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An Overview of Cellulase Production and Industrial Applications

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A B S T R A C T

Cellulose is one of most abundant natural biopolymer found on earth. It is a natural plant biomass made up of D-glucose units linked together by β-1, 4 bonds. Cellulase is an enzyme, which is used for the degradation of cellulosic and lignocellulosic residues into its monomeric units. Different type of cellulase enzymes causes the breakdown of cellulose rich substrates into useful byproducts in a systematic manner. It can be produced by various microbes (anaerobes and aerobes), plants and animals. So, cellulase can be modified for the effective conversion of easily available agrowastes biomass into fermentable sugar, fuels and other products. This demands an appropriate methodology for producing high enzyme titre at low cost and in eco-friendly manner. Cellulase has been a source of attraction due its huge requirements in industries, having very high commercial value and high growth rate. In this review, we will study the types, mechanism and production of cellulase enzyme using solid state (SSF) and submerged (SmF) fermentation along with their various industrial applications such as textile, food, animal feed and paper (pulp) industries. So, this review is a systematic presentation of cellulose, cellulase enzymes and their mechanism of action, types of production (SSF and SmF) as well as applications.

Keywords: Applications, Cellulose, Cellulase, Microorganisms (cellulose producing), Production

1 INTRODUCTION

Cellulose is the most abundant bioresource found in biosphere. It is the primary product of photosynthesis present in the cell wall of terrestrial plants. The source of the cellulose in plants is found as microfibrils (2-20 nm in diameter and 100-40, 000 nm long) [1]. Annually, plants produce 4×10^{9} tons cellulase. It is one of the most important source of carbon on earth and its biosynthesis occurs at a rate of 0.85×10^{11} tons per annum by both land plants and marine [2]. Cellulase is an important class of enzyme that catalyzes the hydrolysis of a single substrate. It degrades insoluble cellulose to soluble sugars. Cellulase is a polysaccharide linked with β-1, 4-glucosidic bond. It is composed of three enzymes: 1, 4 – β-glucanase and β-glucosidase (β-D-glucoside glycohydrolase or cellobiase). Exoglucanase cleaves at the non reducing end of cellulose chain; splits the elementary fibres from crystalline cellulose and β-1, 4-glucosidase hydrolyze cellobiose and cellodextrins (water soluble) to glucose [3].

A large number of microbial enzymes can be produced by fermentation technique. Cellulase plays an important role in producing fermentable sugar from lignocellulosic biomass. It is low-cost, sustainable feedstock for the production of biochemical as it is rich in cellulose content. It comprises of hemicellulose (20-30%), cellulose (30 – 40%), and lignin (20-30%) [4]. The hydrolysis of lignocellulosic material to monosaccharides is a major problem. Therefore, hydrolysis makes it repulsive due to its high cost (Putro et al., 2016). The potential source of enzyme that help in the degradation are wood consuming microbes such as wood borers, termites, beetles etc. as well as cellulolytic fungi, bacteria, actinomycetes and protozoa [5]. Cellulase is extracted from the gut of microorganism like termites (*Isopteran*) and bookworm (*Lepidoptera*) thriving on cellulosic biomass as major feed. The microbial species (termites) plays an important role in the recycling of photosynthetically fixed carbon. Fungi (anaerobic cellulolytic) and bacteria (may be aerobic, anaerobic, thermophilic and mesophilic) are the major source in the production of cellulases [6]. A few species of genera *Aspergillus*, *Penicillium* and *Trichoderma* are used to obtain lignocellulosic biomass. Some bacteria which have high growth rate as compared to fungi have good

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potential to be used in the production of cellulase are *Cellulomonas*, *Cellvibrio*, *Pseudomonas sp*., *Bacillus* and *Micrococcus* [7]. The dairy animals like cow and sheep to produce cellulases have been documented [8]. The yield of enzyme depends on some physiological factors like size of inoculum, temperature, substrate, incubation period, pH [7].

Enzymes are utilized in various industrial processes. Cellulase has created a huge demand as it is broadly applicable in recent years. The development of scientific research on enzyme is mainly based on biotechnology, molecular biology and genetics. Cellulase is mostly used in industrial as well as academic research, as it is easily available [9]. Scientists have shown their keen interest in the research on cellulase due to its high commercial value and importance in various industries [10]. Basic studies have been applied on cellulytic enzyme to demonstrate their biotechnological potential in different industries like food processing, animal feed production, brewery, pulp and paper, and in detergent, textile industries, biorefining, biocoversion of agriculture wastes into sugar and ethanol. As cellulase is used in several industries, its development in biotechnology is yielding new applications for enzyme [11]. It is useful in the production of single cell protein to replace livestock protein using microorganisms obtained from agriculture waste i.e. wheat straw [12]. The production of ethanol from various lignocellulosic materials is one of new technique to produce renewable source of fuel [13]. The potent industrial applications of cellulase enzyme have been critically reviewed. Cellulase is applicable in textile industry for bio polishing as well as laundry as household detergent for improvement of softness and brightness of fabrics [14]. However, the most important use of cellulase is in the bioconversion of plant based cellulosic and lignocellulosic waste as a unique source of renewable fuel [1]. Due to the high demand of cellulase enzyme as it is specific in rapid growth, more stable and highly active will be most storing technology of future. The research for the advancement is desired to achieve improve techno economic appreciable.

2 TYPES AND MODE OF ACTION

Cellulase is composed of three type of enzyme system based on their mode of action [15].

A. Endoglucanase

Endoglucanase is also called 1, 4-β-D-glucan Glucanohydrolase or Carboxymethyl cellulase (EC. 3.2.1.4).It cuts at internal amorphous sites and gives rise to different length of oligosaccharide.

B. Excoglucanase 1, 4-β-D-Glucan Cellobiohydrolases (Cellodextrinases) (EC. 3.2. 1 .9.1)

Exoglucanase acts possessively on the exposed ends of cellulose polysaccharides chains, resulting either glucose or cellobiase as major product.

C .Excoglucanase 1, 4-β-D-Oligoglucan Cellobiohydrolases (Cellodextrinases) (EC. 3.2.1.74)

It is another form of exoglucanase, which acts on the reducing end of cellulose polysaccharide to produce various disaccharides units.

D. B-Glucosidases or β-D-Glucoside Glucohydrolases (EC. 3.2.1.21)

It cleaves at the end of non reducing polysaccharide and hydrolyzes soluble cellodextrins and cellobiose.

3 MICROBES USED FOR PRODUCING CELLULASE

Cellulases are commonly produced by different microbial strains of species including aerobic and anaerobic strains of fungi and bacteria. These strains show higher cellulolytic activity [3]. Cellulases are mostly present as extracellular aggregated structures attached to cells. It can be produced either by cell bound or extracellular [16]. Some microorganism such as *Bacillus*, *Clostridium*, *Cellulomonas*, *Ruminococcus*, *Streptomyces* spp. and Bacillus spp. create differing scope of cellulases remain steady under outrageous condition. Bacillus species such as *B. agardherans* and *B. circulans* have been reported to produce cellulose [17], [18]. *Bacillus amyoliquefacuens* [19], *Bacillus thuringiensis* [20], *Paenibacillus spp.* and *Aeromons spp*. are also documented in literature [21]. Several species of fungi like *Penicillium funicolosum*, *Aspergillus niger*, *Penicillium pinosphilum*, *Penicillium sp. CR-316*, *Trichoderma reesei*. The species of *Trichoderma* were reported as *Fusarium oxysporum, Humicola sp.*, *Glyeophyllum trabeum*, *Malenocarpus sp.* and *Ascomycota*. The organisms such as protists, nematodes, molluscs, crustaceans and insects are also mentioned [22]. The sources of microbial species which are potent to produce cellulase are listed in Table.1.

Table. 1. Sources of some microorganism producing cellulase.

Sr. No.	Various Bacterial Species	Sources	Various Fungal Species	Sources
	Aerobes (free, non-complexed cellulases) **Mesophilic bacteria**		**Mesophilic fungi**	
	Bacillus brevis[a]	Termite gut	*Aspergillus nidulans, A. niger, A. oryzae*	Soil, wood rot

1.	*B. thuringiensis*[a]	Caterpillar gut		
	Bacillus cereus[a], *B. subtilis*[a]	Soil, rumen	*Agaricus bisporus*	Compost
	Cellulomonas fimi[a]	Soil	*Coprinus truncorum*	Soil, compost
	Cellvibrio japonicas	Soil	*Geotrichum candidum*	Soil, compost
	Cytophaga hutchinsonii	Soil, compost	*Penicillium chrysogenum*	Soil, wood rot
	Paenibacillus polymyxa	Compost	*Phanerochaete chrysosporium*	Compost
	Pseudomonas fluorescens	Soil, sludge	*Rhizopus oryzae*	Soil, dead organic matter
	Pseudomonas putida	Soil, sludge	*Trichocladium canadense*	Soil
	Saccharophagus degradans	Rotting marsh grass	*Trichoderma reesei*	Soil, rotting canvas
	Sorangium cellulosum	Soil	*Trichoderma longibrachiatum*	Soil
2.	**Thermophilic bacteria**		**Thermophilic fungi**	
	Acidothermus cellulolyticus	Hot spring	*Chaetomium thermophilum*	Soil
	Thermobifida fusca	Compost	*Corynascus thermophilus*	Mush compost
			Paecilomyces thermophile	Soil, compost
			Thielavia terrestris	Soil
3.	***Anaerobes (complexed or free, non complex cellulases)*** **Mesophilic bacteria**		**Mesophilic fungi**	
	Acetivibrio cellulolyticus	Sewage	*Neocallimastix patriciarum*	Rumen
	Bacteroides cellulosolvens	Sewage	*Orpinomyces joyonii*	Rumen
	Clostridium cellulolyticum	Compost	*Orpinomyces PC-2*	Rumen
	Clostridium cellulovorans	Wood fermenter	*Piromyces equi*	Rumen
	Clostridium josui	Compost	*Piromyces E2*	Feces

	Clostridium papyrosolvens	Mud (freshwater)		
	Clostridium phytofermentans	Soil		
	Fibrobacter succinogenes	Rumen		
	Prevotella ruminicola	Rumen		
	Ruminococcus albus	Rumen		
	Ruminococcus flavefaciens	Rumen		
4.	**Thermophilic bacteria**			
	Caldicellulosiruptor Saccharolyticus	Hot spring		
	Clostridium thermocellum	Sewage, soil, manure		
	Clostridium stercorarium	Compost		
	Thermotoga maritima	Mud (marine)		
	Rhodothermus marinus	Hot spring		
	Anaerocellum thermophilum	Hot spring		

4 PRODUCTION OF CELLULASE

Fermentation technology is widely used in the production of enzymes. The production is considered as cost representative step [22]. It is done by two fermentation techniques: Solid State Fermentation and Submerged Fermentation. The substrate used in the process should be cost effective and highly productive such as wheat, bran straw, rice, bran, green gram, husk etc. [23].

1. Solid State Fermentation (SSF)

This process requires solid substrate (i.e. nutrient rich waste) which can be easily available and recycle for the growth of microorganisms [23]. This technique is more efficient as it has (i) lower consumption of energy and water; (ii) highly concentrated product; (iii) reduced waste stream [24]. It lacks foam build up and reported to be most appropriate process in enzyme production [25]. *Bacillus sp. NZ*, Clostridium *thermocellum*, *B. cereus* and *B. licheniformis* are produced by SSF [22].

2. Submerged Fermentation (SmF)

This process utilizes liquid nutrient rich substrates like molasses and broth for the growth of microorganisms [26]. It is mainly used for purification of enzyme due to higher production rate as well as protein rate. Some microorganisms produced by SmF are *Aspergillus flavus [27]*, *Aspergillus niger* [28], *Bacillus amyloliquefaciens UNPDV-22* [29], *Bacillus sp. SMIA [30]*, *Brucella*, and *B. licheniformis* RT-17 [31].

5 MECHANISM OF CELLULOSE HYDROLYSIS

Cellulolytic microorganisms play an important role in the hydrolysis of lignocellulosic polymer materials. Cellulase enzyme breaks down cellulose into monosaccharides using three major enzymes: Endoglucanase, exoglucanase and β-glucosidase. Endoglucanase randomly cleaves at internal site of cellulose to yield oligosaccharides of different lengths while on the other hand, exoglucanase attacks on the reducing and non-reducing ends of cellulose to liberate glucose, cellobiose and cellooligosaccharides hydrolyzed by β-glucosidases [32]. The efficient hydrolysis requires presence of β-glucosidases as it is inactive against amorphous and crystalline cellulose. The end products of endoglucanase and cellobiohydrolases are cellodextrans and cellobiose respectively which inhibit the enzyme activity [33]. The mechanism of hydrolysis is shown in Fig. 1.

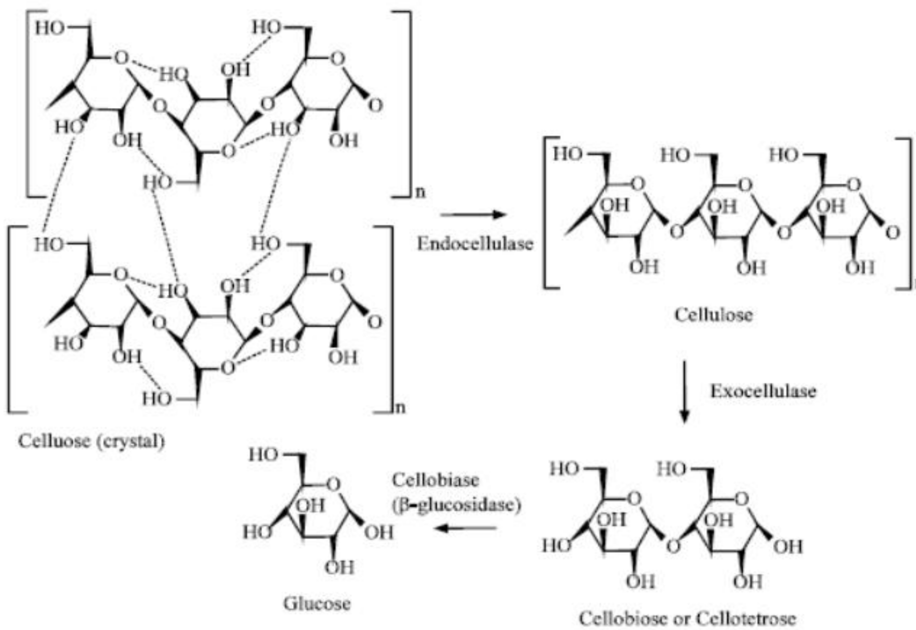

Fig. 1. Mechanism of action of cellulase enzyme

6 OPTIMIZATION OF PHYSIOLOGICAL FACTORS

Cellulase activity is enhanced by various physiological factors like pH, temperature, substrate, carbon source, and nitrogen source and incubation period. These should be optimized over a wide range [34].

A. Effect of pH and temperature

The pH and temperature play a key role in the enzyme production. Their optimization has to be done as it affects the production of enzyme directly. The cellulase activity has been recorded at pH value 5.0 at temperature 30°C for *Penicillium* and pH value 4 at temperature 30°C for *Aspergillus tubingensis KY615746* [22].

B. Effect of carbon source

The carbon sources such as starch, glucose, maltose, lactose and fructose has been used to replace glucose (original carbon source). Glucose has been documented as the highest cellulase production. It has been found to be significant source for production of cellulase enzyme. Ishihara et al. studied the utility of source and found sucrose, glucose and mannitol as suitable source for optimum levels of cellulase production [35].

C. Effect of nitrogen

The nitrogen source are yeast extract, peptone, urea and ammonium sulphate, ammonium chloride. The extracellular cellulase production has shown sensitivity towards nitrogen sources [36]. Mandrels et al. observed the effect of nitrogen in the growth medium. Among nitrogen sources ammonium sulphate has been recorded as good nitrogen source. The maximum activity of cellulase has been obtained on yeast extract and combination of peptone and ammonium molybdate [34].

D. Effect of incubation period

The time taken by microorganism to synthesize enzyme by using medium nutrients is termed as incubation period. It depends on the type of fermentation technique and microorganism used. As SSF take longer time in comparison with SmF. Therefore, bacteria requires less time than fungi for cultivation. Ahmed et al. has been reported 192 hrs and 168 h suitable for fermentation using *Myceliophthora heterothalika* containing wheat bran and sugarcane [22].

7 APPLICATIONS OF CELLULASE

Cellulase is widely used in various industries such as food, animal feed, agriculture and biofuel, textile and laundary. These are discussed below.

1. Food processing industry

Cellulase plays an important role in food industry. It is used to improve nutritive quality of fermented food items and in production of low calorie food ingredient oligosaccharides. It is utilized in the production of food colouring agent as carotenoid which is a group of colouring substance responsible for many plant colours from red to yellow [33]. Macerating enzyme consists of cellulases with pectinase and hemicellulase are used to improve the cloud stability and texture of nectar and purees [37]. Cellulase and pectinase are used to disrupt the cell wall of potato, carrot and orange peel and release carotenoid [38]. It is also used to release antioxidants from fruit and vegetable pomace and helps in controlling atherosclerosis, cronry heart disease and reduce food spoilage. [39].

2. Animal feed industry

The animal feed industry is an important sector of agribusiness comprising poultry, pigs, ruminants, pet foods and fish farming. Cellulase is utilized to improve feed, body weight and feed gain by ruminants [1]. Cellulase degrades certain cereal component and upgrades the nutritional value of feed. The dietary fibres present in feed consist of non – starch polysaccharides such as arabinoxylans, cellulose, other plant component including chitin, β-glucan, waxes, lignin, and oligosaccharides like proteases, amylases and glucanases [40]. In the feed of monogastric animals, β-glucans which helps in decreasing intestinal viscosity and release nutrients from plants (grains), enhance the digestion and absorption of feed and weight gain by chickens and boilers. Cellulase in addition with proteases is significant to improve the quality of pork meat [41].

3. Textile and laundry industry

Cellulase is the third largest group of enzymes used in textile and laundry. It is used to improve the fabric quality by biostoning of denim garments, biopolishing of non-denim fabrics, defibrillation and biofinishing [42]. In biostoning process, cellulases act on cotton fabric and break down small fibre ends results in loosening the dye after washing [38]. This process includes the fibre damage, less work intensive. It increases productivity of machine [32]. The fibres such as cotton, linen, viscose, ramie, and lynocell (pure cellulosic fork of wood) are used in the manufacture of fabric. These fibres form fuzz as well as pilling results in negative impact of cellulosic fibres [1]. Therefore, biopolishing is used to overcome this problem by bleaching, desizing, scouring, bleaching and dying. Cellulase is also used in biofinishing as it is rich in endoglucanase activity. Cellulase along with protease and lipase in detergents widely used as household washing powder [38]. Cellulase is applicable to remove colour brightness, softness in cotton fabrics and helps in removing soil from inter fibril spaces [32], [43].

4. Brewery industry

Cellulase is used in the production of wine and beverages. In wine production, the enzyme such as pectinases and glucanases and hemicellulases play a key role by removing colour extraction skin maceration, filtration and improve the stability and quality of wine [44]. Macerating enzyme also improves settling, press ability and juice yield of grapes for fermentation [38]. Brewing is based on the action of enzyme activated during malting and fermentation. Malting mainly depends on germination of seed which initiates the activation of amylase, biosynthesis carboxypeptudase and cellulases to produce high quality malt [45].

5. Paper and pulp industry

Cellulase has broader applications in paper and pulp industry. It is one of the largest industrial sectors. Cellulase is used for bio-mechanical pulping, bio-modification of fibres, removing of ink coating and toners from paper, improving drainage of paper mills. It is also used in the manufacture of soft paper like sanitary paper, paper towels and biodegradable cardboard [36]. Cellulases are also used to enhance the bioleachability of soft wood kraft pulp [43]. The cellulase alone or combination of cellulose and hemicellulase has been used to improve pupil betability, runnability, paper sheet and trouble free printing process. Cellulase in addition with xylanase is considered as most affective for recycling the waste paper from old books, newspapers, magazines. This process is known as deinking. It leads to the formation of newspaper or ethanol. The enzymative deinking has ability to improve fibre brightness, pulp free and cleanliness, enhance strength properties and reduce fine particles in the pulp [46], [47]. Deinking also prevents the alkaline yellowness, change the ink particle size by using cellulases at acidic pH [42], [44].

6. Agriculture industry

Cellulase is used for many purposes in agriculture industry. It is used for increasing crop growth and in improvement of soil fertility [48]. The use of exogenous cellulase is a potential means to straw decomposition and increase soil fertility [49]. Straw incorporation is strategy to improve soil quality and reduce the dependence on mineral fertilizers. Cellulase is also used as biocontrol agents to provide protection against plant pathogens [1].

7. Biofuel industry

Cellulase is very important in the biofuel production. The consumption of fossil fuels and increasing demand of alternative renewable source of energy has developed a huge interest in it [25]. The biocoversion of lignocellulosic substrates and other enzymes are used for the commercial production of biofuel [50]. Cellulase is used in the conversion of non food biomass such as agriculture waste and energy crops into fermentable sugar for renewable fuel and chemical [51]. The use of agrowastes such as sugarcane, bagasse, rice straw for bioethanol production are invaluable and do not create any problem in the environment.

8 CONCLUSION

Currently, cellulase is gaining huge attention of industries and causing an enormous economic impact. With a view to develop an economically feasible technology for growing demand of cellualse and also to realise its potential in biotechnological research is very crucial. Various industries favour the technology having low cost, readily available carbon sources (agrowastes) and eco-friendly strategies. So, cellulase can be modified for the effective conversion of easily available agrowaste biomass into fermentable sugar, fuels and other products. Therefore, appropriate methodology has to be developed for producing high enzyme titre at low cost and also eco-friendly in nature.

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REFERENCES

1. M. K. Bhat (2000).Cellulase and related enzymes in biotechnology, Biotechnol. Adv.18, 355-383

2. Nowak, J. Florek, M. Kweatek, W. Lekki, J., Chevallier, P. and Zieba E. (2005).Composite structure of wood cells in petrified wood, Mater. Sci. Eng, 25, 9-39

3. Gomashe A. V., Gulhane P. A. and Bezalwar P. M. (2013).Isolation and screening of cellulase degrading microbes from Nagpur soil region, Int. J. of Life Sci., 1(4), 291-293

4. Chang L. Ding M, Bao Letal (2011).Characterization of biofunctional xylanase /endoglucanase from yak rumen microorganisms, Appl. Microbial Biotechnol., 90, 1933-1942.

5. Libin Mathew Varghese, Sharad Agarwal , Divya Sharma, Rishi Pal, Ritu Mahajan (2017).Cost effective screening and isolation of xylano-cellulolytic positive microbes from termite gut and termitarium, Springer –Verlag, Berlin, 7, 108

6. Jungebloud , A., Bohle, K., Gocke, Y., Cordes, C., Hern, H. Hempel, D.C. (2007).Quantification of product – Specific gene expression in biopellets of *Aspergillus niger* with the real-time PCR, Enz. Microb. Techn., 40(4), pp.653-660

7. Immanuel G., Dhanusha R., Prema P., Palavesam A. (2006).Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment, Int J. Environ. Sci. Tech., 3(1), 25-34.

8. Vipul Verma, Alpika Verma and Akhilesh Kushwaha (2012).Isolation and production of cellulose enzyme from agricultural fields in district Hardoi, Advances in Appl. Sci., Res.3 (1), 171-174

9. A.Singh, R.C.Kuhad, O.P.Ward, in: Industrial app. of microbial cellulases (2007).Lignocellulose Biotechnology: Future prospects, I. K. International publishing House, 345-358

10. Anita Singh, Somvir Bajar, Arti Devi, Deepak Pant (2021).An overview on the recent developments in fungal cellulase production and their industrial applications, 14:100652

11. Ashiq Margey, Sanjay Sahay, Ragini Gothalwal (2018).Cellulases for biofuel: A review, Int. J. of Recent Trends in Science and Technol., 18, 17-25

12. Eveliina Voutilanien, Ville Pihlajaniemi, Twre Parvianien (2021). Economic comparison of food protein production with single cell organisms from lignocellulose side stream, Biore. Technol. Reports, 14:100683

13. Lee B.H., Kim B.K., Lee Y.J., Chung C.H., Lee J.W. (2010).Industrial Scale of optimization for the production of Carboxymethylcellulase from rice bran by a marine bacterium *Bacillus subtilis subsp. subtilis A-53*, Enzyme. Microb. Technol., 46(1), 38-42

14. K.Shanmugapriya, P.S. Saravana, Krishnapriya1, Malini Manoharan1, A. Mythili, Sunu Joseph (2012).Isolation, screening and partial purification of cellulase from cellulase producing bacteria, International Journal of Advanced Biotechnology and Research, 3(1), 509-514

15. Sangrila Sadhu and Tushar Kanti (2013).Cellulase Production by Bacteria: A Review, British Microbiology Research Journal, 3(3), 235-25

16. Sonia Sethi, Aparna Datta, B.Lal Gupta, and Saksham Gupta (2013).Optimization of Cellulase Production from Bacteria Isolated from Soil, ISRN Biotechnology, 201, 1-7

17. Li Y.H., Ding M., Wan J., Xu G.J., Zhao F. (2006). A Novel thermoacidic endoglucanase, Ba-EGA, from a New cellulose-degrading bacterium, *Bacillus sp. AC-1*, Applied Microbiology and Biotechnology, 70, 430-436

18. Korpole S., Sharma R,, Verma D. (2011).Characterization and phylogenetic diversity of Carboxymethyl cellulase producing Bacillus species from a landfill ecosystem, Int. J. Microbiol., 51(4), 531-535

19. Bai S., Kumar M.R., Kumar D.J.M., Balashanmugam P., Balakumaran M.D., Kalaichelvan P.T. (2012).Cellulase production by *Bacillus subtilis* isolated from cow dung, Arch. Appl. Sci., 4(1), 269-279.

20. Lin L., Kan X., Yan H., Wang D. (2012).Characterization of extracellular cellulose degrading .Enzymes from *Bacillus thuringiensis* strains, Elect. J. Biotechnol., 15(3), 310-317.

21. Farjana Islam and Narayan Roy (2018).Screening, purification and characterization of cellulase from cellulase producing bacteria in molasses, Islam and Roy BMC Res Notes, 11,445

22. Vijay Pratap Singh and Divya Sharma (2020).Cellulase and its role in industries: A Review, International Journal on Agricultural Sciences, 11(1), 1-7

23. Babu K.R., Satyanarayana T. (1996).Production of Bacterial enzymes by solid state fermentation, J. Sci. Ind., 55, 464-467

24. Zhuang J., Marchant M.A., Nokes S.E., Strobel H.J. (2007).Economic analysis of cellulase production methods for bio-ethanol, Appl. Eng. Agr., 23(5), 679-687.

25. Pandey, A.Selvakumar, C.P., Soccol R, and Nigam P. (1999).Solid State fermentation for the production of industrial enzymes , Current Science, 77(1), 149-162

26. Subramaniyam R., Vimala R. (2012).Solid state and submerged fermentation for the production of bioactive substances: A comparative study, Int. J. Sci. Nature, 3(3), 480-

486.

27. Gomathi D., Muthulakshmi C., Kumar D.G., Ravikumar G., Kalaiselvi M., Uma C. (2012).Submerged fermentation of wheat bran by *Aspergillus flavus* for production and characterization of Carboxymethyl cellulose, Asian Pac. J. Trop. Biomed., 2,67-73

28. Reddy G.P.K., Narasimha G., Kumar K.D., Ramanjaneyulu G., Ramya A., Kumari B.S., Reddy B.R. (2015).Cellulase production by *Aspergillus niger* on different natural lignocellulosic substrates, Int. J. Curr. Microbiol. Appl. Sci., 4, 835- 845

29. Singh S., Moholkar V.S., Goyal A. (2014).Optimization of Carboxymethylcellulase production from Bacillus Amyloliquefaciens SS35. 3 Biotech, 4(4), 411–424

30. Ladeira S.A., Andreia E.C., Delatorre B., Barbosa J.B., Martins M.L.L.(2015).Cellulase production by thermophilic *Bacillus sp. SMIA-2* and its detergent compatibility, Electron J. Biotechnol, 18, 110-115

31. Tariq R., Ansari I., Qadir F., Ahmed A., Shariq M., Zafar U., Ahmad A., Khan S.A., Sohail M. (2018).Optimization of endoglucanase production from thermophilic strain of *Bacillus Licheniformis RT-17* and its application for saccharification of sugarcane bagasse, Pak. J. Bot., 50(2), 807-814

32. R.K.Sukumaran, R.R.Singhania, A. Pandey (2005), J. Sci. Ind, 64,832-844

33. R.Gupta, G.Mehta, D.Deswal, S.Sharma, K.K.Jain, A. Singh, R.C.Kuhad, in: R.C. Kuhad and A.Singh (eds.) (2013).Biotechnology for Environmental Management and Resourse Recovery, 89-106

34. Saini A., Aggarwal N.K., Yadav A. (2017).Cost-effective cellulase Production using *Parthenium hysterophorus* biomass as an unconventional lignocellulosic substrate, 3 Biotech, pp.7-12

35. K.V. Ramana, A. Tomar and L. Singh (2000).Effect of various carbon and nitrogen sources on cellulose synthesis by Acetobacter xylium, World Journal of Microbiol. And Biotechnol., 16(3), pp.245-248

36. Prasanna H.N., Ramanjaneyulu G., Rajasekhar R.B. (2016).Optimization of cellulase production by *Penicillium sp.*, 3 Biotech, 6, 162

37. C.Grassin, P. Fauquembergne, in: T.Godfrey, S.West (Eds.) (1996).Industrial Enzymology, second ed., 226-4

38. R.C.Kuhad, R. Gupta, A. Singh, Enzyme Res. (2011)10

39. A.S. Mayer; S.M. Jepsen, N.S, Sorensen (2010).J. Agric. Food Chem., 46, 2439-2446

40. R.C. Kuhad, R. Gupta, A. Singh (2011), Microbial cellulases and their industrial applications, Res. (2011)10

41. W.D.Cowan, in: T.Godfrey, S.West (Eds) (1996).Industrial Enzymology, Second ed., Macmillan press, 360-371

42. O.Kirk, T.V. Borchert, C.C., Fuglsang (2002).Curr. Opin. Biotechnol., 13, 345-351

43. A.Singh, R.C.Kuhad, O.P.Ward, in: Industrial app. of microbial cellulases (2007).Lignocellulose Biotechnology: Future prospects, I.K.International publishing House, 345-358

44. L. Thomas, H. Ram, A. Kumar, and V.P.Singh (2005), J. Plant Develop. Sci., 5, 237-247

45. R. Gupta, G. Maheta, D. Deswal, S. Sharma, K. K. Jain, A. Singh (Eds) (2013).Biotechnology for environmental management and Resource Recovery, 10, 89-106

46. R.C.Kuhad, R.Gupta, Y.P.Khasa, A. Singh (2010).Bioresource Technol., 101, 8348-8354

47. R.C.Kuhad, G.Meheta, R.Gupta, K.K.Sharma (2010).Biomass Bioenergy 34, 1189-119

48. Escobar MEO, Hue NV (2008).Temporal changes of selected chemical properties in three manure-amended soils of Hawaii, Bioresource Technol, 99(18), 8649-8654

49. W. Han, M. He (2010).Bioresour. Technol., 101, 3724-3721

50. Srivastava N., Rawat R., Sharma R, Oberoi H.S.., Srivastava M. and Singh J. (2014).Effect of nickelcobalite nanoparticles on production and thermostability of cellulases from newly isolated thermotolerant *Eurotiyomycetes sp.*, N. S. Appl. Biochem. Biotechnol., 174, 1092-110

51. Payne C.M., Knott B.C., Mayes H.B., Hansson H, Himmel M.E., Sandgren M., Stanberg J. and Beckham G.T. (2015). Fungal Cellulases, Chem Rev.115, 1308, 1448