm Sci Rev Res 32(2):161–165

Kleerebezem R, van Loosdrecht MCM (2007) Mixed culture biotechnology for bioenergy production. Curr Opin Biotechnol 18(3):207–212. https://doi.org/10.1016/j.copbio.2007.05.001

Klein M, Griess O, Pulidindi IN, Perkas N, Gedanken A (2016) Bioethanol production from Ficus Religiosa leaves using microwave irradiation. J Environ Manage 177:20–25

Klein-Marcuschamer D, Oleskowicz-Popiel P, Simmons BA, Blanch HW (2012) The challenge of enzyme cost in the production of Lignocellulosic biofuels. Biotechnol Bioeng 109(4):1083–1087

Kumar S, Singh SP, Mishra IM, Adhikari DK (2009) Recent advances in production of bioethanol from Lignocellulosic biomass. Chem Eng Technol 32(4):517–526. https://doi.org/10.1002/ceat.200800442

Kumar L, Arantes V, Chandra R, Saddler J (2012) The lignin present in steam pretreated softwood binds enzymes and limits cellulose accessibility. Bioresour Technol 103:201–208. https://doi.org/10.1016/j.biortech.2011.09.091

Lee S-M, Koo Y-M (2001) Pilot-scale production of cellulase using Trichoderma Reesei rut C-30 fed-batch mode. J Microbiol Biotechnol 11(2):229–233

Li XH, Yang HJ, Roy B, Park EY, Jiang LJ, Wang D, Miao YG (2010) Enhanced cellulase production of the Trichoderma Viride mutated by microwave and ultraviolet. Microbiol Res 165:190–198. https://doi.org/10.1016/j.micres.2009.04.001

Liming X, Xueliang S (2004) High-yield cellulase production by Trichoderma Reesei ZU-02 on corn cob residue. Bioresour Technol 91(3):259–262

López-Contreras AM, Gabor K, Martens AA, Renckens BAM, Claassen PAM, Van Der Oost J, De Vos WM (2004) Substrate-induced production and secretion of cellulases by clos-

tridium Acetobutylicum. Appl Environ Microbiol 70(9):5238–5243. https://doi.org/10.1128/AEM.70.9.5238-5243.2004

Lynd LR, Laser MS, Bransby D, Dale BE, Davison B, Hamilton R, Himmel M, Keller M, McMillan JD, Sheehan J (2008) How biotech can transform biofuels. Nat Biotechnol 26(2):169

Ma H, Liu WW, Chen X, Wu YJ, Yu ZL (2009) Enhanced enzymatic saccharification of rice straw by microwave pretreatment. Bioresour Technol 100:1279–1284. https://doi.org/10.1016/j.biortech.2008.08.045

Magnusson L, Islam R, Sparling R, Levin D, Cicek N (2008) Direct hydrogen production from cellulosic waste materials with a single-step dark fermentation process. Int J Hydrogen Energy 33:5398–5403. https://doi.org/10.1016/j.ijhydene.2008.06.018

Marín M, Artola A, Sánchez A (2018) Optimization of down-stream for cellulases produced under solid-state fermentation of coffee husk. Waste Biomass Valorization:1–12

Mohapatra S, Padhy S, Das Mohapatra PK, Thatoi HN (2018) Enhanced reducing sugar production by saccharification of lignocellulosic biomass, Pennisetum species through cellulase from a newly isolated Aspergillus fumigatus. Bioresour Technol 253:262–272

Mrudula S, Murugammal R (2011) Production of cellulase by Aspergillus Niger under submerged and solid state fermentation using coir waste as a substrate. Braz J Microbiol 42(3):1119–1127

Muthuvelayudham R, Viruthagiri T (2007) Optimization and modeling of cellulase protein from *Trichoderma reesei* rut C30 using mixed substrate. Afr J Biotechnol 6(1):41–46

Muthuvelayudham R, Viruthagiri T (2010) Application of central composite design based response surface methodology in parameter optimization and on cellulase production using agricultural waste. Int J Chem Biol Eng: 97–104

Neagu DA, Destain J, Thonart P, Socaciu C (2012) Trichoderma Reesei cellulase produced by submerged versus solid state fermentations. Bull UASVM Agri 69(2):320–326

Nguyen TAD, Kim KR, Han SJ, Cho HY, Kim JW, Park SM, Park JC, Sim SJ (2010) Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars. Bioresour Technol 101:7432–7438. https://doi.org/10.1016/j.biortech.2010.04.053

Passos DDF, Pereira N, de Castro AM (2018) A comparative review of recent advances in cellulases production by Aspergillus, Penicillium and Trichoderma strains and their use for lignocellulose deconstruction. Curr Opin Green Sustain Chem 14:60–66. https://doi.org/10.1016/j.cogsc.2018.06.003

Pirota RDPB, Miotto LS, Delabona PS, Farinas CS (2013) Improving the extraction conditions of endoglucanase produced by Aspergillus Niger under solid-state fermentation. Braz J Chem Eng 30(1):117–123. https://doi.org/10.1590/S0104-66322013000100013

Prasanna HN, Ramanjaneyulu G, Rajasekhar Reddy B (2016) Optimization of cellulase production by Penicillium Sp. 3 Biotech 6(2). https://doi.org/10.1007/s13205-016-0483-x

Radhika R, Roseline Jebapriya G, Joel Gnanadoss J (2013) Production of cellulase and laccase using Pleurotus Sp. under submerged and solid-state fermentation. Int J Curr Sci 6:7–13

Reyes-Ortiz V, Heins RA, Cheng G, Kim EY, Vernon BC, Elandt RB, Adams PD et al (2013) Addition of a carbohydrate-binding module enhances cellulase penetration into cellulose substrates. Biotechnol Biofuels 6:93. https://doi.org/10.1186/1754-6834-6-93

Romaní A, Garrote G, López F, Parajó JC (2011) Eucalyptus globulus wood fractionation by autohydrolysis and organosolv delignification. Bioresour Technol 102:5896–5904. https://doi.org/10.1016/j.biortech.2011.02.070

Saini R, Saini JK, Adsul M, Patel AK, Mathur A, Tuli D, Singhania RR (2015) Enhanced cellulase production by Penicillium oxalicum for bio-ethanol application. Bioresour Technol 188:240–246. https://doi.org/10.1016/j.biortech.2015.01.048

Sánchez C (2009) Lignocellulosic residues: biodegradation and bioconversion by fungi. Biotechnol Adv 27(2):185–194. https://doi.org/10.1016/j.biotechadv.2008.11.001

Saravanan P, Muthuvelayudham R, Viruthagiri T (2013) Enhanced production of cellulase from pineapple waste by response surface methodology. J Eng (United States) 2013. https://doi.org/10.1155/2013/979547

Seidl V, Gamauf C, Druzhinina IS, Seiboth B, Hartl L, Kubicek CP (2008) The Hypocrea Jecorina (Trichoderma Reesei) Hypercellulolytic mutant RUT C30 lacks a 85 Kb (29 gene-encoding) region of the wild-type genome. BMC Genomics 9(1):327

Sethi S, Datta A, Lal Gupta B, Gupta S, Sethi S, Datta A, Lal Gupta B, Gupta S (2013) Optimization of cellulase production from bacteria isolated from soil. Int Sch Res Not 2013(2013):e985685. https://doi.org/10.5402/2013/985685

Shajahan S, Moorthy IG, Sivakumar N, Selvakumar G (2017) Statistical modeling and optimization of cellulase production by bacillus Licheniformis NCIM 5556 isolated from the hot spring, Maharashtra, India. J King Saud Univ Sci 29(3):302–310. https://doi.org/10.1016/j.jksus.2016.08.001

Shao X, Lynd L, Wyman C, Bakker A (2009) Kinetic modeling of cellulosic biomass to ethanol via simultaneous saccharification and fermentation: part I. Accommodation of intermittent feeding and analysis of staged reactors. Biotechnol Bioeng 102:59–65. https://doi.org/10.1002/bit.22048

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 N, Devi A, Jaryal R, Rani K (2018) An ecofriendly and efficient strategy for cost effective production of Lignocellulotic enzymes. Waste Biomass Valorization 9(6):891–898

Singhania RR, Sukumaran RK, Pandey A (2007) Improved Cellulase production by Trichoderma Reesei RUT C30 under SSF through process optimization. Appl Biochem Biotechnol 142(1):60–70

Singhania RR, Sukumaran RK, Patel AK, Larroche C, Pandey A (2010) Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. Enzyme Microb Technol 46:541–549. https://doi.org/10.1016/j.enzmictec.2010.03.010

Sreena CP, Sebastian D (2018) Augmented cellulase production by bacillus Subtilis strain MU S1 using different statistical experimental designs. J Genet Eng Biotechnol 16(1):9–16. https://doi.org/10.1016/j.jgeb.2017.12.005

Srivastava N, Srivastava M, Mishra PK, Gupta VK, Molina G, Rodriguez-Couto S, Manikanta A, Ramteke PW (2018) Applications of fungal cellulases in biofuel production: advances and limitations. Renew Sustain Energy Rev. https://doi.org/10.1016/j.rser.2017.08.074

Su LH, Zhao S, Jiang SX, Liao XZ, Duan CJ, Feng JX (2017) Cellulase with high β-glucosidase activity by Penicillium oxalicum under solid state fermentation and its use in hydrolysis of cassava residue. World J Microbiol Biotechnol 33(2):37. https://doi.org/10.1007/s11274-016-2200-7

Sukumaran RK, Singhania RR, Pandey A (2005) Microbial cellulases-production, applications and challenges. J Sci Ind Res 64(11):832–844

Szambelan K, Nowak J, Czarnecki Z (2004) Use of Zymomonas mobilis and Saccharomyces cerevisiae mixed with Kluyveromyces fragilis for improved ethanol production from Jerusalem artichoke tubers. Biotechnol Lett 26:845–848. https://doi.org/10.1023/B:BILE.0000025889.25364.4b

Tabssum F, Irfan M, Shakir HA, Qazi JI (2018) RSM based optimization of nutritional conditions for cellulase mediated Saccharification by bacillus cereus. J Biol Eng 12(1). https://doi.org/10.1186/s13036-018-0097-4

Thakkar A, Saraf M (2014) Application of statistically based experimental designs to optimize cellulase production and identification of gene. Nat Products Bioprospecting 4(6):341–351. https://doi.org/10.1007/s13659-014-0046-y

Xia Y, Yang L, Xia L (2018) High-level production of a fungal β-glucosidase with application potentials in the cost-effective production of Trichoderma reesei cellulase. Process Biochem 70:55–60

Yin X, You Q, Jiang Z (2011) Optimization of enzyme assisted extraction of polysaccharides from Tricholoma Matsutake by response surface methodology. Carbohydr Polym 86(3):1358–1364

Yu H, Zhang X, Song L, Ke J, Xu C, Du W, Zhang J (2010) Evaluation of white-rot fungi-assisted alkaline/oxidative pretreatment of corn straw undergoing enzymatic hydrolysis by cellulase. J Biosci Bioeng 110:660–664. https://doi.org/10.1016/j.jbiosc.2010.08.002

Zeng J, Singh D, Chen S (2011) Biological pretreatment of wheat straw by Phanerochaete chrysosporium supplemented with inorganic salts. Bioresour Technol 102:3206–3214. https://doi.org/10.1016/j.biortech.2010.11.008

Zhang F, Zhao X, Bai F (2018) Improvement of cellulase production in Trichoderma Reesei rut-C30 by overexpression of a novel regulatory gene Trvib-1. Bioresour Technol 247:676–683. https://doi.org/10.1016/j.biortech.2017.09.126

Zhu SD, Wu YX, Zhao YF, Tu SY, Xue YP (2006) Fed-batch simultaneous Saccharification and fermentation of microwave/acid/alkali/H2O2 pretreated Rice straw for production of ethanol. Chem Eng Commun 193(5):639–648. https://doi.org/10.1080/00986440500351966

Zuroff TR, Curtis WR (2012) Developing symbiotic consortia for Lignocellulosic biofuel production. Appl Microbiol Biotechnol 93:1423–1435. https://doi.org/10.1007/s00253-011-3762-9

Chapter 6
Cellulose as a Potential Feedstock for Cellulose Enzyme Production

Soni Tiwari and Tuhina Verma

6.1 Introduction

Cellulose is the most plentiful renewable natural resource and a cost-effective energy source based on energy content (Lynd et al. 2008; Zhang 2009). The generation of bio-based products and bioenergy from cost-competitive renewable lignocellulosic materials would bring profit to the neighboring economy, atmosphere, and national energy safety (Zhang 2008). High overheads of cellulases are one of the major problems for commercialization of biomass biorefineries since a huge quantity of cellulase is consumed for biomass saccharification (Zhang et al. 2006; Zhu et al. 2009). In order to decline cellulase use, enhance volumetric yield, and diminish center asset, consolidate bioprocessing (CBP) has been planned by integrating cellulase production, cellulose hydrolysis, and ethanol fermentation in a single step (Lynd et al. 2002, 2008). Cellulases are the enzymes that hydrolyze β-1,4 linkages in cellulose polymer. Cellulases are produced by microorganisms (fungi, bacteria, and protozoans), plants, and animals. The catalytic units of cellulases have been classified into several families based on their amino acid sequences and crystal structures (Henrissat 1991). Cellulases comprise noncatalytic carbohydrate-binding modules (CBMs) and other functionally known/unknown units, which may be situated at the N- or C-terminus of a catalytic unit. Complete cellulose hydrolysis is mediated by a grouping of three major types of cellulases: (1) endoglucanases (EC

S. Tiwari
Department of Soil Sciences and Agricultural Chemistry Institute of Agricultural Sciences BHU, Varanasi, India

T. Verma (✉)
Department of Microbiology (Centre of Excellence), Dr. Ram Manohar Lohia Avadh University, Faizabad, India

M. Srivastava et al. (eds.), *Approaches to Enhance Industrial Production of Fungal Cellulases*, Fungal Biology,
https://doi.org/10.1007/978-3-030-14726-6_6

3.2.1.4), (2) exoglucanases (EC 3.2.1.91), and (3) β-glucosidase (BG) (EC 3.2.1.21). Several microorganisms must produce the cellulases that are either free or cell-surface-bound to hydrolyze and metabolize cellulose. Cellulases are ever more being used for a huge range of manufacturing purposes—in the textile, pulp and paper industry, and food industry, as well as an stabilizer in detergents and improving digestibility of animal feeds. Currently, cellulases account for a considerable share of the world's trade enzyme market. The increasing concerns about reduction of crude oil and the discharges of greenhouse gases have forced the generation of bioethanol from lignocellulose, particularly during enzymatic hydrolysis of lignocelluloses resources—sugar platform (Bayer et al. 2007; Himmel et al. 1999; Zaldivar et al. 2001). While expenditure of cellulase for hydrolysis of pretreated lignocellulosic materials require to be reduced, their catalytic competence should be further improved in order to make the process cost-effectively (Sheehan and Himmel 1999). Cellulolytic enzymes with enhanced catalytic competence and improved thermostability are significant to commercialize lignocellulose biorefinery. Individual cellulase can be improved by using either rational design or directed advancement. Though improvements in cellulase presentation have been incremental, no harsh activity enrichment has been reported to time. The further improvement on cellulase presentation desires the improved kind of cellulose hydrolysis mechanisms as well as the association of cellulase molecular structure, function, and substrate characteristics.

6.2 Enzymatic Mechanisms of Cellulases

Glycoside hydrolases cleave glucosidic bonds by using acid–base catalysis. The hydrolysis is carried out by two catalytic residues of the enzyme: a general acid (proton donor) and a nucleophile/base (Davies and Henrissat 1995). Depending on the spatial arrangement of these catalytic residues, hydrolysis happens via retention of the anomeric pattern. For "retaining" cellulases, the anomeric C bearing the target glucosidic bond holds the substituent pattern after a double-displacement hydrolysis with two key glycosylation/deglycosylation steps. By contrast, for "inverting" cellulases, the anomeric C inverts its (substituent) configuration after a single nucleophilic displacement hydrolysis (Vocadlo and Davies 2008) (Fig. 6.1).

6.2.1 Endoglucanase

Endoglucanase (CMCase) randomly cuts β-1,4 bonds of cellulose chains, producing new ends (Fig. 6.2). Different endoglucanases are generated by archaea, bacteria, fungi, plants, and animals with different catalytic unit belonging to families 5–9, 12, 44, 45, 48, 51, and 74. Fungal endoglucanases also have a catalytic unit with or

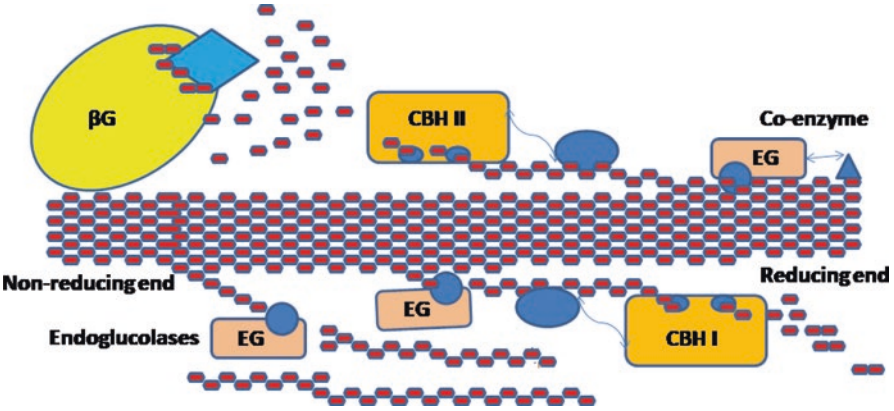

Fig. 6.1 Cellulase enzyme working together to hydrolyze cellulose molecules

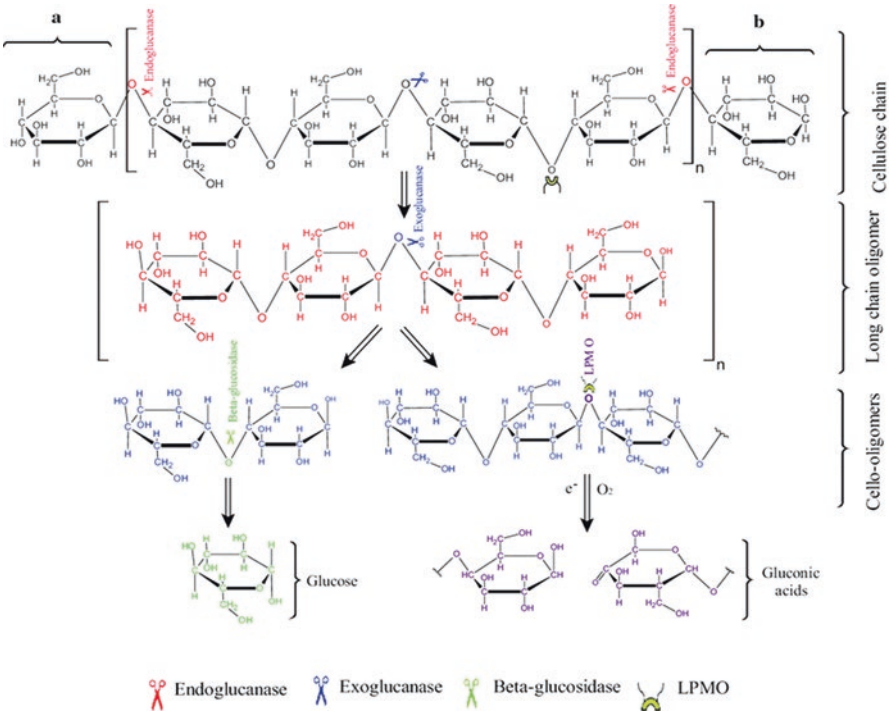

Fig. 6.2 Cellulase hydrolysis theory. (**a**) The nonreducing end; (**b**) the reducing end. Endoglucanase cleaves amorphous sites of cellulose to yield long-chain oligomers; exoglucanase processively attacks crystalline sites to produce cello-oligomers; and β-glucosidase hydrolyzes cellobiose to fermentable sugars. Lytic polysaccharide monooxygenase (LPMO) oxidizes glycosidic linkages along the cellulose chain to yield gluconic acids (Adopted from Obeng et al. 2017)

without a CBM, whereas bacterial endoglucanases may possess numerous catalytic units, CBMs, and other units with unknown function. The catalytic units of most endoglucanases have a cleft-/grove-shaped catalytic site, which permits the endoglucanases to bind and cleave the cellulose chain to produce glucose, soluble cellodextrins, or insoluble cellulose fragment (Fig. 6.2), while several endoglucanases can work "processively," based on their capacity to hydrolyze crystalline cellulose and produce the main yield as cellobiose or longer cellodextrins (Cohen et al. 2005; Li and Wilson 2008; Mejia-Castillo et al. 2008; Parsiegla et al. 2008; Yoon et al. 2008; Zverlov et al. 2005).

6.2.2 Exoglucanase

Exoglucanases perform in a processive mode on the reducing or nonreducing ends of cellulose polysaccharide chains, liberating either cellobiose or glucose as main yield (Fig. 6.2). Exoglucanases can efficiently work on microcrystalline cellulose, presumably peeling cellulose chains from the microcrystalline structure (Teeri 1997). Different CBHs are generated by several microorganisms (bacteria and fungi) with catalytic units belonging to families 5, 6, 7, 9, 48, and 74 glycoside hydrolases. Aerobic fungal CBHs are in families 6 and 7 only; aerobic bacterial CBHs are in families 6 and 48; anaerobic fungal CBHs are in family 48; and anaerobic bacterial CBHs are in family 9 as well as 48. The most important topological aspect of CBHs' catalytic unit is the burrow structure which is created by two surface loops. The burrow may wrap the total (e.g., family 7 CBH) or part of the active site (e.g., family 48 CBH). The crystal structures of family 6 endoglucanase and exoglucanase allocate the interrelated folding, and the catalytic site of endoglucanase is the deep cleft structure, whereas it is a burrow for exoglucanase. The burrow-shaped catalytic site of exoglucanase permits the enzyme to hydrolyze cellulose in a distinctive "processive" way (Koivula et al. 2002; Vocadlo and Davies 2008). The glycoside hydrolase family 48 exoglucanases are generally assumed to take part in crystalline cellulose hydrolysis refereed by bacterial cellulase systems. Their function is assumed to be rather related to that of the *Trichoderma* CBHI (Cel7A) (Teeri 1997; Zhang et al. 2006). Family 48 exoglucanases are central catalytic machinery of cellulosomes, such as *Clostridium cellulolyticum* CelF (Reverbel-Leroy et al. 1997), *Clostridium cellulovorans* ExgS (Liu and Doi 1998), *Clostridium jusui* CelD (Kakiuchi et al. 1998), and *Clostridium thermocellum* CelS (Kruus et al. 1995), or main noncomplexed cellulase components, such as *Cellulomonas fimi* CbhB (Shen et al. 1995), *Clostridium stercorarium* Avicelase II (Bronnenmeier et al. 1991), *Thermobifida fusca* Cel48 (Irwin et al. 2000), *Paenibacillus barcinonensis* BP-23 Cel48C (Sánchez et al. 2003), and *Ruminococcus albus* 8 Cel48A (Devillard et al. 2004; Bayer et al. 1985; Zhang and Lynd 2005).

6.2.3 β-Glucosidase

β-Glucosidases (BGs) that do not have a CBM hydrolyze soluble cellodextrins and cellobiose to glucose (Fig. 6.2). The action of BG on insoluble cellulose is insignificant. BGs cleave cellobiose, which is an identified inhibitor of CBH and endoglucanase. Different BGs are generated by several archaea, bacteria, fungi, plants, and animals, with different catalytic unit belonging to families 1, 3, and 9. Based on either experimental data or structural homology analysis, the stereochemistry of families 1 and 3 BGs is of the retaining type, whereas the stereochemistry of family 9 BG is of the inverting type. Nearly all aerobic fungi generate extracellular BGs, and anaerobic bacteria stay their BGs in cytoplasm. BGs have a pocket-shaped catalytic site, which permits them to bind the nonreducing glucose unit and clip glucose off from cellobiose or cellodextrin.

6.3 Cellulose Source Materials and Their Derivatives

Industrial sustainability aims to achieve sustainable production and processing within the context of ecological and social sustainability (Miyamoto 1997). Compared to conventional production, sustainable processes and production systems should be more profitable because they require less energy, result in less emission of greenhouse gases and other pollutants, enable greater and more efficient use of renewable resources, and lessen dependence on nonrenewable resources. Lignocellulosic biomass is the major sustainable resource, comprising around half of the plant matter produced by photosynthesis and representing the most abundant renewable organic matter. Lignocellulosic residues from wood, grass, agricultural and forestry wastes, and municipal solid wastes are particularly abundant in nature and have a potential for bioconversion, and the production on global scale of these sources are given in Table 6.1. They constitute a renewable resource from which many useful biological and chemical products can be derived (Zosel 1994; Van Berkel 2000; Gavrilescu and Nicu 2004). Industry is truly sustainable only when it is economically viable, environmentally compatible, and socially responsible (OECD 1998; UNEP 1999). Lignocellulosic biomass consists of three types of polymers, cellulose, hemicellulose, and lignin, that are strongly intermeshed and chemically bonded by non-covalent forces and by covalent cross-linkages. The major component is cellulose, followed by hemicellulose and lignin. The composition and proportions of these compounds vary between plants, and chemical composition of some of lignocellulosic residues is given in Table 6.2 (McKendry 2002; Malherbe and Cloete 2002; John et al. 2006, Prassad et al. 2007). Only a small amount of the cellulose, hemicellulose, and lignin produced as by-products in agriculture or forestry is used, the rest being considered waste. Many microorganisms

Table 6.1 Lignocellulosic residues generated from different agricultural sources

Lignocellulosic residues	Ton × 10^6/year
Sugarcane bagasse	317–380
Maize straw	159–191
Rice shell	157–188
Wheat straw	154–185
Soja straw	54–65
Yucca straw	40–48
Barley straw	35–42
Cotton fiber	17–20
Sorghum straw	15–18
Banana waste	13–15
Mani shell	9.2–11.1
Sunflower straw	7.5–9.0
Bean straw	4.9–5.9
Rye straw	4.3–5.2
Pine waste	3.8–4.6
Coffee straw	1.6–1.9
Almond straw	0.4–0.49
Hazelnut husk	0.2–0.24
Sisal a henequen straw	0.77–0.093

are capable of degrading and utilizing cellulose and hemicelluloses as carbon and energy sources. However, a much smaller group of filamentous fungi has evolved with the ability to break down lignin, the most recalcitrant component of plant cell walls. These are known as white-rot fungi, which possess the unique ability of efficiently degrading lignin to CO_2. Accumulation of lignocellulose in large quantities in places where agricultural residues present a disposal problem results not only in deterioration of the environment but also in loss of potentially valuable material that can be used in paper manufacture, biomass fuel production, composting, and human and animal feed, among others.

6.4 Sources of Cellulases

Microbial cellulases are the most economic and available sources to meet the industrial scale, because microorganisms can grow on an inexpensive media such as agriculture and food industry by-products. Certain microbial sources that are studied for cellulase production are listed in Table 6.3.

Table 6.2 Composition of some lignocellulosic materials

Lignocellulosic residues	Lignin (%)	Hemicellulose (%)	Cellulose (%)	Ash (%)
Hardwood stems	18–25	24–40	40–55	NA
Softwood stems	25–35	25–35	45–50	NA
Nut shells	30–40	25–30	25–30	NA
Corn cobs	15	35	45	1.36
Paper	0–15	0	85–99	1.1–3.9
Rice straw	18	24	32.1	NA
Cotton seed hairs	0	5–20	80–95	NA
Newspaper	18–30	25–40	40–55	8.8–1.8
Waste paper from chemical pulps	5–10	10–20	60–70	NA
Waste paper from chemical pulps	5–10	10–20	60–70	NA
Switch grass	12.0	31.4	45	NA
Grasses (average values for grasses)	10–30	25–50	25–40	1.5
Sugarcane bagasse	19–24	27–32	32–44	4.5–9
Wheat straw	16–21	26–32	29–35	NA
Barley straw	14–15	24–29	31–34	5–7
Oat straw	16–19	27–38	31–37	6–8
Rye straw	16–19	27–30	33–35	2–5
Bamboo	21–31	15–26	26–43	1.7–5
Bast fiber kenaf	15–19	22–23	31–39	2–5
Bast fiber jute	21–26	18–21	45–53	0.5–2
Leaf fiber abaca (Manila)	8.8	17.3	60.8	1.1
Leaf fiber sisal (agave)	7–9	21–24	43–56	0.6–1.1
Leaf fiber henequen	13.1	4–8	77.6	0.6–1
Banana waste	14	14.8	13.2	11.4

NA not available

6.4.1 Cellulolytic Organisms

Cellulolytic enzymes are produced by a large number of microorganisms. The cellulolytic organisms are found among fungi and bacteria. The organisms are capable of producing the extracellular hydrolytic enzymes that attack cellulose polymer. Cellulolytic organisms can be found in all biota where cellulosic waste accumulates.

6.4.1.1 Fungi

They usually occur in mixed populations comprising cellulolytic and noncellulolytic species, which often interact synergistically. However, relatively few organisms are able to produce the necessary group of enzymes for degradation of crystalline cellulose (Singh and Hayashi 1995; Beguin and Aubert 1994). Fungi producing the necessary enzymes for the cell-free degradation of crystalline

Table 6.3 Certain microbial sources for cellulolytic enzyme production

Organism	Enzyme
Bacteria	
Acidothermus cellulolyticus	Endoglucanase
Alkalophilic Streptomyces	Cellulase, endoglucanase
Anaerocellum thermophilum	Endoglucanase
Bacillus sp. KSM-S237	Endoglucanase
Caldicellulosiruptor saccharolyticus	Endoglucanase, exoglucanase
Caldocellum saccharolyticum	Endoglucanase
Cellulomonas flavigena	β-D-Glucosidases
Clostridium stercorarium	Endoglucanase, exoglucanase
Clostridium thermocellum	Endoglucanase
Lactobacillus plantarum	β-D-Glucosidase
Pseudomonas fluorescens	Cellulase
Pyrococcus furiosus	Cellulase
Rhodothermus marinus	Endoglucanase
Sulfolobus solfataricus	Cellulase
Fungi	
Aspergillus aculeatus	Cellulase
Aspergillus glaucus XC9	Cellulase (endoglucanase)
Aspergillus nidulans	β-D-Glucosidases
Aspergillus nidulans	β-D-Glucosidases
Aspergillus niger	Cellulase
Aspergillus niger	B-Glucosidase
Aspergillus oryzae	β-D-Glucosidases
Aspergillus sojae	β-D-glucosidases
Chaetomium thermophilum	Endoglucanase
Cladosporium sp.	Endoglucanase, exoglucanase
Humicola insolens	Cellulase
Humicola grisea	Cellulase
Melanocarpus albomyces	Endoglucanase
Metschnikowia pulcherrima	β-D-Glucosidase
Mucor circinelloides	Endoglucanase
Penicillium pinophilum	Cellulase
Rhizopus oryzae	Endoglucanase, β-D-glucosidase
Thermoascus aurantiacus	Cellulase, β-D-glucosidase
Trichoderma reesei	Cellulase
Trichoderma viride	Cellulase

cellulose generally belong to ascomycetes and dueteromycetes groups or to the basidiomycetes (Ljundahl and Eriksson 1985). The white-rot fungi are heterogeneous and include *Phanerochaete chrysosporium* and have in common capability to degrade lignin and other lignocelluloses (Semichaevsky 1989). Among brown-rot fungi, *Poria placenta*, *Lanzitus trabeum*, and *Tyromyces palustris* are the good known examples of cellulolytic organisms (Eriksson et al. 1990). They seem to utilize a mechanism of cellulose degradation different from that operating in *P. chrysosporium*. Soft-rot fungi have the capability to deplete both polysaccharides and lignin; however, the polysaccharides are the main targets for this group. The well-known *Trichoderma reesei* produces a complete enzyme system (Reese et al. 1950; Gilligan and Reese 1954; Reese and Mandels 1971). Further, the complete enzyme system is also reported in *Trichoderma koningii* (Wood and McCrae 1972), *Penicillium pinophilum* (Wood and McCrae 1986; Wood et al. 1989), *Penicillium funiculosum* (Wood and McCrae 1986), *Penicillium citrinum, Aspergillus niger, Aspergillus* species (DeVries and Visser 2001). The anaerobic rumen inhabiting fungi also has capability to degrade cellulose to glucose. Survival, metabolism, and production of hydrolytic enzymes in respect of anaerobic fungi are exclusively reviewed (Tounissen et al. 1993). This group includes *Neocallimastix*, *Piromyces*, *Orpinomyces*, and *Ruminonyces* sp. The major digestion product by the action of *Neocallimastix frontalis* cellulase on microcrystalline cellulose is glucose and not cellobiose. Thermophilic fungi constitute a heterogeneous physiological group of various genera in the *Phycomycetes*, *Ascomycetes*, fungi imperfecti, and mycelia sterilia and have a growth temperature maximum at or above 50 °C (Kvesitadze et al. 1986; Maheswari et al. 2000). *Chaetomium thermophile*, *Sporotrichum thermophile*, and *Thielavia* are the well-reported cellulolytic members of this group. Use of thermophilic fungi for production of enzymes including cellulase is extensively reviewed by Johri and Ahmad (1991).

6.4.1.2 Bacteria

Degradation of cellulose by bacterial systems occurs both aerobically and anaerobically (Singh and Hayashi 1995, Beguin and Aubert 1994). Among aerobic cellulolytic soil bacteria, several species belonging to the genera *Cellulomonas*, *Pseudomonas*, *Thermomonospora*, *Bacillus*, and *Microbispora* are predominant. Bacterial degradation of cellulose seems to take place by a cluster of enzymes rather than individual enzymes as in the case of fungi (Ljundahl and Eriksson 1985). Anaerobic degradation of cellulose occur in a variety of anaerobic biota such as manure, compost, sludge of wastewater treatment plants and marine, freshwater sediments, rumen, and gastrointestinal tract of herbivorous animals (Leschine 1995). *Clostridium thermocellum* (McBee 1959), *Clostridium cellofermentans* (Yanling et al. 1991), *Bacteroides xylanisolvens* (Murray et al. 1984), *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Butyrivibrio fibrisolvens* appear to be cellulolytic anaerobes isolated from the anaerobic biota. Only few microorganisms produce complete set of enzymes capable of degrading native cellulose efficiently. However, a large number of species produce incomplete systems.

6.5 Application of Cellulases in Various Industries

6.5.1 Pulp and Paper Industry

In the last decade, the application of cellulases in the pulp and paper industry has increased significantly (Mai et al. 2004). As compared to the mechanical pulping processes such as refining and grinding of the woody raw material, the biomechanical pulping using cellulases resulted in substantial energy savings (20–40%) during refining and also improvements in hand-sheet strength properties were observed (Singh et al. 2007; Akhtar 1994; Pere et al. 2001; Bhat 2000). Mixtures of cellulases (endoglucanases I and II) and hemicellulases have also been used for biomodification of fiber properties with the aim of improving drainage and beatability in the paper mills before or after beating of pulp (Dienes et al. 2004). Mansfield et al. (1996) studied the action of a viable cellulase preparation on different fractions of Douglas fir kraft pulp and observed that the cellulase treatment decreased the defibrillation reducing the fiber coarseness. While endoglucanases have the ability to decrease the pulp viscosity with a lower degree of hydrolysis (Pere et al. 1995), cellulases have also been reported to enhance the bleachability of softwood kraft pulp producing a final brightness score comparable to that of xylanase treatment (Singh et al. 2007; Suominen and Reinikainen 1993). Cellulases only, or used in mixture with xylanases, are valuable for deinking of various types of paper wastes. Cellulases and hemicellulases are also used for the liberation of ink from the fiber surface by partial hydrolysis of carbohydrate molecules (Kuhad et al. 2010b). It has been postulated that improvements in dewatering and deinking of various pulps result in the peeling of the individual fibrils and bundles, which have high affinity for the surrounding water and ink particles (Kibblewhite et al. 1995). The main advantages of enzymatic deinking are reduced or eliminated alkali usage, improved fiber brightness, enhanced strength properties, higher pulp freeness and cleanliness, and reduced fine particles in the pulp (Kuhad et al. 2010a, b). Furthermore, deinking by enzymes at acidic pH also inhibits the alkaline yellowing, simplifies the deinking process, alters the ink particle size delivery, and cuts the environmental pollution. Though enzymatic deinking can reduce the need for deinking chemicals and inhibit the unfavorable environmental impacts of the paper industry, too much use of enzymes must be avoided, because considerable hydrolysis of the fines could ease the bond ability of the fibers (Stork and Puls 1996; Karnis 1995). Attractively, the exploit of cellulases in humanizing the drainage has also been followed by several mills with the intent to enhance the production rate. Enzyme treatments eliminate some of the fines or rind off fibrils on the fiber surface and dissolved and colloidal substances, which often cause harsh drainage troubles in paper mills. In this part, cellulases have shown significant improvement in the overall performance of paper mills (Bhat 2000; Kantelinen et al. 1995). Enzymatic treatment also destabilizes the lipophilic extractives in the filtrates and facilitates their attachment to thermomechanical pulping fibers. These enzymes are also used in preparation of easily

biodegradable cardboard (Buchert et al. 1998), manufacturing of soft paper including paper towels and sanitary paper (Salonen 1990; Hsu and Lakhani 2002), and removal of adhered paper (Sharyo et al. 1978).

6.5.2 Textile Industry

Cellulases are the most booming enzymes used in textile dripping processing, mainly finishing of cellulose-based textiles (Hebeish and Ibrahim 2007; Karmakar and Ray 2011). Conventional stone washing of jeans entails amylase-mediated exclusion of starch desizing and abrasion of jeans with pumice stone (1–2 kg/pair of jeans) in large washing machines. Cellulases have been effectively applied for the biostoning of jeans and biopolishing of cotton and other cellulosic fabrics. Throughout the biostoning procedure, cellulases work on the cotton fabric and split the small fiber ends on the yarn surface, thus releasing the dye, which is easily removed by mechanical abrasion in the wash cycle. The reward in the substitution of pumice stones by a cellulose-based action comprises less injure of fibers, improved efficiency of the machines, and less work-intensive and environment compassionate (Kuhad et al. 1999; Singh et al. 2007; Uhlig 1998; Galante et al. 1998). The acidic cellulases improve softness and water absorbance assets of fibers, strongly diminish the affinity for pill formation, and grant a cleaner surface structure with less fluff (Sreenath et al. 1996). Cellulase preparations rich in endoglucanases are best suited for biopolishing enhancing fabric look, feel, and color without needing any chemical coating of fibers (Galante et al. 1998). The action of cellulases removes short fibers and surface fuzziness, creates a smooth and glossy appearance, and improves color brightness, hydrophilicity as well as moisture absorbance, and it is also an environmentally friendly process (Bhat 2000). Similarly, endoglucanase activity-rich cellulase is also proved better for biofinishing. Most cotton or cotton-blended garments, during repeated washing, tend to become fluffy and dull, which is mainly due to the presence of partially detached microfibrils on the surface of garments. The use of cellulases can remove these microfibrils and restore a smooth surface and original color to the garments (Hebeish and Ibrahim 2007; Ibrahim et al. 2011). The use of cellulase also facilitates in softening the clothes and in elimination of dust particles intent within the microfibril system. There are several reports where the performance of the complete cellulase preparations was relatively different from the enzyme rich in endoglucanase activity and that the latter offered improved presentation in applications where losses in fabric potency and weight were least. Depilling/cleaning and/or aging effects are the result of the synergistic action of cellulases and mechanical action, simultaneously or consecutively (Baker et al. 2005). Attempts have also been made via cellulase treatment to improve the dimensional stability of cellulosic fabrics and to upgrade the surface and dyeing properties of bleached cotton, mercerized cotton, and cotton/polyester blend fabric (50/50), using the padwet batch technique, followed by subsequent washing under mechanical action (Ibrahim et al. 2011; Cortez et al. 2002).

6.5.3 Bioethanol Industry

Enzymatic saccharification of lignocellulosic materials such as sugarcane bagasse, rice straw, switch grass, saw dust, etc. by cellulases for biofuel production is one of the most popular applications being investigated (Sukumaran et al. 2005; Gupta et al. 2011). Biological conversion of lignocellulosic materials into value products usually requires multistep process (Ghosh and Singh 1993; Wyman et al. 2005). It comprises mechanical, chemical, or biological treatment and hydrolysis of the polymers to generate readily metabolizable molecules, e.g., hexose and pentose sugars. Further, bioconversion of these molecules is done using microbial system, and chemical products are generated, and finally the preferred products are obtained after partition and refinement step. The efficacy rate of enzymatic hydrolysis is more superior as compared to the acid or alkaline hydrolysis because enzyme hydrolysis is frequently conducted at mild conditions (pH 4–6 and temperature 45–50 °C) and does not have deterioration issues (Kuhad et al. 2010a; Gupta et al. 2011). Technologies are now available for the bioconversion of lignocellulose to ethanol and other chemical products (Sun and Cheng 2002; Kuhad et al. 1997; Kuhad and Singh 1993; Mosier et al. 2005). However, some of these technologies must be improved to produce renewable biofuel and other by-products at prices, which can compete with more conventional production systems. Not only the recalcitrance of the substrate but also several other factors also limit cellulase efficiency during the hydrolysis process including end product inhibition, thermal deactivation of the native protein, nonspecific binding to lignin (Yang and Wyman 2004), and irreversible adsorption of the enzymes to the heterogeneous substrate (Taniguchi et al. 2005). To cut the enzyme price in the generation of fuel ethanol from lignocellulosic biomass, two aspects are generally addressed: optimization of the cellulase production and development of a more proficient cellulase-based catalysis method. Protein engineering and directed evolution are potent tools that can assist the growth of more competent thermophilic cellulases (Baker et al. 2005). Strategies for recycling and reusage of the enzymes may also be used to reduce enzymatic hydrolysis costs (Sun and Cheng 2002; Mosier et al. 2005; Lee et al. 1995; Singh et al. 1991). The revival of enzymes is basically subjective by adsorption (enzymes) against the substrate, mainly to lignin and enzyme inactivation. There are numerous reports where the unfocused and unalterable adsorption of cellulase to lignin has been experienced (Bernardez et al. 1993; Yang and Wyman 2004). The compounds that imitate cellulose or the compounds that have high affinity toward lignin have been used to stop the adsorption of cellulases (Yang and Wyman 2006; Kumar and Wyman 2009). Moreover, Scott and coworkers (Scott et al. 2010) have filed a US patent (20100221778) on novel lignin-resistant cellulase enzyme, in which modification in linker peptides has been done to stop their adsorption against lignin and increase the enzyme activity. Among different approaches to improve and reuse the cellulases are concentration of the cellulose fraction by ultrafiltration to remove sugars and other small compounds that may inhibit the action of the enzymes (Tu et al. 2007) and recycling of immobilized enzymes, which enables separation of the enzymes

from the process flow (Mosier et al. 2005; Dourado et al. 2002). However, the recycling techniques are examined at laboratory scale. Therefore, the ability to scale up the techniques, the robustness, and feasibility still needs to be demonstrated.

6.5.4 Wine and Brewery Industry

Microbial glucanases and related polysaccharides play important roles in fermentation processes to produce alcoholic beverages including beers and wines (Kuhad et al. 1999; Singh et al. 2007; Galante et al. 1998; Bamforth 2009). These enzymes can improve both quality and yields of the fermented products (Bamforth 2009). Glucanases are added either during mashing or primary fermentation to hydrolyze glucan, reduce the viscosity of wort, and improve the filterability (Bamforth 2009; Canales et al. 1988). During wine production, pectinases, glucanases, and hemicellulases play an important role by improving color extraction, skin maceration, must clarification, filtration, and finally the quality and stability of wine (Singh et al. 2007; Galante et al. 1998). β-Glucosidases can improve the aroma of wines by modifying glycosylated precursors. Macerating enzymes also improve pressability, settling, and juice yields of grapes used for wine fermentation. A number of commercial enzyme preparations are now available to the wine industry. The main benefits of using these enzymes during wine making include better maceration, improved color extraction, easy clarification, easy filtration, improved wine quality, and improved stability (Galante et al. 1998). Beer brewing is based on the action of enzymes activated during malting and fermentation. Malting of barley depends on seed germination, which initiates the biosynthesis and activation of α- and β-amylases, carboxypeptidase, and β-glucanase that hydrolyze the seed reserves (Bamforth 2009). In an earlier study, Oksanen et al. (1985) observed that endoglucanase II and exoglucanase II of the *Trichoderma* cellulase system were responsible for a maximum reduction in the degree of polymerization and viscosity. Significant and reproducible improvements in grape pressability, settling rate, and total juice yield were achieved by using combination of macerating enzymes. Such improvements were noticeable only with a correct balance of pectinases, cellulases, and hemicellulases. Using three varieties (Soave, Chardonnay, and Sauvignon) of white grapes, Galante et al. (1998) assessed the performance of Cytolase 219 (mixture of cellulase, pectinase, and xylanase) in wine making and reported a 10–35% increase in the extraction of the first wine must, a 70–80% increase in the must filtration rate, 50–120 minutes decrease in pressing time, 30–70% decrease in must viscosity, 20–40% saving of energy during cooling of fermenter, and a significant improvement in wine stability. A range of improved enzymes like cellulase and pectinase that would be exogenously added to the process is expected to enhance the productivity of existing brewing processes in the future (Bamforth 2009).

6.5.5 Food Processing Industry

Cellulases are having large array of application in food biotechnology. Manufacturing of fruit and vegetable juice necessitates better ways for extraction, clarification, and stabilization. Cellulases also have significant use as a part of multienzyme complex (cellulases, xylanases, and pectinases) which is used to obtain fruit and vegetable juices after the extraction and clarification step, and it also increases the final product yield (Minussi et al. 2002; de Carvalho et al. 2008). Use of macerating enzymes enhances the production and performance of the method employed without any additional fund investment. These enzymes are also used to attain better cloud consistency and texture and reduce the viscosity of nectars and purees from tropical fruits such as mango, papaya, plum, peach, pear, and apricot (Singh et al. 2007; Bhat 2000; de Carvalho et al. 2008). Consistency, taste, and aroma properties of fruits and vegetables can be enhanced by dropping the bitterness of citrus fruits by using combination of enzymes such as pectinases and β-glucosidases (Baker and Wicker 1996; Youn et al. 2004; Rai et al. 2007). Multienzyme combination containing pectinases, cellulases, and hemicellulases is also used for better olive oil extraction. Use of such macerating enzymes not only enhanced the cloud stability and texture of nectars and purees but also decreased their viscosity swiftly (Grassin and Fauquembergue 1996). Thus, the macerating enzymes, constituted mainly of cellulase and pectinase, have a significant role in food biotechnology and will be more demanding for extraction of juice from various fruits and vegetables (Dourado et al. 2002). Further, the combination of pectinases and β-glucosidases has been reported to alter the texture, flavor, and other sensory properties such as aroma and volatile characters of fruits and vegetables (Marlatt et al. 1992).

6.5.6 Animal Feed Industry

Use of cellulases and hemicellulases in the feed industry is of great importance because of their ability to enhance feed value and performance of animals (Dhiman et al. 2002). Pretreatment of agricultural silage and grain feed by cellulases or xylanases boosts up the nutritional quality (Godfrey and West 1996). Further, enzymes can reduce the anti-nutritional factors present in the feed grains, improve the nutritional value by degrading some feed constituents, and add some beneficial digestive enzymes such as proteases, amylases, and glucanases. Also, the dietary fiber consists of some non-starch polysaccharides such as arabinoxylans, cellulose, and many other plant components including dextrins, inulin, lignin, waxes, chitins, pectins, β-glucan, and oligosaccharides, which may work as anti-nutritional cause for several animals such as swine (Ali et al. 1995). In such case, the cellulase enzyme hydrolyzes the cellulose (anti-nutritional factor), present in the animal feed, into easily absorbent component and thus improve the animal health. β-Glucanases and xylanases are supplemented in the monogastric animal feeds for hydrolyzing the

non-starch polysaccharides, viz., β-glucans and arabinoxylans. Cellulases, employed as feed additives either alone or along with protease enzyme, can considerably improve the pork meat quality. Glucanases and xylanases have decreased the viscosity of high fiber containing rye- and barley-based feeds of poultry and pig. It has been reported that both the enzymes may also result in the increase in weight among the chickens and piglets as the digestion and absorption of feed materials are improved (Shrivastava et al. 2011). Most of the low-quality feedstuffs have high cellulose concentration, less protein and fat, and comparatively more ash content as compared to that of the high-quality feedstuffs. Cellulases are also utilized for better silage production for cattle feeding, which involves increased rate of the digestive ability of grasses which has large amount of total digestible vital nutrients and energy values along with small quantity of water-miscible carbohydrates. The cereal-based feed of poultry and pigs is comparatively simpler than that of the ruminants, which contains cellulose, hemicellulose, pectin, and lignin. The nutritional qualities of forages are improved by the use of enzyme preparations which contain high amount of cellulase, hemicellulase, and pectinase (Graham and Balnave 1995; Lewis et al. 1996). Nonetheless, scientists have also observed contradictory results when cellulase, hemicellulase, and pectinase enzyme preparations were added to the ruminant diet. The animal feedstock making process usually comprises heat treatments which inactivate the possible viral and microbial contaminants. Use of thermophilic cellulase in the production of feedstock has the ability to decrease pathogenic microbes as well as to increase the digestibility and nutrition quality of the feed, thus facilitating a blending of heat treatment and feed transformation in a single step (Bhat 2000). The cellulase and hemicellulase enzymes have a significant role in the partial hydrolysis of lignocellulosic substances, dehulling of cereal grains, hydrolysis of β-glucans, and enhanced emulsification and flexibility of feed materials, which results in improvement in the nutritional quality of animal feed (Cowan 1996; Paulo and Gubitz 2003). Further, these enzymes may also result in partial hydrolysis of plant cell wall at the time of silage and fodder preservation. The utilization of enzymes to enhance animal nutrition became extremely important when the use of nutritive ionophore antibiotics were prohibitied, which were earlier used in the European countries (Ali et al. 1995). There is wide divergence in the digestive ability of starch substrates. The low digestive ability of some starch-based substrates promotes the emergence of some digestive tract diseases. This is because the non-digested and non-absorbed starch when reaches the large intestine, then it acts as a substrate for bacterial fermentation and promotes the propagation of some hazardous pathogenic bacterial strains (Pascual 2001; Pazarlioglu et al. 2005). It has been observed that sometimes cellulase has a positive effect on the fermentation processes as it increases the propionic acid production, which further acts as a bacteriostatic substance and therefore can reduce the colonization of pathogenic bacteria (Fortun-Lamothe et al. 2001; Pazarlioglu et al. 2005).

6.5.7 Agricultural Industries

Diverse enzymatic preparations that consist of various combinations of cellulases, hemicellulases, and pectinases have possible applications in agriculture for enhancement of the crop growth and controlling plant diseases (Chet et al. 1998; Bhat 2000). Microbial hydrolases are used to produce plant and/or fungal protoplasts. They are of great significance as they are used for producing hybrid strains with desirable properties. Bhat (2000) reported that cellulases and other similar enzymes isolated from some fungi are able to degrade the cell wall of plant pathogens and thus are good means for controlling the plant disease. Also, fungal β-glucanases are competent to control the diseases by degrading the cell walls of plant pathogens. Many cellulolytic fungi, viz., *Trichoderma*, *Geocladium*, *Chaetomium*, and *Penicillium* species, are well reported to have a significant role in agriculture by promoting increased seed germination, rapid plant growth and flowering, improved root system, and better crop yield (Bailey and Lumsden 1998; Harman and Bjorkman 1998). Although these fungal species have both direct and indirect impact on plant growth and yield, still it is not apparent how they cause the improved plant performance.

The β-1,3-glucanase and N-acetylglucosaminidase enzymes from *Trichoderma harzianum* strain P1 inhibited the spore germination and germ tube elongation of *B. cinerea* in a synergistic way (Lorito et al. 1994). Furthermore, in *Trichoderma*, the exoglucanase promoters are used for expression of various enzymes, proteins, and antibodies at enhanced rate. The cellulase enzyme is also used for the development of soil quality. Conventionally, addition of straw is considered as a significant approach to develop better soil quality and has also decreased the use of mineral fertilizers (Ortiz Escobar and Hue 2008; Tejada et al. 2008). Many researchers have attempted to accelerate the straw decomposition rate through microorganisms. Use of cellulolytic fungi such as *Aspergillus*, *Chaetomium*, *Trichoderma*, and actinomycetes has shown potential results (Bowen and Harper 1990; Tiwari et al. 1987; Abdulla and El-Shatoury 2007). Fontaine et al. (2004) reported that the exogenous cellulase addition accelerates the decomposition of cellulose in soil. Thus, the use of exogenous cellulase is perhaps a prospective way to hasten the decomposition of straw and increase soil fertility.

6.5.8 Olive Oil Extraction

Nowadays the olive oil extraction is of high concern in the international market due to its various health claims. Olive oil extraction involves the crushing and grinding of olive fruits in a stone or hammer mill followed by passing the grinded olive paste through a chain of malaxeurs and horizontal decanters and finally high-speed centrifugation for recovery of the olive oil. For the production of good-quality olive oil, fresh, clean, and slightly immature olive fruits are used, and they are mechanically pressed in cold environment (De Faveri et al. 2008). Nevertheless, greater yields are

achieved when extracted from fully ripened fruit which is processed at higher than ambient temperatures, but the oil obtained is slightly acidic and has high rancidity and poor aroma. Thus, a better method for extraction of high-quality olive oil is required in order to meet the rising consumer demand. Olivex was used as a first enzyme mixture (a pectinase preparation with cellulase and hemicellulase from *Aspergillus aculeatus*), for the extraction of olive oil (Fantozzi et al. 1977). Further, the application of macerating enzymes has increased the ratio of antioxidants in extra-virgin olive oil and reduced the rancidity. The foremost advantages of macerating enzymes during olive oil extraction are (1) better extraction yield (up to 2 kg oil per 100 kg olives) in cold processing conditions, (2) improved centrifugal fractionation of the oil, (3) oil having more amount of antioxidants and vitamin E, (4) slow initiation of rancidity, (5) overall increased plant efficiency, and (6) small amount of oil released in wastewater. Similarly, the macerating enzymes have a prominent role in the oil extraction from many different agricultural oilseed crops. Further, the enzymes may additionally be used during olive paste malaxation. The occurrence of secured action of cellulase and hemicellulase nature of the enzymatic formulation ensures a quick and powerful disintegration of the cell walls and membranes of olive fruits, thus favoring the access of noble substances (particularly the polyphenols and aromatic precursors) into the final product. It is also used to reduce olive paste thickness during olive oil production and to strengthen the procedure of extracting the polyphenolic substances present in the olive fruit (Ranalli et al. 2003). Interestingly the preferred enzymes are present naturally inside the olive fruit; however, they are powerfully deactivated all through the pressing step, most likely because of oxidation phenomena (Chiacchierini et al. 2007). So, the substitution of these enzymes is anticipated to be appropriate keeping in view their role in final product quality.

6.5.9 Carotenoid Extraction

In nature carotenoids are the most important factor of coloring substances being accountable for different plant colors from violet to red. Carotenoids are very commonly used as food colorants, and its market is continuously growing due to its various beneficial properties, such as its natural origin, almost negligible toxicity, versatility, easy availability, and both lipo- and hydrosoluble color. Further, the carotenoid pigments have provitamin A activity which has a very significant function in lipid oxidation, and it also harbors anticarcinogenic properties. Generally a blend of cellulolytic and pectinolytic enzymes increase the hydrolysis rate in order to achieve complete liquefaction. Cellulase enzyme aimlessly split the cellulose chains into glucose residues, while the commercially available pectinase enzyme isolated from *Aspergillus niger* has pectinesterase (PE), polygalacturonase (PG), and pectin lyase (PL) activity (Ory and St. Angelo 1977; Inar 2005). The application of pectinase and cellulase enzymes breaks the cell wall of orange peels, sweet potato, carrot, etc. and liberates the carotenoids in the chloroplasts and in the cell

fluids. These pigments stay in their normal state and are bound to the proteins. They prevent the oxidation of pigment and also contribute to color stability (Fennema 1985), while the solvent extraction method dissociates the pigments from its bound proteins and cause water insolubility and ease of oxidation (Bassi et al. 1993).

6.5.10 Detergent Industry

The detergent industry is nowadays using a combination of cellulase, lipase, and protease enzyme during detergent formulation and is thus attempting to develop advance detergents (Singh et al. 2007). The cellulase present in the detergent is capable of modifying cellulose fibrils that can improve color brightness and could enhance the removal of dirt from the cotton blend garments. The commercial application of alkaline cellulases as a potential detergent additive is being keenly followed with a vision to make selective contact of the enzyme with the interior of garment fibers and remove soil/dirt from the interfibril spaces. Nowadays, fluid-based laundry detergents are formulated which contain anionic/nonionic surfactant, citric acid or water-soluble salt, protease, lipase, cellulase, propanediol, and boric acid in order to improve the stability of cellulase. In the modern textile industry, most of the cellulose fibers used are arranged as long, straight chains, and during this some small cotton fibers extend beyond the fabric. The cellulose enzyme is used to eliminate these rough protuberances to get a smooth, glossy, and bright-colored fabric (Karmakar and Ray 2011).

6.5.11 Waste Management

A huge quantity of unutilized or underutilized cellulosic waste is generated through forests, agriculture fields, and agro-industries which is ultimately causing environmental pollution (Milala et al. 2000; Milala et al. 2005). These days, such wastes are sensibly and judiciously used for the production of valuable products, viz., enzymes, sugars, biofuels, chemicals, cheap energy sources for fermentation, improved animal feeds, and human nutrients (Gupta et al. 2009; Gupta et al. 2010).

6.6 Immobilization of Cellulase

In industries the utilization of enzymes of microbial origin is restricted due to some factors, e.g., the elevated cost of enzyme production, its instability, and isolation in small quantity. Moreover, since these enzymes are soluble in liquid media, thus it is not easy to obtain their good yield. These factors limit the soluble enzymes to be employed for batch operations. Over the last few decades, intense research in the

area of enzyme technology has provided many approaches to overcome these limitations to facilitate their practical applications. Among them, the use of immobilized enzymes/biocatalysts offers the opportunity of wider and more cost-effective methods for the use of biocatalysts in various industries, treatment of waste, medicine formulation and manufacturing, and biosensor development. Immobilized biocatalysts can be reused and are thus economically cheap and are used in the development of continuous bioprocesses. The diverse chemical reactions catalyzed by these enzymes have made these biocatalysts a key target for various applications in biotech-based industries. In general, the term immobilization refers to the act of the limiting movement or restricting movement to a confined space. Immobilization of biocatalysts helps in their economic reuse and in the development of continuous bioprocesses. Enzymes can be immobilized either by using the isolated enzymes or the whole cells. A number of techniques that have been used for immobilization of cells/enzymes include cross-linking, physical adsorption, ionic binding, metal binding, covalent binding, and cell/enzyme entrapment. All of these experimental biocatalyst immobilizations can be placed into categories: (1) entrapment of biocatalyst in polymer gels and/or porous supports, (2) bonding on the surface of micro-carrier, and (3) encapsulation of biocatalyst. Occasionally the differences among the various categories are not apparent, depending on the immobilization system to be applied. However, each method has its own advantage and drawback. A large number of enzymes have been immobilized on inorganic carriers like porous glass, ceramics, carbon, and sand by different techniques. The most extensively studied immobilization method is the entrapment of microbial cells/enzymes in polymer matrices. The matrices used are agar, alginate, carrageenan, cellulose and its derivatives, collagen, gelatin, epoxy resin, photo cross-linkable resins, polyacrylamide, polyester, polystyrene, and polyurethane. Among the various matrices reported, the entrapment of cells/enzymes in the alginate gel is well accepted as its use requires mild conditions and the immobilization process is simple. Cellulase and amylase enzymes are employed by various industries for the hydrolysis of cellulose or starch to different products such as dextrins, syrups, and sugars. Such type of reaction signifies the important foremost step for the manufacturing of various chemicals and sweeteners and is also valuable in the pulp and paper industry for modification of fiber. Enzymatic hydrolysis of cellulosics, the most abundant renewable resource on earth, offers an attractive alternative, if the process can be made economically competitive. The earlier studies have considered the benefits of immobilized enzymes with soluble substrates, and in also several studies, the researchers investigated the properties of immobilized enzymes/cells with insoluble substrates. The performance of cellulase and amylase immobilized on siliceous supports was evaluated with respect to immobilization conditions and thermal stability. Enzyme immobilization has improved the thermostability of the enzymes and also affects the binding of substrates and inhibitors to the enzyme. Natural cellulases from most fungal sources are limited in their overall activity by low amounts of β-glucosidase and by cellobiose inhibition of cellobiohydrolase. Although advantageous to the growth of the fungus, these characteristics are inconvenient for the industrial use of cellulase in cellulose processing. However, better hydrolysis of cellulose and

improved glucose yield can be obtained in laboratory or industries by supplementing additional β-glucosidase enzyme along with fungal cellulases. Further, β-glucosidase is a costly enzyme in terms of production and, since it is used in a soluble form and hence cannot be reused/recycled, is therefore not cost-effective when used at large scale. The co-immobilization of cellulase and β-glucosidase enzyme has improved the enzyme kinetic properties and has also enhanced the glucose yield in comparison to the cellulase enzyme when used alone. Thus the use of immobilized enzyme or cells may perhaps present a solution toward reducing the cost of cellulase production.

6.7 Future Perspectives

The lignocellulose is the prospective resource of biofuels, biofertilizers, animal feed, and chemicals, further being the raw material for paper industry. Utilization of this renewable resource needs either chemical or biological treatment of the material, and in the latter circumstance, cellulases have got extensive status above the past several decades. Research has about the mechanisms of microbial cellulase production and has led to the development of technologies for production and applications of cellulose-degrading enzymes for generation of useful metabolites or biofuel. Utilization of the existing viable planning of cellulase for bioconversion of lignocellulosic waste is economically not feasible. The main objectives for upcoming cellulase study would be (1) decline in the price of cellulase production and (2) improvement on the presentation of cellulases to make them other efficient, so that less enzyme is required. The previous assignment may comprise such procedures as optimizing growth conditions or processes, while the concluding need directed efforts in protein engineering and microbial genetics to recover the properties of the enzymes. Optimization of growth conditions and processes has been effort to a large level in improving cellulase production. The section on fermentation production of cellulases describes several of these works mainly commerce with observed optimization of process variables to recover productivity. Many of the existing marketable manufacture technologies operate submerged fermentation technology and employ hyper-producing mutants. In spite of numerous efforts aimed at generating hyperproducers by directed progress, the cost of enzymes has remained elevated. Optional approaches thought of in cellulase production comprise chiefly solid substrate fermentation on lignocellulosic biomass principally by using host-/substrate-specific microorganisms. There are some reports on such use of filamentous fungi in production of optimal enzyme complex for the degradation of host lignocelluloses. Performance of enzyme complexes on lignocellulosic material is superlative when these complexes are ready with the same lignocellulosic material as the host/substrate in fermentation. Another strategy is to use mixed culture in the production of enzyme. Numerous reports have shown that mixed culture gives enhanced production and enzyme complexes with better hydrolytic activity. Thus, SSF may be considered as a cost-efficient way for large-scale production of cellulases which

probably would be severalfold cheaper compared to the present viable preparations. Cellulases are matter to instruction by different factors, and some of the cis-acting promoter elements have been characterized. Dynamic research in this field has led to genetic progress of cellulase production by diverse methods with over-expressing cellulases from the cbh1 promoter of *T. reesei* and production of preferred deviation in the cellulase production profile of organism. The cbh1 and cbh2 promoters of *T. reesei* have also been subjugated for expression of foreign proteins in *Trichoderma*. Feedback inhibition of cellulase biosynthesis by the end products, glucose and cellobiose, generated by endogenous cellulolytic activity on the substrate is one more key crisis encountered in cellulase production. Cellobiose is an enormously potent inhibitor of the CBH and EG biosynthesis. *Trichoderma* and the other cellulose-producing microbes make very little β-glucosidase compared to other cellulolytic enzymes. The low amount of β-glucosidase results in a deficiency of ability to hydrolyze the cellobiose to glucose resulting in a feedback inhibition of enzyme generation and in the case of biomass alteration applications in the inhibition of cellulases. This problem has been addressed by different ways like addition of exogenous β-glucosidases to remove the cellobiose and engineering β-glucosidase genes into the organism so that it is overproduced. More and more study is oriented in genetic manipulations of cellulase producers for improving productivity. The developments in process design and medium formulations have come to an age, and the upcoming absolutely requires controlled genetic interventions into the physiology of cellulase producers to get better production and thereby make the cellulase production process more cost-efficient. The key tasks ahead comprise principal feedback control by glucose and development of incorporated bioprocesses for the production of cellulases. Improvements in cellulase activities or imparting of preferred features to enzymes by protein engineering are perhaps other areas where cellulase research has to progress. Active site modifications can be imparted through site-directed mutagenesis, and the mutant proteins can be used for understanding the mechanisms of action as well as for changing the substrate specificities or improving the activities. There are several reports of developments made in this direction. Such modifications affecting the enzyme properties may be beneficial in improving the overall performance of cellulases and a better understanding of their mode of action, which will enable better utilization of enzymes in biomass conversion. More basic research is needed to make designer enzymes suited for specific applications.

6.8 Conclusion

The natural characteristic of processing of cellulosic biomass becomes the origin of latent learning linking cellulases and cellulolytic microorganisms. Cellulases are being commercially generated by various industries worldwide and are usually being used in food, animal feed, fermentation, agriculture, pulp and paper, and textile purposes. Cellulase research has been concentrated generally in fungi, but there

is increasing attention in cellulase production by bacteria due to their higher growth rate and thermostable and alkali-stable properties. The growth of rapid and consistent methods for the selection of cellulases from microorganisms within unfriendly environments will permit a larger number of novel bacterial cellulases to be isolated with purpose for industrial use. Among current biotechnology apparatus, principally in the area of microbial genetics, novel enzymes and new enzyme applications will become accessible for the several industries. Developments in cellulase activities or informing of desired features to enzymes by protein engineering are perhaps other areas where cellulase research has to progress.

Acknowledgment Authors acknowledge the Department of Microbiology, Dr. Rammanohar Lohia Avadh University, Faizabad, Uttar Pradesh, India, and Government of Uttar Pradesh, India, for awarding the center of excellence to the department.

References

Abdulla HM, El-Shatoury SA (2007) Actinomycetes in rice straw decomposition. Waste Manag 27(6):850–853

Akhtar M (1994) Biochemical pulping of aspen wood chips with three strains of *Ceriporiopsis subvermispora*. Holzforschung 48:199–202

Ali S, Hall J, Soole KL et al (1995) Targeted expression of microbial cellulases in transgenic animals. In: Petersen SB, Svensson B, Pedersen S (eds) Carbohydrate bioengineering, vol 10 of progress in biotechnology. Elsevier, Amsterdam, pp 279–293

Bailey BA, Lumsden RD (1998) Direct effects of *Trichoderma* and *Gliocladium* on plant growth and resistance to pathogens. In: Harman GF, Kubicek CP (eds) Trichoderma & gliocladium—enzymes, vol 2 of biological control and commercial applications. Taylor & Francis, London, pp 327–342

Baker RA, Wicker L (1996) Current and potential applications of enzyme infusion in the food industry. Trends Food Sci Technol 7(9):279–284

Baker JO, McCarley JR, Lovett R et al (2005) Catalytically enhanced endocellulase Cel5A from *Acidothermus cellulolyticus*. Appl Biochem Biotechnol A 121(1–3):129–148

Bamforth CW (2009) Current perspectives on the role of enzymes in brewing. J Cereal Sci 50(3):353–357

Bassi R, Pineau B, Dainese P et al (1993) Carotenoid-binding proteins of photosystem II. Eur J Biochem 212(2):297–303

Bayer EA, Setter E, Lamed R (1985) Organization and distribution of the cellulosome in Clostridium thermocellum. J Bacteriol 16:552–559

Bayer EA, Lamed R, Himmel ME (2007) The potential of cellulases and cellulosomes for cellulosic waste management. Curr Opin Biotechnol 18:237–245

Beguin P, Aubert JP (1994) The biological degradation of cellulose. FEMS Microbiol Rev 13(1):25–58

Bernardez TD, Lyford K, Hogsett DA et al (1993) Adsorption of *Clostridium thermocellum* cellulases onto pretreated mixed hardwood, Avicel, and lignin. Biotechnol Bioeng 42(7):899–907

Bhat MK (2000) Cellulases and related enzymes in biotechnology. Biotechnol Adv 18(5):355–383

Bowen RM, Harper SHT (1990) Decomposition of wheat straw and related compounds by fungi isolated from straw in arable soils. Soil Biol Biochem 22(3):393–399

Bronnenmeier K, Rucknagel KP, Staudenbauer WL (1991) Purification and properties of a novel type of exo-1,4-betaglucanase (Avicelase II) from the cellulolytic thermophile *Clostridium stercorarium*. Eur J Biochem 200(2):379–385

Buchert J, Oksanen T, Pere J, Siika-aho M, Suurnakki A, Viikari L (1998) Applications of *Trichoderma reesei* enzymes in the pulp and paper industry. In: Harman GF, Kubicek CP (eds) Trichoderma & gliocladium—enzymes, vol 2 of biological control and commercial applications, pp 343–363

Canales AM, Garza R, Sierra JA et al (1988) The application of beta-glucanase with additional side activities in brewing. MBAA Tech Q 25:27–31

Chet I, Benhamou N, Haran S (1998) Mycoparatism and lytic enzymes. In: Harman GF, Kubicek CP (eds) *Trichoderma* and *Gliocladium*—enzymes, vol 2 of biological control and commercial applications. Taylor & Francis, London, pp 327–342

Chiacchierini E, Mele G, Restuccia D, Vinci G (2007) Impact evaluation of innovative and sustainable extraction technologies on olive oil quality. Trends Food Sci Technol 18(6):299–305

Cohen R, Suzuki MR, Hammel KE (2005) Processive endoglucanase active in crystalline cellulose hydrolysis by the brown rot basidiomycete *Gloeophyllum trabeum*. Appl Environ Microbiol 71(5):2412–2417

Cortez JM, Ellis J, Bishop DP (2002) Using cellulases to improve the dimensional stability of cellulosic fabrics. Text Res J 72(8):673–680

Cowan WD (1996) Animal feed. In: Godfrey T, West S (eds) Industrial enzymology, 2nd edn. Macmillan Press, London, pp 360–371

de Carvalho LMJ, de Castro IM, da Silva CAB (2008) A study of retention of sugars in the process of clarification of pineapple juice (*Ananas comosus, L. Merril*) by micro- and ultra-filtration. J Food Eng 87(4):447–454

Davies G, Henrissat B (1995) Structures and mechanisms of glycosyl hydrolases. Structure 3(9):853–859

De Faveri D, Aliakbarian B, Avogadro M, Perego P, Converti A (2008) Improvement of olive oil phenolics content by means of enzyme formulations: effect of different enzyme activities and levels. Biochem Eng J 41(2):149–156

Devillard E, Goodheart DB, Karnati SKR et al (2004) *Ruminococcus albus* 8 mutants defective in cellulose degradation are deficient in two processive endocellulases, Cel48A and Cel9B, both of which possess a novel modular architecture. J Bacteriol 186:136–145

DeVries RP, Visser J (2001) *Aspergillus* enzymes involved in plant cell wall polysaccharides. Microbiol Mol Biol Rev 65:497–522

Dhiman TR, Zaman MS, Gimenez RR et al (2002) Performance of dairy cows fed forage treated with fibrolytic enzymes prior to feeding. Anim Feed Sci Technol 101(1–4):115–125

Dienes D, Egyhazi A, Reczey K (2004) Treatment of recycled fiber with *Trichoderma* cellulases. Ind Crop Prod 20(1):11–21

Dourado F, Bastos M, Mota M et al (2002) Studies on the properties of Celluclast/Eudragit L-100 conjugate. J Biotechnol 99(2):121–131

Eriksson KE, Blanchette RA, Ander P (1990) Microbial and enzymatic degradation of wood and wood components. Springer, Berlin/Heidelberg, pp 1–407

Fantozzi P, Petruccioli G, Montedoro G (1977) Trattamenti con additivi enzimatici alle paste di oliva sottoposte ad estrazione per pressione unica: influenze delle cultivars, dell'epoca di raccolta e della conservazione. Grasse 54:381–388

Fennema OW (1985) Food chemistry. Marcel Dekker, New York

Fontaine S, Bardoux G, Benest D et al (2004) Mechanisms of the priming effect in a Savannah soil amended with cellulose. Soil Sci Soc Am J 68(1):125–131

Fortun-Lamothe L, Gidenne T, Debray L, et al 2001 Intake regulation, performances and health status according to feeding strategy around weaning. In: Proceedings of the second meeting of COST 848 working group 4, Gödöllő, pp 40–41

Galante YM, DeConti A, Monteverdi R (1998) Application of *Trichoderma* enzymes in food and feed industries. In: Harman GF, Kubicek CP (eds) *Trichoderma* and *Gliocladium*—enzymes, vol 2 of biological control and commercial applications. Taylor & Francis, London, pp 311–326

Gavrilescu M, Nicu M (2004) Source reduction and waste minimization. Ecozone Press, Iasi

Ghosh P, Singh A (1993) Physicochemical and biological treatments for enzymatic/microbial conversion of lignocellulosic biomass. Adv Appl Microbiol 39:295–333

Gilligan W, Reese ET (1954) Evidence for multiple components in microbial cellulases. Can J Microbiol 1:90–107
Godfrey T, West S (1996) Textiles. In: Industrial enzymology, 2nd edn. Macmillan Press, London, pp 360–371
Graham H, Balnave D (1995) Dietary enzymes for increasing energy availability. In: Wallace RJ, Chesson A (eds) Biotechnology in animal feeds and animal feedings. VHC, Weinheim, pp 296–309
Grassin C, Fauquembergue P (1996) Fruit juices. In: Godfrey T, West S (eds) Industrial enzymology, 2nd edn. Macmillan Press, London, pp 226–264
Gupta R, Sharma KK, Kuhad RC (2009) Separate hydrolysis and fermentation (SHF) of Prosopis juliflora, a woody substrate, for the production of cellulosic ethanol by *Saccharomyces cerevisiae* and *Pichia stipitis*-NCIM 3498. Bioresour Technol 100(3):1214–1220
Gupta R, Mehta G, Khasa YP et al (2010) Fungal delignification of lignocellulosic biomass improves the saccharification of cellulosics. Biodegradation 22(4):797–804
Gupta R, Khasa YP, Kuhad RC (2011) Evaluation of pretreatment methods in improving the enzymatic saccharification of cellulosic materials. Carbohydr Polym 84:1103–1109
Harman GE, Bjorkman T (1998) Potential and existing uses of Trichoderma and Gliocladium for plant disease control and plant growth enhancement. In: Kubicek CP, Harman GE (eds) Trichoderma and Gliocladium, vol 2. Taylor & Francis, London, pp 229–265
Hebeish A, Ibrahim NA (2007) The impact of frontier sciences on textile industry. Colourage 54:41–55
Henrissat B (1991) A classification of glycosyl hydrolases based on amino acid sequence similarities. J Biochem 280(Pt 2):309–316
Himmel ME, Ruth MF, Wyman CE (1999) Cellulase for commodity products from cellulosic biomass. Curr Opin Biotechnol 10:358–364
Hsu JC, Lakhani NN (2002) Method of making absorbed tissue from recycled waste paper. US Patent 6413363
Ibrahim NA, El-Badry K, Eid BM et al (2011) A new approach for biofinishing of cellulose-containing fabrics using acid cellulases. Carbohydr Polym 83(1):116–121
Inar IC (2005) Effects of cellulase and pectinase concentrations on the colour yield of enzyme extracted plant carotenoids. Process Biochem 40(2):945–949
Irwin DC, Zhang S, Wilson DB (2000) Cloning, expression and characterization of a family 48 exocellulase, Cel48A, from *Thermobifida fusca*. Eur J Biochem 267:4988–4997
John F, Monsalve G, Medina PIV et al (2006) Ethanol production of banana shell and cassava starch. Dyna Universidad Nacional de Colombia 73:21–27
Kakiuchi M, Isui A, Suzuki K et al (1998) Cloning and DNA sequencing of the genes encoding *Clostridium josui* scaffolding protein CipA and cellulase CelD and identification of their gene products as major components of the cellulosome. J Bacteriol 180:4303–4308
Kantelinen A, Jokinen O, Sarkki ML, et al (1995) Effects of enzymes on the stability of colloidal pitch. In: Proceedings of the 8th international symposium on wood and pulping chemistry 1, Helsinki, pp 605–612
Karmakar M, Ray RR (2011) Current trends in research and application of microbial cellulases. Res J Microbiol 6(1):41–53
Karnis A (1995) The role of latent and delatent mechanical pulp fines in sheet structure and pulp properties. Paperi Ja PuuPaper Timber 77:491–497
Kibblewhite RP, Bawden AD, Brindley CL (1995) TMP fiber and fines qualities of 13 radiata pine wood types. APPITA 48:367–377
Koivula A, Ruohonen L, Wohlfahrt G et al (2002) The active site of cellobiohydrolase Cel6A from *Trichoderma reesei*: the roles of aspartic acids D221 and D175. J Am Chem Soc 124:10015–10024
Kruus K, Wang WK, Ching J et al (1995) Exoglucanase activities of the recombinant *Clostridium thermocellum* CelS, a major cellulosome component. J Bacteriol 177:1641–1644
Kuhad RC, Singh A (1993) Lignocellulose biotechnology: current and future prospects. Crit Rev Biotechnol 13(2):151–172

Kuhad RC, Singh A, Eriksson KE (1997) Microorganisms and enzymes involved in the degradation of plant fiber cell walls. Adv Biochem Eng Biotechnol 57:45–125
Kuhad RC, Manchanda M, Singh A (1999) Hydrolytic potential of extracellular enzymes from a mutant strain of *Fusarium oxysporum*. Bioprocess Eng 20(2):133–135
Kuhad RC, Gupta R, Khasa YP et al (2010a) Bioethanol production from *Lantana camara* (red sage): pretreatment, saccharification and fermentation. Bioresour Technol 101(21):8348–8354
Kuhad RC, Gupta R, Khasa YP (2010b) Bioethanol production from lignocellulosic biomass: an overview. In: Lal B (ed) Wealth from waste. Teri Press, New Delhi
Kumar R, Wyman CE (2009) Effect of additives on the digestibility of corn stover solids following pretreatment by leading technologies. Biotechnol Bioeng 102(6):1544–1557
Kvesitadze G, Gogilashvidi L, Svaondize P et al (1986) Exogeneous celluloses of thermophilic micromycetes. II. Thermo stability of enzyme preparations. Acta Biotechnol 6:361–367
Lee D, Yu AHC, Saddler JN (1995) Evaluation of cellulase recycling strategies for the hydrolysis of lignocellulosic substrates. Biotechnol Bioeng 45(4):328–336
Leschine SB (1995) Cellulose degradation in anaerobic environments. Annu Rev Microbiol 49:399–426
Lewis GE, Hunt CW, Sanchez WK et al (1996) Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers. J Anim Sci 74(12):3020–3028
Li Y, Wilson DB (2008) Chitin binding by *Thermobifida fusca* cellulase catalytic domains. Biotechnol Bioeng 100:644–652
Liu CC, Doi RH (1998) Properties of *exgS,* a gene for a major subunit of the *Clostridium cellulovorans* cellulosome. Gene 211:39–47
Ljundahl LG, Eriksson KE (1985) Ecology of microbial cellulease degradation. In: Marshall KC (ed) Advances in microbial ecology. Plenum Press, New York, pp 237–299
Lorito M, Hayes CK, Di Pietro A et al (1994) Purification, characterization, and synergistic activity of a glucan 1,3-beta-glucosidase and an N-acetylbeta-glucosaminidase from *Trichoderma harzianum*. Phytopathology 84(4):398–405
Lynd LR, Weimer PJ, Van Zyl WH et al (2002) Microbial cellulose utilization: fundamentals and biotechnology. Microbiol Mol Biol Rev 66:506–577
Lynd LR, Laser MS, Bransby D et al (2008) How biotech can transform biofuels. Nat Biotechnol 26:169–172
Maheswari R, Bharadwaz G, Bhat MK (2000) Thermophilic fungi: their physiology and enzymes. Microbiol Mol Biol Rev 64:461–468
Mai C, Kues U, Militz H (2004) Biotechnology in the wood industry. Appl Microbiol Biotechnol 63(5):477–494
Malherbe S, Cloete TE (2002) Lignocellulose biodegradation: fundamentals and applications. Rev Environ Sci Biotechnol 1(2):105–114
Mansfield SD, Wong KKY, De Jong E et al (1996) Modification of Douglas-fir mechanical and Kraft pulps by enzyme treatment. TAPPI J 79(8):125–132
Marlatt C, Ho CT, Chien M (1992) Studies of aroma constituents bound as glycosides in tomato. J Agric Food Chem 40(2):249–252
McBee RH (1959) The anaerobic thermophilic cellulolytic bacteria. Bacteriol Rev 14:51–63
McKendry P (2002) Energy production from biomass: overview of biomass. Bioresour Technol 83:37–43
Mejia-Castillo T, Hidalgo-Lara ME, Brieba LG et al (2008) Purification, characterization and modular organization of a cellulose-binding protein, CBP105, a processive beta-1,4-endoglucanase from *Cellulomonas flavigena*. Biotechnol Lett 30:681–687
Milala EA, Onyenekwe PC, Ameh DA, et al (2000) Cellulase production from sorghum bran by *Aspergillus niger* SL:1: an assessment of pretreatment methods. In: Proceedings of the international conference on biotechnology: commercialization and food security (ICBCFS '00), Abuja, pp 153–159
Milala MA, Shugaba A, Gidado A et al (2005) Studies on the use of agricultural wastes for cellulase enzyme production by *Aspergillus niger*. Research Journal of Agriculture and Biological Science 1:325–328

Minussi RC, Pastore GM, Duran N (2002) Potential applications of laccase in the food industry. Trends Food Sci Technol 13(6–7):205–216
Miyamoto K (1997) Renewable biological systems for alternative sustainable energy production. http://www.fao.org/docrep/w7241e/w7241e00.htm. #Contents
Mosier N, Wyman C, Dale B et al (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96(6):673–686
Murray WD, Sowden LC, Colvin JR (1984) Bacteroides cellulosolvens sp. nov. a cellulolytic species from sewage sludge. Int J Syst Bacteriol 34:185–187
Obeng EM, Adam SNN, Budiman C, Ongkudon CM, Maas R, Jose J (2017) Lignocellulases: a review of emerging and developing enzymes, systems, and practices. Bioresour Bioprocess 4:16
OECD (1998) Biotechnology for clean industrial products and processes. Towards industrial sustainability. OECD, Paris
Oksanen J, Ahvenainen J, Home S (1985) Microbial cellulase for improving filterability of wort and beer. In: Proceedings of the 20th European Brewery Chemistry Congress, Helsinki, pp 419–425
Ortiz Escobar ME, Hue NV (2008) Temporal changes of selected chemical properties in three manure—amended soils of Hawaii. Bioresour Technol 99(18):8649–8654
Ory R, St. Angelo AJ (1977) Enzymes in food and beverage processing. American Chemical Society, Washington, D.C.
Parsiegla G, Reverbel C, Tardif C et al (2008) Structures of mutants of cellulase Cel48F of *Clostridium cellulolyticum* in complex with long hemithiocellooligosaccharides give rise to a new view of the substrate pathway during processive action. J Mol Biol 375:499–510
Pascual JJ (2001) Recent advances on early weaning and nutrition around weaning. In: Proceedings of the 2nd meeting of COST 848 working group 4, Gödöllő, pp 31–36
Paulo AC, Gubitz GM (2003) Textile processing with enzymes. Woodhead, Cambridge
Pazarlioglu NK, Sariisik M, Telefoncu A (2005) Treating denim fabrics with immobilized commercial cellulases. Process Biochem 40(2):767–771
Pere J, Siika-aho M, Buchert J et al (1995) Effects of purified *T. reesei* cellulases on the fiber properties of Kraft pulp. TAPPI J 78(6):71–78
Pere J, Puolakka A, Nousiainen P et al (2001) Action of purified *Trichoderma reesei* cellulases on cotton fibers and yarn. J Biotechnol 89(2–3):247–255
Prassad S, Singh A, Joshi HC (2007) Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resour Conserv Recyc 50:1–39
Rai P, Majumdar GC, Das GS et al (2007) Effect of various pretreatment methods on permeate flux and quality during ultrafiltration of mosambi juice. J Food Eng 78(2):561–568
Ranalli A, Pollastri L, Contento S et al (2003) Enhancing the quality of virgin olive oil by use of a new vegetable enzyme extract during processing. Eur Food Res Technol 216(2):109–115
Reese ET, Mandels M (1971) Enzymatic degradation. In: Bikaies NM, Segal L (eds) Cellulase and cellulose derivatives. Part 5. Wiley Interscience, New York, pp 1079–1094
Reese ET, Siu RGH, Levin MS (1950) The biological degradation of soluble cellulase derivatives and its relationship to the mechanism of cellulose hydrolysis. J Bacteriol 59:485–497
Reverbel-Leroy C, Pages S, Belaich A et al (1997) The processive endoglucanase CelF, a major component of the *Clostridium cellulolyticum* cellulosome: purification and characterization of the recombinant form. J Bacteriol 179:46–52
Salonen SM (1990) Method for manufacturing paper or cardboard and product containing cellulase. US Patent 4980023
Sánchez MM, Pastor FIJ, Diaz P (2003) Exo-mode of action of cellobiohydrolase Cel48C from *Paenibacillus* sp. BP-23. Eur J Biochem 270:2913–2919
Scott BR, St-Pierre P, Lavigne J, et al (2010) Novel lignin-resistant cellulase enzymes. US Patent 20100221778
Semichaevsky VD (1989) Cellulases of higher basidiomycetes. Mikol Filopatol 23:58–590
Sharyo M, Sakaguchi H, Ohishi M et al (1978) Method of making sanitary paper from chemical pulp using a single component cellulase that does not contain cellulose-building domain. US Patent 6468391

Sheehan J, Himmel M (1999) Enzymes, energy, and the environment: a strategic perspective on the U.S. department of energy's research and development activities for bioethanol. Biotechnol Prog 15:817–827

Shen H, Gilkes NR, Kilburn DG et al (1995) Cellobiohydrolase B, a second exo-cellobiohydrolase from the cellulolytic bacterium *Cellulomonas fimi*. J Biochem 311:67–74

Shrivastava B, Thakur S, Khasa YP et al (2011) White-rot fungal conversion of wheat straw to energy rich cattle feed. Biodegradation 22(4):823–831

Singh A, Hayashi K (1995) Microbial cellulases, protein architecture molecular properties and biosynthesis. Adv Appl Microbiol 40:1–44

Singh A, Kumar PKR, Schugerl K (1991) Adsorption and reuse of cellulases during saccharification of cellulosic materials. J Biotechnol 18(3):205–212

Singh A, Kuhad RC, Ward OP (2007) Industrial application of microbial cellulases. In: Kuhad RC, Singh A (eds) Lignocellulose biotechnology: future prospects. I. K. International Publishing House, New Delhi, pp 345–358

Sreenath HK, Shah AB, Yang VW et al (1996) Enzymatic polishing of jute/cotton blended fabrics. J Ferment Bioeng 81(1):18–20

Stork G, Puls J (1996) Changes in properties of different recycled pulps by endoglucanase treatments. In: Srebotnik E, Mesner K (eds) Proceedings of the international conference on biotechnology in the pulp and paper industry: recent advances in applied and fundamental research, vol 1. Facultas-Universitatsverlag, Vienna, pp 145–150

Sukumaran RK, Singhania RR, Pandey A (2005) Microbial cellulases—production, applications and challenges. J Sci Ind Res 64(11):832–844

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

Suominen P, Reinikainen T (1993) Foundation for biotechnical and industrial fermentation research. In: Proceedings of the 2nd symposium on *Trichoderma Reesei* cellulases and other hydrolases (TRICEL '93), vol 8. Espoo, Finland

Taniguchi M, Suzuki H, Watanabe D et al (2005) Evaluation of pretreatment with *Pleurotus ostreatus* for enzymatic hydrolysis of rice straw. J Biosci Bioeng 100(6):637–643

Teeri TT (1997) Crystalline cellulose degradation: new insights into the function of cellobiohydrolases. Trends Biotechnol 15:160–167

Tejada M, Gonzalez JL, García-Martínez AM et al (2008) Application of a green manure and green manure composted with beet vinasse on soil restoration: effects on soil properties. Bioresour Technol 99(11):4949–4957

Tiwari VN, Pathak AN, Lehri LK (1987) Effect of plant waste incorporation by different methods under uninoculated and inoculated conditions on wheat crops. Biol Wastes 21(4):267–273

Tounissen MJ, Dekont GVM, Op-den Camp HJM et al (1993) Production of cellulolytic and xylanolytic enzymes during growth of anaerobic fungi from ruminant and nonruminant herbivores on different substrates. Appl Biochem Biotechnol 39–40:177–189

Tu M, Chandra RP, Saddler JN (2007) Evaluating the distribution of cellulases and the recycling of free cellulases during the hydrolysis of lignocellulosic substrates. Biotechnol Prog 23(2):398–406

Uhlig H (1998) Industrial enzymes and their applications. Wiley, New York

UNEP (1999) International cleaner production information clearinghouse, CD version 1. United Nations Environment Programme, Division of Technology, Industry and Economics, Paris. www.emcentre.com/unepweb/

Van Berkel R (2000) Cleaner production for process industries. Plenary lecture. Chemeca, Perth

Vocadlo DJ, Davies GJ (2008) Mechanistic insights into glycosidase chemistry. Curr Opin Chem Biol 12:539–555

Wood TM, McCrae SI (1972) Purification and properties of Q component of *Trichoderma koningii* cellulose. J Biochem 128:1183–1192

Wood TM, McCrae SI (1986) The cellulase of Penicillium pinophilum. Synergism between enzyme components solubulising with reference to the involvement of two immunologically distinct cellobiohydrolases. J Biochem 234:93–99

Wood TM, McCrae SI, Bhat MK (1989) The mechanism of fungal cellulase action. Synergism between enzyme components of native cellulose. J Biochem 260:37–43
Wyman CE, Dale BE, Elander RT et al (2005) Coordinated development of leading biomass pretreatment technologies. Bioresour Technol 96(18):1959–1966
Yang B, Wyman CE (2004) Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose. Biotechnol Bioeng 86(1):88–95
Yang B, Wyman CE (2006) BSA treatment to enhance enzymatic hydrolysis of cellulose in lignin containing substrates. Biotechnol Bioeng 94(4):611–617
Yanling H, Youfang D, Yanquan L (1991) Two cellulolytic *Clostridium* species. Clostridium cellulosi sp. nov. and clostridium cellulofermentans sp. Int J Syst Bacteriol 41:306–309
Yoon JJ, Cha CJ, Kim YS et al (2008) Degradation of cellulose by the major endoglucanase produced from the brown-rot fungus *Fomitopsis pinicola*. Biotechnol Lett 30:1373–1378
Youn KS, Hong JH, Bae DH et al (2004) Effective clarifying process of reconstituted apple juice using membrane filtration with filter-aid pretreatment. J Membr Sci 228(2):179–186
Zaldivar J, Nielsen J, Olsson L (2001) Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration. Appl Microbiol Biotechnol 56:17–34
Zhang Y-HP (2008) Reviving the carbohydrate economy via multi-product biorefineries. J Ind Microbiol Biotechnol 35:367–375
Zhang Y-HP (2009) A sweet out-of-the-box solution to the hydrogen economy: is the sugar-powered car science fiction? Energ Environ Sci 2:272–282
Zhang Y-HP, Lynd LR (2005) Regulation of cellulase synthesis in batch and continuous cultures of *Clostridium thermocellum*. J Bacteriol 187:99–106
Zhang Y-HP, Himmel M, Mielenz JR (2006) Outlook for cellulase improvement: screening and selection strategies. Biotechnol Adv 24:452–481
Zhu Z, Sathitsuksanoh N, Zhang Y-HP (2009) Direct quantitative determination of adsorbed cellulase on lignocellulosic biomass with its application to study cellulase desorption for potential recycling. Analyst 134:2267–2272
Zosel T (1994) Pollution prevention in the chemical industry. In: Edgerly D (ed) Opportunities for innovation: pollution prevention. National Institute of Standards and Technology, Gaithersburg, pp 13–25
Zverlov VV, Schantz N, Schwarz WH (2005) A major new component in the cellulosome of *Clostridium thermocellum* is a processive endo-beta-1,4-glucanase producing cellotetraose. FEMS Microbiol Lett 249:353–358

Chapter 7
Cellulose as Potential Feedstock for Cellulase Enzyme Production: Versatility and Properties of Various Cellulosic Biomass – Part II

Swati Madhu and Pramod W. Ramteke

7.1 Introduction

Cellulose is a polysaccharide made up of a linear chain of thousands of β-(1,4)-linked D-glucose units. Being an integral part of structural component of the primary cell wall of green plants, algae, and oomycetes, it is also considered as the most abundant natural biopolymer on earth. Due to increase in population, the use of renewable and nonrenewable source of energy is at its peak. To meet the future demands of population, cellulose which accounts to 50% of the dry cell weight of plant biomass could be exploited as the only anticipatable sustainable source of fuels and for another chemical feedstock (Kuhad and Singh 1993).

The French chemist Anselme Payen coined the term cellulose in 1838 while studying different types of wood and also determined its formula by isolating it from plant matter. Then in 1870, the first successful thermoplastic polymer was produced by Hyatt Manufacturing Company by using cellulose. Cellulose is widely used in textile industry as cotton, linen, and rayon for clothes and in explosive industry as nitrocellulose. Cellulose produced by plants is considered to be native cellulose which is present in two crystalline forms, cellulose I and cellulose II. After treatment with liquid ammonia celluloses I and II gives crystalline cellulose III, which on further heating generate cellulose IV crystal (Lavanya et al. 2015).

S. Madhu
Department of Chemical Engineering, IIT Bombay, Mumbai, India

P. W. Ramteke (✉)
Department of Biological Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Allahabad, Uttar Pradesh, India

M. Srivastava et al. (eds.), *Approaches to Enhance Industrial Production of Fungal Cellulases*, Fungal Biology,
https://doi.org/10.1007/978-3-030-14726-6_7

Cellulose is the building block of plants which is the first link of food chain. Though cellulose is an excipient product but there are numerous sources available from where cellulose can be derived naturally as well as synthetically. It is also used in diverse areas of industry like as an anticaking agent in shredded cheese and as a processing aid for juice filtration. FDA has also approved the use of cellulose as fat substitute and bulking agent in low-calorie foods and as a texturizer and emulsifier in food products. One of the unique features of cellulose is its crystalline structure in which individual microfibrils are packed so tightly so that it does not allow even water to penetrate. However, due to variation in the degree of departure of crystallinity there are cellulose fibers from purely crystalline to purely amorphous. There are many other irregularities associated with cellulose fibers like kinks, twists, voids and capillaries which is responsible for its structural heterogenity. This different kind of structural heterogeneity in the cellulose makes it partially penetrable (Detns et al. 2002).

To study cellulose biosynthesis, *Acetobacter xylinum*-synthesized cellulose is used as a model system. There is a lot of difference between plant and bacterial cellulose in terms of arrangement of glucosyl units within the unit cell of crystallites. There are genetic evidences also which entail the differences in enzymatic machinery at molecular level involved in cellulose synthesis.

7.2 Sources of Cellulose

7.2.1 Natural

The most common natural source of cellulose is plant which includes cotton, jute, flax, ramie, sisal, and hemp. Table 7.1 represents the different natural sources of cellulose with its examples.

Table 7.1 Representation of various sources of natural cellulose

Class	Examples	% of cellulose
Seed fibers	Cotton, kapok	90
Leaf fibers	Sisal, fique, agave	33
Bast fibers	Flax, jute, kenaf	33
Plant skin	Hemp, ramie, rattan, and vine fibers	33
Fruit fibers	Coconut (coir) fiber	30–50
Stalk fibers	Rice, barley, wheat straws, bamboo, grass, tree wood	40–50

7.2.2 *Synthetic*

The fibers coming from synthetic materials such as petrochemicals or manufactured from natural cellulose like rayon are considered to be synthetic fibers. Some synthetic fibers are derived from natural cellulose like lyocell. Lyocell has very similar properties like cellulosic fibers. It has emerged as a promising, environment-friendly, and economically viable alternative for manufacture of cellulose fibers. It does not require derivatization steps like alkalization or xanthation to dissolve the cellulose. In this process, N-methylmorpholine N-oxide monohydrate (NMMO) is used as a solvent, and on industrial scale, it gives recovery rate of 99.3%. NMMO can be recycled which makes the whole process more economic (Lavanya et al. 2015).

7.3 Cellulase and Its Types

Cellulose is hydrolyzed to its simpler form by enzyme cellulase. This enzyme is synthesized by a large diversity of microorganisms ranging from fungi to bacteria grown over cellulosic matters. Table 7.2 enlists the microorganisms having cellulolytic abilities (Kuhad et al. 2011).

From the past twentieth century, cellulase has been studied and considered to be one of the potential enzymes for industrial saccharification. Its action on cellulose is performed basically by three groups of enzymes: (1) endo-(1,4)- β-D-glucanase (EC 3.2.1.4) cleaves the internal β-1-4 linkages; (2) exo-(1,4)-β-D-glucanase (EC 3.2.1.91) attacks on the nonreducing end of cellulose chain; and (3) β-glucosidases (EC 3.2.1.21), also known as cellobiases, break down the building unit, i.e., cellobiose. Fig. 7.1 represents lysis of cellulose by cellulase (Kuhad et al. 2011). Formation of cellobiose and cellodextrins occurs by the action of endoglucanases and cellobiohydrolases which led to feedback inhibition to the enzyme's activity. To make

Table 7.2 List of microorganisms possessing cellulolytic properties

Fungi	*Aspergillus niger*, *A. nidulans*, *A. oryzae*, *A. terreus*, *Fusarium solani*, *Humicola insolens*, *H. grisea*, *Melanocarpus albomyces*, *Penicillium brasilianum*, *P. occitanis*, *P. decumbans*, *Trichoderma reesei*, *Chaetomium cellulyticum*, *C. thermophilum*, *Neurospora crassa*, *P. echinulatum*, *Trichoderma atroviride*, *Tyromyces palustris*, *Fomitopsis* sp., *Sporotrichum thermophile*, *Agaricus arvensis*, *Phlebia gigantea*
Bacteria	*Acinetobacter junii*, *A. amitratus*, *Acidothermus cellulolyticus*, *Bacillus subtilis*, *B. pumilus*, *B. amyloliquefaciens*, *B. circulans*, *B. flexus*, *Eubacterium cellulosolvens*, *Geobacillus* sp.; *Microbispora bispora*, *Paenibacillus curdlanolyticus*, *Pseudomonas cellulosa*, *Clostridium thermocellum*, *C. cellulolyticum*, *C. acetobutylicum*, *Fibrobacter succinogenes*
Actinomycetes	*Cellulomonas fimi*, *C. bioazotea*, *C. uda*, *Streptomyces drozdowiczii*, *S. lividans*, *Thermomonospora fusca*, *T. curvata*

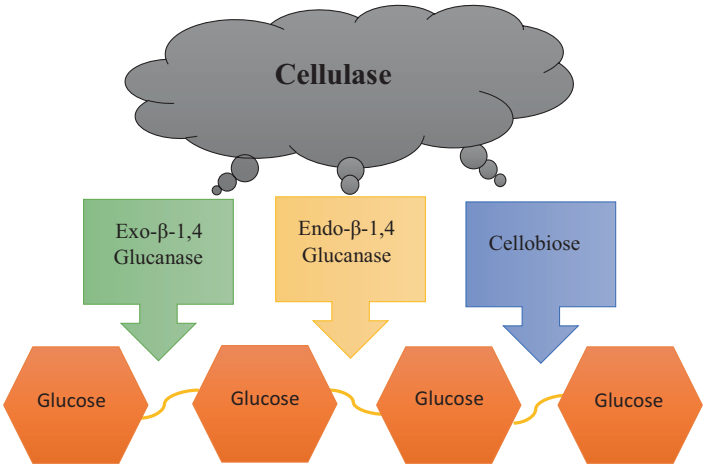

Fig. 7.1 Digestion of cellulose

this whole process energy efficient, microorganisms form glucose monophosphate by digesting cellobiose and cellodextrins by action of cellodextrin phosphorylases or cellobiose phosphorylases (Cavka et al. 2013).

7.4 Effective Ways of Cellulase Production

Cellulose is insoluble in water and highly complex to digest. The effective conversion of these complex biomasses to simple fermentable sugar requires three steps: (1) reduction in size, (2) fractionation, and (3) enzymatic hydrolysis. Commercial production of cellulase requires a robust and cost-efficient method of cellulase enzyme production. Typically, *Trichoderma*, *Aspergillus*, and *Penicillium* species are commonly used for commercial production of cellulases. *Trichoderma reesei* is a well-known fungus used to produce cellulase from renewable resources like spruce, bagasse, wastepaper, dairy manure, and willow. It expresses all enzymes required for cellulase digestion but have low titer of β-glucosidase enzyme which results in the accumulation of cellobiose. So, to enhance the expression of this enzyme, soluble polysaccharide was chosen to induce the production of β-glucosidase enzyme (Zheng et al. 2017).

Due to having short generation time and surveillance to extreme environment, bacteria has also been explored for cellulose hydrolysis. Among them Bacillus, Cellulomonas, Cytophaga, Pseudomonas, *Sporocytophaga*, and *Streptomyces* are favored as cellulolytic bacteria. Among all the potential bacterial strain *Paenibacillus lautus* strain BHU3 has been explored profusely which has discovered its diverse sets of genes for endo-, /exo glucanase and, β-glucosidase. These enzymes are

required for cellulose degradation and thus has been reported as an efficient bacterial system harboring maximum number of glycosyl hydrolase family gene (Yadav and Dubey 2018). The idea of cellulase engineering has also attracted scientists, as it can majorly contribute in the cellulose decomposition. Thus this engineering concept is based on three conception: (1) design of cellulase as per the structure of cellulase and its catalytic mechanism (Wilson 2004); (2) selection of improved catalytic enzymes with directed evolution which will lead to evolve after random mutagenesis and recombination (Arnold 2001); and (3) application of cocktails of cellulase enzyme for efficient hydrolysis of cellulose (Baker et al. 1998).

7.4.1 Production of Cellulase Through Fermentation

Fermentation is extensively used technique for the production of industrially relevant products. With the help of microorganisms, it leads to the conversion of complex substrate into its simpler form. Basically, two types of fermentation strategy got developed for the production of cellulase:

(a) *Solid-State Fermentation (SSF)/Solid-State Cultivation (SSC)*
The principle of this technique is to utilise nutrient rich agricultural waste like bran, bagasse, paddy straw paper pulp, etc as a cheap source of substrate for fermentation. Then the industrially relevant products is extracted from the fermented biomass. It involves those types of fungi and microorganism which require less amount of moisture for its optimum growth and activity

(b) *Submerged Fermentation (SmF)/Liquid Fermentation (LF)*
As the name suggests, this technique utilizes liquid substrates such as molasses and broth as source of substrate and involves microorganisms which have good activity in high moisture content.

7.5 Various Applications of Cellulases

7.5.1 Paper Industry

The biochemical processing has turned out to be substantially energy saving with the use of cellulases. By using mixture of cellulases and hemicellulases in biomodification of fiber, properties not only have improved drainage by removing fine fibrils of fiber as well as beat ability in the paper mills but also have increased the production rate of several mills. Cellulases, in combination with xylanases, are also used in deinking of different types of paper wastes. It has not only eliminated the use of alkali in the whole process but also has improved fiber brightness and enhanced strength properties (Kuhad et al. 2011) (Fig. 7.2).

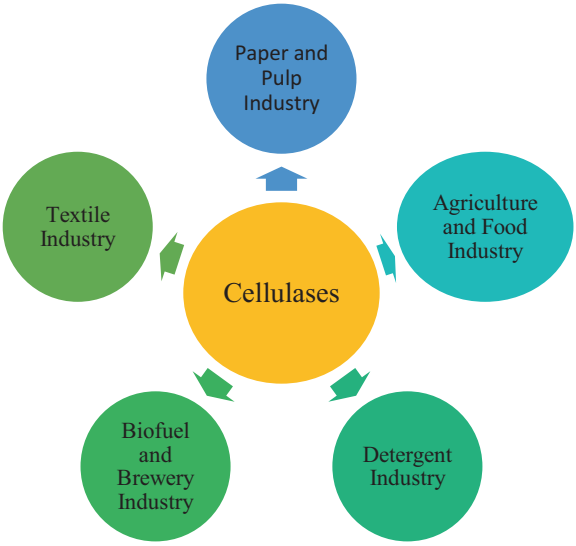

Fig. 7.2 Schematic representation of application of cellulases

7.5.2 Textile Industry

Cellulases are widely used in textile industry for stone washing of jeans and biopolishing of cotton as well as other cellulosic fabrics. Earlier for biostoning, pumice stone was used, but it used to damage the fabric as well as decrease the productivity of machines. In biopolishing the acidic cellulases enhance the softness and water absorbance property of fibers. It is also used in softening the garments by removing the microfibrils which come out due to repetitive washing of cotton fabric. The use of cellulases has been reported to offer better performance in terms of fabric strength loss as well as the stability of cellulosic fabrics (Sreenath et al. 1996).

7.5.3 Biofuel and Brewery Industry

Cellulases are the most popular enzymes used for the bioconversion and enzymatic saccharification of lignocellulosic materials like sugarcane, bagasse, corncob, rice straw, *Lantana camara*, *Prosopis juliflora*, switch grass, and sawdust for biofuel production. The whole process is divided into three parts:

(a) Pretreatment
(b) Hydrolysis
(c) Purification

Similarly, in beverage industry, hemicellulases play an important role. Cellulases are used as macerating enzyme which helps in improving color extraction and enhances wine quality (Mosier et al. 2005).

7.5.4 Agriculture and Detergent Industry

Cellulase is known to improve the soil quality by straw incorporation and reducing the dependence on chemical fertilizers. In combination with other hemicellulases and pectinases, it is also used to enhance the growth of crops and control plant disease. It has been discovered that cellulase-based detergents are recent advancements in detergent industry, which give superior cleaning and retain color brightness of fabric. The presence of anionic and nonionic surfactant, citric acid or a water-soluble salt, protease enhances the stability of cellulases and thus gives excellent cleaning (Bhat 2000).

7.5.5 Food Industry

In food industry cellulases are used for extraction and clarification of fruit and vegetable juices and lead to increase the yield of juices. In animal feed industry as well, it is used to improve the feed value by eliminating antinutritional factors present in the feed grains and by providing supplementary digestive enzyme like proteases, amylases, and glucanases (Chamchong and Athapol 1991). The low-quality feedstuffs contain higher concentration of cellulose and high ash contents as compared to high-quality feedstuffs. In this case cellulase is used in cattle feeding to enhance the digestibility.

7.6 Fine-Tuning the Digestion of Cellulose with a Discovery of Novel Enzymes

Bioethanol is a biofuel which is produced in the biggest volume mainly by degradation of starch from corn and sugarcane. Nevertheless, the corn and sugarcane are potential food sources; efforts are being put to harvest bioenergy from lignocellulosic and algal biomass. Prominently the second-generation bioenergy is produced by thermochemical processes like pyrolysis and biochemical process. Biochemical process involves enzymatic conversion of lignocellulosic biomass into monomeric sugars which caters the central platform chemicals for future biorefinery.

Biochemical process is considered to be the sustainable technology for biomass hydrolysis. It reduces the substrate retaining the actual structure of carbohydrate in

the monomeric sugar. Despite many endeavors, the enzymatic action has not proven to be so efficient. It is because of the heterogeneity of plant cell wall, the impenetrability, and the recalcitrance of its components like hemicellulose and lignin. In 1950, Reese and his coworkers recommended the requirement of a non-hydrolytic component that could disrupt the recalcitrant component thereby increasing the accessibility for hydrolytic enzymes (Reese et al. 1950; Eijsink et al. 2008; Vaaje-Kolstad et al. 2005). Then, later in 2005 CBP21 protein has been discovered from a bacterium that breaks down chitin. This protein potentiates the biochemical action of enzymes by increasing substrate accessibility. This protein was categorized as a family 33 carbohydrate-binding module (CBM33) in the carbohydrate-active enzymes (CAZy) (Cantarel et al. 2009). Basically, CBP21 makes the substrate accessible to hydrolytic enzymes by cleaving glycosidic bonds in chitin in an oxidative manner. This property can be enhanced by adding electron donors like ascorbic acid (Vaaje-Kolstad et al. 2010). GH61 protein was discovered which is structurally similar to CBM33 proteins and acts synergistically with cellulases (Harris et al. 2010; Karkehabadi et al. 2008). The discovery of GH61 and CBM33 has set new paradigm and has created a revolution in the enzyme discovery for biomass degradation. The potential activity of these enzymes is supported by the studies done in transcriptomics and proteomics which tell that the expression of some GH61s and CBM33 protein is induced by cellulose or co-regulated with the expression of cellulases (Adav et al. 2012; MacDonald et al. 2011).

References

Adav SS, Ravindran A, Sze SK (2012) Quantitative proteomic analysis of lignocellulolytic enzymes by Phanerochaete chrysosporium on different lignocellulosic biomass. J Proteome 75(5):1493–1504. https://doi.org/10.1016/j.jprot.2011.11.020

Arnold FH (2001) Combinatorial and computational challenges for biocatalyst design. Nature 409(6817):253–257. https://doi.org/10.1038/35051731

Baker JO, Ehrman CI, Adney WS, Thomas SR, Himmel ME (1998) Hydrolysis of cellulose using ternary mixtures of purified celluloses. Appl Biochem Biotechnol 70–72(1):395–403. https://doi.org/10.1007/BF02920154

Bhat MK (2000) Cellulases and related enzymes in biotechnology. Biotechnol Adv 18(5):355–383. https://doi.org/10.1016/S0734-9750(00)00041-0

Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B (2009) The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. Nucleic Acids Res 37(Database):D233–D238. https://doi.org/10.1093/nar/gkn663

Cavka A, Guo X, Tang SJ, Winestrand S, Jönsson LJ, Hong F (2013) Production of bacterial cellulose and enzyme from waste fiber sludge. Biotechnol Biofuels 6(1):1–10. https://doi.org/10.1186/1754-6834-6-25

Chamchong M, Athapol N (1991) Effect of pH and enzymatic treatment on microfiltration and ultrafiltration of tangerine juice. J Food Process Eng 14(1):21–34. https://doi.org/10.1111/j.1745-4530.1991.tb00079.x

Detns RC, Taken S, Co FS, Smith F, Co C, Titrim- F et al (2002) Pesquisa nacional de saneamento básico 2008. Bioresour Technol 66(3):506–577. https://doi.org/10.1128/MMBR.66.3.506

Eijsink VGH, Vaaje-Kolstad G, Varum KM, Horn SJ (2008) Towards new enzymes for biofuels: lessons from chitinase research. Trends Biotechnol 26(5):228–235. https://doi.org/10.1016/j.tibtech.2008.02.004

Harris PV, Welner D, McFarland KC, Re E, Navarro Poulsen J-C, Brown K et al (2010) Stimulation of lignocellulosic biomass hydrolysis by proteins of glycoside hydrolase family 61: structure and function of a large, enigmatic family. Biochemistry 49(15):3305–3316. https://doi.org/10.1021/bi100009p

Karkehabadi S, Hansson H, Kim S, Piens K, Mitchinson C, Sandgren M (2008) The first structure of a glycoside hydrolase family 61 member, Cel61B from Hypocrea jecorina, at 1.6 Å resolution. J Mol Biol 383(1):144–154. https://doi.org/10.1016/j.jmb.2008.08.016

Kuhad RC, Singh A (1993) Lignocellulose biotechnology: current and future prospects. Crit Rev Biotechnol 13(2):151–172. https://doi.org/10.3109/07388559309040630

Kuhad RC, Gupta R, Singh A (2011) Microbial cellulases and their industrial applications. Enzyme Res 2011:1–10. https://doi.org/10.4061/2011/280696

Lavanya D, Kulkarni PK, Dixit M, Raavi PK, Krishna LNV (2015) Sources of cellulose and their applications: a review. Int J Drug Formul Res 2(November):19–38. https://doi.org/10.1016/j.biotechadv.2011.05.005

MacDonald J, Doering M, Canam T, Gong Y, Guttman DS, Campbell MM, Master ER (2011) Transcriptomic responses of the softwood-degrading white-rot fungus Phanerochaete carnosa during growth on coniferous and deciduous wood. Appl Environ Microbiol 77(10):3211–3218. https://doi.org/10.1128/AEM.02490-10

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

Reese ET, Siu RGH, Levinson HS (1950) The biological degradation of soluble cellulose derivatives and its relationship to the mechanism of cellulose hydrolysis. J Bacteriol 59(4):485–497. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC385789/

Sreenath HK, Shah AB, Yang VW, Gharia MM, Jeffries TW (1996) Enzymatic polishing of jute/cotton blended fabrics. J Ferment Bioeng 81:18–20

Vaaje-Kolstad G, Horn SJ, van Aalten DMF, Synstad B, Eijsink VGH (2005) The non-catalytic chitin-binding protein CBP21 from Serratia marcescens is essential for chitin degradation. *J Biol Chem 280*(31):28,492–28,497. https://doi.org/10.1074/jbc.M504468200

Vaaje-Kolstad G, Westereng B, Horn SJ, Liu Z, Zhai H, Sørlie M, Eijsink VGH (2010) An oxidative enzyme boosting the enzymatic conversion of recalcitrant polysaccharides. *Science 330*(6001):219 LP–219222. Retrieved from http://science.sciencemag.org/content/330/6001/219.abstract

Wilson DB (2004) Studies of Thermobifida fusca plant cell wall degrading enzymes. *Chem Rec 4*(2):72–82. https://doi.org/10.1002/tcr.20002

Yadav S, Dubey SK (2018) Cellulose degradation potential of Paenibacillus lautus strain BHU3 and its whole genome sequence. *Bioresource Technology 262*:124–131. https://doi.org/10.1016/j.biortech.2018.04.067

Zheng W, Chen X, Xue Y, Hu J, Gao MT, Tsang YF (2017) The influence of soluble polysaccharides derived from rice straw upon cellulase production by Trichoderma reesei. *Process Biochem 61*(June):130–136. https://doi.org/10.1016/j.procbio.2017.06.007

Chapter 8
Immobilization Methods of Enzymes: Part I

Kelvii Wei Guo

8.1 Introduction

Enzymes, widely existed in animals, plants, and microorganisms, have the attractive property that can make the chemical and biochemical reactions of efficient catalytic specificity, selectivity, biodegradability, and activity. However, the main drawback of enzymes is they cannot be reused and recovered when they are used after the first run. In order to overcome this issue, the immobilization techniques of enzymes are explored. The advantages of the immobilization of enzymes are enzyme rapid termination, easy recovery, and thermal and pH resistances, repeated usage of assay, and improved stability of the storage. As a result, the immobilized enzymes with the low cost have been broadly adopted in different areas such as bioenergy, medicine, food, and other industry fields (Barbosa et al., 2015; Mehta et al., 2016; Vaz et al., 2016; Grigoras, 2017; Bernal et al., 2018).

The crucial issue of the immobilization is to permanently immobilize enzymes with the activity of enzymes rwetaining. The achievement of the immobilization of enzymes can be achieved by chemical and physical methods, where methods related to physic contain adsorption and gel entrapment. Although there are not additional reagents required and the routine is simple, the physical association between enzymes and electrode surface is weak. However, chemical methods involve covalent bonding between enzyme and support material. Interaction between enzyme and support is strong, but concern grows over the fact that covalent bonding can disrupt enzyme activity. Enzyme immobilization has also been used to provide electrochemical communication between electrode and enzyme active center.

K. W. Guo (✉)
Department of Mechanical and Biomedical Engineering, City University of Hong Kong, Kowloon Tong, Kowloon, Hong Kong

M. Srivastava et al. (eds.), *Approaches to Enhance Industrial Production of Fungal Cellulases*, Fungal Biology,
https://doi.org/10.1007/978-3-030-14726-6_8

The immobilization techniques commonly taken are physical adsorption, covalent attachment/bonding, cross-linking, and entrapment.

It illustrates that not only the method of immobilization but also the support material determines the performance afterward. Mostly, support materials are carbon materials, for example, carbon nanotube or graphene, to usually use as the enzymes' immobilization carrier matrices.

8.2 Considerations of Immobilization of Enzymes

In solution, soluble enzyme molecules behave as any other solute in that they are readily dispersed in the solution and have complete freedom of movement. It is well known that enzyme immobilization is a technique specifically designed to greatly restrict the freedom of movement of an enzyme. The first consideration is to decide on the support material and then the main method of immobilization, taking into account the intended use and application. For physical property, the points for consideration are strength, shape/form (beads/sheets/fibers), pore volume, permeability, density, available surface area, degree of porosity, noncompression of particles, space for increased biomass, flow rate, and pressure drop. As for the chemical characteristics, regeneration/reuse of support, available functional groups for modification, inertness toward enzyme, and hydrophilicity (water binding by the support) should be highlighted. As for the stability, mechanical stability of support material, regeneration and maintenance of enzyme activity, and residual enzyme activity and storage should be pinpointed. For the characteristics of resistance, the effect of disruption by chemicals, pH, organic solvents, proteases, and bacterial/fungal attack shall be addressed. For the reaction consideration, reaction kinetics; side reactions; multiple enzyme systems; enzyme loading and catalytic productivity; flow rate; batch; CSTR, PBR, FBR, ALR, etc.; and diffusion limitations on mass transfer of cofactors, substrates, and products are all definitely premeditated. For the safety, specification of immobilized preparation (GRAS list requirements for FDA approval) for food, pharmaceutical, and medical applications, health and safety for process workers and end product users, toxicity of component reagents, and biocompatiblity should be carefully considered. Also, chemicals, reagents, availability and cost of support, technical skill required, special equipment, industrial-scale chemical preparation, feasibility for scale-up, reusable support, environmental impact, CRL or zero contamination, effective working life, and continuous processing should be checked in the economic viewpoints.

8.3 Methods for Immobilization of Enzymes

8.3.1 Physical Methods

8.3.1.1 Adsorption

Adsorption is currently the common physical way to immobilize enzymes. It includes affinity adsorption, ion adsorption, and physical adsorption, where it is commonly used the physical adsorption. With such kind of method, on the surface of support carriers, enzymes are taken or trapped in the mesoporous materials' pores by van der Waals forces, H bondings, or hydrophobic or electrostatic interactions (Karajanagi et al., 2004; Cracknell et al., 2008). The main benefit is that it does not require any reagents and only needs a minimum of the steps of activation.

The enzyme adsorption on the support matrix is easily realized by mixing with a proper incubation time. As the distinct characteristic of such method, there does not need to add the modification steps and other additional coupling agents.

However, the main defect of the physical adsorption is the reversion and weakness of immobilization support carriers and enzyme interaction. The forces of binding are sensitive to variations at temperature, pH, and ionic strength. As a result, immobilized enzymes' stability is relatively low. Also, the substrate solution can be able to contaminate by the leached enzymes.

8.3.1.2 Gel Entrapment

It is well known that enzymes easily aggregate with the decreased catalytic activities.

Entrapment method for enzyme immobilization is taking the polymeric networks to occlude enzymes economically to prevent enzyme aggregation. The matrix for instant agar, cellulose triacetate, chitosan, polyacrylamide, calcium alginate, collagen, polyvinyl alcohol, and polyurethane is used for the entrapment. Under the effect of the entrapment, the enzyme immobilization is achieved by microencapsulation or the entrapping of gel. The networks keep the enzymes along with the products and substrates passing through. At the same time, the enzyme leaching is decreased, and the stability is improved. Consequently, the catalytic activities are high because there are not any covalent bonds of involved support matrices and enzymes and the maintenance of the enzyme conformations. But, the main drawback of this method is the efficient barriers of diffusion hinder the substrates of macromolecular to flow into the networks.

8.3.2 Chemical Methods

8.3.2.1 Covalent Bonding

For chemical methods, it is traditional to take the covalent binding for enzyme immobilization. Generally, the covalent bonds are realized by the chemical reactions of enzymes and materials supported. Moreover, covalent bonds can avoid the enzyme leakage from the support matrix. Therefore, it is high for the enzyme immobilization stability. But, it is inactivated for the active sites of enzymes because the matrix supported chemically reacts with the molecules of enzymes. As a result, the catalytic activities decrease obviously (Wu et al., 2015; Shen et al., 2016; Wong et al., 2017; Ulu et al., 2018; Souza et al., 2018; Torres et al., 2018; Sharifi et al., 2018; Shinde et al., 2018).

Das et al. (2018) researched the *β*-amylase (bamyl) immobilization (made from peanut (*Arachis hypogaea*)) onto iron oxide nanoparticles (Fe_3O_4), graphene oxide nanosheets (GO), and graphene oxide-carbon nanotube composite (GO-CNT). The relevant immobilization process included product formation and substrate attack, and immobilization is shown in Fig. 8.1.

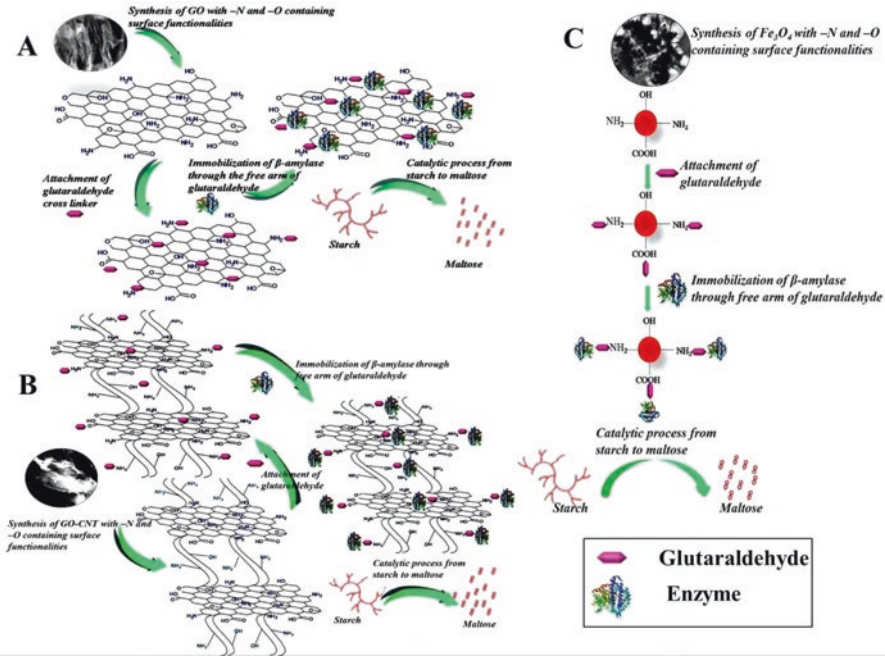

Fig. 8.1 Immobilization process for the functionalized NPs made by nanobiocatalysts for starch applied in industries, where **A**, graphene oxide nanosheets (GO); **B**, graphene oxide-carbon nanotube composite (GO-CNT); C, iron oxide nanoparticles (Fe_3O_4) (Das et al. 2018)

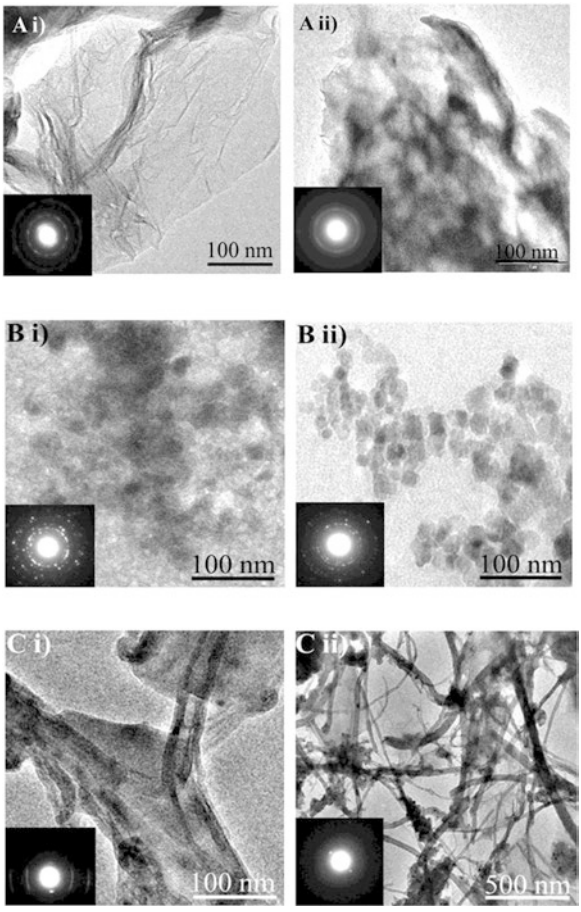

Fig. 8.2 TEM images for (**Ai**) GO native, (**Aii**) immobilized; (**Bi**) Fe_3O_4 native, (**Bii**) immobilized; (**Ci**) GO-CNT native, (**Cii**) immobilized with inset with SAD (Das et al. 2018)

Figure 8.2 shows the bamyl immobilized on the different supports. The immobilized enzyme distributes on the surface of GO, GO-CNT, and Fe_3O_4 with nanostructures in cluster and minute dispersed particles. The image of GO transparent sheet is shown in Fig. 8.2Ai, and the image of GO-CNT embedded by rods separated the sheets is expressed in Fig. 8.2Ci. The image of beads with the small sphere morphology of Fe_3O_4 is illustrated in Fig. 8.2Bi. The images of the immobilized enzyme on the corresponding supports are shown in Fig. 8.2Aii, Bii, and Cii, respectively.

Figure 8.3 shows that the morphology of nanostructures with and without enzyme immobilization is obviously different. A large amount of the crests and troughs distributed on GO and Fe_3O_4 with the distinct roughness variation indicates that the enzyme has been immobilized successfully (Fig. 8.3a, c). For GO-CNT support, the enzyme anchored successfully on GO sheets and CNT rods densely as shown in Fig. 8.3b. Study reveals that the enzyme immobilization is extremely better than that of the comparison counterpart. Moreover, the successive enzyme reuse with immobilization (repeated 10 times) still has almost 80%, 70%, and 50% of bamyl initially

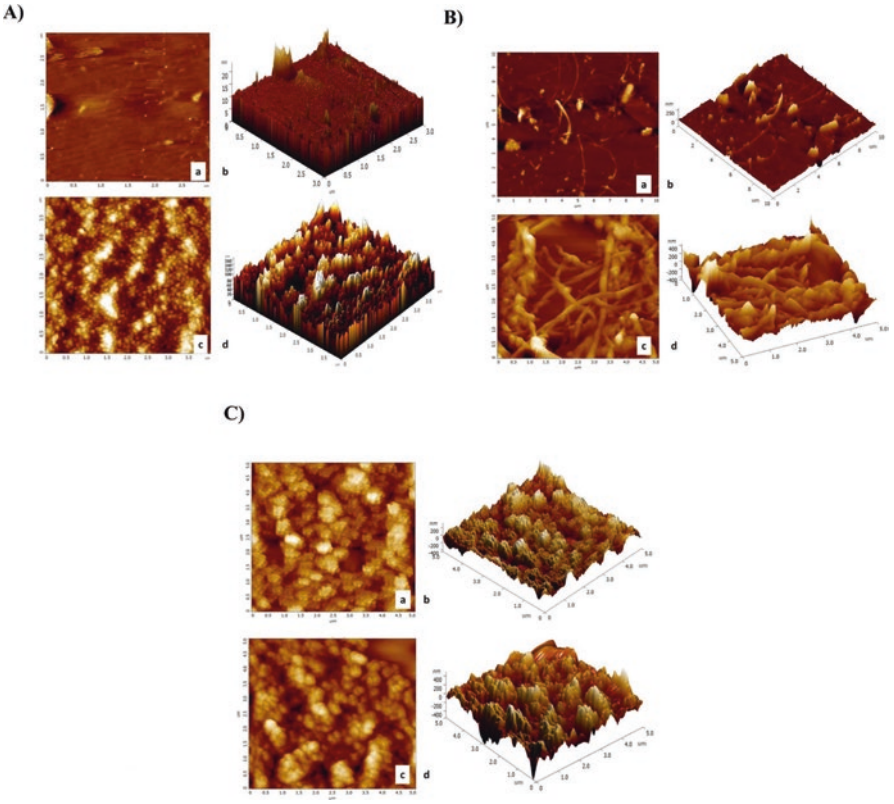

Fig. 8.3 AFM images for β-amylase immobilized nanostructures and bare nanostructures, where native GO (**A**: a, b), GO-CNT (**B**: a, b), and Fe_3O_4 (**C**: a, b) and immobilization of β-amylase on GO (**A**: c, d), GO-CNT (**B**: c, d), Fe_3O_4 (**C**: c, d) (Das et al. 2018)

immobilized activity on Fe_3O_4, GO-CNT, and GO. In addition, covalent attachment with glutaraldehyde has a strong, easy, and nontoxic bonding between nanostructures and enzymes that resulted in the stronger nanobiocatalyst (NBC).

More interestingly, immobilization of the β-amylase onto Fe_3O_4 (bamyl@Fe_3O_4) and GO-CNT (bamyl@GO-CNT) makes the activity retention near to about 70% activity when it is exposed at 65 °C after 100 min.

8.3.2.2 Cross-Linking

According to the intermolecular reactions, the reagents with bifunction are taken to cross-link the enzymes to the matrix supported. With such method, the immobilization of enzymes can be achieved successfully along with the distinctive improvement of the relevant stability and reusability. But, in this processing procedure, the catalytic activities of the enzymes may lose to some extent. To date, it is well known that N, N′-ethylene-bis-maleimide, isocyanate, and glutaraldehyde are always taken

as the bifunctional reagents. Among these, it is usually adopted glutaraldehyde in most processes (Tang et al., 2014; Alamsyah et al., 2017; Voběrková et al., 2018; Müller et al., 2018; Nishida et al., 2018; Khaldi et al., 2017).

Recently, Khaldi et al. (2017) investigated two different methods through the covalent acetylcholinesterase enzyme (AChE) linking by the amide bond formation for acetylcholinesterase immobilization of enzyme on the silicon with porous structure modification. It is reported that the AChE immobilization on the modified mesoporous silicon surface is stable and effective by two precursor surfaces. One is the surface with the acid-terminated (PSi-COOH) which was achieved with the method of the hydrogenated PSi surface by hydrosilylation, and another is the surface with the amine-terminated (PSi-NH_2) which was made with the method of the oxidized PSi surface by silanization along with carboxylic acid functions and amine groups under the effect of the carbodiimide coupling chemistry. The process of the immobilization of AChE on PSi-COOH and PSi-NH_2 surfaces is shown in Fig. 8.4.

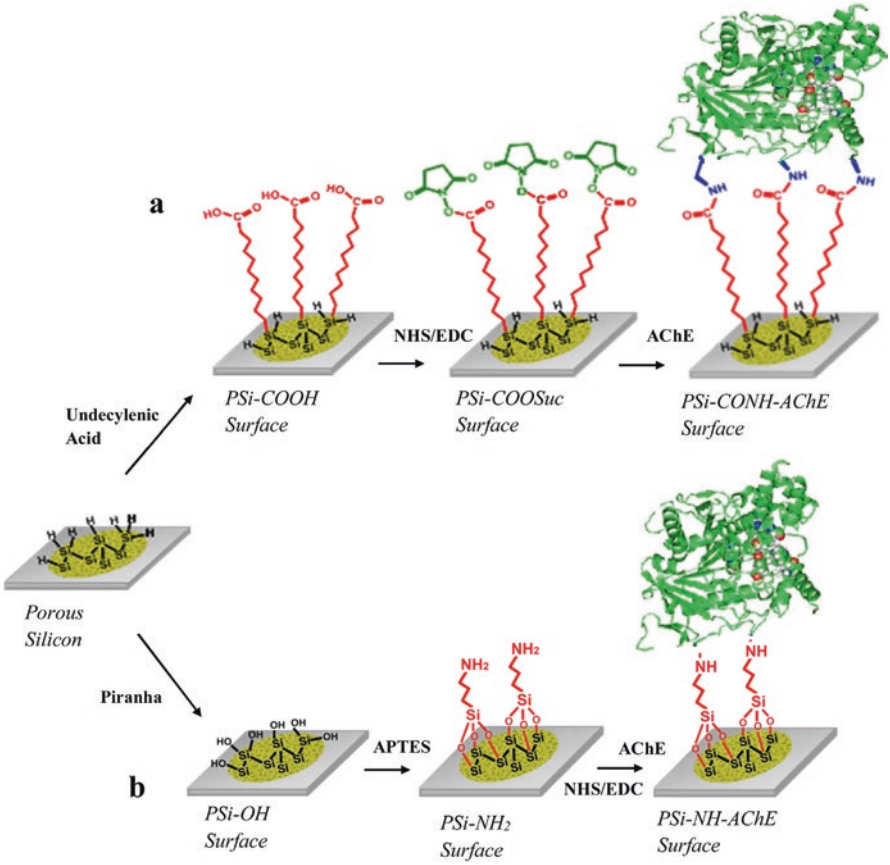

Fig. 8.4 Diagram of the immobilization of AChE on (**a**) PSi-COOH surface and (**b**) PSi-NH_2 surface (Khaldi et al. 2017)

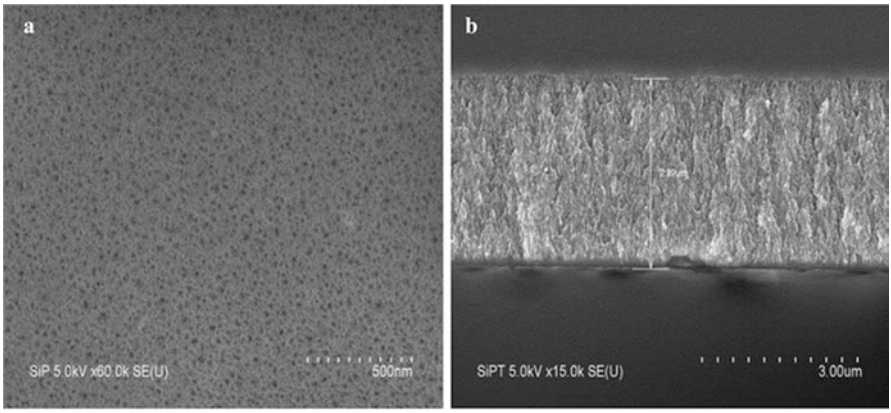

Fig. 8.5 SEM images of mesoporous silicon (**a**) surface view, (**b**) side view (Khaldi et al. 2017)

Figure 8.5 shows the relevant formed mesoporous silicon. It can be seen that the average pore size is less than 15 nm. Meanwhile, the pores are uniformly distributed over the whole surface with the labyrinth shape, and its cross-sectional image is shown in Fig. 8.5b. It illustrates the porous layer with the standard thickness at about 3 μm.

Results reveal that the active enzymes attached to the surface of PSi-NH_2 are superior to that of PSi-COOSuc. Because the surface of PSi-COOSuc is hydrophobic, the biomolecules with the hydrophilic property cannot firmly attach to such surface. For the surface of PSi-NH_2 with the hydrophilic property, the efficiency of the enzyme immobilization is definitely higher. Furthermore, the interaction between AChE and the surface of the hydrophobic Si-COOSuc makes the enzymatic activity decreased. Contrarily, the activity of AChE is distinctively effective on the PSi-NH_2 surface due to the more accessible active sites of enzyme.

To date, technologies for enzyme immobilization evolved steadily for many years after its existence (i) recently due to the research focus on biotechnology expansion, and the investigation on enzyme immobilization keeps accruing from advances in genetic technology, the revitalized enthusiasm for the enzyme immobilization just like injecting stimulants. (ii) Current research and development work have provided a relatively bewildering array of support materials and methods for immobilization. Much of the expansion may be attributed to developments to provide specific improvements for a given application. (iii) Surprisingly, there have been few effectively detailed and comprehensive comparative studies on immobilization methods and supports. Consequently, up to now no ideal support material or method of immobilization has emerged to provide a standard for each type of immobilization. Selection of support material and method of immobilization is made by weighing the various characteristics and required features of the enzyme application against the properties/limitations/characteristics of the combined immobilization/support. A number of practical aspects should be considered before embarking on experimental work to ensure that the final immobilized enzyme is whether suitable for the planned purpose and application or not and will operate at optimum effectiveness.

8.4 Conclusion

Due to the captivating properties of the immobilized enzymes (such as stability enhancement, reusability improvement, thermal resistances and pH extreme, technical and economic advantages), it attracts a great interest. To date, the physical methods are still mainstream of the enzyme immobilization related to the catalytic activity, though the chemical methods are adopted for the enzyme immobilization stability. The hybrid methods should be developed from the viewpoint of good stability and high catalytic activity to take advantage of the attractive property of both physical and chemical methods.

Up to now, the immobilized enzymes are still not easily available for the practical applications easily because of the relevant difficult storage and high price. Moreover, more challenges exist for producing the immobilized enzymes on the large scale because of the current basically homemade situation, especially with the excellent uniformities and activities.

Therefore, keeping efforts are needed to explore the immobilization methods with the extremely improved efficiency to enhance the stability, reusability, and catalytic activity of enzymes immobilized along with the detailed investigation on the relevant characterization.

References

Alamsyah G, Albels VA, Sahlan M, Hermansyah H (2017) Effect of chitosan's amino group in adsorption-crosslinking immobilization of lipase enzyme on resin to catalyze biodiesel synthesis. Energy Procedia 136:47–52

Barbosa O, Ortiz C, Berenguer-Murcia Á, Torres R, Rodrigues RC, Fernandez-Lafuente R (2015) Strategies for the one-step immobilization–purification of enzymes as industrial biocatalysts. Biotechnol Adv 33(5):435–456

Bernal C, Rodríguez K, Martínez R (2018) Integrating enzyme immobilization and protein engineering: an alternative path for the development of novel and improved industrial biocatalysts. Biotechnol Adv 36(5):1470–1480

Cracknell JA, Vincent KA, Armstrong FA (2008) Enzymes as working or inspirational electrocatalysts for fuel cells and electrolysis. Chem Rev 108:2439–2461

Das R, Talat M, Srivastava ON, Kayastha AM (2018) Covalent immobilization of peanut β-amylase for producing industrial nanobiocatalysts: a comparative study of kinetics, stability and reusability of the immobilized enzyme. Food Chem 245:488–499

Grigoras AG (2017) Catalase immobilization-A review. Biochem Eng J Part B 117:1–20

Karajanagi SS, Vertegel AA, Kane RS, Dordick JS (2004) Structure and function of enzymes adsorbed onto single-walled carbon nanotubes. Langmuir 20:11594–11599

Khaldi K, Sam S, Lounas A, Yaddaden C, Gabouze NE (2017) Comparative investigation of two methods for Acetylcholinesterase enzyme immobilization on modified porous silicon. Appl Surf Sci 421:148–154

Mehta J, Bhardwaj N, Bhardwaj SK, Kim KH, Deep A (2016) Recent advances in enzyme immobilization techniques: metal-organic frameworks as novel substrates. Coord Chem Rev 322:30–40

Müller F, Torger B, Allertz PJ, Jähnichen K, Keßler S, Müller M, Simon F, Salchert K, Mäurer H, Pospiech D (2018) Multifunctional crosslinkable itaconic acid copolymers for enzyme immobilization. Eur Polym J 102:47–55

Nishida VS, de Oliveira RF, Brugnari T, Correa RCG, Peralta RA, Castoldi R, de Souza CGM, Bracht A, Peralta RM (2018) Immobilization of *Aspergillus awamori* β-glucosidase on commercial gelatin: an inexpensive and efficient process. Int J Biol Macromol 111:1206–1213

Sharifi M, Robatjazi SM, Sadri M, Mosaabadi JM (2018) Covalent immobilization of organophosphorus hydrolase enzyme on chemically modified cellulose microfibers: statistical optimization and characterization. React Funct Polym 124:162–170

Shen L, Cheng KCK, Schroeder M, Yang P, Marsh ENG, Lahann J, Chen Z (2016) Immobilization of enzyme on a polymer surface. Surf Sci 648:53–59

Shinde P, Musameh M, Gao Y, Robinson AJ, Kyratzis I (2018) Immobilization and stabilization of alcohol dehydrogenase on polyvinyl alcohol fibre. Biotechnol Rep 19:e00260

Souza CJF, Garcia-Rojas EE, Favaro-Trindade CS (2018) Lactase (β-galactosidase) immobilization by complex formation: impact of biopolymers on enzyme activity. Food Hydrocoll 83:88–96

Tang C, Saquing CD, Sarin PK, Kelly RM, Khan SA (2014) Nanofibrous membranes for single-step immobilization of hyperthermophilic enzymes. J Membr Sci 472:251–260

Torres JA, Silva MC, Lopes JH, Nogueira AE, Nogueira FGE, Corrêa AD (2018) Development of a reusable and sustainable biocatalyst by immobilization of soybean peroxidase onto magnetic adsorbent. Int J Biol Macromol 114:1279–1287

Ulu A, Ozcan I, Koytepe S, Ates B (2018) Design of epoxy-functionalized Fe_3O_4@MCM-41 core–shell nanoparticles for enzyme immobilization. Int J Biol Macromol 115:1122–1130

Vaz RP, de Souza Moreira LR, Filho EXF (2016) An overview of holocellulose-degrading enzyme immobilization for use in bioethanol production. J Mol Catal B Enzym 133:127–135

Voběrková S, Solčány V, Vršanská M, Adam V (2018) Immobilization of ligninolytic enzymes from white-rot fungi in crosslinked aggregates. Chemosphere 202:694–707

Wong DE, Senecal KJ, Goddard JM (2017) Immobilization of chymotrypsin on hierarchical nylon 6,6 nanofiber improves enzyme performance. Colloids Surf B Biointerfaces 154:270–278

Wu JCY, Hutchings CH, Lindsay MJ, Werner CJ, Bundy BC (2015) Enhanced enzyme stability through site-directed covalent immobilization. J Biotechnol 193:83–90

Chapter 9
Strategies to Reuse Cellulase: Immobilization of Enzymes (Part II)

Muhammad Irfan, Misbah Ghazanfar, Amad Ur Rehman, and Asma Siddique

9.1 Introduction

The association of humans with enzymes has developed over time. Even the ancient Egypt people used enzymatic fermentation for production of beer and wine long ago, when there was no idea of enzymes. After thousands of years, progress was made in enzymatic studies. Enzymes are proteins in nature, speed up many biochemical reactions, and are natural catalysts widely found in living beings. Improvement in the techniques of extraction and purification of protein make it possible to produce analytical grade pure enzymes for biotechnological and research applications. These enzymes are greatly used in several conventional food procedures, for making of cheese, beer, wine, etc. Advancement in biotechnology certainly in protein engineering builds a way to produce enzyme with better properties leading to the development of new, modified enzymes employed in totally different applications not employed previously. Demand of these enzymes in several industries is growing day by day, particularly during the last two decades. As depicted in Fig. 9.1, the enzymes had wide implementation in different food industries such as baking (Gomes-Ruffi et al. 2012), dairy products (Jaros and Rohm 2015) conversion of starch (Bai et al. 2012), and processing of beverage including fruit and vegetable juice, wine, and beer. Because of their effect on the end products, enzymes have found a special space in textile industries (Schückel et al. 2011). Applications

M. Irfan (✉) · M. Ghazanfar
Department of Biotechnology, University of Sargodha, Sargodha, Pakistan

A. Ur Rehman
Institute of Bioprocess Engineering and Pharmaceutical Technology, Mittelhessen University of Applied Sciences, Giessen, Germany

A. Siddique
Physics of Surfaces, Center of Smart Interfaces, Technical University Darmstadt, Alarich-Weiss-Str., 1664287 Darmstadt, Germany

M. Srivastava et al. (eds.), *Approaches to Enhance Industrial Production of Fungal Cellulases*, Fungal Biology,
https://doi.org/10.1007/978-3-030-14726-6_9

Fig. 9.1 Applications of enzymes in different industries

of enzymes have become certain processing strategy in pulp and paper making industries (Hakala et al. 2013) and detergents (Rao et al. 2009), when ideal end product is required.

Because of specify of enzymes which is an important factor for biosensors, their application in advance industries comprising biosensors is getting better swiftly (Soldatkin et al. 2012). Several other different industries like pharmaceutical and health care (Apetrei et al. 2013) and chemical (Das et al. 2012) manufacturers are getting benefits of these natural catalysts. Since previous several years, researchers are focusing on use of enzymes in biofuels, such as bioethanol and biodiesel and ethanol (Atadashi et al. 2010). In modern life, enzymes are being used for treatment of wastes (Luo et al. 2011) especially for treatment of solid wastes (Tonini and Astrup 2012) and purification of wastewater (Tong et al. 1997). Industrial practices of enzymes in organic solvents were also established in some cases (Gupta and Khare 2009). Furthermore, the enzymes formed from renewable crude materials are easily biodegradable, operated in comparatively simple way by completely controlled equipment. Briefly, we can say that they lessen the utilization of chemicals and energy and formation of associated wastes. But there are also some demerits like lack of shelf life storage, enduring operative strength and difficulty in restoring and use of enzyme again along with all the advantageous characteristics and their wide applications in industries.

Biofuels such as bioethanol, biomethanol, and biodiesel are alternate sources of energy that will probably become more desirable in the future (Nigam and Singh 2011). Previously, plants with high amounts of natural sugars such as sugarcane or corn were being used to produce bioethanol. But due to the growing requirement of these biofuels, competition and hence the costs of food products obtained from these sources also rose. Cellulose is the polysaccharide that is abundantly occurred in the nature. It can be obtained from residues of forest and agriculture, solid waste, and woody and herbaceous crops (Lynd et al. 1991). So the bioethanol produced from cellulose is a very encouraging approach of biofuel because it has no approach or match with food products (Sticklen 2008). Production of bioethanol generally comprises two main parts: first is conversion of cellulose in glucose and the other is fermentation of glucose in ethanol. First part is executed by the enzymatic or chemical process, commonly the fermentation has edge because its process parameters (such as pH, temperature, etc.) can be maintained by adjusting them, and it is also a clean process (Mabee and Saddler 2010).

Cellulase enzymes are found in insects, plants, fungi, and bacteria. Cellulytic enzymes can be produced by both aerobic and anaerobic bacteria as cellulosomes, which are complexes of various enzymes containing several cellulase enzymes, or as single enzyme (Dowe 2009). Cellulase is a group of enzymes that can convert the cellulose into glucose. Usually, it comprises three types including endoglucanase, cellobiohydrolase, and cellobiase (Percival Zhang et al. 2006). Cellobiohydrolase and endoglucanase hydrolyse cellulose into cellobiose which is then broken into glucose by cellobiase (Ortega et al. 2001). Usually, cellulases are employed in several industries like food, brewery, wine, agriculture, textile, detergent, animal feed, pulp and paper industries, etc., as well as in research development (Andriani et al. 2012). These enzymes have applications in various industries like paper and cotton industry, fuel and food industry, extraction and purification of fruit juices, wine and beer industry, detergents, agriculture, research, and animal feed additives (Sajith et al. 2016). Moreover, the demand of cellulolytic enzymes is increasing day by day for bioenergy, agriculture, and biotechnology uses, particularly in the employment of lignocellulosic waste to produce biofuels and other fermentative products of sugar (Verma et al. 2016). Cerveró et al. (2010) described that after hydrolysis, process hinders in isolation, and restoring of free cellulase enzyme from the solution limits the reusability of the cellulase which highly obstructs the levels of this utilization because of the outrageous cost of the enzyme. Immobilization of cellulase is one efficient method which makes it easy to restore and reuse enzyme consequently reducing the cost of bioethanol production (Sheldon 2007).

The production of different compounds from raw materials requires proper choice of process and process parameters. In many cases, the chemical reaction requires high temperature and pressure. Also lower selectivity and yield are cause of concern in some cases. By-products are also problem to tackle in some cases. It is craved to conduct a reaction at medium temperature, low pressure, high yield and selectivity, and high conversion. By using catalysis, the activation energy required for a reaction can be lessen, and the reaction can be made to happen at lower temperature. Enzymes are biocatalysts. The enzymes are specific to substrate and yield highly specific reactions. Various chemicals and compounds produced by enzyme-catalyzed reaction give alternative route to some reactions (Kulkarni 2014). In many

compounds such as starch, ethanol, amino acids, citric acid, and many other pharmaceutical and fine chemicals, intermediates are synthesized in reactions catalyzed by enzymes (Kulkarni et al. 2015). The stability of these enzymes at various pH, temperatures, and thermal conditions is the cause of concern. This problem is almost solved by the immobilization of enzymes. There are different physical and chemical methods used for immobilization.

However, for economic point of view, enzymes' reusability factor becomes compulsory. It is highly demanding to maintain the structural stability of an enzyme during a biochemical reaction. Therefore, in spite of high cost, immobilized enzymes are used as alternatives with practical productivity and better propagation. An enzymes or a whole cell could be immobilized biocatalyst (Kawaguti et al. 2006). Imprisonment of enzyme to a particular phase (support/ matrix) dissimilar from those for substrates and products is actually enzyme immobilization. Inorganic materials and inert polymers are generally employed as carrier matrices. In addition to be economical, a perfect matrix must embrace attributes like immobility, physical power, regenerability, stability, capability of increasing enzyme activity/specificity and limit inhibition of product, contamination by microbes, and nonspecific adsorption (Singh 2009). Continuous affordable operations, high capacity/investment ratio, automation, and restoring of end product with high purity can be achieved by enzyme immobilization (D'Souza 1998).

By immobilization of enzymes, these demerits can be vanquished. In fact, the development of strong, stable, and ideally insoluble biocatalysts is a major demand in industrial biocatalysis.

9.2 Historical Background

Immobilized enzymes were discovered in 1916 (Gomes-Ruffi et al. 2012). It was observed that invertase when absorbed on a support, like aluminum hydroxide or charcoal, showed the same activity, over the base of the reaction container when homogenously dispersed in the solution. Later, this exploration leads to the establishment of present techniques of immobilization of enzyme. Initial approaches of immobilization provided extremely small ladings of enzyme, as compared to accessible surface areas. Various covalent procedures of immobilization of enzyme were developed. Above five thousand publications are published on the techniques of immobilization of enzyme since 1960s. Thousands of enzymes are immobilized in various configurations, and more than 12 of immobilized enzymes such as penicillin G acylase (PGA), lipase, invertase, protease, etc. are being employed as biocatalysts in different industrial approaches. Recent publications denote a continued interest in this field (Hartmann 2005). Hundreds of papers are published on immobilization of enzymes only in the first 6 months of 2010 according to PubMed database. Recently immobilized enzymes have exhibited high efficiency for commercial implementations in different processes. These enzymes are preferred over enzymes in solution because they have characteristics like economically affordable, higher stability, and the easy recovery from the reaction blend which leads to isolation of intact product.

Therefore, an immobilized enzyme is bonded to an inorganic or organic or insoluble (e.g., silica or calcium alginate) or inert material. Moreover, an enzyme attached to a hard support has increased resistance to different environmental fluctuations such as temperature or pH (Cherry and Fidantsef 2003).

9.3 Modes of Immobilization

Conventionally, there are four different modes of immobilization of enzyme known as noncovalent deposition and adsorption, physical entrapment, covalent attachment, and bioconjugation (Fig. 9.2). Attachment of enzyme to support can be chemical or physical and weakly or covalently. Commonly, physical attachment is relatively weak, and in large-scale conditions, it is not too much capable of keeping the enzyme fixed to the support. The carrier or support is generally a synthetic resin, biopolymer, or an inorganic polymer (e.g., silica or zeolite).

In entrapment, an enzyme is incorporated on a membrane apparatus, e.g., a microcapsule or a desolate fiber or in a gel lattice (polymer network) such as a silica sol-gel or an organic polymer. Synthesis of the polymeric network in the existence of enzyme is required for entrapment. The forth mode of immobilization covers the cross-linking of enzyme crystals or aggregates, by utilization of a bifunctional reagent, to construct support-free macroparticles (Cao et al. 2003). Adsorption/carrier-binding method uses water-insoluble carriers such as glass, polysaccharide derivatives, and synthetic polymers (Wu and Lia 2008; Cordeiro et al. 2011). In cross-linking/covalent method, bi-/multifunctional reagents such as glutaraldehyde, bisdiazobenzidine, and hexamethylene diisocyanate are used (Singh 2009). Polymers like cellulose, collagen, and j-carrageenan are employed by entrapment method, while the membrane confinement method includes formulation of liposomes and microcapsules (Jegannathan et al. 2010; Klein et al. 2011). General characterization of immobilization methods are given in Table 9.1.

The immobilization of the enzyme on the interior previous base of a membrane of ceramics with reduced molecular mass that inhibits the enzyme from passing through is a new approach of entrapment. Enzyme immobilization by membrane entrapment method is found to be an efficient bioengineering tool because it retains enzymes in reactors, enhances stability of enzyme, and enables continuous operation. Within a membrane reactor, physical immobilization on a matrix prevents the loss of activity of enzyme and facilitates reuse, hence decreasing the costs. Immobilized enzyme is preferred over its respective free form because of its lengthened availability that reduces not needed subsequential and other purification processes. Another enzyme recovering approach is ultrafiltration (UF) which involves the use of selectively permeable membrane which allows smaller molecules to pass through while keeping the enzyme in the bioreactor. Membrane fouling is an important consideration in the design and operation of membrane systems because it influences pretreatment needs, cleaning requirements, operating cost, conditions, and performance (Ur Rehman et al. 2016).

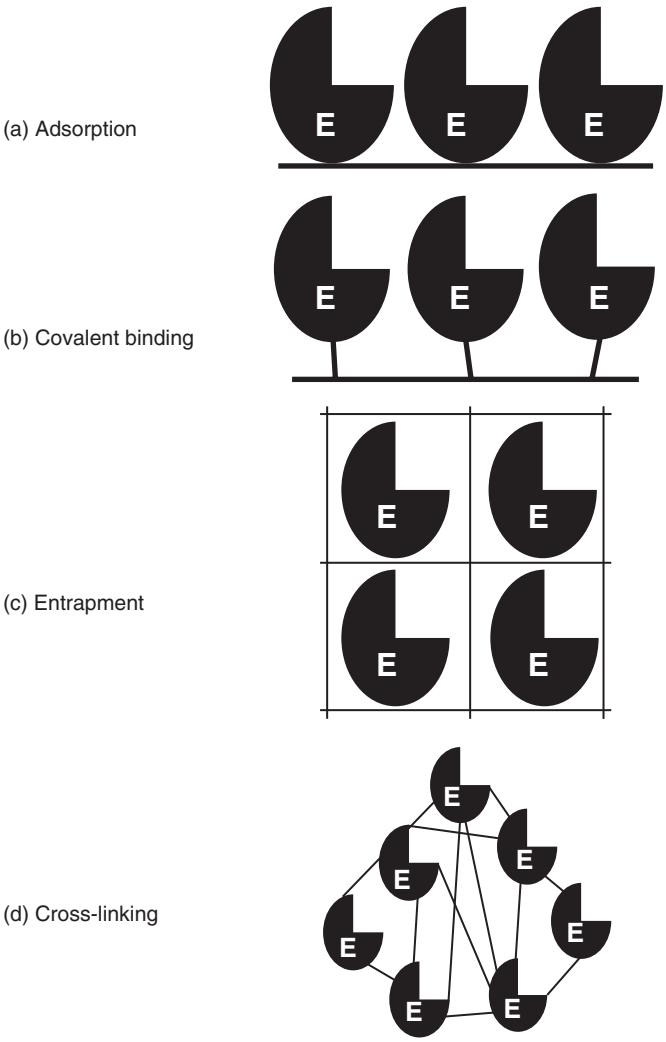

Fig. 9.2 Diagrammatic representation of the various methods of enzyme immobilization (Ahmad and Sardar 2015)

There are also some other methods of immobilization which might be combinations of the ones mentioned earlier, which are specific sometime for a stated matrix or enzyme are being developed. Nonetheless, not a single approach or matrix/support is perfect for all types of enzymes and their utilizations due to the wide range of chemical composition and characteristics of enzymes, difference in the properties of products and substrates, and difference in the uses of the product. However, there may be pros and cons found in all of the methods. Cross-linking and covalent attachments are long-lasting and successful but costly and effortlessly aggravating the

Table 9.1 A generalized characterization of immobilization methods

Parameter	Immobilization	Method	Carrier	Binding	
	Covalent	Ionic	Adsorption	CLEAs, CLECs	Entrapment
Activity	High	High	Low	Intermediate/ high	High
Range of application	Low	Intermediate	Intermediate	Low	Intermediate/ high
Immobilization efficiency	Low	Intermediate	High	Intermediate	Intermediate
Cost	Low	Low	High	Intermediate	Low
Preparation	Easy	Easy	Difficult	Intermediate	Intermediate/ difficult
Substrate specificity	Cannot be changed	Cannot be changed	Can be changed	Cannot be changed	Can be changed
Regeneration	Possible	Possible	Impossible	Impossible	Impossible

implementations of enzyme; adsorption is inexpensive, easy, and productive but commonly reversible; diffusional problems are innate in entrapment, microencapsulation, and membrane reactor-confinement.

9.4 Polymers as Supports

9.4.1 Alginate

Alginate is extensively employed for immobilization as calcium alginate beads, alginate-polyacrylamide gels, and xanthan-alginate beads having better activity of enzyme and reusability. The enzyme stability is enhanced by cross-linking of alginate with glutaraldehyde and divalent ions (Flores-Maltos et al. 2011).

9.4.2 Chitin and Chitosan

Chitin and chitosan are the naturally found polymers that are used as supports for immobilization (Kapoor and Kuhad 2007). The carbohydrate and protein part of enzymes attach to chitosan (Hsieh et al. 2000). Chitosan combined with alginate is used where chitosan-covered enzymes had reduced leaching effect relative to alginate owing to the ionic and physical interactions between the support and enzyme (Betigeri and Neau 2002). Because of hydroxyl and amino groups, a moist complex of clay and chitosan demonstrated to be more effective for enzyme trapping, which attach to enzymes easily, together with high porosity and better hydrophilicity. In the appearance of beads, chitosan can capture double of the enzymes (Chang and Juang 2007).

9.4.3 Carrageenan

For improving stability, carrageenan is being used for immobilization of different enzymes (Tümtürk et al. 2007). It becomes thin during stress conditions and then restores its thickness when the stress or pressure is released, due to its pseudoplastic nature. Jegannathan et al. (2010) achieved an efficiency encapsulation of 42.6% for biodiesel production by the co-extrusion approach employing such support. Carrageenan with better entrapment is inexpensive and reliable support for lactic acid enzyme and galactosidase (Rao et al. 2008).

9.4.4 Starch

Starch is also employed as immobilizer support. For entrapment and surface immobilization of bitter gourd, peroxidase calcium alginate-starch hybrid supports were used. Due to internal carbohydrate moieties, in the presence of denaturants, entrapped enzyme was more stable, while the enzyme immobilized to surface had more activity (Matto and Husain 2009). Grafting of radiations of materials such as dimethylaminoethyl methacrylate and acrylamide on the starch is extensively applied industrial approaches for a high yield of products (Raafat et al. 2012).

9.4.5 Pectin

Pectin is heteropolysaccharide in nature and is utilized as plasticizer to decrease the stiffness of matrix and is applied for immobilization of papain and for the establishment of novel substances for treatment of injury of skin (Ceniceros et al. 2003). According to Satar et al. (2008), calcium pectin alginate and chitin pectin support have improved denaturant and thermal confrontation and catalytic characteristics of entrapped enzymes due to the development of more stable polyelectrolyte complexes between the pectin-coated support and the enzyme.

9.4.6 Activated Carbon

For enzyme adsorption, both hydrochloric acid-modified and natural-activated carbon provide significant support (Alkan et al. 2009). Activated carbon with a significant fraction of its pore volume and a high surface area is suitable immobilization of enzyme (Daoud et al. 2010).

9.5 Immobilization Strategies

As compared to conventional catalysts, enzymes have many advantages, but there are few empirical jeopardies affiliated with their applications in industries. Usually, enzymes are costly than ordinary catalysts because of the immense expenses of their extraction and purification as well. When extracted from their innate environments, enzymes due to protein nature are very sensitive to several denaturing conditions. Thus, their reactivity to process parameters (e.g., pH, substances at microlevels, and temperature) can behave as inhibitors that also increase their prices. Unlike traditional heterogeneous chemical catalysts which lead to the impurity of product leading to the termination of enzyme recovery for reutilization in the functional form from many of the reaction blends, most of the enzymes conduct soluble in water in homogeneous catalysis systems. The most effective method offered to limit these jeopardizes is the implementation of immobilization approach (van de Velde 2002). A technical approach which involves the affixation of enzymes within or to the solid supports that establish a heterogeneous immobilized enzyme system is known as immobilization. Immobilized enzymes are linked to cytoskeleton, cellular membranes, and organelles in living cells where they imitate their natural mode. Generally, the solid support system maintains the activities of enzymes by maintaining their structures. Therefore, immobilized enzymes are more powerful and rebellious to surrounding fluctuations relative to free enzymes in the solution. Additionally, heterogeneous immobilized enzyme systems permit the recovery of product and enzyme easy, reuses of enzymes for multiple time, quick ending of reactions, uninterrupted operation of enzymatic processes, and extensive types of bioreactor designs (Kress et al. 2002). Nowadays, immobilized enzymes are being studied to explore their potential (Bommarius and Riebel 2004). Immobilized enzymes are usually easier to manage and more stable as compared to their free forms, and the product of reaction is not enzyme contaminated thus particularly beneficial in the pharmaceutical and food industries (Massolini and Calleri 2005). These fluctuations are caused by changes in the structure induced in the enzyme molecule through the application of immobilization method and by the development of a microenvironment where the enzyme is working, dissimilar from the major solution. This results in a pure product which is feasible to extract from the solution. Now the linked enzyme is prepared for the following reactions lacking the requirement of rerunning, time gobbling, and expensive isolation and purification processes. Immobilization of enzymes is done by various methods, which can be categorized as chemical, where enzyme and matrix are covalently bonded and physical, where enzyme and support are linked by weak interactions. In recent years, particularly the establishment and employment of site exacting immobilization of protein have experienced effective advancements in current era. Establishment of some highly efficient, site-specific, powerful, and significant implementations of harboring proteins onto the matrix is the result of developments in organic chemistry and molecular biology. The preparation and exploitation of protein microarrays are made possible by the enhanced procedures for high output relative to input

development and purification of huge numbers of proteins. However, many studies have reported that protein attachment procedures which cause non-specific immobilization may be covalently bonded by epoxy-derivative surfaces, aldehyde, and amine or by adsorption on hydrogel-, polylysine-coated or nitrocellulose slides. Various other approaches have exhibited applying corresponding reagents that link specific tags or epitopes on enzymes and reveal them in an accurately aligned pattern, like nickel nitrilotriacetic acid (Ni-NTA)-coated slides, which are utilized to link streptavidin (or avidin) and histidine-tagged proteins (Ismail and Nielsen 2010). Bioconjugation is a significant feature of the life sciences and fundamental to the implementations discussed above; as a result, many procedures have been reported through recent years. Out of these, a few numbers have suited for vast applications because of their pliability, relieve in use, and commercial availability of reagent. Generally, these procedures depend on physical and chemical adsorption occurrence or on functional groups which are found in proteins naturally. Therefore, these are very simple to use and can be applied to almost all proteins, both modified and native. Table 9.2 illustrates cellulases obtained from different sources immobilized on various nanosupports and their enhanced applications/properties.

Phadtare et al. (2004) conducted research on formation of polyurethane microsphere-AuNP "core-shell" structures, and this nanocomposite (NC) was utilized for the endoglucanase immobilization. Interaction of NPs and nitrogen in the polymer causes the binding of AuNPs on the polymer microsphere surface. Hence, endoglucanase attaches to the AuNPs fixing up polyurethane microspheres pursued by a very stable enzyme with extensive reusability. Immobilized cellulase keeps its activity and illustrated enhanced thermal stability as compared to free enzyme. Immobilized enzyme is made up of "quasi-free" by the high surface area of the host AuNPs and simultaneously keeping advantages of immobilization like relive in reuse and improved thermal stability, etc. In some investigations carbodiimide (CDI)-activated Fe_3O_4 MNPs were used for covalent immobilization of cellulase. It was observed that at loadings of 1–2 mg enzyme, highest binding was 90%, and enzyme-to-matrix saturation point supervenes at a weight ratio of 0.02. Immobilized enzyme exhibited optimal temperature of 50 °C and showed a great storage and thermal stability. The shifting of optimum pH from 4.0 to 5.0 is the result of the ionic forces between support and enzyme (Jordan et al. 2011). In 2014, Jordan and Theegala also conducted research to investigate the cellulase immobilization on CDI-activated MNPs of 13 nm in diameter). The immobilized enzyme was reused six times successfully by losing some of its activity after each reuse while keeping 30.2% of the initial activity. After initial hydrolysis reaction, there was a reduction of 47.5% activity. After each repeated use, a protein assay revealed fluctuating degrees of enzyme disengagement. Immobilized enzymes show extensive stability as compared to the free enzyme, and attached enzyme maintained 57.9% of its activity, which was little better than the 51.2% kept by free enzyme. After 96 h, enzyme attached to NPs developed 76.8% of total reducing sugars that is formed by free enzyme in a single reaction. Cellulase is also immobilized by polyvinyl alcohol (PVA)/Fe_2O_3 NPs, a novel soluble and easily operable nanocarrier. In a buffer of pH 6.0, the activity retention of immobilized enzyme was 42%. After five repeated uses, the immobilized cellulase retained 50% activity (Liao et al. 2008).

Table 9.2 MNP immobilized cellulases, their modes of immobilization, and enhanced properties (Husain 2017)

Cellulase	CDI-Fe3O4 MNPs	Covalent binding	Better catalytic efficiency, stability, and reusability
Cellulase	PVA)/Fe2O3	Adsorption	Good reusability
Cellulose	Functionalized MWCNTs	Adsorption	High-binding efficiency and loading, reusability
Cellulase	Supermagnetic MNPs and GA	Covalent binding	High affinity and activity in a broad range of pH and temperature; effectively hydrolyzed steam-exploded corn stalks
Cellulase	Molecular imprinted supermagnetic Fe3O4@ SiO2 NPs	Adsorption	Higher catalytic efficiency and temperature optima, better thermal stability
Cellulase	CS-MNPs	Covalent binding	High heat and storage stability
Cellulase	CS-Fe3O4 and GA	Covalent binding	Very high loading, stability over a broad range of pH and temperature, and reusability
Cellulase from *T. reesei*	Activated magnetic support	Covalent binding	Km decreased, high temperature-optima, good reusability
Cellulase	Functionalized magnetic nanosphere, APTES and GA	Entrapment	Very high loading, stability and reusability, effective use in biofuel production
Cellulase from *A. niger*	β-Cyclodextrin-MNPs via silanization and reductive amidation	Covalent binding	Increased continuous hydrolysis of raw straw, high binding efficiency, stability, and reusability
Cellulase *A. fumigates*	MnO2 NPs	Adsorption	High stability in a broad range of pH and temperatures, high heat stability, reusability, and cellulose hydrolysis
Cellulase	AgNPs and AuNPs	Adsorption	High heat stability and reusability
Cellulase *A. niger*	TiO2 NPs and 3-APTES	Adsorption and covalent binding	Covalently bound enzyme found superior in stability and reusability than adsorbed enzyme
Cellulase	Fe3O4@SiO2 NPs	Adsorption	High immobilization yield, half-life, and reusability
Cellulase	CLEA-amine functionalized Fe_3O_4-@ silica coreshell MNPs	Covalent binding	Improved heat and operational stability and reusability
Cellulase	Vinyl functionalized cubic MS	Adsorption	NPs support far superior in activity, stability, and reusability
T. reesei cellulase	CS-MNPs and GA	Covalent binding	Better thermal and storage stability and very high CMC hydrolyzing reusability
Cellulase	MNPs	Adsorption	Effective bioethanol production from *Sesbania aculeata* biomass

(continued)

Table 9.2 (continued)

Cellulase	CDI-Fe3O4 MNPs	Covalent binding	Better catalytic efficiency, stability, and reusability
Cellulase	Nano-PEGylated GO	Covalent binding	Efficiently hydrolyzed raw straw slurry
Cellulase	AF-$CoFe_2O_4$-MNPs, EDS, and NHS	Covalent binding	Superior thermal stability and good reusability
Cellulase	Silica-coated MNPs	Adsorption	High colloidal stability, loading, and reusability
Cellulase	CS-Fe3O4	Covalent binding	Improved stability and reusability
Cellulase	Attapulgite@CS (ATP@CS) NC and GA	Covalent binding	High pH and heat stability and reusability, effective hydrolysis of wheat straw
Cellulase	AgNPs	Adsorption	Quite efficient in cellulose hydrolysis

9.6 Conclusion

Today, immobilized enzymes of various types are used in various tasks such as manufacturing food, clothes, fuel, and other products of daily use. Still, many drawbacks such as low reaction yields, enzyme cost, and low biodiversity need to be worked upon to tackle these drawbacks. In the future, there will be world of immobilized enzymes. Immobilized enzymes are going to play an extraordinary role in almost all the industries including food, fuel, and chemical as well as pharmaceuticals. While biochemical analysis, immobilized cellulases could be successfully reused by using glutaraldehyde as cross-linking agent. It confirms the striking advantages of enzyme immobilization for a wide range of industrial scale. According to market analysis, the global enzyme market is expected to reach US$ 4.3 billion by 2015 and will definitely cross this number in the coming years.

References

Ahmad R, Sardar M (2015) Enzyme immobilization: an overview on nanoparticles as immobilization matrix. Biochem Anal Biochem 4(2):1

Alkan S, Gür A, Ertan M, Savran A, Gür T, Genel Y (2009) Immobilization of catalase via adsorption into natural and modified active carbon obtained from walnut in various methods. Afr J Biotechnol 8(11):2631–2635

Andriani D, Sunwoo C, Ryu HW, Prasetya B, Park DH (2012) Immobilization of cellulase from newly isolated strain *Bacillus subtilis* TD6 using calcium alginate as a support material. Bioprocess Biosyst Eng 35(1–2):29–33

Apetrei IM, Rodriguez-Mendez ML, Apetrei C, De Saja JA (2013) Enzyme sensor based on carbon nanotubes/cobalt (II) phthalocyanine and tyrosinase used in pharmaceutical analysis. Sensors Actuators B Chem 177:138–144

Atadashi IM, Aroua MK, Aziz AA (2010) High quality biodiesel and its diesel engine application: a review. Renew Sust Energ Rev 14(7):1999–2008

Bai Y, Huang H, Meng K, Shi P, Yang P, Luo H et al (2012) Identification of an acidic α-amylase from Alicyclobacillus sp. A4 and assessment of its application in the starch industry. Food Chem 131(4):1473–1478

Betigeri SS, Neau SH (2002) Immobilization of lipase using hydrophilic polymers in the form of hydrogel beads. Biomaterials 23(17):3627–3636

Bommarius AS, Riebel BR (2004) Biocatalysis: fundamentals and applications. Wiley. Appl Organometalic Chem 18: 373

Cao L, van Langen L, Sheldon RA (2003) Immobilised enzymes: carrier-bound or carrier-free? Curr Opin Biotechnol 14(4):387–394

Ceniceros ES, Ilyina A, Esquivel JC, Menchaca DR, Espinoza JF, Rodriguez OM (2003) Entrapment of enzymes in natural polymer extracted from residue of food industry: preparation methods, partial characterisation and possible application. Becth Mock 44:84–87

Cerveró JM, Skovgaard PA, Felby C, Sørensen HR, Jørgensen H (2010) Enzymatic hydrolysis and fermentation of palm kernel press cake for production of bioethanol. Enzym Microb Technol 46(3–4):177–184

Chang MY, Juang RS (2007) Use of chitosan–clay composite as immobilization support for improved activity and stability of β-glucosidase. Biochem Eng J 35(1):93–98

Cherry JR, Fidantsef AL (2003) Directed evolution of industrial enzymes: an update. Curr Opin Biotechnol 14(4):438–443

Cordeiro AL, Lenk T, Werner C (2011) Immobilization of Bacillus licheniformis α-amylase onto reactive polymer films. J Biotechnol 154(4):216–221

D'Souza SF (1998) Immobilized enzymes in bioprocess. Curr Sci 77:69–79

Daoud FBO, Kaddour S, Sadoun T (2010) Adsorption of cellulase Aspergillus niger on a commercial activated carbon: kinetics and equilibrium studies. Colloids Surf B 75(1):93–99

Das R, Ghosh S, Bhattacharjee C (2012) Enzyme membrane reactor in isolation of antioxidative peptides from oil industry waste: a comparison with non-peptidic antioxidants. LWT-Food Sci Technol 47(2):238–245

Dowe N (2009) Assessing cellulase performance on pretreated lignocellulosic biomass using saccharification and fermentation-based protocols. In: Biofuels. Humana Press, Totowa, pp 233–245

Flores-Maltos A, Rodríguez-Durán LV, Renovato J, Contreras JC, Rodríguez R, Aguilar CN (2011) Catalytical properties of free and immobilized *Aspergillus niger* tannase. Enzym Res 2011:768183.

Gomes-Ruffi CR, da Cunha RH, Almeida EL, Chang YK, Steel CJ (2012) Effect of the emulsifier sodium stearoyl lactylate and of the enzyme maltogenic amylase on the quality of pan bread during storage. LWT-Food Sci Technol 49(1):96–101

Gupta A, Khare SK (2009) Enzymes from solvent-tolerant microbes: useful biocatalysts for non-aqueous enzymology. Crit Rev Biotechnol 29(1):44–54

Hakala TK, Liitiä T, Suurnäkki A (2013) Enzyme-aided alkaline extraction of oligosaccharides and polymeric xylan from hardwood kraft pulp. Carbohydr Polym 93(1):102–108

Hartmann M (2005) Ordered mesoporous materials for bioadsorption and biocatalysis. Chem Mater 17(18):4577–4593

Hsieh HJ, Liu PC, Liao WJ (2000) Immobilization of invertase via carbohydrate moiety on chitosan to enhance its thermal stability. Biotechnol Lett 22(18):1459–1464

Husain Q (2017) Nanomaterials immobilized cellulolytic enzymes and their industrial applications: a literature review. Biol 4(3):1029

Ismail B, Nielsen SS (2010) Invited review: plasmin protease in milk: current knowledge and relevance to dairy industry. J Dairy Sci 93(11):4999–5009

Jaros D, Rohm H (2015) Enzymes exogenous to Milk in dairy technology: transglutaminase. Reference Module in Food Science. 10.1016/B978-0-08-100596-5.21158-X.

Jegannathan KR, Jun-Yee L, Chan ES, Ravindra P (2010) Production of biodiesel from palm oil using liquid core lipase encapsulated in κ-carrageenan. Fuel 89(9):2272–2277
Jordan J, Theegala C (2014) Probing the limitations for recycling cellulase enzymes immobilized on iron oxide (Fe 3 O 4) nanoparticles. Biomass Conv Biorefinery 4(1):25–33
Jordan J, Kumar CS, Theegala C (2011) Preparation and characterization of cellulase-bound magnetite nanoparticles. J Mol Catal B Enzym 68(2):139–146
Kapoor M, Kuhad RC (2007) Immobilization of xylanase from *Bacillus pumilus* strain MK001 and its application in production of xylo-oligosaccharides. Appl Biochem Biotechnol 142(2):125–138
Kawaguti HY, Manrich E, Sato HH (2006) Production of isomaltulose using Erwinia sp. D12 cells: culture medium optimization and cell immobilization in alginate. Biochem Eng J 29(3):270–277
Klein MP, Scheeren CW, Lorenzoni ASG, Dupont J, Frazzon J, Hertz PF (2011) Ionic liquid-cellulose film for enzyme immobilization. Process Biochem 46(6):1375–1379
Kress J, Zanaletti R, Amour A, Ladlow M, Frey JG, Bradley M (2002) Enzyme accessibility and solid supports: which molecular weight enzymes can be used on solid supports? An investigation using confocal Raman microscopy. Chem Eur J 8(16):3769–3772
Kulkarni SJ (2014) Use of biotechnology for synthesis of various products from different feedstocks-a review. Int J Adv Res Biotechnol 2(2):1–3
Kulkarni SJ, Shinde NL, Goswami AK (2015) A review on ethanol production from agricultural waste raw material. Int J Sci Res Sci Eng Technol 1:231–233
Liao HD, Yuan L, Tong CY, Zhu YH, Li D, Liu XM (2008) Immobilization of cellulase based on polyvinyl alcohol/Fe2O3 nanoparticles. Chem J Chin Univ 29:1564–1568
Luo K, Yang QI, Yu J, Li XM, Yang GJ, Xie BX, Zeng GM (2011) Combined effect of sodium dodecyl sulfate and enzyme on waste activated sludge hydrolysis and acidification. Bioresour Technol 102(14):7103–7110
Lynd LR, Cushman JH, Nichols RJ, Wyman CE (1991) Fuel ethanol from cellulosic biomass. Science 251(4999):1318–1323
Mabee WE, Saddler JN (2010) Bioethanol from lignocellulosics: status and perspectives in Canada. Bioresour Technol 101(13):4806–4813
Massolini G, Calleri E (2005) Immobilized trypsin systems coupled on-line to separation methods: recent developments and analytical applications. J Sep Sci 28(1):7–21
Matto M, Husain Q (2009) Calcium alginate–starch hybrid support for both surface immobilization and entrapment of bitter gourd (*Momordica charantia*) peroxidase. J Mol Catal B Enzym 57(1–4):164–170
Nigam PS, Singh A (2011) Production of liquid biofuels from renewable resources. Prog Energy Combust Sci 37(1):52–68
Ortega N, Busto MD, Perez-Mateos M (2001) Kinetics of cellulose saccharification by Trichoderma reesei cellulases. Int Biodeter Biodegr 47(1):7–14
Phadtare S, Vyas S, Palaskar DV, Lachke A, Shukla PG, Sivaram S, Sastry M (2004) Enhancing the reusability of endoglucanase-gold nanoparticle bioconjugates by tethering to polyurethane microspheres. Biotechnol Prog 20(6):1840–1846
Raafat AI, Araby E, Lotfy S (2012) Enhancement of fibrinolytic enzyme production from Bacillus subtilis via immobilization process onto radiation synthesized starch/dimethylaminoethyl methacrylate hydrogel. Carbohydr Polym 87(2):1369–1374
Rao CS, Prakasham RS, Rao AB, Yadav JS (2008) Functionalized alginate as immobilization matrix in enantioselective L (+) lactic acid production by Lactobacillus delbrucekii. Appl Biochem Biotechnol 149(3):219–228
Rao CS, Sathish T, Ravichandra P, Prakasham RS (2009) Characterization of thermo-and detergent stable serine protease from isolated Bacillus circulans and evaluation of eco-friendly applications. Process Biochem 44(3):262–268
Sajith S, Priji P, Sreedevi S, Benjamin S (2016) An overview on fungal cellulases with an industrial perspective. J Nutr Food Sci 6(1):461

Satar R, Matto M, Husain Q (2008) Studies on calcium alginate-pectin gel entrapped concanavalin A-bitter gourd (*Momordica charantia*) peroxidase complex. J Sci Ind Res 67:609–615

Schückel J, Matura A, Van Pee KH (2011) One-copper laccase-related enzyme from Marasmius sp.: purification, characterization and bleaching of textile dyes. Enzym Microb Technol 48(3):278–284

Sheldon RA (2007) Enzyme immobilization: the quest for optimum performance. Adv Synth Catal 349(8–9):1289–1307

Singh BD (2009) Biotechnology expanding horizons. Kalyani, Ludhiana

Soldatkin OO, Kucherenko IS, Pyeshkova VM, Kukla AL, Jaffrezic-Renault N, El'Skaya AV et al (2012) Novel conductometric biosensor based on three-enzyme system for selective determination of heavy metal ions. Bioelectrochemistry 83:25–30

Sticklen MB (2008) Plant genetic engineering for biofuel production: towards affordable cellulosic ethanol. Nat Rev Genet 9:433–443

Tong Z, Qingxiang Z, Hui H, Qin L, Yi Z (1997) Removal of toxic phenol and 4-chlorophenol from waste water by horseradish peroxidase. Chemosphere 34(4):893–903

Tonini D, Astrup T (2012) Life-cycle assessment of a waste refinery process for enzymatic treatment of municipal solid waste. Waste Manag 32(1):165–176

Tümtürk H, Karaca N, Demirel G, Şahin F (2007) Preparation and application of poly (N, N-dimethylacrylamide-co-acrylamide) and poly (N-isopropylacrylamide-co-acrylamide)/κ--Carrageenan hydrogels for immobilization of lipase. Int J Biol Macromol 40(3):281–285

Ur Rehman A, Kovacs Z, Quitmann H, Ebrahimi M, Czermak P (2016) Enzymatic production of fructooligosaccharides from inexpensive and abundant substrates using a membrane reactor system. Sep Sci Technol 51(9):1537–1545

van de Velde F, Lourenço ND, Pinheiro HM, Bakker M (2002) Carrageenan: A Food Grade and Biocompatible Support for Immobilisation Techniques. Adv Synth & Catal 344(8):815–835

Verma ML, Puri M, Barrow CJ (2016) Recent trends in nanomaterials immobilised enzymes for biofuel production. Crit Rev Biotechnol 36(1):108–119

Wu SC, Lia YK (2008) Application of bacterial cellulose pellets in enzyme immobilization. J Mol Catal B Enzym 54(3–4):103–108

Zhang YHP, Himmel ME, Mielenz JR (2006) Outlook for cellulase improvement: screening and selection strategies. Biotechnol Adv 24(5):452–481

Chapter 10
Current Advancements in Recombinant Technology for Industrial Cellulases: Part-I

Abhishek Dutt Tripathi, Suresh Kumar Srivastava, Kamlesh Kumar Maurya, Sadhna Mishra, and Diksha Shaw

10.1 Introduction

The increasing world population is suffering vast difficulty in catering its demand of food, feedstock, reagents, energy, and nonrenewable resources while considering the environmental parameters into consideration. The rapid population growth is putting load on the consumption of nonrenewable resources such as petroleum oils and natural gas. However, since the consumption of this nonrenewable source is very high, there is a need to look for some alternate energy means such as agro-industrial wastes which are being dumped in huge quantity worldwide annually. These agro-wastes particularly cereal by-products and fruit and vegetable peels serve as potential substrate being used in bioprocess technology for the production of value-added products with wide application in food, pharma, medical, and agriculture. Although these resources are rich in lignocellulosic materials, deriving energy from such biomass is a tedious job. One of the major obstacles in the conversion of these potential substrates to value-added products is the inefficient hydrolysis of lignocellulosic components to simpler sugars. The lignocelluloses comprise 30–50% cellulose, 20–35% hemicelluloses, and 15–25% lignin. Although polysaccharide composition may vary among plant species and tissues, it has been previously reported that polysaccharide composition may vary in different varieties of corncob which depends on genetic expression and environmental factors (Templeton et al. 2009). In 2006, the worldwide lignocellulosic biomass production by plant source was found to be 100–500 million tons (Sticklen 2006). This renewable

A. D. Tripathi (✉) · K. K. Maurya · S. Mishra · D. Shaw
Centre of Food Science and Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India

S. K. Srivastava
School of Biochemical Engineering, Indian Institute of Technology (BHU), Varanasi, India

M. Srivastava et al. (eds.), *Approaches to Enhance Industrial Production of Fungal Cellulases*, Fungal Biology,
https://doi.org/10.1007/978-3-030-14726-6_10

biomass can be decomposed to simpler pentoses and hexoses which serve as precursor for different industrial products at commercial level. This can be achieved by enzymatic treatments which hydrolyze the plant biomass owing to the production of by-products which are noninhibitory and harmless effluents upon complete hydrolysis. Certain lytic enzymes such as cellulases and xylanases which convert lignocellulosic waste to fermentable sugars by different microbes produce biofuels and other valuable products. However, the comparatively elevated production cost of such enzymes poses a major barrier in their marketable acceptability. Although in the last few years, noteworthy decline has been made in the production expenditure of these enzymes. This can be minimized by selecting the potent strains capable of producing the cellulase enzyme, by selecting the inexpensive and cheaper substrates, and by adopting efficient recovery strategies. In addition to this, an industrial process can become reasonably feasible which causes enzymatic breakdown of lignocellulose to fermentable sugars that must occur rapidly with maximum conversion leading to enhanced production. This chapter describes the application of recombinant DNA technology for the development of potent strains with maximum cellulase yield utilizing cheaper and easily available raw materials.

10.2 Cellulase System and Control of Cellulose Gene Expression

The chief constituent of the plant cell wall comprises the aromatic polymer lignin, hemicelluloses, and cellulose. In the history of the past few years, many researchers have done extensive studies lying on the bioconversion of plant polysaccharides because these organic compounds have impending application in many industries like fuel and pulp, food, feed, and paper industries. In the ecosystem the biomass of the different varieties of the cellulolytic organisms has played major role in recycling of the carbon. The utilization of the cellulase in cellulolytic microorganisms comprises two strategies. The first one comprises discrete noncomplex cellulases, and the second one includes complex cellulases. The deprivation of the cellulose by the aerobic cellulolytic microorganisms is carried out by secretion of a set of individual cellulases, and each one of these set of cellulase contains a module known as CBM (carbohydrate-binding module) joined to the catalytic module by a bendable linkage peptide, whereas in anaerobic microorganisms, the large multienzyme complexes of more than one million molecular mass are produced and are called as cellulosomes.

In cellulosomes, only a few enzymes contain a CBM; however, most of them are linked with a scaffold protein which contains a CBM. Cellulases or the glycoside hydrolases cleave the glycosidic bonds by the use of acid-base catalysis. The two catalytic residues of the enzyme glycoside hydrolases carried out the hydrolysis of the glycosidic bonds. One of the catalytic residues is a proton donor (general acid), and another one is base/nucleophile (Zhang and Zhang 2013).

The breakdown of cellulose polymers is carried out by cellulase enzyme which hydrolyzes the ß-1,4-glycosidic linkage. Formation of glucose by the dilapidation

of the cellulose is performed by the synergetic effect of three enzymes. The endoglucanases (EG; endo-1,4-b-D-glucanase, EC 3.2.1.4) are accountable for the disruption of the internal links of the glycan chain and yield reducing and nonreducing terminals for cellobiohydrolases (EC 3.2.1.91, exoglucanase, 1,4-b-D-glucan-cellobiohydrolase). The major product which is formed by the hydrolysis of cellulose is cellobiose, produced by the proccessive achievement of cellobiohydrolases. At the end of the glucose production from the cellulose is carried out by the ß-glucosidase (BG; cellobiose, ß-D glucoside glucanohydrolase, EC 3.2.1.21). The synergetic effect of cellobiohydrolases and endoglucanases has high impact on the cellulose hydrolysis. Cellobiose and the cellodextrans which are produced by the action of the cellobiohydrolase and endoglucanase enzymes show inhibitory effect on the enzyme activity. Consequently, for the proficient hydrolysis of the cellulose, ß-glucosidases are required because it cleaves the final glycosidic bonds for the production of glucose.

The gene expression can be regulated at the transcriptional level which is accomplished by the gene regulatory proteins that are the products of regulatory genes. These act as activator or repressor which binds with the regulatory sequences located near the beginning of transcription units. The binding of the activator protein to the promoter site regulates the gene expression positively (positive regulation), whereas the binding of the repressor to the operator site which is also located within the promoter prevents the gene from being expressed (negative regulation) (Fig. 10.1).

The relative transcription ratio of the cellulase genes is different in higher producer mutants and is regulated in an unswerving way possibly payable by the promoter titration effects. The identification of cellulose coding genes has been one of the major achievements in the last one decade. At the moment, three positive transcriptional activators which are concerned for the transcriptional regulation of cellulase gene expression (HAP2/3/5 complex, *xyr*1 in addition to Ace2) and the two repressors named as Cre1 and AceI (carbon catabolite repressor) have been confirmed. In fungi, the Ace2 (isolated from the *Trichoderma* spp.) is the cellulase activator that translates a protein that fits to the zinc binuclear group protein. On culturing the cellulolytic organisms in cellulose-containing media, the activity of the cellulase is reduced up to 70% because of the removal of Ace2 gene which is responsible for the decreased cellulase gene expression. In in vitro condition, the DNA-binding domain of the Ace2 gene binds to the 5'-GGCTAATAA-3′ site existing in the promoter called as *cbh*1. The main cellulase gene promoter (*cbh*1) contains the AceI gene which encodes the narrative cellulase regulator. This Ace1 gene contains three-zinc-finger motif, Cys2-His2-type which was first discovered in a transcription factor TFIIIA. It was found that under in vitro condition, the zinc finger motif binds to the eight sites of the *cel*7*a* promoter in which the basic

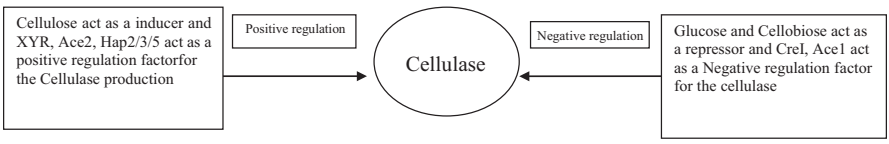

Fig. 10.1 Inducer and repressor of the cellulase gene regulation

sequences 5'-AGGCA-3′ are dispersed along the 1.15-kb. In some mutant strains, the cellulase gene expression and some other gene expression like those genes coding for xylanase enzyme increase by the removal of the Ace1 gene in cultures which contain sophorose and cellulose as substrate. This signifies that the function of cellulase repressor and other enzyme expression is performed by AceI of cellulase. Migl is a transcription factor which is isolated from *Saccharomyces cerevisiae* that have an ortholog called as CreA (transcription factor), which mediates carbon catabolism suppression (CCS) in filamentous fungi, although its removal results in the increase in the expression and secretion of cellulase. *Aspergillus nidulans* contain three other genes named as CreB, CreC, and CreD along with the CreA, which also contribute in CCS (Ali et al. 2014). The ruling of gene expression in cellulolytic organisms of fungal and bacterial origin is shown in Fig. 10.2a and b.

10.3 Characteristics of Host Strains

Cellulases which are produced by the cellulolytic organisms find wide application in food, chemical, textile, fuel, and paper and pulp industries and are also used as animal feed. The cost of the production of the cellulase in cellulolytic organisms of the fungal origin is high as compared with cellulolytic organisms of the bacterial origin because of slow fungal growth. The cellulase production by cellulolytic microorganism of bacterial origin is cost-effective and finds efficient application with wide prospects. With the few exceptions, the cellulolytic organisms of bacterial origin isolated from the different effluent show that these are gram positive (endospore former) having rough, opaque, and gray or yellowish surface colonies. This clearly indicates that these bacteria are aerobic, motile, and rod shaped (bacilliform bacteria). The cellulolytic microbes show positive biochemical reactions with the amylase, gelatinase, oxidase, Voges-Proskauer, protease, ß-galactosidase, citrate utilization and catalase activity which is used for their characterization. The cellulolytic bacterium gives negative test with methyl red, rhamnose, adonitol, indole tests, hydrogen sulfide reduction, citrate, malonate, ornithine, sorbitol, raffinose, and arginine. These microbes can ferment the simpler sugars like fructose, sucrose, and glucose but are not able to metabolize other sugar moieties such as lactose and mannitol. The selection of cellulase-producing bacterial strains can be done on the basis of their ability to form apparent region in the region of the bacterial colonies on the petri plates which contain selective media in cellulolytic activity test. The formation of clear zone is due to the degradation of CMC (Carboxymethyl cellulose) present in minimal growth media and confirms the cellulose activity. The cellulolytic activity varies in individual isolates, and each isolate possesses different cellulolytic index.

The isolated cellulolytic organisms are confirmed at the molecular level by the 16S rDNA sequencing by the use of bioinformatics tools with different kinds of bioinformatics software like Gen Bank/EMBL/DDBJ. Researchers can analyzed

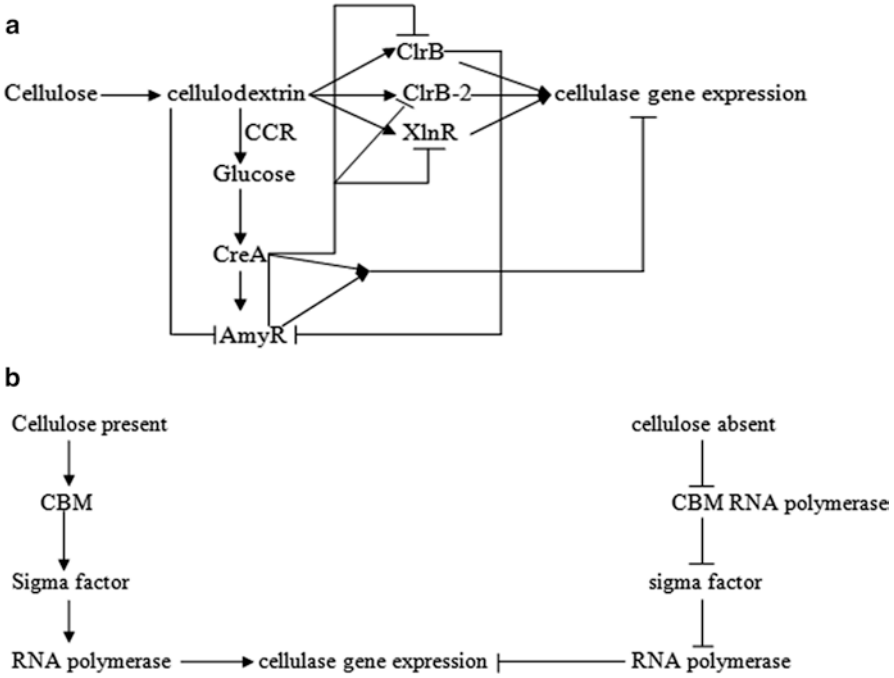

Fig. 10.2 (**a**) Positive and negative regulation of cellulase gene regulation in cellulolytic organisms of fungal origin. (**b**) Effect of the presence and absence of cellulose on the positive and negative regulation of cellulase gene in cellulolytic organisms of bacterial origin. In Fig. 10.2a, the transcription regulation in fungi shows that the CreA gene product and the AmyR gene product negatively regulate the gene expression of the cellulase genes, whereas the transcription factors named as ClrB, ClrB-2, and XlnR have positive effect on regulation of cellulase gene transcription (Zhonghai et al. 2015). In the cellulolytic organisms of bacterial origin, the regulation of the cellulase gene expression during transcription depends upon the attendance of polysaccharides like cellulose. When cellulose is present in the culture media, it activates the cellulase-binding module (CBM), and this CBM further activates the sigma factor which binds with the RNApol. The gene expression of the cellulase genes starts when the sigma factor binds to the RNA pol, although in the absence of the polysaccharides, the cellulase-binding module remains in inactive form; the anti-sigma factor which is formed in the absence of cellulose binds with the sigma factor and prevents its binding to the RNA polymerase and hence represses cellulase gene expression (Nataf et al. 2010)

the phylogenetic analysis by using the data which exists in NCBI (National Centre for Biotechnology Information) with the comparison of the 16S rDNA sequences of all cellulolytic organisms. The DNA extraction and purification can be done through the use of a DNA mining kit. The whole length sequencing of 16SrRNA can be acquired by using the primers, R'-1489 (5′-TACCTTGTTACGACTTCA–3′) and F′-27 (5′-GTTTGATCCTGGCTCAG–3′), which are positioned at 1489–1506 bp and 11–27 bp, respectively. Along with the above, there are some other universal primers which are named as 1500R (5′-GGTTACCTTGTTACGACTT-3′) and 20F

(5′-AGAGTTTGATCATGGCTCAG-3′) should also be used for the whole length sequencing of the 16SrRNA. There is automated DNA sequencer named as 3130xl Applied Bio-systems ABI prism which is used for the sequencing of 16S rRNA gene. BLAST search analysis on EzTaxon-e server is used for the identification of the 16SrRNA gene sequences of different strains (Reddy et al. 2017). Different strains of bacterial and fungal origin exhibit different levels of similarity in their sequence homology. Phylogenetic analysis of the 16SrRNA is done by using targeted sequences. The phylogenetic hierarchy should be constructed by the arrangement of 16S rRNA gene sequences by using the software called MEGA 6.0. The Carbohydrate-Active Enzymes database (CAZy) is used for updating the three-dimensional structure of cellulases family (CAZy; http://www.cazy.org). Figure 10.3 represents the cellulolytic gene expression and repression.

10.4 Individual Strains (Bacteria, Yeast, and Molds Involved in Cellulose Production)

The cellulase production occurs in different strains of bacteria, yeast, and molds. The prominent bacterial strains which are responsible for the cellulase production include *Escherichia coli*, *Anaerocellum thermophilum*, *Bacillus subtilis*, *Cellulomonas flavigena*, *Bacillus pumilus*, *Spirochaeta thermophila*, *Fervidobacterium islandicum*, *Ruminococcus albus*, *R. flavefaciens*, *Bacillus*

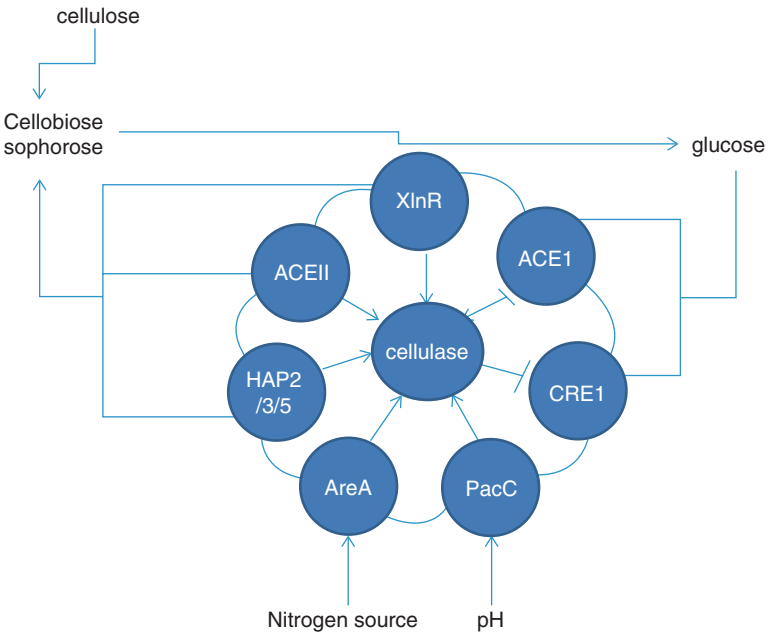

Fig. 10.3 Diagrammatic illustration of cellulase gene expression and its repression (Behera et al. 2017)

mojavensis, *Bacillus cereus*, *Bacillus megaterium*, *C. cellovorans*, *C. uda*, *C. fulvus*, *C. gilvus*, *C. hutchinsonii*, *Erwinia carotovora*, *M. chalcae*, *P. fluorescens*, *Sporocytophaga* sp., *S. reticuli*, *T. fusca*, *Acetivibrio cellulolyticus*, *Butyrivibrio fibrisolvens*, *Caldicellulosiruptor saccharolyticus*, *Eubacterium cellulosolvens*, *Fibrobacter succinogenes*, *Halocella cellulolytica*, *Anaerovorax odorimutans*, *Thermotoga neapolitana*, *Paracoccus yeei*, *Clostridium phytofermentans*, *C. phytofermentans*, *Clostridium thermocellum*, *C. thermocellum, Clostridium cellulolyticum*, *Cellulomonas fimi*, *Thermobifida fusca*, *Geobacillus stearothermophilus*, *Eubacterium siraeum*, *Erysipelothrix rhusiopathiae*, *and Sporobacter termitidis* (Reddy et al. 2017; Ferbiyanto et al. 2015; Zhonghai et al. 2015). The organisms which generate the enzymes cellulase and hemicellulase include bacteria and insects; along with these organisms, some cellulolytic organisms with fungal origin include *Trichoderma reesei* (filamentous fungi), *Aspergillus niger*, and *Penicillium oxalicum* which show tremendous role in the degradation of plant polysaccharides and cellulase production (Ali et al. 2014).

10.4.1 Construction of Recombinant Production Strains

The filamentous fungi *T. reesei* is widely used in the production of enzyme like cellulase which is further used for the dilapidation of polysaccharides like cellulose. In *T. reesei*, the frequently used strong promoter for the heterologous expression is known as cbh1. However, this promoter also has a number of sites for binding of which reduce the expression level. An additional fundamental spot to augment the construction of heterologous enzymes is the steadiness of recombinant mRNA and the anticipation of protein dilapidation within the ER, more than ever for the enzymes of bacterial origin. The efficiency of the cbh1 promoter can be increased by the replacement of the CREI binding sites with the transcription activator ACEII and the HAP2/3/5 complex within it. A stretchy a rigid a-helix linker and polyglycine linker and is used for the construction of merger gene between cbh1 (*T. reesei*) and e1 which encodes endoglucanases (*Acidothermus cellulolyticus)* for auxiliary improvement of bacterial genes within *T. reesei* by the heterologous expression efficiency. The bacterial cellulases have fascinating properties, but they have stability problems at the time of heterologous expression in *T. reesei* because of the fungal proteases which are present in the fungal host. The stability problem can be overcome by fusing the recombinant protein with the native protein which serves like a transporter for the translocation of unfamiliar protein by the secretory corridor. By the process of secretion, it protects the heterologous part of the protein from degradation. The thermophilic bacterium *Acidothermus cellulolyticus* secretes endoglucanase E1 which have high potential for the heterologous expression arrangement within *T. reesei*. At high temperature, the thermostable endoglucanase E1 shows outstanding synergistic activity with cellulases of *T. reesei* with its robustness. Additionally, the heterologous expression of e1 in corn has exposed to assist renovation of pretreated corn stover (PCS) into glucose.

In the case of *T. reesei*, when the catalytic province of e1 was transcribed, the hydrolytic efficiency of PCS is increased by 30% at 55 °C and after that merged through the catalytic province of cbh1. However, the merged protein possibly will get better thermostability of the entire cellulase multifaceted from *T. reesei* is still unidentified. There are two cbh1 promoters which are recently engineered and were obtained by the site unambiguous mutagenesis: one is pcbh1m1 promoter possesing−724 CREI motif that would be altered in the track of the fastening position of transcription factor ACEII (5′-GGCTAA-3′), whereas inside an additional promoter which is named as pcbh1m2, the two CREI motifs which are found at −698 and −690 within the pcbh1m1 promoter were altered within the direction of the binding site of the HAP2/3/5 protein complex (5′-CCAAT-3′). The position explicit mutagenesis would be established by sequencing. The strength of the promoters which are created by site explicit mutagenesis would compare with its wild-type cbh1 promoter by placing the improved green fluorescent protein reporter gene (egfp) at the back of every promoter which resulted in three expression vectors named as pDHt/sk-pcbh1, pDHt/sk-pcbh1m1, and pDHt/sk-pcbh1m2. The vector DH5a from *E.coli* served like cloning host, whereas *A. tumefaciens* AGL1 was used as a T-DNA donor in the direction of maintenance of the constructs and for fungal transformation. Researcher screened the RC30-8 strain of the *T. reesei* from mutants of Rut-C30 which are maintained in laboratory and used for the heterologous expression. The vector pDHt/sk which derived from the T-DNA which containing the hph, codes for hygromycin B phosphotransferase (the trpC promoter and terminator of the *Aspergillus nidulans* controls the expression level of the vectors) was used for the construction of the transformation vectors. The thermostable endocellulase E1 was obtained from the *A. cellulolyticus* strains using primers EIGF, EIHF, EISF, and EIR (Liu et al. 2018).

10.4.2 Transformation and Identification of Transformed Strains

Selection of successful transformants were observed as viable colonies when grown on hygromycin B and cefotaxime containing potato dextrose agar (PDA) by researchers. All successful transformant was used to create single conidial cultures for genetic stability, and real-time PCR was used for the confirmation of single-copy integration of egfp. All of the fungal strains including *T. reesei* strains were allowed to grow on PDA plates at 28 °C for several days and were further stored at 4 °C for conidial growth. Once the conidia formation is accomplished, these conidia are separated from PDA plates and are again inoculated into the SDB (Sabouraud dextrose broth). These strains are then again cultured at temperature of 28 °C in an incubator plus shaker at a revolving speed of 200 rpm in rotary shaker for expression of genes to achieve desirable proteins. Further, several drops of the culture are transferred to another flask which contains the minimal medium incorporated with

different carbon resources comprising cellulose powder (3%, w/v) and wheat bran (2%, w/v) as an inducer. MSM also contains glucose (2%, w/v) as repressor. It is then incubated in incubator plus shaker kept at 28 °C with an agitation speed of 200 rpm. After few days of culture, the mycelia of transformants are rinsed with sterilized water several times and are collected for total RNA extraction and its visualization under epifluorescent microscope. In order to extract the total RNA from transformed mycelia, the culture is blended with guanidinium thiocyanate reagent (TRIzol reagent) and then homogenized in a benchtop homogenizer. The cDNA for real-time PCR (RT-PCR) was extracted by PrimeScript RT reagent Kit. The relative expression levels of egfp in comparison with the expression of *act* gene encoding actin were carried out by RT-PCR using primers GFPrtF, GFPrtR, actF, and actR. The fusion proteins obtained from the cultured filtrate were separated and further purified by using Ni-NTA HisBind through gradient elution. In the final step, the residues were washed with imidazole. The washouts were then pooled, and the buffer was interchanged with sodium hydrogen phosphate for removal of imidazole using an ultrafilter. The fusion protein produced by recombinant strain was then characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot techniques in anti-his antibody (Zou et al. 2012).

10.5 Fermentative Production of Cellulase Enzyme

Cellulases represent a cluster of enzymes comprising endoglucanases, exoglucanases, and β-glucosidases which act synergistically on cellulose that causes its hydrolysis leading to generation of oligosaccharides with smaller glucose subunit chain (Beckham et al. 2010; Kubicek et al. 2009). Cellulase enzymes are synthesized by different microbes including bacteria, fungus, and yeast. It has been previously deduced that filamentous fungi, e.g., *Trichoderma reesei* and *Aspergillus* species, act as major players in commercial cellulase production. The main reason of using filamentous fungi for industrial cellulase production is its easy handling and requirement of less expensive minimal media for culture maintenance and cellulase production with safe application in different industries making the overall production process economical. The data availability of entire genome sequence of these strains makes its screening easier which can be used for hierarchy studies (Le Crom et al. 2009; Martinez et al. 2008). The cellulase production is performed in both submerged and solid-state fermentation (Singhania et al. 2010). Both of these fermentation technologies exhibit certain advantages and some disadvantages; of these two different fermentation technologies being adopted in cellulase production, SSF is considered as cheaper and simpler and does not require skilled personal for its handling. The major advantage of SSF is its ability to use inexpensive and insoluble substrates such as lignocellulosic feedstock for cellulase enzyme production at industrial level (Pandey 2003) (Table 10.1).

Table 10.1 Fermentative production of cellulose by different microorganisms

Microorganisms	Temperature (°C)	Types of substrates used	PH	Type	References
Anaxybacillus flavithermus EHP2	75	Carboxymethyl cellulose (CMC)	7.5	SmF-	Ibrahim and El-diwany (2007)
Botryosphaeria sp. AM01 and Saccharicola sp. EJC04	28	Sugarcane bagasse and soybean	4.8	SSF--	Marques et al. (2018)
Bacillus sp.	50	Sugar cane bagasse	45–55	SSF	Patel et al. (2005)
Trichoderma reesei Rut-C30	28	Corn stover	4.8	SSF	Zhang et al. (2018)
Bacillus sp. NZ	50	Agricultural residues	9–10	SSF	Nizamudeen and Bajaj (2009)
Bacillus cereus	50	Palm kernel cake	9–10	SSF	Lah et al. (2012)
T. reesei zxy-2	28	Glucose	4.8	SmF	Zhang et al. (2017)
Clostridium thermocellum	50–55	Cellulose and paper pulp	7	SmF and SSF	Zhuang et al. (2007)
Cytophaga hutchinsonii NCIM 23	50–55	Paddy straw	7	SmF	Mishra and Lata (2007)
Anoxybacillus sp. 527	70	Crystalline cellulose	6.0	SmF	Liang et al. (2010)
Bacillus sp. AC-1	70	CMC	4.5–6.5	SmF	Li et al. (2006)
Trichoderma reesei	30	Wheat bran	4.8	SSF	Idris et al. (2017)

10.5.1 Cellulase Production by Bacteria

A. **Cellulase production by *Bacillus subtilis*—***B. subtilis*** MU S1 is used for cellulase production using CMC (carboxymethyl cellulose) as the substrate. *B. subtilis* strain was cultivated in a media comprising carboxymethyl cellulose (CMC), yeast concentrate, and NaCl as potential carbon source, nitrogen source, and growth factor. The physical operating conditions constituted an agitation speed of 150 rpm, temperature 40 °C, and pH 7. The optimized media comprised 13.46 mg/ml of carboxymethyl cellulose, 8.38 mg/ml yeast extract, and 6.31 mg/ml sodium chloride. The optimized media gave more than threefold increase in cellulase activity in comparison to unoptimized media (Sreena and Sebastian 2018).

B. **Cellulose production by *Bacillus licheniformis* NCIM 5556—**Thermophilic bacteria *B. licheniformis* is a thermophilic bacterium and is one of the major cellulase-producing microbes. *Bacillus licheniformis* NCIM 5556 cultivated in a minimal media comprising CMC (carboxymethyl cellulose), Tween 20, and

$CaCl_2$ gave maximum cellulase yield. The media components were optimized by varying their concentration at different levels, and their interactive effect on cellulase yield was observed. The optimized media for cellulase production comprised $CaCl_2$•$6H_2O$ 25.06 mg/L, Tween 20 2.96 mL/L, and temperature 43.35 °C. The optimized cultural condition was further scaled up in 7 L bioreactor where maximum cellulase yield of 42.99 U/ml was obtained using recombinant *Bacillus* strain (Shajahan et al. 2017).

C. **Cellulase production by *Bacillus velezensis* ASN1**—Cellulase production by recently screened *B. velezensis* ASN1 utilizing paper waste as feedstock showed maximum cellulose yield after optimizing the cultural condition using a statistical software tool. Different media components including waste office paper, Tween 80, yeast extract, $NaNO_3$, $MgSO_4$, KCl, and KH_2PO_4, physical parameters (pH, temperature, and time), and inoculum size were optimized utilizing two-degree full factorial design to examine the key point significantly influencing the cellulase manufacturing. The optimum media comprised waste office paper (9 mg/ml), $NaNO_3$ (0.35 g/L), pH 4.72, and inoculum size (6.56%, v/v). *Bacillus velezensis* ASN1 gave maximum cellulase yield of 2.42 U/ml under optimized conditions (Nair et al. 2018).

10.5.2 Cellulase Production by Fungus

A. **Production by *Trichoderma reesei***—The production of cellulase by *Trichoderma reesei* RUTC-30 utilizing cellulose and wheat bran as substrate under solid-state fermentation was optimized employing statistical design of test. Optimization of process criterion arises in more than three times increase in carboxymethyl cellulase production to 959.53 IU/gDS. The procedure was analyzed at pilot scale in tray bioreactors, and maximum cellulase production was 457 IU/gDS at pH 4.8, using the lab conditions (Idris et al. 2017).

B. **Production by *Botryosphaeria* and *Saccharicola* sp.**—Certain recombinant strains like *Botryosphaeria* sp. AM01 and *Saccharicola* sp. EJC04 gave maximum cellulases yield under SSF utilizing agro-industrial wastes like sugarcane bagasse, molasses, and soybean meal as potential substrates for cellulase production. The optimization study performed on these recombinant strains showed that fermentation time, inoculum size, and moisture content of the substrates affected the overall cellulase yield. The recombinant strains gave maximum cellulase yield of 184.74 ± 6.0 U/g under SSF after 8 days of cultivation while keeping the temperature and pH at 28 °C and 4.8, individually (Marques et al. 2018).

C. **Production by mutant *Trichoderma reeseizxy-2***—A mutant *Trichoderma reeseizxy*-2 showed maximum growth and cellulase enzyme production when glucose (10 mg/ml) was utilized as individual substrate. A cellulase activity of 2.63 IU/mL and cell mass content of 9.53 mg/ml were observed after 48 h of

cultivation at pH 5 and temperature of 28 °C causing *Trichoderma reeseizxy*-2 mutant. The mutant strain showed maximum productivity of 54.79 IU/L/h which was higher in comparison to the previous reports (Zhang et al. 2017).

D. **Production of cellulose by *Trichoderma reesei* Rut-C30**—*T. reesei* Vib-1 is reported to possess enormous cellulase-producing potentials. The cellulolytic activity of *T. reesei* Vib-1 was in advance matched with that of *T. reesei* Rut-C30 utilizing cellulose as the individual carbon source. *T. reesei* Vib-1 showed better cellulase enzyme-producing ability in comparison to *T. reesei* Rut-C30. The *T. reesei* Vib-1 showed maximum cellulase activity (3.3 IU/mL) within 7 days of cultivation which was two-fold more than the cellulase activity of *Trichoderma reesei* Rut-C30. For increasing cellulase yield, the physical parameters such as temperature and pH were retained at 28 °C and 4.8, respectively. The cellulase yield increased in the existence of unmixed cellulose in association with varied other soluble inducers (Zhang et al. 2018).

E. **Production by mixed culture of recombinant fungus**—It has been found in previous researches that the cellulase enzyme present in the Trichoderma reesei does not possess β-glucosidase and cellobiohydrolase II enzyme activity, which delays the integration of cellulase parts leading to a reduced cellulose-lysing efficiency. In order to enhance the cellobiohydrolase II and cellulase-producing genes, *T. reesei* was genetically engineered. However, it deduced decreased β-glucosidase activity. The recombinant strain possessing desirable genes of *T. reesei* and *A. niger* for cellulase production utilized lignocellulosic resources such as corn more efficiently, which enhanced the cellulose production and further enzymatic hydrolysis. The recombinant *T. reesei* strain possessed enormous cellulase activity whose FPA activity was found to be 12.17 ± 1.18 FPIU/mL under acidic condition (pH 4.8) (Zhao et al. 2018).

F. **Production by textile waste**—The textile industry waste is posing lots of environmental threats owing to its expensive disposal. However, the presence of lignocellulosic source makes it suitable source for cellulase production at industrial level. It has been reported that a recombinant strain *A. niger* CKB efficiently utilized waste produced by textile industry for cellulase making under solid-state fermentation. The recombinant fungal strain showed maximal cellulase action of 1.56 FPU g/1 under solid-state fermentation after 144 h of cultivation. The optimal cellulase production by *A. niger* occurred at 28 °C temperature and pH 7.29, respectively. The recombinant strain efficiently utilized the cotton and polyester present in the textile waste for cellulase production with less labor and cheaper production strategy (Hu et al. 2018).

10.5.3 Cellulase Production by Yeast

Certain filamentous yeast strains like MK-157 and MK-118 and bacteria showed huge potential to produce cellulase enzyme utilizing sugarcane industry waste, i.e., sugarcane bagasse under solid-state fermentation. Previously, Qadir et al. 2018

reported two recombinant yeast strains, MK-118 and MK-157, which showed maximum cellulase activity of 9.81 IU/mL when co-cultured under solid-state fermentation utilizing sugarcane bagasse as raw material. The maximum cellulase activity was achieved after 4 days of cultivation using recombinant yeast strain grown at 35 °C temperature (Qadir et al. 2018).

10.6 Application of Cellulases

Cellulases have shown marked application and wide accessibility in all the industries including food, pharma, agriculture, and medical sector in the last three decades. Cellulases have been a subject of research for both academicians and industry personal owing to its wide applicability. The new researches on cellulase have depicted their possible use in different industries comprising food, animal feed, brewing and wine production, fruit and vegetable processing industries, biomass filtering, paper and pulp industry, and material and washing industries (Table 10.2).

10.6.1 Paper and Pulp Industry

During the last decade, enthusiasm for the utilization of cellulases in pulp and paper industry has expanded significantly due to their industrial advantage. Fineness and firmness of pulp are achieved by pulping process through mechanical means such as refining and grinding. In contrary, pulping utilizing cellulases under biomechanical process resulted in considerable amount of energy saving approximately 20–40% during processing of pulp (Singh et al. 2007; Bhat 2000).

The cellulosic material inherent in the paper pulp can be successfully used for the production of cellulase. Conventional pulp degradation requires increased used of water and ink removal from various paper pulps which causes the peeling of the individual paper fibrils and packets, showing remarkable water and dye molecule adsorption properties (Kibblewhite et al. 1995). The major steps of enzymatic deinking involve rapid soluble base consumption, increased fiber brilliance with improved quality attributes along with more cleanliness, and decreased presence of fine particles in the mashed pulp to be used in fermentative production of cellulases (Kuhad et al. 2010a, b). In addition to this, cellulases are also utilized in commercial production of cardboards with biodegradability (Buchert et al. 1998). This has been also successfully used in the grounding of delicate papers such as sanitary papers and paper towels (Hsu and Lakhani 2002) and expulsion of adhered sanitary papers (Sharyo et al. 2002).

Table 10.2 Application of cellulase in different industries

Industry	Function	Application	References
Food industry	Hydrolysis of cell wall components decreasing the viscosity and maintaining the texture of fruit juice	Extraction of juice from organic products, food-coloring agent; alteration of the tangible properties of fruit and vegetables, and oil from olives and soups; controlling coronary illness and atherosclerosis; reducing food spoilage	Bhat (2000)
Animal feed	Pretreatment of agricultural silage and grain feed for partial hydrolysis of lignocellulosic materials	Change in the healthful nature of animal feed; weight gain by broiler chickens and hens; diminishing colonization of pathogenic microscopic organisms in the large intestine	Cowan (1996), Godfrey et al. (1996)
Beer and wine	Hydrolysis of plant cell wall polysaccharides, modification of aromatic residues	Improvement in skin maceration and color extraction of grapes; quality, stability and clarification and aroma of wines	Galante et al. (1998a)
Textile and laundry	Act on the cotton fabric and break off the small fiber ends on the cotton fabric, thereby loosing the dye after washing; prevention or permanent removal of fuzz formation and pilling; biopolishing of cotton and nondenim fabrics; defibrillation of lyocell	Bio-stoning of denim fabrics; Biopolishing of non-denim fabrics; defibrillation of lyocell-containing fabrics and biofinishing; production of high-quality and environmentally friendly washing powders; production of high-quality fabrics	Kirk et al. (2002)
Pulp and paper	Mechanical pulping, biomodification of fibers, removing of ink coating and toners from paper	Increasing tensile strength and high fiber qualities, energy consumption reduced, improving drainage of the paper mills, and manufacturing of soft papers like paper towels and sanitary papers	Kuhad et al. (2010a, b, c)
Agriculture industry	Solubilization of plant or fungal cell walls, inhibition of spore germination, germ tube elongation, and fungal growth	Production of plant or fungal protoplasts, hybrid and mutant strains, soil fertility, plant growth	Beguin and Aubert (1994)
R&D industries	Affinity tag, affinity systems, conjugation and gene fusion, expression of heterologous proteins and enzymes	Affinity purification; immobilization and fusion of proteins, enzymes, and antibodies; production of hybrid molecules for various applications; production of high levels of proteins, enzymes, and antibodies	Svensson et al. (1995)

(continued)

Table 10.2 (continued)

Industry	Function	Application	References
Biofuel industry	Conversion of cellulosic material to glucose and other fermentable sugars	Production of single-cell protein or fermentation products like ethanol	Sukumaran et al. (2005), Kuhad et al. (2011)
Pharmacy industries	Digestion of cellulose fiber	Preparation of digestin; rapid hydrolysis of cellulose, hemicellulose, and beta-glucan polymers in food	Gupta et al. (2013)
Waste management	Degradation of cellulosic wastes	Reduction of environmental pollution	Milala et al. (2005)

10.6.2 Textile Industry

Potential application of cellulase enzymes involves wet processing of fabrics, predominantly cellulose-based material finishing, with the main focus on increased texture and look (Hebeish and Ibrahim 2007; Karmakar and Ray 2011). Cellulases have been efficiently used for the cotton biopolishing and jeans biostoning along with other cellulose-based clothes. After the biostoning step, cellulases act on the maintenance of cotton texture and cut the small fiber protruding outside of the fabric, which leads to release of the color, which is easily removed mechanically from scraped area of the clothes during washing. The main objective for the pumice stone substitution by enzymatic treatment is to reduce the filament usage with more profit and less labor intensity and no damaging properties posed on the environment (Sukumaran et al. 2005; Singh et al. 2007. Cellulase with enhanced endoglucanase activity is better suited for biofinishing. It has been observed that in most of the cotton or cotton-mixed garments, rapid and continuous washing causes soft and cloudy appearance, which is primarily due to origin of irregular and disconnected small fibrils present on the exterior of cloth pieces. The utilization of cellulases can expel these disconnected microfibrils and produces glossy exterior that provides a specific color to the clothings (Ibrahim et al. 2011). It has been experimentally deduced that cellulose also helps in garments softening and also in the expulsion of the dirt particles present inside the microfibrilar network. There are several reports which deduced that the application of the entire cellulase pattern was specific in respect to the endonuclease activity, and this is quiet effective in maintenance of texture quality and appearance.

10.6.3 Bioethanol Industry

One of the major applications of cellulases is in the bioconversion of materials rich in lignin and cellulose residues like rice straw, sugarcane molasses, bagasse, corncob, switch grass, and wood residues in bioethanol through enzymatic saccharification by cellulases (Kuhad et al. 2010a; Gupta et al. 2011a, b). Extensive researches

are being carried out for effective and efficient strategies for the conversion of lignocellulosic residues to valuable product such as bioethanol and biodiesel (Sun and Cheng 2002; Mosier et al. 2005). The conversion of lignocellulosic substances to biofuel through enzymatic process will overcome the demand of conventional petroleum, and the process would lead to the production of less expensive fuel. The inflexibility of the substrate and other factors hampers the cellulase activity, and the hydrolysis of lignocellulosic components is decreased (Yang and Wyman 2004). Similarly, recombinant cellulase inhibits the substrate binding and its irreversible adsorption on heterogeneous substrate with multiple binding site leading to increased enzyme activity (Taniguchi et al. 2005).

10.6.4 Wine and Brewery Industry

Microbial glucanases show vital role in wine and brewery industry as they act on wide polysaccharides and undergo fermentation to produce the fermented alcoholic beverages like beer and wine (Bamforth 2009; Singh et al. 2007). Recombinant glucanases showed tremendous improvement in quality and yield of alcoholic beverages utilizing agro-industrial waste (Bamforth 2009). Glucanases are involved in either squashing or performing the hydrolysis of glucan which minimizes the consistency of wort and its clarity (Bamforth 2009). Certain food grade enzymes like β-glucosidases can increase the odor of alcoholic beverages such as wines by altering glycosylated residues. Similarly, certain enzymes performing maceration during brewing are responsible for improvement in wine quality by enhancing the juice yield, pressability of wort and its settling during maturation and aging. A variety of enzyme series are currently involved in quality wine production which are produced by recombinant strains. The major benefits of utilizing these enzymes in wine production include improved maceration, increased wort extraction, and easier juice extraction and filtration process which leads to wine with improved grade and higher security (Galante et al. 1998a, b).

Brewing industries rely on synergistic enzyme activity which causes effective malting and fermentation process. The malting of cereal grains relies on germination of seeds, which begins the biosynthesis activity of certain enzymes like carboxypeptidase, β-glucanase, and α- and β-amylases which hydrolyze the substrates present in seeds (Bamforth 2009). In a previous report of Oksanen et al. (1985), it was deduced that the two enzymes in cellulase framework, namely, exoglucanase II and endoglucanase II, produced by *Trichoderma* sp. were responsible for the decrease in the viscosity of wort and polymerization. This caused improvement in grape squeezing and higher juice yield and settling due to mixture of macerating enzymes produced by recombinant *Trichoderma* sp. However, this improvement in wine quality was also dependent on suitable composition of complex polysaccharides such as hemicellulases, cellulases, and pectinases. In a study of Galante et al. (1998a, b), three white grape varieties, namely, Sauvignon, Chardonnay, and Soave, comprising a blend of xylanase, pectinase, and cellulase as cytolase 219 showed

approximately up to 35% enhancement in juice removal during wine making. Similarly, up to 80% increase in the must filtration rate and reduction in squeezing time along with 30–70% decrease in must viscosity were observed. Besides that, an energy saving of 20–40% which was required during bioreactor cooling was observed making the overall wine making process economical. The fermented beverage yield can be further improved by using the recombinant strains which also secrete the pectinase in addition to cellulase for effective utilization of fruit and vegetable wastes such as orange peels (Bamforth 2009).

10.6.5 Food Processing Industry

Cellulases also find wide application in the food processing industries specially in fruit and vegetable processing industries for the production of value-added functional food using recombinant strains produced by genetic engineering. Cellulases are utilized in the fruit and vegetable juice production which also facilitates the removal process, juice refining, and its stability. The recombinant strains express the genes which are required for the production of enzyme network (cellulases, xylanases, and pectinases) which are required during maceration and are widely used for extraction and brilliance of leafy food juices and also to enhance the juice harvest (De Carvalho et al. 2008). The complex enzymes used in maceration are used to prevent the cloudiness in the juices and provide stability and texture with decreased consistency of the sweet liquid secretion (nectars) and purees obtained from tropical organic products, such as pear, papaya, mango, and apricot (Singh et al. 2007; De Carvalho et al. 2008). Sensorial attributes like flavor, texture, aroma, and flavor of fruit- and vegetable-based products may be changed by minimizing the effect of bittering compounds of citrus fruits by incorporation of certain enzymes such as pectinases and β-glucosidases (Rai et al. 2007). Certain enzyme mixture comprising pectinases, cellulases, and hemicellulases is also applied for enhanced olive oil removal. The application of enzymes involved in maceration not merely increases the turbidity, but it also changes the surface areas of nectars and purees which maintain their consistency for longer duration during storage. This clearly suggests that the macerating enzymes comprising pectinase and cellulase play a vital role in sustainable development, and this leads to its further application in different varieties of fruits and vegetables for juice extraction that can be used in preparation of value-added diversified food products (Dourado et al. 2002).

10.6.6 Animal Feed Industry

The major application of cellulases and hemicellulases in the feed industry has established noteworthy care owing to their capability to increase the feed quality with more acceptability by cattles improving the milk and meat product yield

(Dhiman et al. 2002). It has been reported that nutritional quality of cattle feeds such as farming silage and grains can be improved by cellulase pretreatment. These enzymes are also involved in the removal of anti-nutritional factors (ANF) which are prevalent in the feed grains. The supplementation of certain enzymes such as glucanases, amylases, and proteases also minimizes the effect of certain anti-nutritional factors present in the animal feeds which are responsible for elevation of certain digestive enzymes such as amylases and proteases. The assimilation of hydrolytic enzymes such as cellulases and glucanases in the animal feed can enhance the nutritious quality of pork meat. The supplementation of certain enzymes like glucanases alters the physicochemical characteristics such as decrease in viscosity of barley-based feeds and high fiber served to pigs and poultry. Similarly, these enzymes are responsible for increased body mass in piglets and chickens by increasing the assimilation and immersion of feed components (Singh et al. 2007; Karmakar and Ray 2011; Shrivastava et al. 2011).

The partial hydrolysis of lignocellulosic substance brought by cellulases and hemicellulases causes removal of the hulls from bean seeds and oat grains. These are also involved in the hydrolytic cleavage of β-glucans and higher blurriness and adaptableness of feed components, which causes alteration in the nutritional properties of animal feeds (Galante et al. 1998a, b). Besides this, these enzymes are also involved in conversion of silage and fodder during fractional hydrolysis of plant cell wall.

10.6.7 Agriculture-Based Industries

Certain enzyme preparations comprising blend of cellulases, hemicellulases, and pectinases showed profound application in agricultural farming to increase the food yield (Bhat 2000). The recombinant strains containing hydrolases can be inserted in plant or fungal protoplasts for expression of desirable attributes using direct gene transfer method. There are certain reports which suggest that cellulases and related enzymes can be obtained from specific microorganisms and can be cloned in plants to prevent diseases arising due to plant pathogens (Bhat 2000). The β-glucanases are suitable for controlling plant pathogens by disrupting their cell walls. Several fungi including *Penicillium* sp., *Geocladium* sp., *Trichoderma* sp., and *Chaetomium* sp. show cellulase activity which plays significant role in agribusiness by improving germination of seeds, early plant growth and flourishing, and enlarged root network with higher product yields (Harman and Kubicek 1998). Besides that, these enzymes have been used in altering the soil characteristics making them suitable for specific crop production. In general, straw union is adopted as an effective tool to advance the soil value with lesser reliance on chemical fertilizers (Escobar and Hue 2008; Tejada et al. 2008). Previous reports have focused on the utilization of straw decay by using recombinant microbial strains. Certain fungus species such as *Aspergillus* sp., *Chaetomium* sp., *Trichoderma* sp., and actinomycetes have revealed outstanding outcome in soil quality improvement (Bowen and Harper 1990; Abdulla and El-Shatoury 2007).

10.6.8 Extraction of Olive Oil

Olive oil extraction involves utilization of newly picked, spotless, and young fruits under cooled pressurized conditions (De Faveri et al. 2008). Although, significant olive oil recovery can be achieved by using fully matured fruit at higher temperatures than from immature fruits, extraction at higher temperature is not desirable as it leads to recovery of olive oil with higher acidic behavior, rancid flavor, and poor fragrance. Therefore, an advanced technique for the removal of olive oil from oilseed crop was required to meet consumer needs. An improved recombinant strain of *Aspergillus aculeatus* possessing pectinase, hemicellulose, and cellulase activity can be utilized for removal of olive oil and is sold under the brand name OliveX.

10.6.9 Extraction of Carotenoid Pigments

Cellulase also finds wide application in extraction of coloring compounds such as carotenoids from plant and microbial source. The carotenoids constitute color pigments from red to yellow. In general, the mixture of cellulolytic and pectinolytic enzymes fastens the hydrolysis rate in order to achieve complete liquefaction. Cellulases are accountable for splitting the cellulose chains into smaller monosaccharides subunits. In contrary to that, commercial pectinase extracts obtained from fungus such as *Aspergillus niger* possess pectin esterase, polygalacturonase, and gelatin lyase activity (Çinar 2005). The application of pectinase and cellulase enzymes is done in order to extract the carotenoid pigments from agro-waste including orange peel, sweet potato, and carrot pomace through bioconversion of complex carbohydrates to simpler ones which is performed by a wide range of microorganisms. These carotenoid pigments exist in their natural form while adhered with proteins. The carotenoid- and protein-linked framework restricts the pigment oxidation and color deterioration, while solubilized recovery separated the pigment molecules from the protein residues which leads to water insolubility and easier oxidation procedure (Çinar 2005).

10.6.10 Detergent Industry

The combined usage of cellulases, protease, and lipase in the detergents is supposed to be new novelty in the detergent industry (Singh et al. 2007). Cellulase framework is responsible for changing cellulose fibrils which can increase the color brightness, intensity, texture, and filth particle elimination from the cotton-blended clothes. The usage of soluble cellulases as a possible detergent supplement is observed due to its ability to penetrate the inner surface of cellulose fibrils and remove the soil residues present there when added with other traditional detergent ingredient (Singh et al. 2007).

Nowadays, the majority of textile industries are utilizing the cellullase enzymes to produce the clothes with more fine and smooth texture as these cellulases act on the cellulose fibrils located in the interior of garments. The cellulases are connected to expel these harsh bulges for an unwrinkled, shining, and more brightness-shade fabric (Karmakar and Ray 2011).

10.6.11 Waste Management

A large amount of cellulose prevalent in woods is obtained from cultivating lands which remains either unutilized or underutilized and poses environmental threat upon disposal (Milala et al. 2005). Currently, these feasible waste products are proficiently used to produce significant bioproducts such as biocatalysts, sugar, biofuels, synthetic compounds, and inexpensive substrates for fermentation, to increase animal feeds, and as growth supplements for human population (Kuhad et al. 2010a, b, c; Karmakar and Ray 2011; Gupta et al. 2009, 2011a, b).

10.7 Future Prospects

During the past one decade, significant advancement in recombinant DNA technology has created an opportunity to convert the non-cellulolytic organisms to cellulose-degrading microbes which show potential conversion of cellulosic substrates into simpler sugar units which can be easily fermented. The usage of recombinant DNA technology in the cellulase production is an effective tool as it screens and utilizes enzymes not apparently present in a chosen host microorganism which is easily cultivable and requires minimal growth media for its maintenance and product formation. In addition to that, it is previously stated that due to the complex structure of cellulose and hemicelluloses with crystallinity, it requires many enzyme activities in order to accomplish the complete bioconversion. The potential recombinant strain possessing cellulase enzyme complex contributes to vast extent in different industries pertaining to the positioning and composition of individual enzymes in the complex with specific purpose. Although progress has been made in the area of recombinant DNA technology, still more efforts are required to achieve the economical cellulose transformation into value-added compounds such as biofuels, non-biofuel hydrocarbon compounds, and acids to achieve an industrial standard requirement. The recombinant strain developed by genetic engineering means would be able to efficiently utilize the complex and cheaper substrate for its conversion to value-added products. The gene construct with overexpression in suitable host will enhance the cellulose yield while decreasing the production cost. Due to gradual depletion of nonrenewable substrates such as petroleum compounds and other vestige fuels, it is essential to focus on this enzyme and develop recombinant strain with enhanced cellulose activity for efficient utilization of cellulosic material.

References

Abdulla HM, El-Shatoury SA (2007) *Actinomycetes* in rice straw decomposition. Waste Manag 27(6):850–853

Ali N, Athar MA, Khan YH, Idrees M, Ahmad D (2014) Regulation and improvement of cellulase production: recent advances. *Nat Resour* 5:857–863

Bamforth CW (2009) Current perspectives on the role of enzymes in brewing. J Cereal Sci 50(3):353–357

Beckham GT, Bomble YJ, Matthews JF, Taylor CB, Resch MG, Yarbrough JM, Decker SR, Bu L, Zhao X, McCabe C, Wohlert J, Bergenstrahle M, Brady JW, Adney WS, Himmel ME, Crowley MF (2010) The O-Glycosylated linker from the Trichoderma reesei family 7 cellulase is a flexible, disordered protein. Biophys J 99:3773–3781

Béguin P, Aubert JP (1994) The biological degradation of cellulose. FEMS Microbiol Rev 13(1):25–58

Behera BC, Sethi BK, Mishra RR, Dutta SK, Thatoi HN (2017) Microbial cellulases–Diversity & biotechnology with reference to mangrove environment: a review. J Genet Eng Biotechnol 15(1):197–210

Bhat MK (2000) Cellulases and related enzymes in biotechnology. Biotechnol Adv 18(5):355–383

Bowen RM, Harper SHT (1990) Decomposition of wheat straw and related compounds by fungi isolated from straw in arable soils. Soil Biol Biochem 22(3):393–399

Buchert J, Oksanen T, Pere J, Siika-Aho M, Suurnäkki A, Viikari L (1998) Applications of *Trichoderma reesei* enzymes in the pulp and paper industry. *Trichoderma* Gliocladium 2:343–363

Çinar I (2005) Effects of cellulase and pectinase concentrations on the colour yield of enzyme extracted plant carotenoids. Process Biochem 40(2):945–949

Cowan WD (1996) Animal feed,[w:]. In: Godfrey T, West S (eds) Industrial enzymology, 2nd edn. Macmillan Press, London, UK, pp 360–371

De Carvalho LMJ, De Castro IM, Da Silva CAB (2008) A study of retention of sugars in the process of clarification of pineapple juice (Ananas comosus, L. Merril) by micro-and ultrafiltration. J Food Eng 87(4):447–454

De Faveri D, Aliakbarian B, Avogadro M, Perego P, Converti A (2008) Improvement of olive oil phenolics content by means of enzyme formulations: effect of different enzyme activities and levels. Biochem Eng J 41(2):149–156

Dhiman TR, Zaman MS, Gimenez RR, Walters JL, Treacher R (2002) Performance of dairy cows fed forage treated with fibrolytic enzymes prior to feeding. Anim Feed Sci Technol 101(1–4):115–125

Dourado F, Bastos M, Mota M, Gama FM (2002) Studies on the properties of Celluclast/Eudragit L-100 conjugate. J Biotechnol 99(2):121–131

Escobar MO, Hue NV (2008) Temporal changes of selected chemical properties in three manure–amended soils of Hawaii. Bioresour Technol 99(18):8649–8654

Ferbiyanto A, Rusmana I, Raffiudin R (2015) Characterization and identification of cellulolytic bacteria from gut of worker Macrotermes gilvus. HAYATI J Biosci 22:197–200

Galante YM, De Conti A, Monteverdi R (1998a) Application of *Trichoderma* enzymes in food and feed industries. In: Harman GF, Kubicek CP (eds) *Trichoderma* and *gliocladium*—enzymes, vol. 2 of biological control and commercial applications. Taylor & Francis, London, pp 311–326

Galante YM, De Conti RA, Harman GF, Kubicek CP (eds) (1998b) *Trichoderma* & *gliocladium*—enzymes, biological control and commercial applications. Taylor & Francis, London, pp 327–342

Godfrey T, Godfrey T, West S (1996) Industrial enzymology, 2nd edn. Macmillan Press, London, pp 360–371

Gupta R, Sharma KK, Kuhad RC (2009) Separate hydrolysis and fermentation (SHF) of Prosopis juliflora, a woody substrate, for the production of cellulosic ethanol by *Saccharomyces cerevisiae* and *Pichia stipitis*-NCIM 3498. Bioresour Technol 100(3):1214–1220

Gupta R, Khasa YP, Kuhad RC (2011a) Evaluation of pretreatment methods in improving the enzymatic saccharification of cellulosic materials. Carbohydr Polym 84(3):1103–1109
Gupta R, Mehta G, Khasa YP, Kuhad RC (2011b) Fungal delignification of lignocellulosic biomass improves the saccharification of cellulosics. Biodegradation 22(4):797–804
Gupta R, Mehta G, Deswal D, Sharma S, Jain KK, Kuhad RC, Singh A (2013) Cellulases and their biotechnological applications. In: Biotechnology for environmental management and resource recovery. Springer, India, pp 89–106. https://doi.org/10.1007/978-81-322-0876-1_6
Harman GE, Kubicek CP (1998) *Trichoderma* and *gliocladium*: enzymes, vol. 2 of biological control and commercial applications. Taylor & Francis, London
Hebeish A, Ibrahim NA (2007) The impact of frontier sciences on textile industry. Colourage 54(4):41–55
Hsu JC, Lakhani NN (2002) U.S. Patent No. 6,413,363. U.S. Patent and Trademark Office, Washington, DC
Hu Y, Du C, Pensupa N, Lin CSK (2018) Optimisation of fungal cellulase production from textile waste using experimental design. Process Saf Environ Prot. https://doi.org/10.1016/j.psep.2018.06.009
Ibrahim ASS, El-diwany AI (2007) Isolation and identification of new cellulases producing thermophilic bacteria from an Egyptian hot spring and some properties of the crude enzyme. Aust J Basic Appl Sci 1(4):473–478
Ibrahim NA, El-Badry K, Eid BM, Hassan TM (2011) A new approach for biofinishing of cellulose-containing fabrics using acid cellulases. Carbohydr Polym 83(1):116–121
Idris ASO, Pandey A, Rao SS, Sukumaran RK (2017) Cellulase production through solid-state tray fermentation, and its use for bioethanol from sorghum Stover. Bioresour Technol 242:265–271
Karmakar M, Ray RR (2011) Current trends in research and application of microbial cellulases. Res J Microbiol 6(1):41
Kibblewhite RP, Bawden AD, Brindley CL (1995) TMP fibre and fines qualities of thirteen radiata pine wood types. Appita J 48(5):367–377
Kirk O, Borchert TV, Fuglsang CC (2002) Industrial enzyme applications. Curr Opin Biotechnol 13:345–351
Kubicek CP, Mikus M, Schuster A, Schmoll M, Seiboth B (2009) Metabolic engineering strategies for the improvement of cellulase production by Hypocrea jecorina. Biotechnol Biofuels 2(1):19
Kuhad RC, Gupta R, Khasa YP (2010a) Bioethanol production from lignocellulosic biomass: an overview. Teri Press, New Delhi
Kuhad RC, Gupta R, Khasa YP, Singh A (2010b) Bioethanol production from Lantana camara (red sage): pretreatment, saccharification and fermentation. Bioresour Technol 101(21):8348–8354
Kuhad RC, Mehta G, Gupta R, Sharma KK (2010c) Fed batch enzymatic saccharification of newspaper cellulosics improves the sugar content in the hydrolysates and eventually the ethanol fermentation by *Saccharomyces cerevisiae*. Biomass Bioenergy 34(8):1189–1194
Kuhad RC, Gupta R, Singh A (2011) Microbial cellulases and their industrial applications. Enzyme Res 2011:10. https://doi.org/10.4061/2011/280696. (Article ID 280696)
Lah TNT, Rahman NNN, Nama MB (2012) Cellulase activity and glucose production by Bacillus cereus monoculture and co-culture utilizing palm kernel cake (PKC) under solid state fermentation. In International conference on environment, energy and biotechnology, Singapore (Vol. 33, pp. 172–177)
Le Crom S, Schackwitz W, Pennacchio L, Magnuson JK, Culley DE, Collett JR et al (2009) Tracking the roots of cellulase hyperproduction by the fungus Trichoderma reesei using massively parallel DNA sequencing. Proc Natl Acad Sci 106(38):16151–16156
Li YH, Ding M, Wang J, Xu GJ, Zhao F (2006) A novel thermo acidophilic endoglucanase, Ba-EGA, from a new cellulose-degrading bacterium, Bacillus sp. AC-1. Appl Microbiol Biotechnol 70(4):430–436
Liang Y, Feng Z, Yesuf J, Blackburn JW (2010) Optimization of growth medium and enzyme assay conditions for crude cellulases produced by a novel thermophilic and cellulolytic bacterium, *Anoxybacillus sp.* 527. Appl Biochem Biotechnol 160(6):1841–1852

Liu ZL, Li HN, Song HT, Xiao WJ, Xia WC, Liu XP, Jiang ZB (2018) Construction of a tri functional cellulase and expression in *Saccharomyces cerevisiae* using a fusion protein. BMC Biotechnol 18(43):01–07

Marques NP, de Cassia Pereira J, Gomes E, da Silva R, Araújo AR, Ferreira H et al (2018) Cellulases and xylanases production by endophytic fungi by solid state fermentation using lignocellulosic substrates and enzymatic saccharification of pretreated sugarcane bagasse. Ind Crop Prod 122:66–75

Martinez D, Berka RM, Henrissat B, Saloheimo M, Arvas M, Baker SE et al (2008) Genome sequencing and analysis of the biomass-degrading fungus Trichoderma reesei (syn. Hypocrea jecorina). Nat Biotechnol 26(5):553–560

Milala MA, Shugaba A, Gidado A, Ene AC, Wafar JA (2005) Studies on the use of agricultural wastes for cellulase enzyme production by *Aspergillus niger*. Res J Agric Biol Sci 1(4):325–328

Mishra BK, Lata AP (2007) Lignocellulolytic enzyme production from submerged fermentation of paddy straw. Indian J Microbiol 47(2):176–179

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

Nair AS, Al-Battashi H, Al-Akzawi A, Annamalai N, Gujarathi A, Al-Bahry S et al (2018) Waste office paper: A potential feedstock for cellulase production by a novel strain Bacillus velezensis ASN1. Waste Manag 79:491–500

Nataf Y, Bahari L, Raifer HL, Borovok I, Lamed R, Bayer EA, Sonenshein AL, Shoham Y (2010) Clostridium thermocellum cellulosomal genes are regulated by extracytoplasmic polysaccharides via alternative sigma factors. *PNAS* 43(107):18646–18651

Nizamudeen S, Bajaj BK (2009) A novel thermo-alkali tolerant endoglucanase production using cost-effective agricultural residues as substrates by a newly isolated *Bacillus sp*. NZ. Food Technol Biotechnol 47(4):435–440

Oksanen J, Ahvenainen J, Home S (1985) Microbial cellulose for improving filterability of wort and beer. In Proceedings of the 20th European brewery chemistry congress, Helsinki, Finland, pp. 419–425

Pandey A (2003) Solid-state fermentation. Biochem Eng J 13(2-3):81–84

Patel MA, Ou MS, Ingram LO, Shanmugam KT (2005) Simultaneous saccharification and co fermentation of crystalline cellulose and sugar cane bagasse hemicellulose hydrolysate to lactate by a thermotolerant acidophilic Bacillus sp. *Biotechnol Prog* 21(5):1453–1460

Qadir F, Shariq M, Ahmed A, Sohail M (2018) Evaluation of a yeast co-culture for cellulase and xylanase production under solid state fermentation of sugarcane bagasse using multivariate approach. Ind Crop Prod 123:407–415

Rai P, Majumdar GC, Gupta SD, De S (2007) Effect of various pretreatment methods on permeate flux and quality during ultrafiltration of mosambi juice. J Food Eng 78(2):561–568

Reddy KV, Vijayalashmi T, Pabbati R, Maddela NR (2017) Characterization of some efficient cellulase producing bacteria isolated from pulp and paper mill effluent contaminated soil. Braz Arch Biol Technol 60:1–6

Shajahan S, Moorthy IG, Sivakumar N, Selvakumar G (2017) Statistical modeling and optimization of cellulase production by *Bacillus licheniformis* NCIM 5556 isolated from the hot spring, Maharashtra, India. J King Saud Univ Sci 29(3):302–310

Sharyo M, Sakaguchi H, Ohishi M, Takahashi M, Kida K, Tamagawa H, et al (2002) U.S. Patent No. 6,468,391. U.S. Patent and Trademark Office, Washington, DC

Shrivastava B, Thakur S, Khasa YP, Gupte A, Puniya AK, Kuhad RC (2011) White-rot fungal conversion of wheat straw to energy rich cattle feed. Biodegradation 22(4):823–831

Singh A, Kuhad RC, Ward OP (2007) Industrial application of microbial cellulases. In: Lignocellulose biotechnoloy: future prospects. Anshan, Tunbridge Wells, pp 345–358

Singhania RR, Sukumaran RK, Patel AK, Larroche C, Pandey A (2010) Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. *Enzyme Microb Technol* 46:541–549

Sreena CP, Sebastian D (2018) Augmented cellulase production by Bacillus subtilis strain MU S1 using different statistical experimental designs. J Genet Eng Biotechnol 16(1):9–16

Sticklen M (2006) Plant genetic engineering to improve biomass characteristics for biofuels. Curr Opin Biotechnol 17:315–319

Sukumaran RK, Singhania RR, Pandey A (2005) Microbial cellulases-production, applications and challenges. J Sci Ind Res 64(11):832–844

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

Svensson B, Frandsen TP, Matsui I, Juge N, Fierobe HP, Stoffer B, Rodenburg KW (1995) Mutational analysis of catalytic mechanism and specificity in amylolytic enzymes. In: Petersen SB, Svensson B, Pedersen S (eds) Carbohydrate bioengineering. Elsevier, Amsterdam

Taniguchi M, Suzuki H, Watanabe D, Sakai K, Hoshino K, Tanaka T (2005) Evaluation of pre-treatment with Pleurotus ostreatus for enzymatic hydrolysis of rice straw. J Biosci Bioeng 100(6):637–643

Tejada M, Gonzalez JL, García-Martínez AM, Parrado J (2008) Application of a green manure and green manure composted with beet vinasse on soil restoration: effects on soil properties. Bioresour Technol 99(11):4949–4957

Templeton DW, Sluiter AD, Hayward TK, Hames BR, Thomas SR (2009) Assessing corn stover composition and sources of variability via NIRS. Cellulose 16:621–639

Yang B, Wyman CE (2004) Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose. Biotechnol Bioeng 86(1):88–98

Zhang XZ, Zhang YHP (2013) Cellulases: characteristics, sources, production, and applications. In: Bioprocessing technologies in biorefinery for sustainable production of fuels, chemicals, and polymers, vol 1. John Wiley & Sons, Inc., Hoboken, pp 131–146

Zhang X, Li Y, Zhao X, Bai F (2017) Constitutive cellulase production from glucose using the recombinant Trichoderma reesei strain overexpressing an artificial transcription activator. Bioresour Technol 223:317–322

Zhang F, Zhao X, Bai F (2018) Improvement of cellulase production in *Trichoderma reesei* Rut-C30 by overexpression of a novel regulatory gene Trvib-1. Bioresour Technol 247:676–683

Zhao C, Deng L, Fang H (2018) Mixed culture of recombinant *Trichoderma reesei* and *Aspergillus niger* for cellulase production to increase the cellulose degrading capability. Biomass Bioenergy 112:93–98

Zhonghai L, Guangshan Y, Ruimei W, Liwei G, Qinbiao K, Meng L, Piao Y, Guodong L, Yuqi Q, Xin S, Yaohua Z, Xu F, Yinbo Q (2015) Synergistic and dose-controlled regulation of cellulase gene expression in Penicillium oxalicum. PLoS Genet 11(9):e1005509

Zhuang J, Marchant MA, Nokes SE, Strobel HJ (2007) Economic analysis of cellulase production methods for bio-ethanol. Appl Eng Agric 23(5):679–687

Zou G, Shi S, Jiang Y, Brink JVD, Vries RPD, Chen L, Zhang J, Ma L, Wang C, Zhou Z (2012) Construction of a cellulase hyper-expression system in Trichoderma reesei by promoter and enzyme engineering. Microb Cell Factories 11(21):01–12

Chapter 11
Current Advancements in Recombinant Technology for Industrial Production of Cellulases: Part-II

Gurudatta Singh, Amit Kumar Patel, Akanksha Gupta, Deepak Gupta, and Virendra Kumar Mishra

11.1 Introduction

11.1.1 Cellulase

Cellulases are the enzymes which decompose cellulose and some other related polysaccharides. These enzymes have been produced by bacteria, fungi and some protozoans. They are also used for decomposition of cellulosic component contained within any material. Cellulases are multienzyme systems having different types of enzyme, i.e. cellobiose, cellobiohydrolases and carboxymethyl cellulase (CMCase). The significance of using carboxymethyl cellulase is that it has highly different hydrolytic activities compared to other enzymes. Cellulase enzymes hydrolysed the glycosidic bonds of the cellulosic polymer. The catalytic components of cellulases have been categorized into several groups, based on sequences and crystal structures of the amino acid (Gusakov 2011). Cellulases constitute non-catalytic carbohydrate-binding modules and/or other functionally known or unknown units, which might be present at C- or N-terminal end of catalytic units.

11.2 Types of Cellulase

On the basis of types of reaction catalysed, cellulases are categorized into five different types; these include:

G. Singh · A. K. Patel · A. Gupta · D. Gupta · V. K. Mishra (✉)
Institute of Environment and Sustainable Development Banaras Hindu University, Varanasi, India

M. Srivastava et al. (eds.), *Approaches to Enhance Industrial Production of Fungal Cellulases*, Fungal Biology,
https://doi.org/10.1007/978-3-030-14726-6_11

11.2.1 Endocellulases

The internal bonds of the cellulose are randomly cleaved by the endocellulases at amorphous locations, thus forming different chain ends, for example, endoglucanases (EC 3.2.1.4).

11.2.2 Exocellulases

The chain formed by the endocellulases is further broken down into 1–3 units near the end of the exposed chain, resulting in tetrasaccharides or disaccharides, i.e. cellobiose. Exocellulases are again classified into two classes: i.e. type I, these enzymes work on the end of the polymeric chain which are reducing in nature, and type II, enzymes which are included in this class act on the non-reducing site present at the end of cellulose polymer.

11.2.3 Cellobiases

Cellobiases are also called as β-glucosidases which digest exocellulase by hydrolysing them into monosaccharide units.

11.2.4 Oxidative Cellulases

This class of cellulases acts on unfolding of the polymeric chain which works on the radical reaction mechanism, where cellobiose dehydrogenase is used as an acceptor molecule.

11.2.5 Cellulose Phosphorylases

Cellulose phosphorylases are the enzymes which depolymerize the substrate; here, they use phosphates in place of water.

Cellulases can also be divided into different types on the basis of their characteristics such as nature of cleavage and bonding (Table 11.1).

Some enzyme belongs to cellulase family which are Exo-1, 4-β-D-glucanase (EC 32.1.91), *endo*-1, 4-β-D-glucanase (EC 3.2.1.4) and β-glucosidases (EC 3.2.1.21). The ends of the cellulosic chain can be digested by exoglucanase and produce β-cellobiose as an end product of the reaction; endoglucanases (EG)

Table 11.1 Types of cellulase and its basis of classification

Cellulase types	Basis of classification	Examples
Type I	According to cleavage site of cellulose	β-1,4 endoglucanase β-1,4 exocellobiohydralase
Type II	Presence of the carbohydrate binding modules (CBM) within their molecules	Glucosidase
Type III	It is classified on their primary structure	Glycoside hydrolases

Table 11.2 Presence of cellulase enzyme in the different habitats

Habitat	Organisms	Cellulase enzyme
Aquatic	Plants	Endocellulase, β-glucosidase
	Fungi	Endocellulase
	Eubacteria	Endocellulase, cellobiohydrolases, β-glucosidase
	Invertebrate animals	Endo/exocellulase
	Archaea	Endocellulase, β-glucosidase
Terrestrial	Fungi	Endocellulase, cellobiohydrolases, β-glucosidase
	Plants	Endocellulase
	Eubacteria	Endocellulase, cellobiohydrolases β-glucosidase
	Invertebrate animals	Endo/exocellulase
	Archaea	Endocellulase, β-glucosidase

aimlessly break O-glycosidic bonds internally, which resulted in the production of glucan chains with varying lengths; glucose can be produced by the action of β-glycosidase which use oligosaccharides, disaccharides and β-cellobiose as its substrate. The production of cellulase enzyme depends upon habitat and types of producing organism. Some organisms have the potential to produce either endo- or exocellulase, while few organisms have capability of producing both (Table 11.2).

11.3 Sources of Cellulase

All the microorganisms have potential to degrade the carbohydrates, but only some of them have the capability to degrade cellulose. Only a number of microorganisms have the potential to make cellulase naturally, so they have the ability to hydrolyse/consume cellulose as well. However, there are several studies which show that animals like molluscs, Nudibranchia, *Littorina littorea*, snails and few organisms of bivalves also have the potential to make cellulase. *Microbispora* and *Thermomonospora* are the actinomycetes that produce cellulase. In *Microbispora bispora*, cellulases were found to contain 5–7 types of enzymes, whereas in *Thermomonospora fusca*, cellulases have shown five types of enzymes. Accounting these reports, we can say that cellulases can be produced by different consortia of bacteria, plant cells, different fungal species and protozoans, etc. Fungi as well as

bacteria are the microorganisms which can be used for mass-scale production of cellulase as they produce higher amount of enzymes and have the capacity to secrete the whole enzyme complex extracellularly (Table 11.3). Some of the important fungal species used for cellulase production are *Penicillium pinophilum*, *P. funiculosum*, *Trichoderma reesei* and *Fusarium oxysporum*; apart from these species *Phanerochaete chrysosporium*, *Melanocarpus albomyces*, *Talaromyces emersonii* and some other fungi which are anaerobic in nature belonging from the genera *Caecomyces*, *Neocallimastix* and *Orpinomyces* have been well-known for their ability to produce cellulases.

11.3.1 Fungi

Most of the studies done on the fungi revealed that these are the main source of cellulase production and degradation of cellulose. The soft-rot fungi *Penicillium funiculosum*, *Trichoderma reesei*, *T. koningii*, *Fusarium solani* and *Talaromyces emersonii* and the fungal species *Phanerochaete chrysosporium* are intended for the production of cellulase (Saranraj et al. 2012). Presently, nearly all the cellulases that are produced at industrial level are mainly from aerobic cellulolytic fungi, for example, *Humicola insolens* or *Hypocrea jecorina* (*T. reesei*) (Wilson 2009).

The fungi which belong to white-rot group are to a certain extent heterogeneous; still they have the skill to reduce lignin in addition to extra lignocelluloses constituents (Rosenberg 1978). *Sporotrichum pulverulentum* is well-known white-rot fungus which produces one *exo*-1, 4-β-glucanase, two 1, 4-β-glucosidase and five *endo*-1, 4-β-glucanases as reported by Rosenberg (1978). Brown-rot fungi are deficient of *exo*-1, 4-β-glucanase activity; due to this reason, they do not degrade the crystalline cellulose but have the capability to depolymerize the cellulose (Rosenberg 1978). *Tyromyces palustris*, *Lanzites trabeum* and *Poria placenta* are the well-known among the brown-rot fungi that produced cellulase (Nurul and Kikuchi 2011; San Ryu et al. 2011).

The soft-rot fungus has the ability to diminish lignin as well as polysaccharides; though, the later ones are the core goals. The capability of this fungal sp. differs in generation of important extracellular enzymes for reducing the crystalline cellulose. According to Gusakov (2011), *Trichoderma reesei* is a popular complete enzyme-producing fungus. Other soft rot fungi which are *Penicillium citrinum*, *P. funiculosum*, *Fusarium oxysporum*, *F. solani* and *Trichoderma koningii* are widely utilized for the production of cellulase (Bansal et al. 2012; Bhatti et al. 2013; Ghoshal et al. 2013; Maeda et al. 2013; Wang et al. 2014; Xiros et al. 2011). The huge quantity of various cellulases secreted by *T. reesei* produced β-1, 4-glucosidases, two *exo* β-1, 4-glucanases and three *endo* β-1, 4-glucanases by solubilizing the crystalline cellulose with their mutual act (Peterson and Nevalainen 2012).

The requirement of special culturing and induction conditions restricts the application of traditional fungus for cellulolysis. To solve these limitations, nowadays, people have started adopting new strategies to enhance the quantity of production of

Table 11.3 Major microorganisms used in cellulase enzyme production (Kuhad et al. 2011; Salem et al. 2016; Singhania et al. 2017)

Group of microorganisms	Genus of organism	Species of organism
Fungi		
	Aspergillus	*A. niger*
		A. phoenicis
		A. aculeatus
		A. fumigates
		A. terreus
		A. saccharolyticus
		A. sydowii
		A. versicolor
	Humicola	*H. insolens*
		H. grisea
	Fusarium	*F. solani*
		F. fusosporium
		F. oxysporum
	Penicillium	*Penicillium occitanis*
		Penicillium brasilianum
		Penicillium decumbans
		Penicillium purpurogenum
		Penicillium janthinellum
		P. echinulatum
		P. fumigosum
	Phanerochaete	*P. chrysosporium*
	Neurospora	*N. crassa*
	Trichoderma	*Trichoderma reesei*
		Trichoderma harzianum
		Trichoderma viride
	Talaromyces	*T. emersonii*
	Paecilomyces	*P. thermophila*
		P. inflatus
	Gloeophyllum	*G. trabeum*
	Wolfiporia	*W. cocos*
	Laetiporus	*L. sulphurous*
	Piptoporus	*P. betulinus*
	Ceriporiopsis	*C. subvermispora*
	Psedotremella	*P. gibbosa*
	Irpex	*I. lacteus*
	Trichaptum	*T. biforme*
	Psedotremella	*P. gibbosa*
	Trametes	*T. versicolor*
		T. trogii
		T. ochracea
		T. pubescens

(continued)

Table 11.3 (continued)

Group of microorganisms	Genus of organism	Species of organism
	Funalia	*F. trogi*
	Pleurotus	*P. pulmonarius*
		P. sajor-caju
		P. dryinus
		P. ostreatus
	Coriolopsis	*C. polyzona*
	Cerrena	*C. maxima*
	Bjerkandera	*B. adusta*
	Lentinus	*L. edodes*
		L. tigrinus
	Pycnoporus	*P. sanguineus*
		P. coccineus
	Chaetomium	*C. cellulyticum*
		C. thermophilum
	Thermoascus	*T. aurantiacus*
	Coniophora	*C. puteana*
	Sporotrichum	*S. thermophile*
	Poria	*P. placenta*
	Tyromyces	*T. palustris*
	Fomitopsis	*F. cajanderi*
		F. rosea
		F. palustris
		F. incarnates
	Lenzites	*L. trabeum*
	Agaricus	*A. arvensis*
	Acremonium	*A. cellulolyticus*
	Daldinia	*D. eschscholtzii*
	Phlebia	*P. gigantea*
	Piromyces	*P. communis*
	Sclerotium	*S. rolfsii*
Bacteria		
	Acinetobacter	*A. amitratus*
		A. junii
	Acidothermus	*A. cellulolyticus*
	Clostridium	*C. acetobutylicum*
		C. cellulolyticum
	Cellulomonas	*C. biazotea*
	Bacillus	*B. subtilis*
		B. amyloliquefaciens
		B. licheniformis
		Bacillus flexus
		Bacillus circulans
	Pseudomonas	*P. cellulose*

Table 11.3 (continued)

Group of microorganisms	Genus of organism	Species of organism
	Cellvibrio	*C. gilvus*
	Eubacterium	*E. cellulosolvens*
	Rhodothermus	*R. marinus*
	Microbispora	*M. bispora*
	Paenibacillus	*P.curdlanolyticus*
	Salinivibrio	*S. costicola*
	Acetivibrio	*A. cellulolyticus*
	Butyrivibrio	*B. fibrisolvens*
	Fibrobacter	*F. succinogenes*
	Ruminococcus	*R. albus*
	Cellulomonas	*C. uda*
		C. fimi
Actinomycetes		
	Streptomyces	*S. lividans*
		S. cellulolyticus
		S. drozdowiczii
		S. antibioticus
	Thermomonospora	*T. fusca*
		T. curvata

cellulases in cellulolytic fungus to lower the manufacturing costs and to also optimize the recombinant expression system in microbes and plants (Lambertz et al. 2014). The earlier will enable to create the microbial strains by combination of different strains of microbes (Tsai et al. 2009) or inside the microbe's cell (Mazzoli et al. 2012) that expresses synergistically active sets of enzymes leading to low-cost enzyme production.

11.3.2 Bacteria

The enzyme produced by fungal cells has been more subject of study than those which are originated from bacteria, as it is well-known fact that the fungal-originated enzymes are more in quantity. Both anaerobic and aerobic bacteria are well-known cellulase producers. A great difference is seen between anaerobic and aerobic microbes with respect to their cellulase system, end products of biomass degradation and cell mass yield (Wei et al. 2009; Ariffin et al. 2008; Balasubramanian and Simões 2014; Ekperigin 2007). *Cellulomonas* (Lo et al. 2010; Rajoka and Malik 1997), *Acinetobacter* (Gupta et al. 2015) and *Clostridium* (Chinn et al. 2008; Desvaux et al. 2000; Dharmagadda et al. 2010; Lo et al. 2009; Thomas et al. 2014) are the higher cellulase-producing bacteria. Typically, 90–95% bacterial cellulose degradation is done by aerobic bacteria so it plays an important role in natural

system, while the leftover degradation is done by different bacterial strain under non-aerobic conditions (Carere et al. 2008). It is also shown that rumen bacteria produce cellulase enzymes which have the ability to disrupt the constituents of cell wall (Carere et al. 2008). *Fibrobacter succinogenes* of class Fibrobacteria (Béra-Maillet et al. 2009) and *Ruminococcus* albus from class Clostridia (Ohara et al. 2000) are the major reviewed rumen bacteria. The recent report has shown that thermophilic bacteria such as *Anoxybacillus* sp. (Liang et al. 2010), *Geobacillus* spp. (Rastogi et al. 2010), *Bacillus* spp. (Rastogi et al. 2010) and *Bacteroides* spp. (Ponpium et al. 2000) are having cellulolytic activity.

It was observed that *Bacillus subtilis* Pa5 expresses a high level of endoglucanases (approximately 46–49 IU/g of the biomass) and cellobiose (~152–155 IU/g of biomass) (Shu-bin et al. 2012). *E. coli* ATCC25922, *Rhizobium* sp. DASA23010 and *Bacillus subtilis* ATCC6633 were grown on diverse farming litters such as vegetable residue, pineapple peel and corncob to compare the productivity of cellulases. The production of cellulase was found maximum when corncob was used as a substrate (Sudto et al. 2008). The maximum activity equivalent to CMCase (9.6 IU/g), FPAse (2.8 IU/g) and cellobiose (4.5 IU/g) was obtained when grown on a lignocellulosic material. With the use of the same material, Krishna (1999) has obtained 12 times increased yield in the production with SSF in comparison to SmF.

11.4 Synthesis of Cellulase

Cellulases act on cellulose, oligosaccharides and cellobiose for their breakdown and on other carbohydrate metabolisms which act as inducers for cellulose-producing microorganisms (Szakmary et al. 1991). Enzyme produced from conidia of *Trichoderma* commences the degradation of cellulose by the use of cellobiohydrolases that produce cellobiose; this cellobiose is utilized by fungus to induce cellulase synthesis and secretion (Kubicek et al. 1993); however, it is supposed that the induction may be due to β-glycoside, but there is no proof in this regard. Some inducer molecules such as sophorose are vital at lower concentrations, while other inducing molecules are required at higher concentrations (Ryu and Mandels 1980). In bacteria, cellulases are cell associated with unit accumulates known as cellulosomes (Lamed et al. 1983). Around 0.1 g/L concentration of glucose acts as repressor for expression of cellulases (Ryu and Mandels 1980). According to El-Gogary et al. (1989), repression is supposed to take place during pre-translational steps. When *Trichoderma reesei* was cultured in the presence of glucose (5–7 g/L), cellulase production is observed after complete consumption of glucose. Repression in the case of *Acidothermus cellulolyticus*, a type of thermophilic bacterium, caused by sugars metabolized products like glucose, which is relieved by adding of exogenous cAMP (Shiang et al. 1991).

11.5 Cellulase Production Technologies

There are various technologies for cellulase production from different sources, which vary from cellulase production through improved cellulose-producing organisms, batch cellulase production, fed-batch cellulase production, etc. However, high cost of the different methods is a main factor which hinders the industrial cellulase production (Fig. 11.1).

11.5.1 Fermentation for Cellulase Production

Fermentation is one of the methods which is used for production of different forms of cellulases. Fermentations are a metabolic process that converts sugar to alcohol, gases, or acids; it occurs in bacteria and yeast. The process of fermentation is strictly a temperature and pH dependent, and its efficiency is maximum at a particular temperature. Approximately 28 °C is the ideal temperature for the production of fungal cellulase, since most of the cellulase-producing fungi are mesophilic, whereas thermophilic bacteria prefer high temperatures for the growth and development around 52–56 °C, but more inconsistencies are found in the pH value, which varies between 3.1 and 6.9. The production of cellulases varies with a change in pH, e.g. mutant variety of *Trichoderma reesei* Rut C30 has high efficiency of cellulase production at the pH of 5; contrary to this, efficiency of an actual cellulase component, β-glucosidases, was maximum at 6.2 (Tangnu et al. 1981).

11.5.2 Cellulase Production Through Improved Cellulase-Producing Organisms

This technology is making an attempt to make the production of cellulases more economical through hyperproducing mutant form of cellulolytic microorganisms which have been generated with the help of mutagenic factors or ultraviolet (UV) light. These results in high enzyme production as maximum 428 IU/L h^{-1} have been achieved through fed-batch fermentations of *Trichoderma reesei* (Watson et al. 1984). Amylases have 100-fold higher specific activities as compared to cellulases. Thus, work is carried out to increase the activity by mutation of strain to make more active cellulase enzymes, catalytic turnover number of active sites raised by site-specific mutagenesis and collection of novel microbes. Specific activity of a mutant

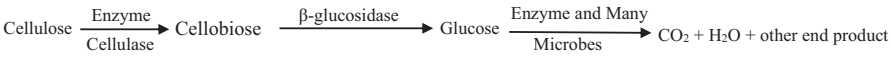

Fig. 11.1 Mechanism and production of enzyme

strain of *Trichoderma* was threefold higher for β-glucosidases as compared to parental strain. Besides these, another mutant strain of *Trichoderma reesei* has been identified to produce cellulase 3–7 times higher rates without any effect on the composition of cellulase enzyme (0.5–2% β-glucosidases, 35% endocellulase and 65% exocellulase).

11.5.3 Batch Cellulase Production Process

This technology is the most common process for the cellulase production because it is easy to operate and control. Concentrations of cellulases are about 14,000–16,000 IU/L, and the observed enzymatic harvests were 290 IU/g of cellulose (Table 11.4). The activity of enzyme was maximum when *T. reesei* was grown at 59 g/L concentration of roll-milled cotton cellulose. In the presence of lactose (60 g/L), a sole C-source, and yeast extract (1 g/L), N-source, *Trichoderma reesei* CL-847 was studied for cellulase productivity on pilot scale in 3000-L fermenter, and in addition to these, cellulose (5 g/L) is also used as inducer. After 1 week of culture, overall yield of enzyme was 162 IU/g, and carbon source and concentration of cellulase reached up to 10,500 IU/L (Knappert et al. 1980). *Acidothermus cellulolyticus* has been considered for the process of batch cellulase production (Shiang et al. 1990). Various sources of carbon were tested for cellulase production with thermophilic bacteria, and it was found that it produced cellulase enzymes only when cultivated on xylose, cellobiose and cellulose with specific growing rates. Productivity and concentrations of bacterial cell have capacity to increase cellulase production proportionally to the concentration of cellobiose (Table 11.5).

11.5.4 Fed-Batch Cellulase Production Technology

This technology is very popular for producing cellulase; it has several advantages over the continuous or batch processes such as high productivity and yield per unit mass of cellulosic biomass used and high concentration of cellulase enzyme. These are mostly used due to additional capacity to use cellulosic system in the fed-batch process by altering the feeding rates of the medium and cellulosic biomass/systems.

Table 11.4 Cellulase production on various cellulosic systems using batch fermentation process

Cellulosic system	Cellulase concentration (IU/mL)	Cellulase enzyme yields (IU/g)	Productivity (IU/L h^{-1})
Printed news paper	0.7	48	19
Mixed waste paper	1.1	64	17
Municipal waste	2.5	130	29
Wheat straw	1.9	151	31
Wheat straw (NaOH treated)	2.0	133	25

Table 11.5 Production of cellulase by using thermophilic bacteria and fungi grown on some cellulosic biomass

Microorganism	Cellulosic biomass	Cellulase concentration (IU/L)	Cellulase productivity (IU/L h^{-1})
Trichoderma reesei L27	Avicel	18,000	93.7
Trichoderma reesei Q M 9414	Solka floc	2600	21.7
Acidothermus cellulolyticus	Solka floc	105	1.5
Thermomonospora fusca	Avicel	150	5.1
Thermomonospora curvata	Cellulose	100	1.4

Higher cellulosic biomass concentration can be observed in batch fermentation process significantly decreased the feeding rate of cellulosic biomass to the fermentation process. These processes are applied by using microorganism with cellulosic biomass to produce cellulase enzyme and check their productivity and yields.

11.5.5 Continuous Cellulase Production

Some researchers have interest to apply continuous processes for attending high efficiency and productivity than batch process for cellulase production. Researchers investigated the cellulase production by using two-stage continuous processes and the productivity of cellulases via *Trichoderma reesei* MCG-77 cultured in 50–100 g/L defined medium of lactose, $(NH_4)_2SO_4$ and mineral salts in 15-L fermenters (Ryu and Mandels 1980). In two-stage cellulase production, different experimental setup conditions used were (1) for growing of cell 89.6 °F temperature, pH 4.5 (acidic), 650–850 revolution per minute (rpm) agitation and 0.4–0.6 vvm aeration and (2) for synthesis of cellulase 82.4 °F temperature, pH 3.5 (acidic), 450–650 revolution per minute (rpm) agitation and 0.2–0.3 vvm aeration. Cell recycle is an alternate option for increasing the cellulase productivity at high rates of dilution, 0.065 and 0.028, for one- and two-stage continuous system, respectively (Watson et al. 1984). When it operates at high rates of dilution, then its residence times and cost are reduced.

11.5.6 Downstream Process for Production of Cellulase

In present scenario, industrial cellulase production is practiced worldwide, mainly in the suspension cultures, while the use of solid-state fermentations is regularly being eliminated (Esterbauer et al. 1991). In large- and pilot-scale fed and batch

processes, the fermentation completed soon after peak productions have been achieved, and the broth is quickly frozen to 5–10 °C for preserving activities of cellulase (Ryu and Mandels 1980). Several types of downstream processing used for the cellulases depend on the intended use for cellulase. Schell et al. (1990) demonstrated in *T. reesei* that the introduction of whole broth in SSF units was valuable.

11.6 Uses of Cellulase in Industries

Cellulases have progressively vast area of application including in various industries, i.e. food, textile, pulp and paper industries; besides this, they have been used for many other purposes like for improving the digestibility of animal feeds and as an additive to detergents (Table 11.6). This much use of single group of enzyme gives it a momentous share in the enzyme industry globally. In present scenario, the production of cellulase from different sources was done by recombinant technology, and it is proved as one of the best technologies for producing cellulase. Following are the important activities which increase the cellulase production by input of recombinant technology. Cellulases decrease viscosity, accelerate colour release, improve flavour, improve filtration, improve taste, improve maceration, enhance aroma and enhance fermentation processes and products.

The requirements of cellulases are regularly increasing day by day as of its various utilities. Now, many industries are being tangled in manufacturing of cellulase for different uses in different industries.

11.7 Need of Recombinant Technology

Some of the technologies used for the cellulase production by different researchers and industries have many limitations (Singhania et al. 2007). These limitations demand for the evolution of novel techniques of cellulase production. Recombinant technologies have capability to improve the production of biological materials which have been tested in the past, and this technology can be helpful in increasing the production of cellulases. Researchers and industries are focussing on recombinant technology because with this technology, one can achieve gigantic amount of product at larger scale. With the help of this technology, the quality and quantity of cellulases can be enhanced. Cellulases are also a matter of interest in agriculture, bioenergy and biotechnology (Phitsuwan et al. 2013), especially in the production of nonconventional liquid biofuels such as biodiesel, butanol, ethanol or other fermentative sugar products by the use of cellulosic biomass (Bhat 2000). Therefore, cellulase has the capacity to become a major group of commercially used cellulase enzyme worldwide (Wilson 2009). Cellulosic biomass is one of the three main components of lignocelluloses (lignin and hemicelluloses are other two components) which perform a key character as a substrate in the process of constructing biofuel,

Table 11.6 Uses of cellulases in various industries

Industry	Function	Application
Agriculture	Fungal or plant cell wall solubilisation, inhibits the elongation of germ tube, spore germination and fungal growth	Plant or fungal protoplast production, mutant and hybrid strain constructions, increase fertility of soil and plant growth, protection of plants from pathogen and diseases, enhance flowering and plant growth
Brewery and wine	Hydrolysis of polysaccharide present in cell wall of plants, alteration of aromatic remainder	Enhancement of colour; extraction and skin maceration of grapes; stability, quality, clarification and filtration of wines; enhance aroma
Food industry	Cell wall constituent hydrolysis, decreases the viscosity and maintains the quality of juice	Fruit juice extraction, in colouring agent of food, development of quality of bakery products and texture, enhance flavour, volatile properties of vegetables and fruits, controlling sourness of citrus fruits
Pulp and paper	Alteration of characteristics of hand sheet strength and coarse mechanical pulp; in the removal of ink from fibre and hydrolysis of carbohydrate molecules	Enhance drainage of the paper mills, improve the high fibre qualities and tensile strength, construction of soft sanitary papers and towels
Textile and laundry	Break the tiny fibre ends available on the cotton fabric, removal of dye, removal of pilling and fuzz formation, bio-polishing of non-denim fabric and cotton	Bio-stoning of jeans, garments softening, enhance ability of cellulosic fabrics, recyclable washing powders, making of good standard fabrics, biofinishing
Animal feed	Partially hydrolyse the lignocellulosic materials, cleavage of β-glucans, better flexibility and emulsification of nourishing materials	Enhancement of the dietary standard of animal fodder, large intestine decreases the colonization of pathogenic bacteria, to gain the high weight by hens and broiler chickens
R&D industries	Gene fusion and conjugation, heterologous expression of proteins, affinity systems	Fusion and immobilization of proteins, enzymes and antibodies; affinity purification, for various use hybrid molecules produced, high-level production of proteins, enzymes and antibodies
Biofuel industry	Glucose and other fermentable sugars are formed from cellulosic material with the help of cellulase	In the production of fermentation products like ethanol and single cell protein
Waste management	Degrade cellulosic wastes	In the reduction of pollution
Pharmacy industries	Digest the cellulosic fibre	Digestion preparation, quickly hydrolysed the hemicellulose, cellulose and β-glucan polymers present in food

Modified from Behera et al. (2017)

i.e. biodiesel and bioethanol, due to the availability of ample raw material throughout the world. The group of cellulase enzymes is ubiquitous that exists in plants, bacteria, insects and fungi (Duan and Feng 2010; Fischer et al. 2013; Watanabe and Tokuda 2010).

Cellulolytic enzyme which is produced by aerobic or anaerobic bacteria either as single enzyme or in cellulosome form that is complex of multienzyme constitutes numerous cellulolytic cellulase enzymes, sticked to skeleton protein (Bayer et al. 2007). However, most common industrially available cellulases which were isolated from fungal cells especially from *Trichoderma* or *Aspergillus* species are noncomplex native enzyme mixtures. Fungi as enzyme producers have more advantages in production and secretion pathways for cellulose production (Phitsuwan et al. 2013). From engineered *Trichoderma reesei* strains, about 100–110 grams (g) of crude cellulases are obtained per litre (Wilson 2009; Peterson and Nevalainen 2012). In addition, some species of other genera of fungi such as *Chrysosporium*, *Penicillium* and *Acremonium* are also used as alternative to *Trichoderma* (Gusakov 2011). In the transfiguration of biomass to biofuels at industrial level, numerous difficulties need to be defeated. The United States National Renewable Energy Laboratory (NREL) evaluated that continued enhanced manufacturing cost of cellulases constitutes up to 20% of the entire ethanol production (Phitsuwan et al. 2013) and minimizes production efficiency on a commercial level. Traditionally used fungal strains for degradation of cellulase need special conditions to grow and face restriction by induction. To get over these limitations, scientists are trying their level best for optimum production at lowest cost. The overall scenario of cellulase production demands for application and use of recombinant technology to enhance the cellulase production.

11.8 Application of Recombinant Technology in Cellulase Industries

The ability of isolation, characterization and modification of genes has become a very powerful method to study the living organisms and their functional elements. Because these methods directly concern with the gene (DNA) and the enzyme (polypeptide) so it has certain limitation. More information is needed concerning the molecular biology and biochemistry of a given system to ensure the successful use of recombinant DNA technology (Knowles et al. 1987). Random mutagenesis, site-specific mutagenesis or its combined effect has been utilized for enhancing the manufacturing of cellulase at industrial scale (Dalby 2007). Consequently, different protocols are being developed and are under supervision for cellulases and glycosyl hydrolase (GH) (Table 11.7). Many mutant strains of *T. reesei* (*RUT C 30* and *CL-847*) are used for mass production of cellulase; these mutant strains were originated from the wild strain of *Trichoderma reesei* QM 6a, which was first isolated by the United States Army Research Laboratory (Esterbauer et al. 1991). Few recent

Table 11.7 Expression of protein in different types of host organisms and its advantages, disadvantages and challenges

Organism	Example	Protein expression	Advantages	Disadvantages	Expression challenges
Bacteria	*E. coli* (gram-negative bacterium)	Protein expression up to 11.23–90.10 mg/L (Garvey et al. 2013)	1. Used by industries 2. *E. coli* is a commercially available strain and can be cloned in most vectors 3. It can be modified for protein engineering and has well-characterized genetic material	1. The outer membrane of gram-negative bacteria confines the protein excretion	1. In case of multidomain cellulase, linker sequences are degraded 2. Inclusion bodies formed 3. Often wrong transportation occurs through outer membrane, and the specific activity of enzyme also decreased
Yeast	*Saccharomyces cerevisiae*	Approximately 1000 mg/L crude enzyme solution (Garvey et al. 2013)	1. Protein secretor 2. Due to surface display characteristics, it is mostly used in industry	1.Hyperglycosylation 2.The expression rates of recombinant are lower than the native systems	1. Inducible systems are greatly organized; however it can be costly 2. Due to greater epitomal gene copy numbers, protein production is high, but a continuous selection is required
Plants	1. *Zea mays*	Around 0.47% of dry weight (Hood et al. 2012)	1. Low-cost protein produced 2. Biomass and enzyme produced instantaneously 3. Used in biofuel production	1. Carrying of genetic information by pollen grains 2. Transformation procedure long	1.Glycosylation may occur 2. For expression efficiency, subcellular targeting is very important inside the plant cell
	2. *Nicotiana tabacum*	Approximately 41% of total solubilized protein, depending on the subcellular targeting within plant cell (Garvey et al. 2013)	1. Production in one system 2. Non-edible 3. Low-cost protein production	1. Genetic information transport through pollen grains (if not transplastomic) 2. Transformation required long time	1. For expression efficiency, subcellular targeting is very important inside the plant cell 2. Possibly affects the plant growth behaviour 3. Possible glycosylation may occur

examples of recombinant cellulases are described in Table 11.8. To increase the cellulase production, various exercises have been done. The molecular modelling of *Egl-237* (this is an endoglucanase isolated from a *Bacillus* strain KSMS-237) was by using the homology modelling and crystalline structure of alkali cellulase (*Bacillus* sp. *KSM635*); six amino acids from 357 to −362 (G-K-S-N-A-T) were substituted by three-amino acid peptide chain in the loop region.

Three different peptide chains were used for generating enzyme options; these peptide chains were containing series of amino acids A-R-A, A-G-A and A-H-A. The short peptide chains which were expressed in the *B. subtilis* were isolated and tested for their enhanced properties. The wild-type variants of the enzyme show maximum activity at pH 9, while the improved enzyme having A-G-A amino acid sequence obtained its highest activity at pH 10. Leftover two cellulase variants showed 95% improvement in specific activity and were active at pH 9.6.

The new variety of cellulase 11AG8 was discovered from *Actinomycetes* sp. and recognized as a CMCase having 386 long amino acid chains with catalytic core and a CMB. The catalytic core which consists of 221 amino acid sequences has the cellulosic function, so this particular sequence was expressed in the *Streptomyces* sp. for use in textile work (Wang and Bao 2009). In another study, a heterologous gene construct was made with catalytic core and linker of *T. reesei cbh1* joined with *Aspergillus cellulolyticus* endoglucanase catalytic core and functionally expressed in *Trichoderma reesei*. This recombinant form of protein was functionally more active which can convert 20% cellulose within 6 h (Bower et al. 2012). A new strain of *Trichoderma reesei* was created by Zhang et al. (2010) with the use of overexpression of β-glucosidase under the control of cbh 1 promoter. These recombinants show enhanced levels of β-glucosidase production and have FPase activity. Likewise, Miettinen-Oinonen et al. (2005) have reported 1.5 times and 4 times enhancement in the expression of CBHI and CBHII, respectively; here two cellobiohydrolases (CBHI and CBHII) were overexpressed with extra copies of genes under control of the cbh 1 promoter. When endoglucanases isolated from different microbes, i.e. *Aureobasidium pullulans*, *Gloeophyllum trabeum* and *Sporotrichum thermophile*, were inserted in *A. niger*, they have shown higher expression activity (≥ 0.4 g/L). ApCel5A (isolated from *Aureobasidium pullulans*) hydrolysed carboxymethyl cellulose (CMC) five times faster, while StCel5A hydrolysed two times faster, as compared to *Trichoderma reesei* endoglucanases Cel5A.

The recombinant form of these strains might be a good source for cellulolytic enzyme development as evident from the works of Tambor et al. (2012). In *Aspergillus niger*, Cel7A exoglucanase from the *Trichoderma reesei* was expressed, and *Aspergillus niger* was capable to develop on consumed bagasse hydrolysate and consumed *Picea* hydrolysate with higher endoglucanases activity than *Trichoderma reesei*. The cellulase gene cloned in *Aspergillus niger* was under constitutive promoter to get rid of the glucose repression in *Trichoderma reesei* strains (Alriksson et al. 2009). In *Penicillium funiculosum*, three *N*-glycan sites were removed by rCel7A which results in 35% increase in enzyme activity, whereas in *A. niger*, 85% activity is enhanced by adding of *N*-glycan at Asp-195 through mutation of Ala-196 to serine. Jeon and co-workers (2009) reported the expression of BGL1 from *S. fibu-*

Table 11.8 Expression of cellulase and their activity levels when produced in different organisms like bacteria, fungi and plants

Recombinant expression host	Gene	Type of enzyme	Source organism	Vector used	Enzyme property	Reference
Bacteria						
E. coli BL21	*CelDR*	Endoglucanase	*B. subtilis DR*	pET-28a	1. The expression of enzyme increases from 0.27 U/ml to 0.82 U/ml 2. Size 55 kDa and maximum activity at 50 °C under pH 6.5	Li et al. (2008)
E. coli	*Xf818*	Endoglucanase	*Xylella fastidiosa*	pET20b and pET28b	1. Activity 2.39 μKat 2. Size 60 kDa, optimum temperature 65 °C and pH 5.2	Wulff et al. (2006)
E. coli	*exg*	Exoglucanase	*Azoarcus* sp. *strain BH72*	pUC19	Activity 30 U/mg of protein	Reinhold-Hurek et al. (1993)
E. coli DH5α	*EglA*	Endoglucanase	*Bacillus* sp.	pUC18	1. 5.2-fold higher activity than the wild type 2. Size 65 kDa; the enzyme was stable up to 70 °C and pH 6.0–8.0	Tang et al. (2009)
E. coli BL21	*EG1*	Endoglucanase	*Rhizopus stolonifer* var. *reflexus TP -02*	pET 20b	1. Activity 0.715 IU/ml 2. Size 40 kDa	Tang et al. (2009)
E coli JM109	*lacZ*	β-galactosidase	*Planococcus*	pΔα18	1. Size 75 kDa, optimum pH 6.5 and temperature 42 °C; enzyme remained active at high salt concentrations, makes reporter enzyme halo tolerant	Sheridan and Brenchley (2000)
E. coli EC100	*cel1A*	CMCase	*Neocallimastix* sp.	pCT	1. Activity 2.06 U/mg 2. Size 44.8 kDa, optimum temp. 50 °C and pH 6.0	Comlekcioglu et al. (2010)
E. coli BL21	*Cel I 15*	Cellulase	*Bacillus subtilis I 15*	pET 25b	1. Wild type show 2.82 U/ml activity, while recombinant show three times higher, i.e. 8.54 U/ml 2. Size 52 kDa, optimum temp. 60 °C and pH 6.0	Yang et al. (2010)

(continued)

Table 11.8 (continued)

Recombinant expression host	Gene	Type of enzyme	Source organism	Vector used	Enzyme property	Reference
B. subtilis	*cel12A*	CMCase	*Streptomyces* sp.	pHPLT	1. Activity 8–10 units/ml 2. Size 38.5 kDa, optimum temp. 50 °C and pH 8	Solingen et al. (2001)
E. coli DH5α	*Cel5D*	Endoglucanase	*Martilella mediterranea*	pUC 18/pGEX	1. Activity 1.6 U/mg 2. Size 40.5 kDa, at temp. 60 °C and pH 5.0, enzymes show maximum activity	Dong et al. (2010)
Fungi						
Pichia pastoris	*CsCelA*	Endocellulase	*Ciboria shiraiana*	pPIC9K	1. Activity 17.44 U/ml 2. Size 55.3 kDa, optimum temp. 45 °C and pH 4.0–9.0	Lü et al. (2015)
Pichia pastoris	*bgl1*	β-Glucosidase	*Thermoascus aurantiacus*	pPICZα	1. Activity 3.9 U/ml 2. Size 116 kDa, optimum temp. 70 °C and pH 5	Hong et al. (2007)
Trichoderma reesei	*cel3a*	β-Glucosidase	*Talaromyces emersonii*	Lambdagem-11	1. Activity 2.7 mg/l 2. Size 90.59 kDa, optimum temp. 71.5 °C and pH 4.02	Murray et al. (2004)
Pichia pastoris	*cbh3*	Cellobiose hydrolase	*Chaetomium thermophilum*	pMD 18 T	1. Activity 2.5 units/ml 2. Size 48 kDa, optimum temp. 60 °C and pH 5.0	Li et al. (2009)
Pichia pastoris	egl5A	Carboxymethyl cellulase	*Cryptococcus* sp. S-2	pPIC3	1. Activity 4.36 U/mg of protein (657-fold higher) 2. Size 34 kDa. Optimum temp 40–50 °C and pH 3.5	Thongekkaew et al. (2008)
P. pastoris X33	bgl3	β-Glucosidase	*Aspergillus fumigatus Z5*	pPICZaA,	1. Activity 101.77 ± 5.2 U/mg of protein (3.5-fold) 2. Size 91.47 kDa optimal activity at pH 6.0 and 60 °C	Liu et al. (2012)

Recombinant expression host	Gene	Type of enzyme	Source organism	Vector used	Enzyme property	Reference
Plants						
Nicotiana tabacum and *Zea mays*	Cel5A	Endocellulase, E1	*Acidothermus cellulolyticus*	*A. tumefaciens*	1. Activity 3.1 µg/mg biomass 2. Optimal activity at 80 °C	Brunecky et al. (2011)
Oryza sativa L	*E1* gene	β-1, 4-endoglucanase	*Acidothermus cellulolyticus*	*A. tumefaciens*	1. Recombinant shows 20-fold higher expression 2. Size 38 kDa optimal temp. 81 °C	Chou et al. (2011)

ligera and EglE of *Clostridium thermocellum* in the budding yeast. After preculturing in artificial galactose nutrient media and excess washing in minimal nutrient media, the resulting yeast strain can make ethyl alcohol from β-D-glucan (9.78 g/L in 16 hours), CMC (8.56 g/L in 16 hours) and phosphoric acid swollen cellulose (7.16 g/L in 36 hours). By means of yeast which co-displayed the *Aspergillus oryzae* gene BGL and an EGL, SSF study (Kotaka et al. 2008) reported from barley β-D-glucan, 7.94 g/L ethyl alcohol was produced during 24 hours. Transgenic plant of tobacco (*Nicotiana tabacum* cv. SR1) developed by Jung et al. (2013) accumulated *BglB* cellulose of *Thermotoga maritima* which was driven by *Medicago sativa* RbcsK-1A promoter and enclosed a small subunit of the rubisco complex transpeptide. This enhancement in *BglB* expression which takes place by transgenic plants illustrates the probability in increment of yield of cellulases in plants.

11.9 Future Use of Recombinant Technology in Cellulase Industries

The demand for cellulases is increasing due to the rise in oil price and volatile political scenario; therefore, researchers nowadays are interested to use the cellulases in the formation of biofuel (biodiesel, bioethanol) from cellulosic biomass (Kuhad et al. 2016). Identification of current systems for cellulase expression might be the crucial factor in the success of second-generation biofuel production. It will help to produce good quality of products in industries. Codon optimizes co-expression of helper proteins, screening multicopy strains with high expression levels and cell organelle; engineering all these strategies is worth considering to improve the production of enzyme level; therefore, industries keep focusing on this technology (Juturu and Wu 2014).

11.10 Conclusion

Cellulase is one of the most important groups of enzymes catalysing conversion of cellulase into glucose. Because of its diverse application and utility, the demands for cellulases are increasing, while the production is facing some serious limitations. Various technologies are available in the market; however, these technologies are quite costly. For the quick and efficient production of cellulases, the application of recombinant technology is getting the attention of researchers worldwide. Application of this technology is opening a diverse area for cellulase production. Under several studies, scientists were able to incorporate foreign genes from bacteria and fungi to enhance the production of cellulase. Major contributions are made

through the intended recombinant cellulosomes for optimizing the ratio and arrangement of enzymes through artificial complexes.

Although there are several paths to transform cellulose as useful products, still there is gap in advanced and efficient process for conversion of cellulose to industrial products. By the help of the recombinant technology, bacteria expressed the cellulase protein up to 11.2–90 mg/L, and yeast expressed the cellulase approximately 1000 mg/L; out of the soluble protein, only 40% is expressed as cellulase in plants. Encouragement in the production of celluloses by means of recombinant technology is significant and effective by characterization and utilization of non-native enzymes in desired organism. The application of this technology will reduce the crisis of fuel production.

References

Alriksson B, Rose SH, van Zyl WH, Sjöde A, Nilvebrant NO, Jönsson LJ (2009) Cellulase production from spent lignocellulose hydrolysates by recombinant *Aspergillus niger*. Appl Environ Microbiol 75(8):2366–2374

Ariffin H, Hassan MA, Shah UK, Abdullah N, Ghazali FM, Shirai Y (2008) Production of bacterial endoglucanase from pretreated oil palm empty fruit bunch by *Bacillus pumilus* EB3. J Biosci Bioeng 106(3):231–236

Balasubramanian N, Simões N (2014) *Bacillus pumilus* S124A carboxymethyl cellulase; a thermo stable enzyme with a wide substrate spectrum utility. Int J Biol Macromol 67:132–139

Bansal N, Tewari R, Soni R, Soni SK (2012) Production of cellulases from *Aspergillus niger* NS-2 in solid state fermentation on agricultural and kitchen waste residues. Waste Manag 32(7):1341–1346

Bayer EA, Lamed R, Himmel ME (2007) The potential of cellulases and cellulosomes for cellulosic waste management. Curr Opin Biotechnol 18(3):237–245

Behera BC, Sethi BK, Mishra RR, Dutta SK, Thatoi HN (2017) Microbial cellulases diversity & biotechnology with reference to mangrove environment: a review. J Genet Eng Biotechnol 15 (1):197–210

Béra-Maillet C, Mosoni P, Kwasiborski A, Suau F, Ribot Y, Forano E (2009) Development of a RT-qPCR method for the quantification of *Fibrobacter succinogenes* S85 glycoside hydrolase transcripts in the rumen content of gnotobiotic and conventional sheep. J Microbiol Methods 77(1):8–16

Bhat MK (2000) Cellulases and related enzymes in biotechnology. Biotechnol Adv 18(5):355–383

Bhatti HN, Batool S, Afzal N (2013) Production and characterization of a novel (beta)-glucosidase from *Fusarium solani*. Int J Agric Biol 15(1):140–144

Bower BS, Larenas EA, Mitchinson C, inventors; Danisco US Inc, assignee (2012) Exo-endo cellulase fusion protein. United States patent US 8,097–445

Brunecky R, Selig MJ, Vinzant TB, Himmel ME, Lee D, Blaylock MJ, Decker SR (2011) In planta expression of *A. cellulolyticus* Cel5A endocellulase reduces cell wall recalcitrance in tobacco and maize. Biotechnol Biofuels 4(1):1

Carere C, Sparling R, Cicek N, Levin D (2008) Third generation biofuels via direct cellulose fermentation. Int J Mol Sci 9(7):1342–1360

Chinn MS, Nokes SE, Strobel HJ (2008) Influence of moisture content and cultivation duration on *Clostridium thermocellum* 27405 end-product formation in solid substrate cultivation on Avicel. Bioresour Technol 99(7):2664–2671

Chou HL, Dai Z, Hsieh CW, Ku MS (2011) High level expression of *Acidothermus cellulolyticus* β-1, 4-endoglucanase in transgenic rice enhances the hydrolysis of its straw by cultured cow gastric fluid. Biotechnol Biofuels 4(1):58

Comlekcioglu U, Ozkose E, Tutus A, Akyol I, Ekinci MS (2010) Cloning and characterization of cellulase and xylanase coding genes from anaerobic fungus *Neocallimastix sp.* GMLF1. Int J Agric Biol 12(5):691–696

Dalby PA (2007) Engineering enzymes for biocatalysis. Recent Pat Biotechnol 1(1):1–9

Desvaux M, Guedon E, Petitdemange H (2000) Cellulose catabolism by *Clostridium cellulolyticum* growing in batch culture on defined medium. Appl Environ Microbiol 66(6):2461–2470

Dharmagadda VS, Nokes SE, Strobel HJ, Flythe MD (2010) Investigation of the metabolic inhibition observed in solid-substrate cultivation of *Clostridium thermocellum* on cellulose. Bioresour Technol 101(15):6039–6044

Dong J, Hong Y, Shao Z, Liu Z (2010) Molecular cloning, purification, and characterization of a novel, acidic, pH-stable endoglucanase from *Martelella mediterranea*. J Microbiol 48(3):393–398

Duan CJ, Feng JX (2010) Mining metagenomes for novel cellulase genes. Biotechnol Lett 32(12):1765–1775

Ekperigin MM (2007) Preliminary studies of cellulase production by *Acinetobacter anitratus* and *Branhamella* sp. Afr J Biotechnol 6(1):28–33

El-Gogary S, Leite A, Crivellaro O, Eveleigh DE, El-Dorry H (1989) Mechanism by which cellulose triggers cellobiohydrolase I gene expression in *Trichoderma reesei*. Proc Natl Acad Sci 86(16):6138–6141

Esterbauer H, Steiner W, Labudova I, Hermann A, Hayn M (1991) Production of *Trichoderma* cellulase in laboratory and pilot scale. Bioresour Technol 36(1):51–65

Fischer R, Ostafe R, Twyman RM (2013) Cellulases from insects. In: Yellow biotechnology II. Springer, Berlin, Heidelberg, pp 51–64

Garvey M, Klose H, Fischer R, Lambertz C, Commandeur U (2013) Cellulases for biomass degradation: comparing recombinant cellulase expression platforms. Trends Biotechnol 31(10):581–593

Ghoshal G, Banerjee UC, Shivhare US (2013) Optimization of cellulase (EC 3.2. 1: 4) production using *Penicillium citrinum* MTCC 9620 in solid state fermentation. Br Biotechnol J 3(4):509–523

Gupta M, Sharma M, Singh S, Gupta P, Bajaj BK (2015) Enhanced production of cellulase from *Bacillus licheniformis* K-3 with potential for saccharification of rice straw. Energ Technol 3(3):216–224

Gusakov AV (2011) Alternatives to Trichoderma reesei in biofuel production. Trends Biotechnol 29(9):419–425

Hong J, Tamaki H, Kumagai H (2007) Cloning and functional expression of thermostable β-glucosidase gene from *Thermoascus aurantiacus*. Appl Microbiol Biotechnol 73(6):1331–1339

Hood EE, Devaiah SP, Fake G, Egelkrout E, Teoh K, Requesens DV, Hayden C, Hood KR, Pappu KM, Carroll J, Howard JA (2012) Manipulating corn germplasm to increase recombinant protein accumulation. Plant Biotechnol J 10(1):20–30

Jeon E, Hyeon JE, Suh DJ, Suh YW, Kim SW, Song KH, Han SO (2009) Production of cellulosic ethanol in *Saccharomyces cerevisiae* heterologous expressing Clostridium thermocellum endoglucanase and *Saccharomycopsis fibuligera* β-glucosidase genes. Mol Cells 28(4):369

Jung S, Lee DS, Kim YO, Joshi CP, Bae HJ (2013) Improved recombinant cellulase expression in chloroplast of tobacco through promoter engineering and 5′ amplification promoting sequence. Plant Mol Biol 83(4–5):317–328

Juturu V, Wu JC (2014) Microbial cellulases: engineering, production and applications. Renew Sust Energ Rev 33:188–203

Knappert D, Grethlein H, Converse A (1980) Partial acid hydrolysis of cellulosic materials as a pretreatment for enzymatic hydrolysis. Biotechnol Bioeng 22(7):1449–1463

Knowles J, Lehtovaara P, Teeri T, Penttilä M, Salovuori I, Andre L (1987) The application of recombinant-DNA technology to cellulases and lignocellulosic wastes. Philos Trans R Soc 321(1561):449–454

Kotaka A, Bando H, Kaya M, Kato-Murai M, Kuroda K, Sahara H, Hata Y, Kondo A, Ueda M (2008) Direct ethanol production from barley β-glucan by sake yeast displaying *Aspergillus oryzae* β-glucosidase and endoglucanase. J Biosci Bioeng 105(6):622–627

Krishna C (1999) Production of bacterial cellulases by solid state bioprocessing of banana wastes. Bioresour Technol 69(3):231–239

Kubicek CP, Messner R, Gruber F, Mach RL, Kubicek-Pranz EM (1993) The *Trichoderma* cellulase regulatory puzzle: from the interior life of a secretory fungus. Enzym Microb Technol 15(2):90–99

Kuhad RC, Deswal D, Sharma S, Bhattacharya A, Jain KK, Kaur A, Pletschke BI, Singh A, Karp M (2016) Revisiting cellulase production and redefining current strategies based on major challenges. Renew Sust Energ Rev 55:249–272

Kuhad RC, Gupta R, Singh A (2011) Microbial cellulases and their industrial applications. Enzym Res 1–10

Lambertz C, Garvey M, Klinger J, Heesel D, Klose H, Fischer R, Commandeur U (2014) Challenges and advances in the heterologous expression of cellulolytic enzymes: a review. Biotechnol Biofuels 7(1):135

Lamed RA, Setter E, Kenig RI, Bayer EA (1983) The cellulosomes—a discrete cell surface organelle of *Clostridium thermocellum* which exhibits separate antigenic, cellulose-binding and various cellulolytic activities. Biotechnol Bioenergy Symp 13:163–181

Li W, Zhang WW, Yang MM, Chen YL (2008) Cloning of the thermostable cellulase gene from newly isolated *Bacillus subtilis* and its expression in *Escherichia coli*. Mol Biotechnol 40(2):195–201

Li YL, Li H, Li AN, Li DC (2009) Cloning of a gene encoding thermostable cellobiohydrolase from the thermophilic fungus *Chaetomium thermophilum* and its expression in *Pichia pastoris*. J Appl Microbiol 106(6):1867–1875

Liang Y, Feng Z, Yesuf J, Blackburn JW (2010) Optimization of growth medium and enzyme assay conditions for crude cellulases produced by a novel thermophilic and cellulolytic bacterium, *Anoxybacillus* sp. 527. Appl Biochem Biotechnol 160(6):1841–1852

Liu D, Zhang R, Yang X, Zhang Z, Song S, Miao Y, Shen Q (2012) Characterization of a thermostable β-glucosidase from *Aspergillus fumigatus* Z5, and its functional expression in *Pichia pastoris* X33. Microb Cell Factories 11(1):25

Lo YC, Saratale GD, Chen WM, Bai MD, Chang JS (2009) Isolation of cellulose-hydrolytic bacteria and applications of the cellulolytic enzymes for cellulosic biohydrogen production. Enzym Microb Technol 44(6–7):417–425

Lo YC, Lu WC, Chen CY, Chen WM, Chang JS (2010) Characterization and high-level production of xylanase from an indigenous cellulolytic bacterium *Acinetobacter junii* F6-02 from southern Taiwan soil. Biochem Eng J 53(1):77–84

Lü R, Zhao A, Li J, Liu C, Wang C, Wang X, Wang X, Pei R, Lu C, Yu M (2015) Screening, cloning and expression analysis of a cellulase derived from the causative agent of hypertrophy sorosis scleroteniosis, *Ciboria shiraiana*. Gene 565(2):221–227

Maeda RN, Barcelos CA, Santa Anna LM, Pereira N Jr (2013) Cellulase production by *Penicillium funiculosum* and its application in the hydrolysis of sugar cane bagasse for second generation ethanol production by fed batch operation. J Biotechnol 163(1):38–44

Mazzoli R, Lamberti C, Pessione E (2012) Engineering new metabolic capabilities in bacteria: lessons from recombinant cellulolytic strategies. Trends Biotechnol 30(2):111–119

Miettinen-Oinonen A, Paloheimo M, Lantto R, Suominen P (2005) Enhanced production of cellobiohydrolases in *Trichoderma reesei* and evaluation of the new preparations in biofinishing of cotton. J Biotechnol 116(3):305–317

Murray P, Aro N, Collins C, Grassick A, Penttilä M, Saloheimo M, Tuohy M (2004) Expression in *Trichoderma reesei* and characterisation of a thermostable family 3 β-glucosidase from the moderately thermophilic fungus *Talaromyces emersonii*. Protein Expr Purif 38(2):248–257

Nurul K, Kikuchi T (2011) Analysis of expressed sequence tags from the wood-decaying fungus *Fomitopsis palustris* and identification of potential genes involved in the decay process. J Microbiol Biotechnol 21(4):347–358

Ohara H, Karita S, Kimura T, Sakka K, Ohmiya K (2000) Characterization of the cellulolytic complex (cellulosome) from *Ruminococcus albus*. Biosci Biotechnol Biochem 64(2):254–260

Peterson R, Nevalainen H (2012) *Trichoderma reesei* RUT-C30–thirty years of strain improvement. Microbiology 158(1):58–68

Phitsuwan P, Laohakunjit N, Kerdchoechuen O, Kyu KL, Ratanakhanokchai K (2013) Present and potential applications of cellulases in agriculture, biotechnology, and bioenergy. Folia Microbiol 58(2):163–176

Ponpium P, Ratanakhanokchai K, Kyu KL (2000) Isolation and properties of a cellulosome-type multienzyme complex of the thermophilic *Bacteroides* sp. strain P-1. Enzym Microb Technol 26(5–6):459–465

Rajoka MI, Malik KA (1997) Cellulase production by *Cellulomonas biazotea* cultured in media containing different cellulosic substrates. Bioresour Technol 59(1):21–27

Rastogi G, Bhalla A, Adhikari A, Bischoff KM, Hughes SR, Christopher LP, Sani RK (2010) Characterization of thermostable cellulases produced by *Bacillus* and *Geobacillus* strains. Bioresour Technol 101(22):8798–8806

Reinhold-Hurek B, Hurek T, Claeyssens M, Van Montagu M (1993) Cloning, expression in *Escherichia coli*, and characterization of cellulolytic enzymes of *Azoarcus* sp., a root-invading diazotroph. J Bacteriol 175(21):7056–7065

Rosenberg SL (1978) Lignin biodegradation and the production of ethyl alcohol from cellulose. Lignin biodegradation: microbiology, chemistry and potential applications. US Forest Products Laboratory, Madison, WI, May 9–11, 2013

Ryu DD, Mandels M (1980) Cellulases: biosynthesis and applications. Enzym Microb Technol 2(2):91–102

Salem KS, Rashid TU, Islam MM, Khan MN, Sharmeen S, Rahman MM, Haque P (2016) Recent updates on immobilization of microbial cellulase. In New and future developments in microbial biotechnology and bioengineering, pp 107–139. Elsevier

San Ryu J, Shary S, Houtman CJ, Panisko EA, Korripally P, John FJ, Crooks C, Siika-aho M, Magnuson JK, Hammel KE (2011) Proteomic and functional analysis of the cellulase system expressed by *Postia placenta* during brown rot of solid wood. Appl Environ Microbiol AEM-05496:7933–7941

Saranraj P, Stella D, Reetha D (2012) Microbial cellulases and its applications. Int J Biochem Biotech Sci 1:1–2

Schell DJ, Hinman ND, Wyman CE, Werdene PJ (1990) Whole broth cellulase production for use in simultaneous saccharification and fermentation. Appl Biochem Biotechnol 24(1):287

Sheridan PP, Brenchley JE (2000) Characterization of a salt-tolerant family 42 β-galactosidase from a psychrophilic Antarctic *Planococcus* isolate. Appl Environ Microbiol 66(6):2438–2444

Shiang M, Linden JC, Mohagneghi A, Rivard CJ, Grohmann K, Himmel ME (1990) Cellulase production by *Acidothermus cellulolyticus*. Appl Biochem Biotechnol 1:223–235

Shiang M, Linden JC, Mohagheghi A, Grohmann K, Himmel ME (1991) Regulation of cellulase synthesis in *Acidothermus cellulolyticus*. Biotechnol Prog 7(4):315–322

Shu-bin L, Ren-Chao Z, Xia L, Chu-yi C, Ai-lin Y (2012) Solid-state fermentation with okara for production of cellobiase-rich cellulases preparation by a selected *Bacillus subtilis* Pa5. Afr J Biotechnol 11(11):2720–2730

Singhania RR, Sukumaran RK, Pandey A (2007) Improved cellulase production by Trichoderma reesei RUT C30 under SSF through process optimization. Appl Biochem Biotechnol 142(1):60–70

Singhania RR, Adsul M, Pandey A, Patel AK (2017) Cellulases. In: Current developments in biotechnology and bioengineering. Elsevier, Amsterdam, pp 73–101

Solingen P, Meijer D, Kleij WA, Barnett C, Bolle R, Power SD, Jones BE (2001) Cloning and expression of an endocellulase gene from a novel streptomycete isolated from an East African soda lake. Extremophiles 5(5):333–341

Sudto A, Punyathiti Y, Pongsilp N (2008) The use of agricultural wasted as substrates for cell growth and carboxymethyl cellulase (CMCase) production by *Bacillus subtilis*, *Escherichia coli* and *Rhizobium* sp. Curr Appl Sci Technol 8(2):84–92

Szakmary K, Wotawa A, Kubicek CP (1991) Origin of oxidized cellulose degradation products and mechanism of their promotion of cellobiohydrolases I biosynthesis in *Trichoderma reesei*. Microbiology 137(12):2873–2878

Tambor JH, Ren H, Ushinsky S, Zheng Y, Riemens A, St-Francois C, Tsang A, Powlowski J, Storms R (2012) Recombinant expression, activity screening and functional characterization identifies three novel endo-1, 4-β-glucanases that efficiently hydrolyse cellulosic substrates. Appl Microbiol Biotechnol 93(1):203–214

Tang B, Pan H, Zhang Q, Ding L (2009) Cloning and expression of cellulase gene EG1 from *Rhizopus stolonifer* var. *reflexus* TP-02 in *Escherichia coli*. Bioresour Technol 100(23):6129–6132

Tangnu SK, Blanch HW, Wilke CR (1981) Enhanced production of cellulase, hemicellulose, and β-glucosidase by *Trichoderma reesei* (Rut C-30). Biotechnol Bioeng 23(8):1837–1849

Thomas L, Joseph A, Gottumukkala LD (2014) Xylanase and cellulase systems of *Clostridium* sp.: an insight on molecular approaches for strain improvement. Bioresour Technol 158:343–350

Thongekkaew J, Ikeda H, Masaki K, Iefuji H (2008) An acidic and thermostable carboxymethyl cellulase from the yeast *Cryptococcus* sp. S-2: purification, characterization and improvement of its recombinant enzyme production by high cell-density fermentation of *Pichia pastoris*. Protein Expr Purif 60(2):140–146

Tsai SL, Oh J, Singh S, Chen R, Chen W (2009) Functional assembly of minicellulosomes on the *Saccharomyces cerevisiae* cell surface for cellulose hydrolysis and ethanol production. Appl Environ Microbiol 75(19):6087–6093

Wang H, Bao K, inventors; Danisco US Inc, assignee (2009) Neutral cellulase catalytic core and method of producing same. United States patent application US 11/784-926

Wang Z, Bay H, Chew K, Geng A (2014) High-loading oil palm empty fruit bunch saccharification using cellulases from *Trichoderma koningii* MF6. Process Biochem 49(4):673–680

Watanabe H, Tokuda G (2010) Cellulolytic systems in insects. Annu Rev Entomol 55:609–632

Watson TG, Nelligan I, Lessing L (1984) Cellulase production by *Trichoderma reesei* (RUT-C30) in fed-batch culture. Biotechnol Lett 6(10):667–672

Wei H, Xu Q, Taylor LE II, Baker JO, Tucker MP, Ding SY (2009) Natural paradigms of plant cell wall degradation. Curr Opin Biotechnol 20(3):330–338

Wilson DB (2009) Cellulases and biofuels. Curr Opin Biotechnol 20(3):295–299

Wulff NA, Carrer H, Pascholati SF (2006) Expression and purification of cellulase Xf818 from *Xylella fastidiosa* in *Escherichia coli*. Curr Microbiol 53(3):198–203

Xiros C, Katapodis P, Christakopoulos P (2011) Factors affecting cellulose and hemicellulose hydrolysis of alkali treated brewers spent grain by *Fusarium oxysporum* enzyme extract. Bioresour Technol 102(2):1688–1696

Yang D, Weng H, Wang M, Xu W, Li Y, Yang H (2010) Cloning and expression of a novel thermostable cellulase from newly isolated *Bacillus subtilis* strain I 15. Mol Biol Rep 37(4):1923–1929

Zhang J, Zhong Y, Zhao X, Wang T (2010) Development of the cellulolytic fungus *Trichoderma reesei* strain with enhanced β-glucosidase and filter paper activity using strong artificial cellobiohydrolase 1 promoter. Bioresour Technol 101(24):9815–9818

Index

M. Srivastava et al. (eds.), *Approaches to Enhance Industrial Production of Fungal Cellulases*, Fungal Biology,
https://doi.org/10.1007/978-3-030-14726-6