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Review Article

Bacterial Cellulases and its Applications: A Review

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ABSTRACT

The most abundant biomass on earth is cellulose. Lignocellulosic biomass is the alternative of fossil fuels and can be used in the production of Biofuels. Degradation of this cellulolytic biomass requires cellulases produced by microorganisms including Bacteria, Fungi and actinomycetes. A wide variety of microorganisms are available in nature that can be isolated from different environment. Endoglucanases obtained from bacteria are able to degrade the ß-1, 4-glucan linkages present in amorphous cellulose whereas exoglycanases help in cleaving the remaining chain of Oligosaccharide. Bacterial Cellulases are reported to have higher growth rate and versatility in genetic composition which makes them advantageous. These enzymes have several applications in industries worldwide which include Fermentation, Textile, Paper and pulp Agriculture and Food. This review summarizes the various applications of bacterial cellulases with future challenges.

Keywords: Bacterial cellulases; Cellulose; Exoglycanases; Microorganisms; Lignocellulosic biomass

INTRODUCTION

In India, lignocellulosic biomass is found in abundant quantities. Lignocellulosic biomass is composed of cellulose, hemicellulose and lignin, and ash in a variable amount. Cellulose is a major part of biomass, which contains long chains of glucose sugar. Hemicellulose is made up of a combination of sugars like xylose, arabinose, and mannose. Lignin is a complex polymer that gives strength to the biomass and highly crosslinked phenols are present in it. The composition, however, has interactions of these components with highly resistant and recalcitrant structure. This biomass includes sugarcane bagasse, wheat straw, castor plant waste, rice husk and other agricultural waste.

Cellulase is commonly used for the degradation of Cellulose. Cellulases are widely spread in nature, predominantly produced by microorganisms, like molds, fungi and bacteria [1]. Cellulase is the enzyme that hydrolyzes the ß-1, 4-glycosidic bonds in the polymer to release glucose units. It is a multienzyme system composed of several enzymes with numerous isozymes, which act in synergy having a wide range of industrial applications such as textile, laundry, pulp and paper, fruit juice extraction, and animal feed additives as well as in bioethanol production [2]. Cellulose is the most ubiquitous form of fixed carbon and a possible source of renewable organic energy in the eco-system. It is a fibrous, tough and water insoluble substance that offers mechanical strength as well as chemical stability to plants and thus helps to maintain the plant cell wall structure. Cellulose and hemi-cellulose represent the most abundant and potential source of fermentable sugar to produce various value-added products and bio-active compounds of great interest for biotechnology [3]. The characterization of Bacterial cellulases is not fully determined due to the mechanism of biosynthesis and regulation of this enzyme even after proving cellulose as important source for industries. There is study showing the synthesis of cellulose in Gluconacetobacter xylinum, which is very well elaborated. Scientific community in recently attracted to this matter as well [4].

LITERATURE REVIEW

One of the main reasons for bact0065ria to survive is through the production of cellulose. Cellulases are also responsible for providing the strategies of survival to bacteria. It is also reported that many bacterial species exhibit the property of degrading biomass obtained from plant like Cellulases, Hemicellulases and pectinases since they possess these genes [5]. There are several studies reporting the production of cellulases by bacteria and also the characterization of cellulose biosynthesis in *Pseudomonas* spp., *Rhizobium leguminosarum*, *Escherichia coli, Salmonella spp, Gluconoacetobacter hansenii* and *Rhodococcus sphaeroides* [6-10].

Cellulose Microfibrils having ß-1,4-glucan linkages are degraded by cellulases. The presence of these microfibrils is seen in the biomass obtained from plants, cell wall of microorganisms which includes

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pathogens. Studies have proved the presence of different enzymes responsible for degradation of polysaccharide through bacterial genome sequencing. The inherent resistance of cellulosic biomass assembly to microbial and enzymatic deconstruction is the prime impediment for its economic transformation into high value-added bio-products. The factors responsible for the biomass recalcitrance include structural complexity due to micro fibrils and matrix polymers, agglomeration of lignin, presence of dense vascular bundles, thick wall tissues and crystalline nature of cellulose. Owing to these constraints, bio-processes techniques employed in biomass degradation face some hurdles at an industrial scale [11-13].

MECHANISM OF ACTION OF CELLULASE

Cellulase catalyzes the decomposition of cellulose polysaccharide by simply breaking down β-1,4-glycosidic bonds. Three major types of enzymes are generally involved in hydrolyzing cellulose microfibrils in the plant cell wall: endoglucanase, exoglucanase, and β-glucosidase. Complete cellulose hydrolysis is mediated by the combination of these three main types of enzymes. Endoglucanase usually attacks amorphous areas of cellulose. The random attack of this enzyme on internal bonds of loosely bound, amorphous areas of cellulose creates new chain ends. These new chain ends are then easily attacked by other types of enzymes. The highest activity of this enzyme usually occurs against soluble cellulose forms or acid-treatedamorphous cellulose. The function of exoglucanase is to produce glucose or cellobiose units by attacking the reducing or nonreducing end of cellulose chains.

Endoglucanase is different from exoglucanase because it is usually very active against crystalline cellulose substrates such as avicel or cellooligosaccharides. Finally, ß-glucosidase can hydrolyze cellobiose to glucose from the no reducing ends, and it is inactive against amorphous or crystalline cellulose. Although an exact mechanism is not yet finalized, fragmentation of cellulose aggregations into short fibers has been observed and reported during the beginning of cellulose hydrolysisprior to releasing any detectable amount of reducing sugars. This is known a morphogenesis. There are two catalytic mechanisms of cellulases. They are simply introduced as retaining mechanisms and inverting mechanisms. Cellulases cleave glucosidic bonds by using acid-based catalysis. The hydrolysis is performed by two catalytic residues of the enzyme: a general acid (proton donor) and a nucleophile/base. The catalytic mechanism which occurs depends on the spatial position of the catalytic residues. The retention and inversion of the anomeric configuration of cellulose are the two mechanisms which hydrolyze cellulose. The "retaining" cellulases retain the same configuration of anomeric C bearing the target glucosidic bond even after a double-displacement hydrolysis with two key glycosylation or deglycosylation steps. "Inverting" cellulases inverts the configuration of the anomeric C configurationafter a single nucleophilic displacement hydrolysis [14].

APPLICATIONS

Cellulase is the most frequently used group of enzymes in various industries due to its innumerous applications (Figure 1). It is used in food and wine biotechnology, biofuel production, bio-de-inking, textile and laundry industry, pulp and paper production, conversion of cellulosic biomass and applications in research and development and also, in agriculture and medicine (Table 1) [15-17].

Table 1: Bacterial cellulase enzyme system [16].

Enzyme	E. C. number	Reaction	Other names
i) Endo -1,4 β-D-glucanglucanohydrolase	E.C.3.2.1.4	glucanohydrolase E. C. 3. 2. 1. 4 Cut at random at internal amorphous sites of cellulose generating oligosaccharides of various lengths. It acts on Endo-1, 4-beta-D-glucosidic linkages in cellulose, lichenin and cereal beta-D-glucans.	Endoglucanase ,Endo- 1,4-β–glucanase, Carboxymethyl cellulase, β -1,4-endoglucon hydrolase, Endocellulase
ii)Exoglucanase or 1,4-β-Dglucan cellobiohydrolases (cellobiohydrolases)	E.C.3.2.1.91	Hydrolysis of 1,4-beta-Dglucosidiclinkages in cellulose and cellotetraose, releasing cellobiose from the non-reducing ends of the chains	Exoglucanase, Exocellobiohydrolase, 1, 4-β-cellobiohydrolase.
iii) Exoglucanases or 1,4-βD-oligoglucan cellobiohydrolases	E.C 3.2.1.74	Removal of cellobiose from cellooligosaccharide or from p-nitrphenylβ-D-cellobioside	Cellodextrinases
iv) β - Glucosidases or β-Dglucoside gluco-hydrolases	E.C.3.2.1.21	Hydrolysis of terminal non-reducing betaD-glucose residues with release of betaD-glucose	Gentobiase, Cellobiase, Amygdalase
v)Cellobiose: orthophosphate alfa–Dglucosyl transferase	E.C. 2.4.1.49	It catalyzes the reversible phosphorolytic cleavage of cellobiose	Cellobiose phosphorylase
vi)1,4-β-Doligoglucan:orthophosphate alfa –D-glucosyl transferase	E.C. 2.4.1.20	It catalyzes the reversible phosphorolytic cleavage of cellodextrins ranging from cellotriose to cellohexoses.	Cellodextrin phosphorylase
vii) Cellobiose 2- epimerase	EC 5.1.3.11	It catalyzes the cellobiose into 4-O- β-Dglucosylnannose	Cellobiose 2- epimerase
viii) Complete Cellulase system	-	Catalyzes extensive hydrolysis of crystalline cellulose	Total cellulase

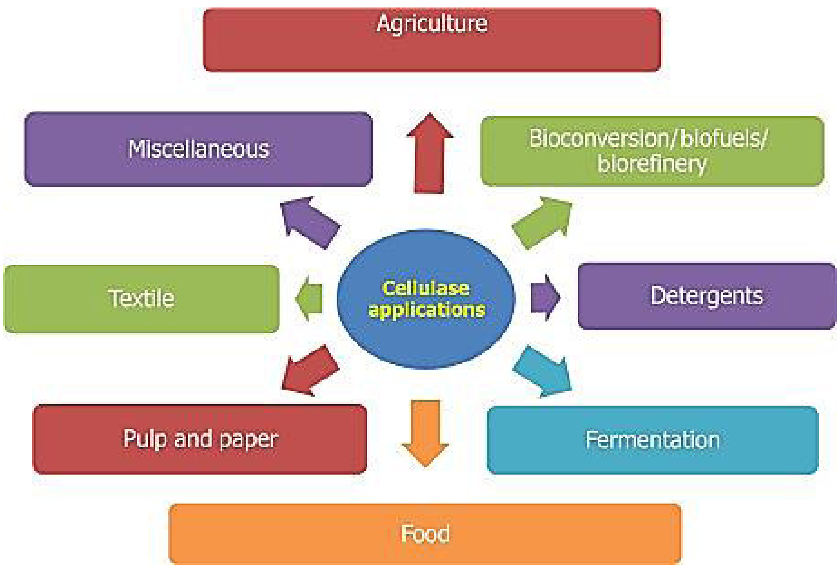

Figure 1: (A) UV–Vis absorption spectrum of CdTe quantum dots.

Bacterial fermentation for cellulase production

Several cellulase-producing bacteria such as *Acidothermus cellulolyticus, Bacillus subtilis, Bacillus coagulans, Bacillus pumilus,Clostridium acetobutylicum, Clostridium thermocellum, Cellulomonas fimi, Cellulomonas bioazotea, Cellulomonas uda*, etc., have been isolated from different sources and selected due to their xylan-degrading properties (Figure 2). In order to explore the possibility of using banana waste as solid substrate for the production of cellulases, Some researchers have designed and tested a bioprocess to ascertain the suitability of banana fruit stalk as a solid substrate for SSF employing *Bacillus subtilis* (CBTK 106) [18,19]. Some have also designed a statistical optimization of xylanase activity by an amazon environment isolated strain of *B. coagulans* grown in SSF, using an industrial fibrous soybean residue as substrate. Moreover, *Acidothermus* cellulolyticus is a highly thermotolerant hot spring bacterium that has been investigated as a source of enzyme for biomass hydrolysis. Production of xylanase and cellulase from SSF of switch grass colonized by A. cellulolyticus has also been reported [20]. Considering the potent bacterial strains for the production of cellulose enzyme, Singh and Kaur, isolated an efficient strain, *Bacillus sp*. JS14 from a total 30 bacterial isolates and claimed a highest enzyme activity for the production of cellulase [21-25]. This review paper highlights the various advances of cellulases in different fields.

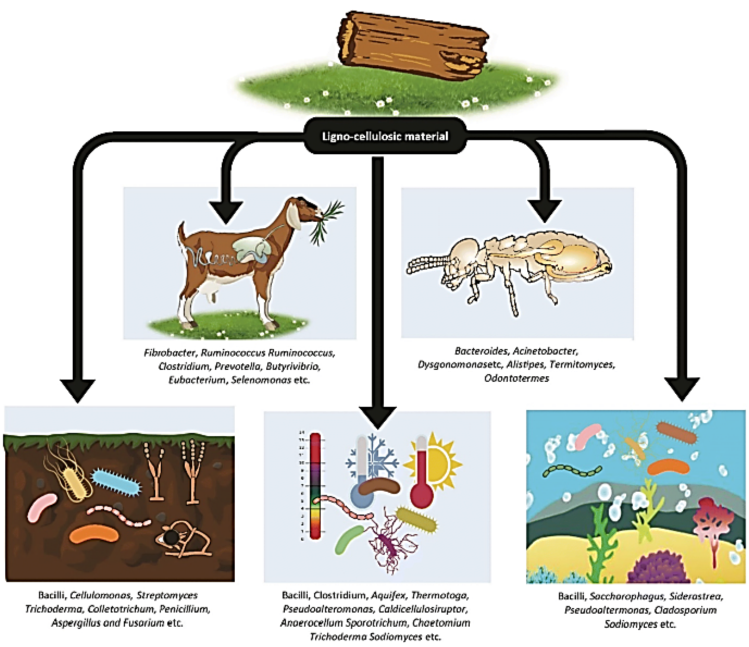

Figure 2: (A) UV–Vis absorption spectrum of CdTe quantum dots.

Industrial applications

In recent times, Industries have started focusing of production processes which are low cost, efficient and have specific applications in major sectors. To obtain this outcome, novel strain and improved cellulases are needed. For this purpose, identification of better and stable cellulases is to be obtained from various sources as well as optimization of various parameters is essential.

Textile and detergent industries

Cellulases play a major role in textile industries in the production of denims which are stonewashed and in biopolishing of fabrics. To improve the brightness and softness of cotton fabrics, it is also used in laundry detergents. Various features are considered for a good detergent which includes, color stability, cleansing agent and entire deposition for better washing. Moreover, compatibility with other ingredients present in formulations, temperature stability, and activity under alkaline conditions are all important performance characteristics for cellulases, which are to be employed in textile and detergent applications [26,27].

Cellulases which are commonly used for in textile industries can be of obtained from fungi, bacteria and actinomycetes which includes *T. resei*, *Streptomyces, Thermobifida, Pseudomonas, Sphingomonas* [28]. Under thermophilic and alkalinophilic conditions, cellulases along with other enzymes are added to detergents for breaking the chemical bonds [29]. Alkaline and neutral conditions are required for the processing of denim fibres and the alkaline-stable endoglucanase of *Thermonospora* sp. is used as commercial fungal cellulases. *Bacillus* has also been reported to show high potential in producing cellulases that that has applications in various industries [30]. Major part of enzyme activity is retained after incubating at different temperatures and in the presence of commercial detergents of different brands. In one of the study, it is also revealed that the cotton fabrics can be softening by using a recombinant endoglucanase obtained from a species of genus *Bacillus* [31].

Food/animal feed processing industries

In food processing and animal feed processing industries, cellulase is widely used in combination with enzymes like hemicellulases and pectinases. There are numerous uses of this enzyme including the production of juices from fruits and vegetables as well as in wine and beer industries for producing carotenoids and for degrading plant cell walls. For clarifying fruit juices, cellulases obtained from *Bacillus* and *Paenibacillus* are used as accessory enzymes apart from enzymes obtained from fungal origin [32]. The degradation of orange, carrot and sweet potato cell walls can be accelerated by the combination of cellulases with pectinases. Through this, extraction of carotenoids can be achieved by using cellulases which can be used as colouring agent in food industries [33]. It also has other industrial applications that involves production of sugars by degradation of grapefruit peels and in extraction of phenolic compounds from grape promace [34,35].

Several applications were also reported by Bamforth about endo-1,4-ß-D glucanases, obtained from unknown origin and playing essential role in degradation of cell wall and Barley malting [36]. Cellulases are also widely used for obtaining high quality of forages with increased nutritive values and also in enhancing the digestibility of cereal based food [37-39]. Cellulases obtained from *Bacillus subtilis* can be used in the degrading the hull of soya grain for enriching the nutritional value in animal feed [40]. Through all these studies, it can be concluded that cellulases have innumerous applications in the Industries processing food and animal feed that will provide us ample opportunities in the future to explore approaches to identify bacterial cellulases. To meet these challenges, metagenomics can be used to search new glycosyl hydrolases [41].

DISCUSSION

Endoglucanase showing halotolerance properties, obtained from soil and belonging to GH5 family was cloned and identified by using Metagenomics .This enzyme showed similarity with the cellulase obtained from *Cellvibrio mixtus* [42]. Some metagenomic studies also helped in characterization of cellulases obtained from soil samples and botanical gardens of aquatic communities. These cellulases showed similarities with those obtained from Cellvibrio japonicas and it belonged to GH5 and GH9 families [43]. New discoveries are needed to identify novel Bacterial species producing cellulases that can be used in various processes and metagenomics is one such tool that can be a boon for various food processing industries.

Paper and pulp industries

The application of cellulases especially alkaline cellulases in paper and pulp industries is specified by several studies and patents mainly in the process of de-inking papers, in improvement of drainage, in modification of fibre and recycling of paper. However, use of these enzymes is not much desirable because of its possibility of degrading the fibre and losing viscosity [44-46]. Mostly fungal cellulases are used in these industries where the formulations are commercially the coctails of enzymes obtained from *Trichoderma reseei* and Aspergillus niger. There are very few studies revealing the use of Bacterial cellulases. One such study has revealed that cellulase identified from *Paenibacillus sp.* BP-23, named CelB and 171 AIMS Bioengineering Volume 2, Issue 3, 163-182.is being used in improvement of paper properties and drainage process [47]. Thus, the potential applications of Bacterial cellulases needs to be explores in these industries.

Biorefineries and biofuels

Plant Biomass degradation is an expensive process and can be achieved via 3 steps viz. physicochemical pretreatments, enzymatic hydrolysis and fermentation. Renewable lignocellulosic biomass is abundant in nature and cellulases play a vital role in the process of bioconversion of these biomass. The ß-glycoside bonds of internal regions of cellulose which are amorphous and can be hydrolyzed by endoglucanases to obtain simple chains of oligosaccharides having varied degree of polymerization [48]. Bioprocessing and eliminating pretreatments can be done by using cellulolytic microbes with the estimated reduction in cost by 40 percent [49]. The permanence and viability of commercial cellulase production can be ensured by significant reduction in the costs. For this purpose cellulases obtained from bacteria or cellulolytic bacteria is cost effective only with proper compatibility between processing parameters. For bioconversion of lignocellulosic biomass, a variety of fungal strain have been used to produce biofuels. Cellulases and hemicellulase are used widely in depolymerizing plant biomass to obtain sugars and this is achieved by *Trichoderma reseei* which acts as main industrial source [50].

Bioconversion of lignocellulosic biomass into bioethanol and biofuels can be brought about by thermophilic bacteria that are acting as efficient sources to identify and characterize cellulases. A review is also being published that addresses the potential of thermophilic strains to exhibit high cellulolytic activity [51]. There are other reports as well which suggests the production of cellulase by thermophilic isolates such as *Geobacillus* sp. T1, *Bacillus sp.* SMIA-2, clostridia consortium, marine extremophiles, anaerobic thermophilic hydrogen-producer Thermosipho sp. and many more [52-56]. Moreover, *Bacillus vallismortis* RG-07 which produces solvent-thermo stable alkalophilic cellulases can be used in future for various applications. These novel and potential bacteria can be used for bioconversion of lignocellulosic biomass using various substrates like baggasses, straws etc [57]. It is also reported that *Caldicelluloseruptor bescii* can be used for bioconversion of lignocellulosic biomass to bioethanol directly [58].

Medical applications

In the field of medicine, several evidences help us understand that cellulases play a vital role directly or indirectly mainly on degrading chitosan with the help of chitinases and lysozymes and are mostly of fungal origin. The derivatives of chitosan has several application including surgical sutures, bone rebuilding, production of artificial skin, anticoagulant, antibacterial agent, hemostatic dressings, anticancer and anti-diabetic agents (in combination with metals), hypo cholesterolemic effectors (LMWCs), elaboration of cosmetics, production of biopharmaceutics and encapsulation of diverse materials (Table 2) [59-62].

Table 2: Industrial applications cellulases in various ways [62].

Industry	Technical roles
Agriculture	Cellulases are involved in mechanism of defense against various pests and insects, activation of phytochrome, metabolism of pigment , cell wall and repining of fruits.
Paper and pulp industry	Fibre softness, flexibility, water retention capacity is improved by the use of Cellulases
Animal feed	Cellulases enhance the degradation of fibre in diets and also helps to improve the efficiency of utilizing feed efficiency.
Textiles and polymers	Starch size, cotton softening, fabric softening, vivid softness can be achieved by Cellulases.
Biofuel industry	Cellulosic biomass can be converted into feedstock

CONCLUSION AND FUTURE PERSPECTIVES

With the increase in advancement of Biotechnological applications, the market of industrial cellulases will be increasing in the near future. The success of various industries will be determined by the strategies of using cellulase and the parameters to be considered for optimization .This will play pivotal role in minimizing the cost of production. Still there is a need to find the novel strain with maximum potential to bring biodegradation of Lignocellulosic biomass and efforts are needed to develop engineered and thermo/ alkalinostable cellulases.

REFERENCES

1. Pérez J, Munoz-Dorado J, De la Rubia TD, Martinez J. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. Int. Microbiol. 2002 Jun;5(2):53-63.
2. Bhat M. Cellulases and related enzymes in biotechnology. Biotechnol. Adv.. 2000;18(5):355-383.
3. Ross P, Weinhouse H, Aloni Y, Michaeli D, Weinberger-Ohana P, Mayer R, et al. Regulation of cellulose synthesis in Acetobacter xylinum by cyclic diguanylic acid. Nature. 1987;325(6101):279-281.
4. Misra P, Shukla PK, Rao KP, Ramteke PW. Genetic Engineering Applications to Improve Cellulase Production and Efficiency: Part II. InNew and Future Developments in Microbial Biotechnology and Bioengineering 2019;227-260.
5. Ude S, Arnold DL, Moon CD, Timms-Wilson T, Spiers AJ. Biofilm formation and cellulose expression among diverse environmental *Pseudomonas* isolates. Environ. Microbiol. 2006;8(11):1997-2011.
6. Zogaj X, Nimtz M, Rohde M, Bokranz W, Römling U. The multicellular morphotypes of Salmonella typhimurium and Escherichia coli produce cellulose as the second component of the extracellular matrix. Mol. Microbiol. 2001;39(6):1452-1463.
7. Römling U. Molecular biology of cellulose production in bacteria. Res. Microbiol. 2002;153(4):205-12.
8. Solano C, García B, Valle J, Berasain C, Ghigo JM, Gamazo C, et al. Genetic analysis of Salmonella enteritidis biofilm formation: critical role of cellulose. Mol. Microbiol. 2002;43(3):793-808.
9. Mohite BV, Patil SV. Impact of microbial cellulases on microbial cellulose biotechnology. InNew and Future Developments in Microbial Biotechnology and Bioengineering 2016;31-40.
10. Morgan JL, Strumillo J, Zimmer J. Crystallographic snapshot of cellulose synthesis and membrane translocation. Nature. 2013;493(7431):181-186.
11. Himmel, Michael E, Shi-You Ding, David K. Johnson, William S. Adney, Mark R. Nimlos, John W. Brady, and Thomas D. Foust. "Biomass recalcitrance: engineering plants and enzymes for biofuels production." science 315,5813 (2007): 804-807.
12. Hosseini M. A perspective on bioprocessing for biofuels, bio-based chemicals, and bioproducts. InAdvanced Bioprocessing for Alternative Fuels, Biobased Chemicals, and Bioproducts.Woodhead Publishing. 2019;1-11.
13. Dragone G, Kerssemakers AA, Driessen JL, Yamakawa CK, Brumano LP, Mussatto SI. Innovation and strategic orientations for the development of advanced biorefineries. Bioresour Technol. 2020;302:122847.
14. Vocadlo DJ, Davies GJ. Mechanistic insights into glycosidase chemistry. Curr Opin Chem Biol.2008;12(5):539-555.
15. Sadhu S, Maiti TK. Cellulase production by bacteria: a review. Int J Microbiol Res. 2013:235-258.
16. Bhat M. Cellulases and related enzymes in biotechnology. Biotechnol. Adv.. 2000;18(5):355-383.
17. Kuhad RC, Gupta R, Singh A. Microbial cellulases and their industrial applications. Enzyme Res. 2011;2011.
18. Thapa S, Mishra J, Arora N, Mishra P, Li H, O′ Hair J. Microbial cellulolytic enzymes: diversity and biotechnology with reference to lignocellulosic biomass degradation. Rev Environ Sci Biotechnol. 2020;19:621-648.
19. Krishna C. Production of bacterial cellulases by solid state bioprocessing of banana wastes. Bioresour Technol. 1999;69(3):231-239.
20. Rezaei F, Joh LD, Kashima H, Reddy AP, VanderGheynst JS. Selection of conditions for cellulase and xylanase extraction from switchgrass colonized by Acidothermus cellulolyticus. Appl. Biochem. Biotechnol. 2011;164(6):793-803.
21. Heck JX, Flôres SH, Hertz PF, Ayub MA. Optimization of cellulase-free xylanase activity produced by *Bacillus* coagulans BL69 in solid-state cultivation. Process Biochem. 2005;40(1):107-12.
22. El-Bakry M, Abraham J, Cerda A, Barrena R, Ponsá S, Gea T, et al. From wastes to high value added products: novel aspects of SSF in the production of enzymes. Crit Rev Environ Sci Technol.2015;45(18):1999-2042.

23. Juturu V, Wu JC. Microbial cellulases: engineering, production and applications. Renew Sustain Energy Rev.2014;33:188-203.

24. Phitsuwan P, Laohakunjit N, Kerdchoechuen O, Kyu KL, Ratanakhanokchai K. Present and potential applications of cellulases in agriculture, biotechnology, and bioenergy. Folia Microbiol. 2013;58(2):163-176.

25. Heck JX, Flôres SH, Hertz PF, Ayub MA. Optimization of cellulase-free xylanase activity produced by *Bacillus* coagulans BL69 in solid-state cultivation. Process Biochem. 2005;40(1):107-112.

26. Adrio JL, Demain AL. Microbial enzymes: tools for biotechnological processes. Biomolecules. 2014;4(1):117-139.

27. Cherry JR, Fidantsef AL. Directed evolution of industrial enzymes: an update. Curr Opin Biotechno. 2003;14(4):438-443.

28. McMullan G, Meehan C, Conneely A, Kirby N, Robinson T, Nigam P, et al. Microbial decolourisation and degradation of textile dyes. Appl Microbiol Biotechnol. 2001;56(1):81-87.

29. Adrio JL, Demain AL. Microbial enzymes: tools for biotechnological processes. Biomolecules. 2014;4(1):117-139.

30. Ladeira SA, Cruz E, Delatorre AB, Barbosa JB, Leal Martins ML. Cellulase production by thermophilic *Bacillus sp*: SMIA-2 and its detergent compatibility. Electron J Biotechnol. 2015;18(2):110-115.

31. Yu M, Qiu Y, Chen W, Zhao F, Shao J. Action modes of recombinant endocellulase, EGA, and its domains on cotton fabrics. Biotechnol. Lett. 2015;37(8):1615-1622.

32. Singh K, Singh RK. "Role of Enzymes in Fruit juices Clarification during Processing: A review." Int J Biol Technology 6 (2015): 114-124.

33. Çinar I. Effects of cellulase and pectinase concentrations on the colour yield of enzyme extracted plant carotenoids. Process Biochem. 2005;40(2):945-949.

34. Wilkins MR, Widmer WW, Grohmann K, Cameron RG. Hydrolysis of grapefruit peel waste with cellulase and pectinase enzymes. Bioresour. Technol. 2007;98(8):1596-1601.

35. Meyer AS, Jepsen SM, Sørensen NS. Enzymatic release of antioxidants for human low-density lipoprotein from grape pomace. J Agric Food Chem. 1998;46(7):2439-2446.

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

37. Himmel ME, Ruth MF, Wyman CE. Cellulase for commodity products from cellulosic biomass. Curr. Opin. Biotechnol. 1999;10(4):358-64.

38. Dhiman TR, Zaman MS, MacQueen IS, Boman RL. Influence of corn processing and frequency of feeding on cow performance. Int J Dairy Sci. 2002;85(1):217-26.

39. Beauchemin KA, Colombatto D, Morgavi DP, Yang WZ. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J Anim Sci. 2003;81:E37-47.

40. Wongputtisin P, Khanongnuch C, Kongbuntad W, Niamsup P, Lumyong S, Sarkar PK. Use of B acillus subtilis isolates from T ua-nao towards nutritional improvement of soya bean hull for monogastric feed application. Lett Appl Microbiol. 2014;59(3):328-333.

41. Sathya TA, Khan M. Diversity of glycosyl hydrolase enzymes from metagenome and their application in food industry. J. Food Sci.. 2014;79(11):R2149-156.

42. Voget S, Steele HL, Streit WR. Characterization of a metagenome-derived halotolerant cellulase. J. Biotechnol. 2006;126(1):26-36.

43. Pottkämper J, Barthen P, Ilmberger N, Schwaneberg U, Schenk A, Schulte M, et al. Applying metagenomics for the identification of bacterial cellulases that are stable in ionic liquids. Green chemistry. 2009;11(7):957-965.

44. Eriksson KEL (1990) Biotechnology in the pulp and paper industry. Wood Sci Technol 24: 79– 101.

45. Viesturs U, Leite M, Eisimonte M, Eremeeva T, Treimanis A. Biological deinking technology for the recycling of office waste papers. Bioresour. Technol. 1999;67(3):255-265.

46. Bajpai P. Application of enzymes in the pulp and paper industry. Biotechnol. Prog. 1999;15(2):147-157.

47. Garcia O, Torres AL, Colom JF, Pastor FI, Diaz P, Vidal T. Effect of cellulase-assisted refining on the properties of dried and never-dried eucalyptus pulp. Cellulose. 2002;9(2):115-125.

48. Hasunuma T, Okazaki F, Okai N, Hara KY, Ishii J, Kondo A. A review of enzymes and microbes for lignocellulosic biorefinery and the possibility of their application to consolidated bioprocessing technology. Bioresour. Technol.. 2013;135:513-522.

49. Lynd LR, Laser MS, Bransby D, Dale BE, Davison B, Hamilton R, et al. How biotech can transform biofuels. Nat Biotechnol.. 2008;26(2):169-172.

50. Kanafusa-Shinkai S, Wakayama JI, Tsukamoto K, Hayashi N, Miyazaki Y, Ohmori H, et al. Degradation of microcrystalline cellulose and non-pretreated plant biomass by a cell-free extracellular cellulase/hemicellulase system from the extreme thermophilic bacterium Caldicellulosiruptor bescii. J Biosci Bioeng. 2013;115(1):64-70.

51. Scully SM, Orlygsson J. Recent advances in second generation ethanol production by thermophilic bacteria. Energies. 2015;8(1):1-30.

52. Assareh R, Zahiri HS, Noghabi KA, Aminzadeh S. Characterization of the newly isolated *Geo*bacillus sp. T1, the efficient cellulase-producer on untreated barley and wheat straws. Bioresour. Technol. 2012;120:99-105.

53. Ladeira SA, Cruz E, Delatorre AB, Barbosa JB, Leal Martins ML. Cellulase production by thermophilic *Bacillus sp*: SMIA-2 and its detergent compatibility. Electron. J. Biotechnol. 2015;18(2):110-115.

54. Dipasquale L, Romano I, Picariello G, Calandrelli V, Lama L. Characterization of a native cellulase activity from an anaerobic thermophilic hydrogen-producing bacterium Thermosipho sp. strain 3. Ann. Microbiol. 2014;64(4):1493-1503.

55. Dalmaso GZ, Ferreira D, Vermelho AB. Marine extremophiles: a source of hydrolases for biotechnological applications. Mar Drugs. 2015;13(4):1925-1965.

56. Kinet R, Destain J, Hiligsmann S, Thonart P, Delhalle L, Taminiau B, et al. Thermophilic and cellulolytic consortium isolated from composting plants improves anaerobic digestion of cellulosic biomass: toward a microbial resource management approach. Bioresour. Technol. 2015;189:138-144.

57. Gaur R, Tiwari S. Isolation, production, purification and characterization of an organic-solvent-thermostable alkalophilic cellulase from *Bacillus vallismortis* RG-07. BMC Biotechnol. 2015;15(1):1-2.

58. Chung D, Cha M, Guss AM, Westpheling J. Direct conversion of plant biomass to ethanol by engineered Caldicellulosiruptor bescii. PNAS. 2014;111(24):8931-8936.

59. Rinaudo M. Chitin and chitosan: Properties and applications. Prog Polym Sci. 2006;31(7):603-632.

60. Pillai CK, Paul W, Sharma CP. Chitin and chitosan polymers: Chemistry, solubility and fiber formation. Prog. Polym. Sci.. 2009;34(7):641-678.

61. Zhang J, Xia W, Liu P, Cheng Q, Tahi T, Gu W, Li B. Chitosan modification and pharmaceutical/biomedical applications. Mar Drugs. 2010 Jul;8(7):1962-1987.

62. Sadhu S, Maiti TK. Cellulase production by bacteria: a review. Microbiol. 2013;235-258.