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# **Cellulase: Distribution, Production, Characterization and Industrial Applications**

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### **Authors' contributions**

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## **ABSTRACT**

Cellulase enzyme complex is comprised of three enzymes namely exo-glucanase, endo-glucanase and  $\beta$ -glucosidase which act synergistically to deconstruct cellulosic biomass in order to produce fermentable sugars. The enzymes are produced naturally by the living organisms such as bacteria, fungi and algae. The majority of microorganisms that live in extreme environments including hot/cold springs, rumen stomach, deep ocean trench, acidic/alkaline pH environment, have been regarded as appealing producers of cellulase. Cellulases produced by microorganisms have enormous applications in different industries such as agriculture, food and feed production, brewing, textile, laundry and biofuel production. Scientists as well as industry researchers consider cellulases as a prospective candidate for further studies due to the intricacy of the enzyme system and massive industrial potential. Scientific belief in its production and further studies challenges are receiving greater attention these days, notably in the intent of decreasing its production cost at the industrial scale. In this review, future possibilities of using cellulase for various industrial applications are also addressed.

*Keywords: Cellulase; cellulolytic enzymes; microbes; bioenergy; biofuels; detergents.*

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## 1. INTRODUCTION

The massive demand for commercially sustainable enzymes is steadily increasing and leading to the need for imperishable methods. Enzymes are specific for bio-chemical reactions, also cost-effective, minimize the energy consumption, environmental stewardship, and superior efficiency. They are becoming increasingly popular due to these distinct characteristics. Various studies are done to optimize their production and as a result, to grow new products and services for various commercial bio-processes. Considering the positive aspects of specific enzymes, much efforts are being done to search novel biological enzymes [1,2].

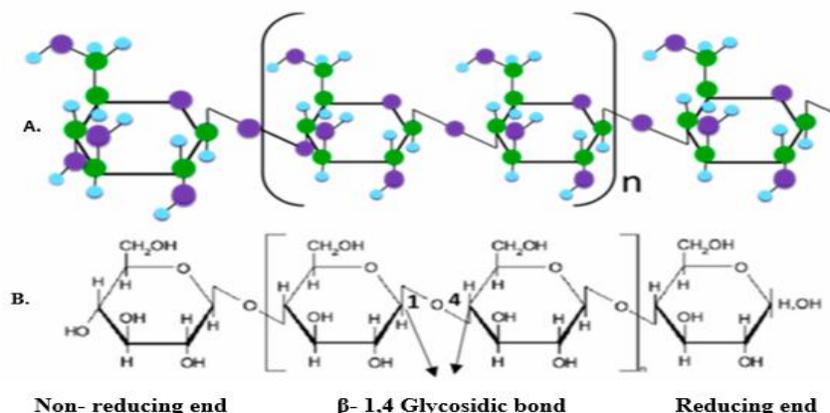
Cellulose is the most ample carbohydrate on earth which is synthesized mainly by the plants in the process of carbon assimilation. It is considered to be a structural carbohydrate which by being part of cell wall, has mostly protective function. It is a linear polysaccharide composed of glucosyl moieties linked through  $\beta$ -1, 4 glycosidic linkages [3]. The plant cell wall has mainly ligno-cellulosic biomass, where cellulose contents range from 35 to 50% in different plant species. The percent composition of hemicelluloses having mainly pentosyl moieties in their structure has been reported to be between 20 to 35%. The lignin contents vary from 5 to 30 % [4].

Cellulases (sometimes also called as cellulosome) are considered to be a multi-enzyme complex and comprised of more than

one enzyme that act synergistically to hydrolyze cellulose. The cellulosome is comprised of at least three enzymes namely, endo-1, 4- $\beta$ -D-glucanase (also known as carboxymethyl cellulase; EC 3.2.1.4), exo-1, 4- $\beta$ -D-glucanase (also referred as cellobiohydrolase (CBH); EC 3.2.1.91) and  $\beta$ -glucosidase (EC 3.2.1.21), respectively [5]. The endo-1,4- $\beta$ -D-glucanase randomly cleaves  $\beta$ -1,4 glycosidic linkages in cellulose producing oligosaccharides of glucosyl moieties linked by  $\beta$ -1,4 linkages. The exo-1, 4- $\beta$ -D-glucanase, also commonly called as exoglucanase splits  $\beta$ -1,4 glycosidic linkage alternately generating  $\beta$ -cellobiose as the product; and  $\beta$ -glucosidase acts directly on  $\beta$ -cellobiose (disaccharide) and releases  $\beta$ -D-glucose [6,7]. Cellulase enzymes are hydrolytic enzymes and being important from both an industrial and biotechnological standpoint, have much demand in the global market. Microbes seem to be the most prominent source for industrial enzymes because of their wide biochemical diversity and the ease through which they can be produced on a large scale [1,2]. Cellulase occurs in nature and primarily secreted by the microbes like moulds, fungi and bacteria [8].

## 2. CHEMICAL STRUCTURE OF CELLULOSE

As indicated in Fig.1, each repeat unit comprises of three hydroxyl groups. These hydroxyl groups and their capacity to make hydrogen bonds among cellulose chains regulate the physical properties of cellulose [9].



**Fig.1. Biochemical structure of the cellulose unit. A. 3D structure of cellulose B. Structural formulae of cellulose [9]**

### 3. CELLULOLYTIC ENZYMES

Cellulases are inducible enzymes that hydrolyze  $\beta$ -1,4 linkages in cellulose chains, and are synthesized by a number of microorganisms throughout their growth on cellulose enriched substances [10,11]. The fungal parasites which attack the plants, secrete cellulases to invade the plant tissue. The organisms involved in the senescence of dead plant tissues also secrete this enzyme to break the plant cell wall. Cellulases in their structure are consisted of modules having folded, functionally and structurally distinct domains [12]. Most cellulases have one catalytic domain (CD) and also a

cellulose-binding domain (CBD) responsible for cellulose chain hydrolysis and supports cellulase binding to cellulose, respectively [13]. A schematic degradation of cellulose by cellulase has been indicated in Fig. 2. It is proposed that cellulase binds on the microfibrils of cellulose and loosened them to convert cellulose microfibrils in the amorphous form. Thereafter, endo- and exo-cellulases are accessible to cellulose polymer and fragment it into smaller chained oligomeric molecules. Thereafter, these oligomers get further fragmented into cellobiose. The cellobiose moieties get converted into glucosyl moieties by the  $\beta$ -glucosidase enzyme [14].

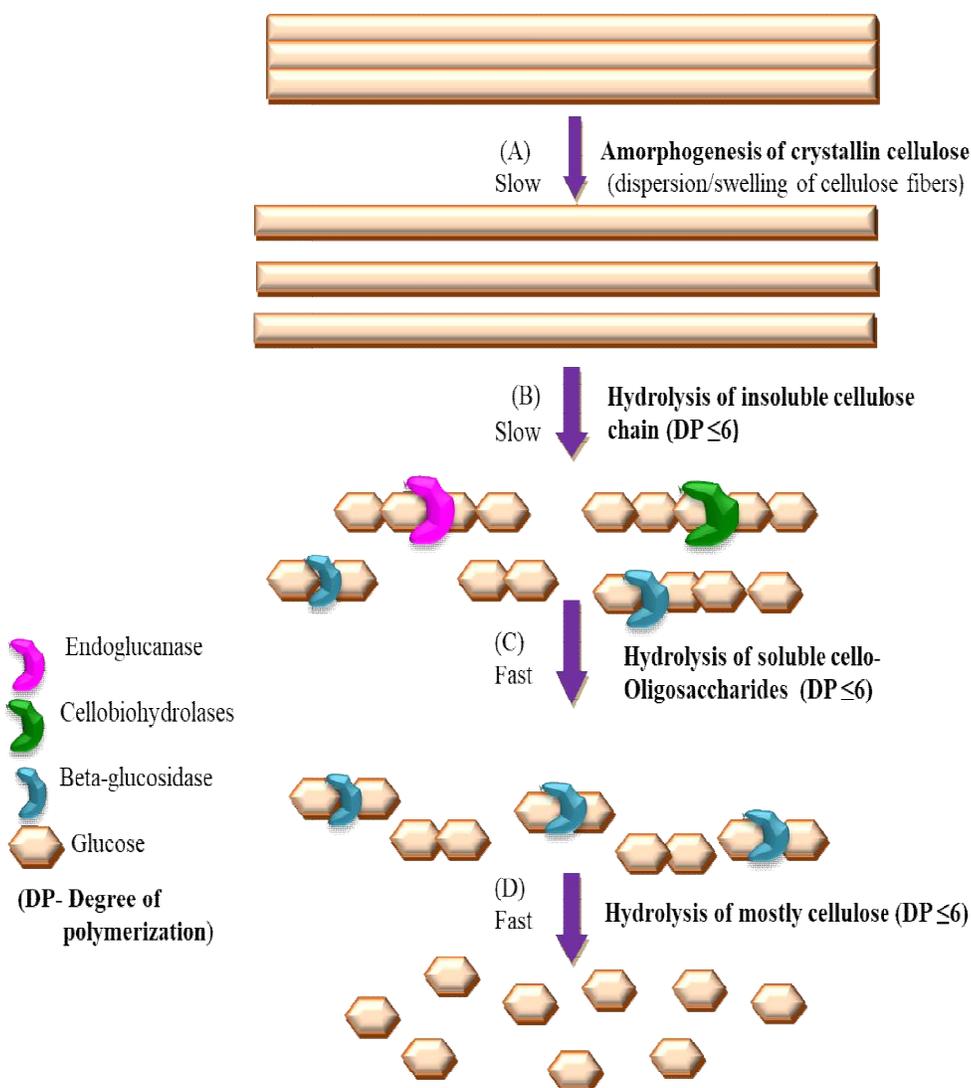


Fig. 2. A schematic degradation of cellulose by cellulase [14,15]

**Table 1. Bacterial cellulase enzyme systems [16]**

<b>Enzyme</b>	<b>EC. number</b>	<b>Reaction</b>	<b>Other Names</b>
Endo 1,4 $\beta$ -D-glucanglucano-hydrolase	EC. 3.2.1.4	Oligosaccharides of different lengths are formed by cutting randomly at internal amorphous sites of cellulose.	Endoglucanase; Endo 1,4- $\beta$ -glucanase; Carboxymethyl cellulase; $\beta$ -1,4 endoglucan hydrolase, Endocellulase
Exoglucanase or 1,4- $\beta$ -D-glucan cellobiohydrolases (Cellobiohydrolases)	EC.3.2.1.91	In cellulose and cellotetraose, hydrolyzes 1,4--D glucosidic linkages, and produces cellobiose as from non-reducing end of a chain.	Exoglucanase; Exocellobiohydrolase; 1, 4- $\beta$ -cellobiohydrolase
Exoglucanases or 1,4- $\beta$ -D-oligoglucan cellobiohydrolases	EC. 3.2.1.74	Cellobiose is produced from cellooligosaccharides or p-nitrophenyl—D-cellobioside.	Cellodextrinases
$\beta$ - Glucosidases or $\beta$ -D-glucoside gluco-hydrolases	EC. 3.2.1.21	Hydrolysis of D-glucosyl residues from the non-reducing end of the chain leading to the release of D-glucose.	Gentiobiase; Cellobiase; Amygdalase
Cellobiose: orthophosphate $\alpha$ -D-glucosyl transferase	EC. 2.3.1.20	It converts cellobiose into D-glucose-1-phosphate and D-glucose in the presence of Pi (phosphorolytic cleavage of cellobiose).	Cellobiose phosphorylase
1,4- $\beta$ -D-oligoglucan orthophosphate $\alpha$ -D-glucosyl transferase	EC. 2.3.1.49	It causes the cleavage of glucosyl moieties from cellodextrins as glucose-1-phosphate in the presence of Pi.	Cellodextrin phosphorylase
Cellobiose 2-epimerase	EC. 5.1.3.11	It hydrolyses the cellobiose into D-glucosyl-D-mannose.	Cellobiose 2- epimerase
Complete Cellulase system	-	Substantial hydrolysis of crystalline cellulose is catalysed by this enzyme.	Total cellulase

#### 4. CLASSIFICATION OF CELLULASES

The cellulases are classified on their catalytic action. Microorganisms produce extracellular cellulases which are either free or associated to hydrolyze insoluble cellulose and metabolize it. The biochemical study of cellulase systems from various microbes during last three decades has been thoroughly reviewed [16].

Degradation of cellulose has been shown to be a complex process that requires the synergistic intervention of several glycoside hydrolases (GH) families [12]. The GH belong to a superfamily of an inclusive group of plant cell wall degrading enzymes. The GH act on glycosidic bonds of carbohydrate or between carbohydrates and non-carbohydrate moieties, and catalyze many different reactions [17]. Based on the mechanism of catalysis, cellulase systems have been categorized and shown in Table 1.

#### 5. MICROBIAL SOURCES FOR CELLULOLYTIC ENZYMES

The abundance of microbial cellulolytic enzymes seems to be everywhere and distributed over the world's most varied and extreme environments on the planet. A huge diversity has been found among cellulase producing microorganisms along with their extreme habitat such as the rumen of ruminants, a marine or saltwater environment, soil, insects and intestines of termites [18]. The key producers of cellulolytic microorganisms are fungi, bacteria, and actinomycetes. There are large number of reports on various types of microbes which cause hydrolysis of cellulose, [8,19]. Some of the recently discovered bacterial and fungal species for cellulase production are mentioned in Tables 2 and 3, respectively. Many reports are in the literature on detection and functional characterisation of microbial enzymes inhabiting extreme environmental habitats [20].

##### 5.1 Rumen Microflora

The ruminant diet is mainly composed of cellulose and it plays a crucial role in rumen fermentation [21]. The breakdown of lignocellulosic feed in ruminants is feasible due to the enzymes secreted by the rumen's microbial population. These enzymes allow consumption of more lignocellulosic feed, and therefore, animals have the access to the nutrients and energy stored in the ligno-cellulosic feed [22]. The rumen microbiota is considered to

degrade ten thousand million tonnes of cellulosic materials worldwide [23]. The key producers of cellulolytic enzymes are bacteria and fungi [24]. The metagenomic researchers got attention to learn more about microbial ecology and enzymatic diversity exploring rumen microbiome since it is a hub of cellulolytic microbes. Several metagenomic studies have found a variety of CAZymes (carbohydrate active enzymes) in various ruminants like Holstein–Friesian cross bred steers [25,26], buffalo [27], Saudi sheep [28], camel [29] and goat [18]. In rumen microbiota, bacteria (both Gram-negative and Gram-positive species) are predominantly present in the rumen environment. The most common ruminal cellulolytic bacteria are *Ruminococcus flavefaciens*, *Fibrobacter succinogenes* and *Ruminococcus albus*, which produce enzymes effective for degrading crystalline cellulose [30]. Besides, other bacteria like *Clostridium lochheadii*, *Clostridium longisporum*, *Eubacterium ruminantium*, *Butyrivibrio fibrosolvans*, *Eubacterium cellosolvans* and *Prevotella ruminicola* are also found in rumen capable to lyse the fibers [31]. Anaerobic fungi found in Herbivores' rumens, produce hydrolytic enzymes which degrade plant fiber [32]. Fungal biomass accounts for 8–20% of the total rumen microbial biomass [33]. Some examples of rumen fungi are *Neocallinastix frontalis* MCH3, *Piromyces* (*Piromonas*) *communis* FL, and *Caecomyces* (*Sphaeromonas*) *communis* FG10 [34].

##### 5.2 Microbes in guts of Insects and Termites

Woody plant components are consumed by a variety of invertebrates. Termites, wood borers and beetles are examples of pests. These pests usually have a diversified and ecologically abundant microbiome consisting of bacteria, archaea, and protists [35]. A cellulase secreting *Bacillus* sp. BMP01 has been isolated from the gut of the termite, *Cryptotermes brevis* [36]. Similarly, *Bacillus*, *Paenibacillus*, and *Flexibacter* groups capable of secreting cellulase have been dominantly found in termites, *Zootermopsis angusticollis* and *Nasutitermes lujae* [37]. The presence of endo  $\beta$ -1, 4 glucanase in the digestive system of the wood-eating termite, *Coptotermes formosanus* Shiraki has been described by Nakashima et al. [38]. Besides, many other cellulolytic bacteria including *Acinetobacter*, *Pseudomonas*, *Streptomyces*, *Bacillus*, *Clostridium*, *Ochrobactrum*, *Paenibacillus*, *Brevibacillus*, *Cellulomonas*,

*Citrobacter*, *Eubacterium*, *Serratia*, *Paenibacillus*, *Klebsiella*, *Brevibacillus*, *Eubacterium*, *Serratia*, *Citrobacter* have been shown to be present in the guts of termites [36].

Besides, cellulolytic yeasts have also been found in the guts of termites [39]. Other cellulolytic yeast strain (ZM1, ZM2 and ZM3) are found in *Zootermopsis nevadensis* which belongs to the genus *Sporothrix* [37].

### 5.3 Soil Microbial Diversity

Soil is often considered to be a significant source of cellulose, which is found in the state of decaying plant biomass in both top soil layer and beneath the top soil layer [40,41]. The degradation of this cellulosic material is known to be aided by cellulolytic microorganisms including fungi, bacteria and actinomycetes. The abundance and functions of cellulolytic microorganisms are largely determined by the soil composition. For instance, it has been revealed that the abundance of cellulolytic bacteria is higher in forest soil than other types of soils like composted, garden, agricultural, arid and dry soils [42].

The fungi namely *Trichoderma*, *Penicillium*, *Aspergillus*, *Fusarium* and *Colletotrichum* are of industrial importance due to magnificent production of cellulase [43,44]. The *Trichoderma reesei* is the most employed species out of all *Trichoderma* species for different types of industrially important cellulases [45]. In addition, *Aspergillus* and *Penicillium* are also significant cellulase producers and robustly utilised in biomass degradation and biofuel manufacturing [46].

It has been well documented that among the soil cellulolytic bacteria, *Bacillus sp.* (*B. licheniformis*, *B. subtilis* and *B. cereus*), *Serratia* and *Pseudomonas sp.*, are important which significantly utilize cellulose present in various forest and agricultural wastes, and transform it into high-value products [47]. Another significant class of soil microorganisms is *Actinobacteria*, known for producing a substantial amount of cellulase. There are many species of *Cellulomonas* such as *C. terrae*, *C. iranensis*, *C. pachnodae*, *C. fimi*, *C. aurantiaca* and *C. uda*, and of *Streptomyces* such as *S. olivochromogenes*, *S. lividans* and *S. flavogriseus*, which are reported to secrete cellulases [48-56].

### 5.4 Extremophiles

Extremophiles are the microorganisms that live in a diverse variety of extreme climatic environments including temperature, pressure and extreme radiations, as well as geochemical inordinates like salinity, acidic or alkaline pH, ionic strength and oxygen species [57]. The majority of researches have been conducted on extremophiles such as thermophiles, alkalophiles, acidophiles, halophiles and sychrophiles. The enzymes isolated from extremophiles are shown to be active and stable under adverse conditions. Consequently, extremeophiles derived enzymes are effective biocatalysts for a variety of commercial bioprocesses including metabolising sugars, polysaccharides, and plant biomass under a variety of environmental conditions [18].

### 5.5 Thermophiles

Thermophiles are the microbes which grow optimally at higher temperatures ranging from 60 to 108°C [58]. Beguin et al. [59] were the pioneer in studying cellulase coding gene in the thermophilic bacteria, *Clostridium thermocellum*. The cellulase secreted by the thermophilic *Clostridium* species is important for fuels like n-butanol and isobutanol [60]. The *Bacillus* is another notable genus that has been associated with thermophilic cellulases. In the detergent industries, thermophilic *Bacillus* has been considered to be important which produces exo- and endocellulases with nearly 100% efficiency at 60°C [61]. Thermophilic bacteria such as *B. licheniformis* and *B. coagulans*; *Geobacillus thermoleovorans*, and *Paenibacillus* are found to have cellulolytic activity at temperatures of 50°C and higher [62-65]. There are reports indicating that hyperthermophilic bacteria such as *Aquifex aeolicus*, are able to grow at 95°C and they have thermostable endoglucanase having optimum temperature between 80 and 90°C. The *Thermotoga sp.* secretes cellulase at 100 –106 °C growth temperature [66- 68].

In addition to bacteria, several thermophilic fungi like *Sporotrichum pulverulentum*, *Aspergillus* (*A. versicolor*, *A. terreus*, *A. wentii*), *Myceliophthora thermophila*, *Chaetomium thermophilum* and *Humicola insolens* are also reported to secrete thermophilic cellulases having optimum temperature in the range of 60 to 65°C temperature [69,70].

## 5.6 Psychrophiles/cryophiles

Psychrophiles are cold-loving extremophilic microbes or archaea which generally grow in the temperature range of 20°C to 0°C with an ideal growth temperature of 15°C [71]. Cold-active cellulases are also being studied for their capability to breakdown of cellulosic materials. The psychrophiles, *Pseudoalteromonas* (*P. haloplanktis*) and *Flavobacterium* sp. AUG42 are documented to secrete cellulases having good activity at low temperatures [72,73]. Recently, a novel fungal species, *Aureobasidium paleasum* sp. nov. is shown to have a promising possibility for straw degradation. There are reports that psychrotrophic fungi exhibited strong cellulolytic activities at lower temperatures, with good thermal tolerance from 5 to 50 °C [73].

## 5.7 Acidophiles

Cellulases from acidophiles have a high economic value. Certain acidophilic bacteria have been identified to secrete acid-tolerant cellulases. Besides, some acidophilic bacteria are also reported to be thermoacidophiles [75-77]. Kusube et al. [78] reported a cellulase from a thermoacidophile bacteria, *Alicyclobacillus cellulosilyticus* having maximum activity at pH 4.8 and 55°C temperature. A thermostable and salt tolerant cellulase has been reported from a marine *Bacillus* sp. having optimal activity at pH 6.5 and 60°C temperature [79]. They also reported a cellulase secreted by the fungus, *Paenibacillus* sp. which had good activity at pH range 4.0 to 4.5 and at a temperature, 20°C. There are reports that certain thermoacidophilic fungi secrete cellulase in acidic pH range and comparatively higher temperature range. For instance, *Aspergillus fumigatus* isolated from sugarcane bagasse, has significant cellulase activity at pH 2.0 and 65°C [80,81]. Similarly, a thermoacidophilic cellulase has been reported from *Pleurotus ostreatus* exhibiting maximum activity at pH 4.0 and 55°C. A cellulase from white-rot fungus, *Inonotus obliquus* also has good catalytic activity at a temperature range, 40 to 60°C and pH 3.0 to 4.5. [82,83].

## 5.8 Alkaliphiles

Cellulases having activity in alkaline condition are employed in detergent industries [84]. Hakamada et al. [85] reported thermostable endo-1,4-β-glucanase having good activity in

wide pH range of 6 to 10 from alkaliphilic *Bacillus agaradhaerens* and *B. pseudofirmus*.

Several *Bacillus* sp. cellulases may exhibit both pH and thermostable characteristics [86]. Aikawa et al. [87] reported a cellulase having activity at pH 9.0 from an alkaliphilic bacteria, *Clostridium alkalicellum*. Thapa et al. [18] reported cellulolytic activity in *Herbivorax saccincola* A7 strain having optimum pH 9.0 and optimum temperature, 55°C. The fungi, *Aspergillus* and *Penicillium*, have also been exploited to get alkaline cellulase from their spores [88]. Dutta et al. [89] showed production of an alkaline and thermostable endoglucanase having good activity in the wide pH range of 5.5 to 8.0 from an alkali tolerant *Penicillium citrinum* (MTCC 6489).

## 5.9 Marine Biome

Marine microorganisms grow in extremes of physico-chemical conditions. As a result, marine bio-resources must be studied not just for their prominent function in aquatic food webs and biogeochemical cycles, but as a source of several biochemical catalysts [90]. The enzymes secreted by the marine microbial sources are thought to be more powerful and exhibit a wider range of biochemical characteristics. However, Balabanova et al. [91] reported that marine microbes grown on cellulosic biomass production are similar to their terrestrial equivalents. Dos Santos et al. [92] reported cellulase production from marine microbe, *Bacillus* sp. SR22 isolated from Cabo Branco coral reefs. Garsoux et al. [93] studied cellulase production by *Pseudoalteromonas haloplanktis* isolated from Antarctica vicinity. Zeng et al. [94] reported cellulase from *Pseudoalteromonas* sp. DY3 isolated from the sea bottom. Trivedi et al. [90] showed that marine fungus, *Cladosporium sphaerospermum* from Sediment of Arabian sea is a significant cellulase producer.

## 6. CELLULASE PRODUCTION

Cellulase has much higher industrial applicability, therefore its large-scale production at low production cost and its efficient downstream processing are important parameters. Many microbes secrete this enzyme in the surrounding culture medium, and therefore, are for cellulase production [135]. Two most used methods for production of microbial cellulase are solid substrate fermentation (SSF) and submerged fermentation (SmF). The submerged fermentation has been used for production of enzymes at the commercial level due to ease of

**Table 2. Some recently studied cellulase secreting bacterial species.**

Microorganism	Source of isolation	Property	Industrial Applications	Reference
<i>Bacillus albus</i> SCB9	Simlipal biosphere reserve	Biomass saccharification	bioethanol production	[95]
<i>Bacillus licheniformis</i> A5 and <i>Bacillus subtilis</i> B2	NS	Degradation of grains	Liquor industry	[96]
<i>Aneurinibacillus aneurinilyticus</i> BKT-9	Urban fresh water lake	Biomass saccharification	Food processing and biofuels	[97]
<i>B. methylophilicus</i> 1EJ7	Rotten wood	Bio-pretreatment of cellulose	Biofuels	[98]
<i>Bacillus licheniformis</i> Bi1	Soil	Biomass saccharification	Biofuels	[99]
<i>Paenibacillus</i> sp. C1	Sugar industry waste	High substrate specificity	Various industrial applications	[100]
<i>Streptomyces glaucescens</i> SK91	Land	Biomass saccharification	Biofuels	[101]
<i>Streptomyces rochei</i> SK78L				
<i>Bacillus zhangzhouensis</i> MCCC 1A08372	Romanian Hypersaline Lake	Tolerance to high salt concentrations	Various biotechnological applications	[102]
<i>Streptomyces thermoalkaliphilus</i> sp. nov. 4-2-13 <sup>T</sup>	tropical rain forest soil	Alkaline thermophilic cellulase	NS	[103]
<i>Bacillus Amylolyticus</i> <i>efaciens</i> AK9	Hot water spring	Conversion of lignocellulosic biomass	Biofuels	[104]
<i>Geobacillus</i> sp. HTA426	Hot water spring	Thermostable	Biofuels	[105]
<i>Streptomyces argenteolus</i> AE58P	Dept. of Agri.Sci., divi. of Microbiol of the Univ.of Naples Federico II	Conversion of lignocellulosic biomass	Biofuels and biochemical production	[106]
<i>Pseudomonas fluorescens</i>	Rice paddy field soil	Pretreatment and biomass saccharification	Lignocellulosic biorefineries	[107]
<i>Bacillus</i> sp. K1	Rotten wood	NS	Biorefining	[108]
<i>Bacillus vallismortis</i> RG-07	Soil	Thermostable-alkalophilic	Biofuels	[109]
<i>Paenibacillus terrae</i> ME27-1	Natural reserves of subtropical region of china	Diversity of cellulose-degrading bacteria	NS	[110]
<i>Bacillus subtilis</i> BY-2	Tibetan pig's intestine	NS	Animal food	[111]
<i>Pseudomonas fulorescens</i>	Soil	stability between neutral to alkaline pH	Detergent, food, pharmaceutical	[112]
<i>Pseudomonas aeruginosa</i>	Extreme ecological habitats (Soil and water)	Thermostable	Biofuels	[113]
<i>Bacillus subtilis</i>	Industrial waste	Thermostable	Biofuels	[114]

NS\* Not Specified

**Table 3. Some recently studied cellulase secreting fungal species**

Microorganism	Source of isolation	Property	Industrial Applications	Reference
<i>Aspergillus oryzae</i> RIB40	Water Hyacinth	NS	Agriculture	[115]
<i>Fusarium oxysporum</i> VSTPDK	Soil	Stable at alkaline pH	Paper and pulp	[116]
<i>Pestalotiopsis microspora</i> TKBRR	Thalakona forest soil	Degradation of casein	Biotechnological applications.	[117]
<i>Aspergillus niger</i> MK543209	Egyptian soils	NS	Bioenergy	[118]
<i>Aspergillus sp.</i> (CBMAI 1926)	Soil	NS	Biotechnological application	[119]
<i>Aspergillus niger</i> - MR2	Municipal solid wastes	Organic municipal solid waste degradation	Waste Management	[120]
<i>Trichoderma viride</i>	Municipal solid wastes	Organic municipal solid waste degradation.	Waste Management	[121]
<i>Periconia epilithographicola</i> sp. nov. and <i>Coniochaeta cipronana</i> sp.	Ancient lithographs	NS	Textile laundry detergents, biofuels, bioremediation	[122]
<i>Aspergillus niger</i> S2	Fruit litter	NS	Waste management	[123]
<i>Emericellavariecolor</i> NS3	Orange peel waste	NS	Waste management	[124]
<i>Cochliobolus</i> sps.	Plastic dumped soil	NS	Waste management	[125]
<i>Aspergillus fumigatus</i> AA001	Rice	Thermostable and stable at alkaline		
	Straw	pH	Biofuel	[126]
<i>Chaetomium dolichotrichum</i>	Deteriorated papers	NS	Waste Management	[127]
<i>Aspergillus foetidus</i> TISTR 3159	Soil	NS	Food processing and others	[128]
<i>Mucor racemosus</i> Fresenius 1850	Swabbed from the historical books.	NS	Waste management	[129]
<i>Aspergillus flavus</i> BS1	Woodyards	NS	<i>Bioethanol</i>	[130]
<i>Aspergillus fumigatus</i> ABK9	Agriculture Waste	NS	Pulp and Paper	[131]
<i>Aspergillus niger</i> N402	Agriculture Waste	Higher hydrolysis efficiency	Biological pre-treatment	[132]
<i>Aspergillus terreus</i>	Rice Straw	NS	Biofuels	[133]
<i>Aspergillus fumigatus</i> P40M2	Soybean bran	Thermostable and stable at acidic pH	Biomass conversion	[134]

NS\* Not Specified

operation and controlled physical parameters. It employs liquid components in the culture medium. Generally, broth and/ or molasses are used in the culture medium [136]. Solid substances serve as sources of minerals, carbon, nitrogen, and growth factors, and are capable of absorbing water to make it available for microbes with native habitat and growth requirements. The solid substances generally used in SSF are various agricultural wastes and paper pulp [137]. Cellulase production is an inducible process that is profoundly influenced by physical parameters like incubation time, temperature, inoculum size and pH. Similarly, requirements of nutrition (carbon, nitrogen and mineral sources) must be regulated and optimized to boost the effectiveness of cellulase production [138]. Optimum fermentation parameters for cellulase secretion optimized for different microorganisms are shown in Tables 4 and 5.

### 6.1 Production of Cellulase by Statistical Modeling

A statistical design of experiments is a factorial cum statistical model used to achieve fast and accurate results. A two levels factorial model is much more informational and easier to determine different variables [138]. Response surface methodology (RSM) is the most commonly used designs among the various Design of experiments (DoE) and has been often used for the design, production, enhancement and optimization of different processes and for the development of new products and the upgrade of current ones [139]. The RSM is a series of mathematical and statistical techniques focused on the fit of a polynomial equation to the experimental data to explain the data. It has been practically observed that predictions of various parameters implied in a process are important to enhance the enzyme production [140]. A statistical design of experiments could be implemented at any stage of an optimization technique, and for screening experiments to check for the optimum conditions for targeting response(s). Recently, statistically analysed findings have been analysed by anticipated studies that are better documented than standard OFAT methods [142]. It is very important to choose parameters or variables since it is a crucial step in all approaches and their levels, that have to be studied. The RSM employs statistical models such as Box-behnken design (BBD), Central composite design (CCD), Plackett-Burman design, Face centered design

(FCCD) etc. and these models are provided by different statistical packages software namely Minitab, Design-Expert, Stat-ease etc. Most commonly, CCD and BBD are employed for optimization of nutritional requirements (culture medium) of microbes and process parameters for cellulase production [142]. The statistical software used for optimization of cellulase production are mentioned in Table 6.

## 7. ENZYME CHARACTERIZATION

The, characterization of cellulases is obligatory to understand the physiochemistry in cellulose hydrolysis. The studies on the enzyme are done to elucidate catalytic, structural and kinetic properties. The enzyme stability at various temperatures ranging from low to higher temperature, enzyme activity- pH variability relationship, effect of unresolvable cellulosic substrate on activity and product inhibition are important to study. Many laboratories are engaged in the study of cellulases [84, 143-152]. The standard protein purification techniques such as ammonium sulfate fractionation, ion exchange chromatography, chromatofocusing, hydrophobic interaction chromatography and gel filtration chromatography have been frequently. [153-155]. The cellulase from fungal strains namely *Thermomonospora curvata* [156], *Trichoderma viride* [157], *Fusarium oxysporum* [158], *Aspergillus tubingensis* [159] and *Thermomonospora fusca* [160], has been purified and characterized. Fungi have been studied comparatively more for cellulase production, isolation and characterization compared to bacterial sources [161]. Although, cellulase has been analysed from bacterial sources but their number is lesser compared to their abundance in nature. The bacterial cellulase has been studied and purified from *Bacillus subtilis* [162,163] *Paenibacillus* sp. [164], *Bacillus licheniformis* [165], *Caldibacillus cellulovorans* [166], *Thermotoga maritima* [167], *Thermobifida fusca* [168], *Acidotherrmus cellulolyticus* [169] and *Rhodothermus marinus* [170]. The comparative characteristics of purified cellulases from various microbial sources are summarized in Table 7.

## 8. INDUSTRIAL APPLICATIONS OF CELLULASES

Cellulases have become an objective for various researches since last few decades because cellulase has a huge scope in diverse applications. Cellulase has applications in textile desizing, monogastric feed production for

Table 4. Optimum fermentation parameters for cellulase secretion in different fungi

Microorganism	Substrate	Moisture (%)	pH	Temp (°C)	Inoculum Level	Particle Size (mm)	Incubation Time (h)	Reference
<i>Fusarium oxysporum</i> VSTPDK	Rice Straw	-	8.5	45	-	-	192	[171]
<i>Aspergillus fumigatus</i> JCM 10253	Ragi husk	-	2-4	50	-	1.33	168-192	[172]
<i>Aspergillus tubingensis</i> MN239975	Sorghum straw	70.5	5.5	27.5	7.5%	-	168	[173]
<i>Aspergillus niger</i> CCUG33991	Wheat bran	70	-	33	$2 \times 10^7$ - $4 \times 10^7$	30 and 50	51	[174]
<i>Myceliophthora thermophila</i> BJTLRMDU3	Rice straw	1:7 (w/v)	7.0	45	$12 \times 10^6$ /ml	-	96	[175]
<i>Aspergillus niger</i> NFCCI 4113	Wheat bran	70	7.0	30	-	250–1400 $\mu$ m	144	[176]
<i>Bacillus subtilis</i> PS-5CM-UM3	Citrus sinensis bagasse	60 $\pm$ 5	7.0	37	40%	-	72	[177]
<i>Trichoderma sp.</i> RCK65	<i>Prosopis juliflora</i>	20%	4.5	30	-	-	72	[178]
<i>Aspergillus niger</i> CICC 41258	Rapeseed cake	60	-	34	$1 \times 10^7$ /ml	40 mesh	72	[179]

Table 5. Optimum fermentation parameters for cellulase secretion in different bacteria

Microorganism	Carbon	Nitrogen	pH	Temperature (°C)	Inoculum Level	Agitation Speed (rpm)	Incubation Time (h)	Reference
<i>Bacillus subtilis</i> CD001	Galactose	Sodium Nitrate	7.0	45	10%v/v	120	72	[180]
<i>B. licheniformis</i> BCLLNf-01	CMC	–	–	40	5.5%	500	96	[181]
<i>B. licheniformis</i> Bi1	CMC	Peptone	6.5	40	1.8%	150	72	[182]
<i>Penicillium oxalicum</i> JG	Wheat bran	soy bean	Initial 1.5	40	108 spores/10 0 mL	200	Within 168	[183]
<i>Streptomyces sp.</i> NAA2	<i>Parthenium hysterophorus</i> & CMC	–	6.5	40	$5 \times 10^7$ spores/ml	120-150	192	[184]
<i>Bacillus cereus</i>	poplar waste biomass	Yeast extract	9.0	37	2%	120	24	[185]
<i>Bacillus subtilis</i> MU S1	CMC	Peptone Yeast extract	7.0	40	–	150	24	[186]
<i>Bacillus subtilis</i> K-18	Potato peel powder	Yeast extract	5.0	50	2%	120	24	[187]
<i>Bacillus licheniformis</i> 2D55	Sugarcane bagasse and rice husk	Peptone	3.5	60	3%	180	18	[188]
<i>Bacillus stratosphericus</i> N12.	Lactose	–	8.0	30	10%	200	72	[189]

**Table 6. Statistical software used for optimization of cellulase production**

Microorganism	Design	No. of Variables	Experiment Runs	Software	Yield Improvement	Reference
<i>B. Subtilis</i> CD001	Box-Behnken	9	46	Design expert 11	3	[180]
<i>Trichoderma Stromaticum</i> AM7	(ANN-GA)	4	28	Matlab R 2016a	31.58	[190]
<i>Bacillus licheniformis</i>	Central composite	4	36	Design expert 8.0.1	25	[182]
<i>Bacillus subtilis</i>	(ANN-GA)	3	20	Matlab R2018a	16.09	[191]
	CCRD	3	20	Stat-Ease	15.73	
<i>Bacillus licheniformis</i> Hi-08	Box-Behnken	4	29	Design expert 11.0	1.8	[192]
<i>Bacillus subtilis</i> S1	Placket Burman	3	15	Design expert triall version 7	1.43	[193]
<i>Bacillus licheniformi</i> NCIM 5556	FCCCD	4	30	Design expert 8	3.0	[138]
<i>Aspergillus niger</i>	CCD	4	20	Design expert	-	[194]
<i>Penicillium oxalicum</i> IODBF-5	Box-Behnken	3	29	Design expert 7.0	1.87	[195]

**Table 7. Biochemical properties of some of the recently discovered cellulase from various sources**

Organism/ Source	Temp (°C)	pH	MW (kDa)	Metal Ion (Divalent)	Km, Vmax	Inhibitors	Substrate Specificity Natural	Substrate Specificity Synthetic	References
<i>Caulobacter crescentus</i>	40	5.5-6.0	73	Mn, Sn, Co	0.66 mg/mL 2.41 U/mg/ min.	Hg <sup>+2</sup> , Ag <sup>+2</sup>	-	CMC	[196]
<i>Bacterial sp.</i>	40	6.5	74.5	Ca <sup>2+</sup> , Zn <sup>2+</sup>	400 mM, 18, 33 µM/min	Mg <sup>2+</sup>	-	ONPG	[197]
<i>Trichoderma longibrachiatum</i> KM274 866	45	4.8	67	Ca <sup>2+</sup> , Mg <sup>2+</sup> , Fe <sup>2+</sup>	0.121 mg/ml, 0.421 µmmol/min	K <sup>+</sup>	-	CMC	[198]
<i>Aspergillus flavus</i>	60	10	55	-	3.02 mg mL <sup>-1</sup> 37.87 mol min <sup>-1</sup> mg <sup>-1</sup>	EDTA	-	CMC	[199]

Organism/ Source	Temp (°C)	pH	MW (kDa)	Metal Ion (Divalent)	Km, Vmax	Inhibitors	Substrate Specificity Natural Synthetic	References
<i>Pseudomonas sp.</i>	50	7.0	50	Fe <sup>2+</sup> , Mn <sup>2+</sup>	-	SDS, Tide, surf Excel	- CMC	[200]
<i>Schizophyllum commune</i> NAIMCC -F-03379	25	5	~ 60	-	0.0909 mg/mL 45.45 µmol/ min/mg	-	- CMC	[201]
<i>Paenibacillus sp.</i>	40	.70	67	-	-	-	- CMC	[202]
<i>Bacillus pantothenicus</i>	60	4.5	51.48	-	1.167 mg/ml, 0.833 µg/ml/min	NaCl <sub>2</sub> , NiCl <sub>2</sub>	- CMC	[203]

ruminants and detergent formulations. Besides, its application in biorefinery is much remarkable. Much work is focused on cellulases involved saccharification of biomass in last couple of decades. However, researchers are looking forward for affordable commercial production method for cellulase to overcome various cost and technical constraints [204,19]. The plausible industrial applications of cellulases are described below:

### 8.1 Cellulases in Agriculture

Enzyme cocktails with cellulases, hemicellulases, pectinases have possible uses in farming to improve crop growth and control diseases of plants [205, 206]. Cellulases and associated enzymes from several fungi have been known to deform the outer coat of plant pathogens in plant disease control [205]. The  $\beta$ -glucanases have been isolated from various fungi like *Trichoderma sp.*, *Penicillium sp.*, and *Chaetomium sp.* There are evidences that  $\beta$ -glucanases have important vital roles in agriculture through the enablement of increased germination of seeds, rapid growth and flowering of plants, strengthened the root system and enhanced yields of crops [207, 208]. The cellulases are also used to boost the consistency of the soil. The inclusion of straw is conventionally an effective approach to enhance the quality of soil and reduce the reliance on mineral fertilizers [209, 210].

Cellulase has also been employed in olive oil extraction to produce top notch quality of olive oil. In industries, enzyme preparations used have specific combination of enzymes. For example, Olivex is a mixture of pectinase, cellulase and hemicellulase from *Aspergillus aculeatus*. This is used in maceration process to enhance the antioxidants in mechanically extracted olive oil and to avoid the process of rancidity. Ultimately, it enhances the extraction of the oil from olives [211,212]. Similarly, Olivex has also been found useful in carotenoid extraction. The enzymes present in it disrupt the outer coat of sweet potato, carrot and orange peel, and subsequently, carotenoids present in the plastids and cell fluids are released and help to stand their natural state. These pigments still bind strongly to proteins. Oxidation of pigments is inhibited by a bonded structure resulting in colour stability. Furthermore, carotene is enriched in provitamin A. The function of provitamin A has been shown in the oxidation of lipids. Besides, these carotenes have also been shown to exhibit anticarcinogenic properties [213, 214].

### 8.2 Cellulases in Animal Feed Industries

Cellulase and hemicellulases are found to exhibit uses in the feed market. These enzymes are capable to increase the feeding value and also the animals' productivity [215]. The cellulase and xylanase enzymes are also employed in the pre-treatment of agricultural ensilage and feed grain. This pre-treatment enhances the nourishing property of the animal feed [212].

In the feed grain, there are some antinutritional factors. For example, dietary fibers have many indigestible substances called as antinutritional. These enzymes can degrade antinutritional feed components resulting in increase of the nutritional value. On feeding treated feed grains to animals, there is secretion of certain supplementary digestive enzymes which enhance the digestion, and subsequently, there is strengthening of absorption process. Ultimately, there is improvement in animal health and performance [75, 216, 217].

Cellulases and especially thermophilic cellulases are also important in cecal fermentation processes to avoid viral and microbial contaminants on heat treatments of animal feed stock. As a result, there is increase in propionic acid production. It leads in formation of bacteriostatic material which may result in reduction of the pathogenic bacteria colonization [205, 212, 213].

### 8.3 Cellulases in Food Processing Industries

Cellulases also have a diverse array of advancement in food technology. These enhance extraction, clarification, and stabilisation processes of juices from various plant products like vegetables and fruits. The cellulases do have a significant application as macerating enzyme complex. The enzymatic maceration process improves fruits extraction and clarification process resulting in enhancement of fruits juice yield [218, 219]. These macerating enzymes boost texture and cloud stability, and also reduce the viscosity of purees and nectars from tropical fruits such as mangoes, papayas, peaches, plums, pear and apricot [19, 205, 219, 220]. There are certain fruits and vegetables which have specific characteristic aroma, texture and flavor. However, in many cases, intense bitter taste is there. These specific characteristics may be enhanced by reducing bitter taste by the use

of enzymes like  $\beta$ -glucosidases and pectinases [221, 222].

#### 8.4 Cellulases in Wine and Brewery Industries

The procedure to produce alcoholic drinks, including beers and wine involves fermentation where microbial glucanases are used prominently [19, 211, 218, 220].

Yeast and certain enzymes play important role to produce high-quality products during fermentation. In oenological procedures, mainly two methods namely cold prefermentative and maceration are in practice. The inclusion of macerating enzymes responsible for increasing the secretion of grape polyphenols, and sustaining the color, facilitate the attempts to ameliorate yeast strains. Influence of fermentation of yeast in the protein reservoir of grape juice has been in use for decades before and after alcoholic fermentation [224, 178].

Wine makers and customers both are a bit skeptical about using exogenous enzymes already characterized by them. During wine-making, the use of macerating enzymes makes improved skin maceration and excellent extraction of colour, which is especially necessary in red wine production. Besides, it enhances clarity, filtration, and also the wine's overall consistency and stability [211]. The outer coat of grape berries releases carbohydrate polymer fraction in wines [225].

Galante et al. [211] studied wine making from three different varieties of white grapes using a mixture cellulase, xylanase and pectinase in a commercial preparation, Cytolase 219. They found up to 35% enhancement in the solubilization of first wine must, and also up to 80% enhancement in the removal of particles by filtration, much reduction in the pressing time and must viscosity, resulting in up to 40% saving of energy during cooling of fermenter, and enhancement in wine stability.

#### 8.5 Cellulases in Biofuel and Bioethanol Industries

Biofuels are the fuels derived from biomass. Unlike fossil fuels, biofuels being renewable energy, are carbon neutral and emit much lesser greenhouse gases. From the point of cleaner environment, biofuels have become the need of

the day and therefore, are being accepted by the society [226].

Based on the feedstock, biofuels have been categorized into different generation biofuels. At present, most researches are being carried out on second and third generation biofuels [226].

First generation biofuels are produced from starchy crop plants like corn. However, after the concept of biofuel from edible starchy cash crop, there was debate globally since there are so many deaths due to hunger especially in poor and developing countries.

Second generation biofuels are the biofuels derived from ligno-cellulosic biomass. It is considered that lignocellulosic biomass is comparatively lesser expensive and is readily available. For example, there is plenty of sugarcane straw and bagasse and other plant based waste which predominantly consisted of cellulose (30% to 50%), hemicellulose (15% to 35%), and lignin (10-20%) [228]. There is debate on second generation biofuels also since people started to use more space for growing specific plants important for biofuels. With increasing population, there is already shortage of space for accommodations.

Third generation biofuels are derived mainly from algal biomass. Algae are grown in aquatic spheres. Currently, they are subjected to comprehensive study to enhance the metabolic production of fuels. Much emphasis is on the processes of separation of bio-oil to remove components that are not fuel and further reduce the costs of production [229]. Microalgae are often seen as potential candidates for fuels due to their high photo-assimilation rate. There are many methods for producing renewable energy using algal biomass. The biochemical conversion, thermochemical conversion and chemical reactions including specific combustion are regarded as necessary for biomass processing in order to produce high-value chemicals such as bioethanol [230].

For production of fourth generation biofuels, genetically modified (GM) algae are normally used. For fourth generation biofuels, open-pond model is an affordable option for large-scale microalgae cultivation. However, issues related to health and environmental threats and related severe restrictions must not be ignored.

Although extensive research on genetic engineering and other advancements aimed at increasing productivity of algae strain, have been undertaken, only a few of them deal with the regulatory restrictions placed on the exploitation and processing of GM algae. Besides, there are some legislative limitations in fourth generation biofuels production [231].

To increase the yield for second generation bioethanol, a crucial step is pre-treatment of ligno-cellulosic biomass, and subsequently enzymatic saccharification for release of fermentable sugar [232].

Enzymatic hydrolysis is a costly step, and therefore, more research must be carried out in order to get cheaper enzymes, improving the efficiency of hydrolysis and increasing the production of fermentable sugar which leads to productivity improvements [233, 234].

The degradation of lignocellulosic materials into goods of useful and better worth requires multi-step processes [235,236]. These mechanisms involve pretreatment and hydrolysis of the readily formed polymers molecules that are metabolizable (e.g., hexose and pentose sugars). In addition to bioconversion to simple sugars and/or production of chemical products, separation of the products and their purification is of interest.

At present, enzymatic hydrolysis is more expensive and also slow process compared to acid or alkaline hydrolysis. Currently, it is performed under moderate conditions (pH 4 to 6 and temperature, 45-50°C) which doesn't have problems of corrosion [235, 237].

The certain modifications in proteins and guided evolution are significant techniques which could promote production of even more thermophilic cellulases [238]. Recycling and methods related to reuse of enzymes are of interest to decrease enzymatic hydrolysis expenses [239-242]. In addition, there are reports that certain compounds or imitate cellulose have a very strong affinity for lignin and are able to inhibit the adsorption of cellulases to lignin [243-246]. Immobilized enzymes can be recovered when recycling from the production process. There are recycling methods which are mostly tested on a lab scale [241, 247]. Consequently, it is always important to scale up the methods, the reproducibility and viability.

In biomass hydrolysis, the major issue is obstinate behavior of cellulose to complete degradation. New biocatalytic procedures are needed to boost enzymatic hydrolysis overcoming this problem [248]. The total cellulose enzymatic degradation into sugar monomers is being considered theoretically by integrating enzymes of various families, such as glycosidases, hydrolases, and oxidases. By merging these enzymes, a modest improvement in hydrolysis efficiency has been achieved. However, kinetic ambiguity is there due to cellulose crystallinity and the inhibition of enzymes [248].

## 8.6 Textile Industry

The role of enzymes in multiple procedures in the textile industry is increasing on an enormous scale. Because of their biodegradable nature, non-toxicity and environmental amiability, they have been favored in textiles [249].

Cellulases are the most productive in the wet processing of textiles, notably finishing cellulose dependent textiles [206, 250]. Almost all of the substances used in fabric manufacturing are dependent on cellulosic fibers which tend to shape down (short fibers coming from the surface) and lint (loose down stuck to the surface). Traditional methods of extracting protruding fibers use a highly harmful burning procedure or chemical methods, significantly toxic and after just a few washes, the fibers revert to just the surface creating a fluff [251]. Biopolishing is a biological procedure wherein cellulases function upon the surface of the tissue, eliminating any fluff or lint protruding from such a surface. This provides clean surface to enhancing the texture, smoothness, appearance, hydrophilic properties, brightness and intensity of colors. It also provides full resistance to fiber while reducing the propensity to form lint [252].

After cellulases work upon this cotton fabric during the biostoning process, and cut off the tiny fiber ends on the yarn surface, results in easing the dye, which is extracted by physical abrasion in the cycle of cleaning.

The benefits provided in replacing pumice stones with a cellulose-based procedure include lesser fiber disruption, improved efficiency of the appliances, least labor-intensive and environment friendly [19, 211, 220, 253]. Although biopolishing normally takes place during the process, the wet processing phases

involve desizing, scouring, bleaching, dyeing and finishing. Acidified cellulase enhances the properties of softness and water absorption of fibers. The propensity for pill forming is strongly diminished and provides a smoother surface and less fuzz [254]. The cellulase- endoglucanase enriched formulations are the most ideal for biopolishing to improve the look, feel and color of the fabric without obligation of any chemical fiber coating. Cellulase intervention reduces short fibers and surface fuzziness, and provides a smooth and shiny look as well as enhances the clarity of the color, hydrophilicity, absorption of moisture and the eco- acceptable process [205]. Cellulases' synergistic function and mechanical action causes the de-pilling/cleaning and/or ageing of the product which occurs concurrently or successively [235].

### 8.7 Cellulases in Paper Manufacturing Industry

Substantially, over the past decade attention seeking the application of cellulase throughout has arisen in the paper manufacturing industry.

Nowadays, 90% paper pulp is made up of wood. The recycling of one ton of newspapers and magazines saves the use of one ton of wood. Similarly, recycling of one ton of printing or copying paper saves nearly two tons of wood, [256]. The paper manufacturing industry significantly uses the lignocellulose-containing sources. Lignocellulosic material has constituents of lignin, hemicellulose and cellulose, that could be broken down into smaller components and used as feedstocks for their efforts towards valorization. So many of these substances are found in streams including waste materials that are underused, along with black liquor, pulp, sludge and log, and wastewater. Bacterial fermentation procedures have the magnificent capacity for upgrading lignocellulosic biomass, and the value-added chemicals found in these streams. A sustainable and economically viable conversion by bacteria enables the valorization of these streams, which helps and extend in pulp and paper industry applications [257].

The paper is manufactured in a three-stage process that includes pulping, bleaching and the processing or finishing of paper or paper making [258]. The forms of wood species which are used to produce pulp are softwoods like fir, spruce and pine, and hardwoods like eucalyptus, aspen, and ash. Along with global rise in requirements, also

environmental protection concerns, apart from forests, other substances like rice straw and waste paper are currently used for pulp manufacturing [258,259].

Pulping includes use of chemical, mechanical and /or biological processes to break woody substance bonds and detach cellulose fibers from lignite fibers [260]. Mechanical pulping involves wood refining, slicing, mechanical shearing and disintegration of different fibers [261].

A chemical pulping is used to solubilize lignin. It facilitates separation of fiber [261]. The complete chemical pulping technique (Kraft pulping process) involves use of sodium hydroxide and sodium sulfide at a temperature of 155 to 180°C and at a pH over 12, with 800 kPa steam pressure as the key cooking conditions to break down wood chips into pulp [262].

The biological pulping procedure involves organic and non-degradable fiber raw materials in natural conditions. Biological pulping requires wood chips processing and also requires biological nutrients (primarily white-rot fungi) to loosen and eliminate lignin [263, 264].

Bio-bleaching using enzymes is an important replacement to avoid environmental pollution. The elimination of intransigent lignin is carried out from the pulp, a process known as bleaching used to make the paper brighter and whiter paper [256]. Many paper mills globally use chlorine dioxide ( $\text{ClO}_2$ ) as a bleaching agent for the manufacturing of top-quality white paper. However, there are more environmentally sustainable bleaching options available to pulp mills. The substitutes of  $\text{ClO}_2$  are prolonged cooking, oxygen, hydrogen peroxide or delignification based on ozone. However, adoption of these alternatives when requires moderations to the procedure and is regarded a higher cost proposal on a broad scale. Xylanases and laccases are environmentally-sound for the bleaching purposes [256].

Deinking is a crucial process where cellulase is suitably used in paper recycling, and that implies the separation of printing ink from the previously used paper to achieve brighter pulp. Deinking procedure involves selecting the factors such as process of printing and type of ink. Electrophotography printing has 5 phases namely imaging, writing, toner transfer (printing), fixing or fusing, and cleaning (conditioning) [265].

In electrophotography printing, the ink (toner) used comprises thermoplastic polymers, in addition to carbon black. This process of ink removal is laborious and also not viable economically. Although the standard deinking methods need expensive wastewater treatment since hazards, and other chemical agents which are not environment friendly are present in it [266]. On the contrary, deinking by enzymatic methods are novel methods to mitigate this problem. This method allows pollutant free discharging since enzymatic mechanism forwards ink separation from the paper fibers [267].

Recently, upcoming future possibilities for new pulping procedures are prompted by the demand for new bio-based products to substitute the fossil-derived products while still lowering pulping costs [268].

### 8.8 Cellulase in Detergent Industries

Cellulase is important in the detergent industries with other enzymes like lipase, protease and amylase. Nowadays, during manufacture of detergents, these enzymes are normally mixed in the liquid detergent and detergent powder to replace the toxic compounds such as phosphates and silicates to lower the energy demand and cost effectiveness of formulation of the detergents. Cellulolytic microbes are secreting extracellular enzymes for commercial purposes. Because of its applicability, cellulase is a demanding enzyme in the detergent industries also [269].

Enzyme cocktails having lipase, proteases, amylase and cellulase are applied to detergents to improve washing performance. Detergents containing cellulase can stabilize the brightness and color of the fabrics, helping to reduce the formation of fluff and pills in woollen fabrics. In cotton and cotton blends, the enzyme can enhance brightness of color by modifying the cellulose fibrils. It also assists in the absorption of soil and stains by specifically attacking the cellulose fibrils internally washing fibrils and withdrawing dirt of inter-fibrils in which other components in the detergent also play role [270].

The presence of cold-active cellulase to detergent improves the detergent's washing quality, reducing water usage and resulting in substantial energy savings [271]. Cold active enzymes are isolated from the microorganisms at different geographical areas varying from

extreme temperatures (hot to cold). These are studied for compatibility tests as detergent additives. Enzymes having activity under cold conditions with high catalytic activity are present in psychrophiles which thrive in cold environments, and their resilience under harsh conditions makes them ideal eco-friendly and cost-effective detergent additives.

Modern genomics and proteomics approaches created an opportunity for a more thorough view of the efficiency of cold-active enzymes for detergent additives. Molecular techniques are important –to unravel the riddle regarding these enzymes' alkaline stability and chemical compatibility with oxidising agents are important [75, 272, 273].

### 8.9 Cellulase in Waste Management

There is plenty of waste produced from forests, farm lands, and agro-industries. These wastes have maximum amount of cellulose which is discarded or underused, resulting pollution in the atmosphere [274,275]. Researchers have shown that characterization of cellulase producing bacteria and their genome functional analyses help to improve waste management [276]. All the wastes are now wisely used to manufacture useful goods such as enzymes, carbohydrates, biofuels, chemicals, low-cost energy fermentation streams, enhanced cattle diets, and human nutrition dietary supplements [204, 277,278].

### 8.10 Cellulase Market Scenario

As per worldwide cellulase (CAS 9012-54-8) market Analysis, the animal feed industry accounts for about 30% of the overall cellulase market, whereas food and beverages, and textile industries retain about 26% and 14%, respectively [279].

Use of such cellulase/xylanase enzymes in biofuel industries is important to rise at a faster rate. In the next few years, the economy will grow by around 7 to 10%. In 2019, the worldwide cellulase enzyme market was valued at around US\$ 1500 million, and it has been speculated to grow to US\$ 2320 million in the year 2024 [18]. By 2024, such biotechnological and pharmaceutically important enzymes are predicted to grow at the fastest pace. North America is expected to dominate the cellulase/xylanase enzyme market in terms of regional spread owing to the drastic advancement in manufacturing technology and

its use in various industries [280]. The cellulase/xylanase enzyme industries are quite likely to be projected in the Asia Pacific region, with China and India expected to get the largest demand of these enzymes in the upcoming future. According to a systematic quantitative and qualitative evaluation, nearly three-fourth of the enzyme industry is distributed in this market. Some of the cellulase industries include Worthington Biochemical Corporation, Sigma-Aldrich Co. LLC, Amano enzyme USA, BIOCATALYSIS LTD., and DONG Energy, GmbH, Prozmix LLC and MP Biomedicals LLC [281, 282].

## 9. FUTURE RECOMMENDATIONS AND CONCLUSION

Production of thermostable enzymes is important to improve cellulosic bioconversions. New approaches are intended to produce commercially important products with better-quality especially in terms of eco-friendly and green products. Besides, these approaches must cause lesser loss of the substrate during the bioconversion of cellulosic biomass. The search for a stable and consistent naturally occurring microbial cellulolytic enzymes in diverse range of environments, might be a factor which may help for implementing effective use, especially for the widely accessible cellulosic substrates in the bio-refineries. Several intrinsic catalytic properties of presently available cellulolytic enzymes, like inefficiency, nonstability and end-product inhibition, impede their large-scale use in industries. A basic understanding of functional genomics and proteomics techniques for analysing microbial diversity from various habitats and ecosystems might help to explore cellulolytic enzymes. To understand the cellulolytic enzyme production from different microbial sources, synthetic and system biology, along with omics methods and bioinformatics algorithms can assist to certain extent. The possibility of enhanced enzyme characteristics with a higher efficiency and physical stability potentially may influence the cellulolytic enzyme industries. For exploring the novel cellulolytic enzymes with improved functional efficiencies, state of the art technologies are needed. The use of a diverse set of molecular and genetic techniques is important, and genetic engineering is one among them. However, it is not the only one for strain improvement by mutagenesis, and the structure guided recombination approach (SGRA), Genome-scale modeling, multi-plex CRISPR/CAS9 in association with the synthetic

expression system (SES), fast expectation-maximization microbiological source tracking (FEAST), statistical optimization, solid-state fermentation, and consolidated bio-processing concepts might help researchers to get better knowledge of the potential microbes to secrete cellulolytic enzymes and how to produce them efficiently in future. These strategies may be useful in resolving challenges of efficacy of cellulose degrading enzymes currently in the market. There are several approaches to be researched, as well as innovative methods to be found in order to turn the most ubiquitous bioresource in to the value-added green products that may be used for a variety of purposes in industries.

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## DISCLAIMER

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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