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Developments in Cellulase Activity Improvements Intended Towards Biofuel Production

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Lignocellulosic biomass represents a huge potential for energy production worldwide. However, it has not been harnessed for biofuel and biogas production at industrial scale due to the problems in digestibility of the lignocellulosic materials and the inaccessibility of the polymers to the enzymes for degradation. There are various methods of pretreatment which can be classified into physical, physicochemical, chemical, and biological pretreatments. For physical pretreatment, available methods are microwave irradiation, ultrasound irradiation, and pulsed electric fields are reviewed. For physicochemical pretreatment, ammonia fiber explosion, supercritical carbon dioxide explosion, and steam explosion are discussed. For chemical pretreatment, ammonia recycled percolation, organosolv process, and oxidative processes such as ozonolysis and wet oxidation methods are available. Thus cellulases (EC 3.2.1.4) has been classified into three major categories according to action over cellulose, Endoglucanase (EC 3.2.1.4), Exoglucanases, (sometime known as cellodextrinases) (EC 3.2.1.74) and 1,4-ß-D- glucancellobiohydrolases (cellobio-hydrolases) (EC 3.2.1.91), and ß-Glucosidases or ß-glucosideglucohydrolases (EC 3.2.1.21). Many microbes are reported to have either more than one endoglucanase system or more of exoglucanase over endoglucanase or only one out of both. Sometime, different microbes are mixed to get the unique combinations so that to get better hydrolysis results. The efficient catalysis of cellulase is possible because of presence of a unique cellulose binding module called as Carbohydrate Binding Modules (CBMs) which is a more often a common feature of fungal cellulases and while bacterial cellulase have similar dominant counterpart named as cellulosome, which bestows additional carbohydrate binding properties to the enzyme by bringing the catalytic domaincloser to the substrate,thus increasing more chances of successful conversion of carbohydrate cellulose. Cellulosome itself reported to have different type of glycosyl hydrolase including cellulase, hemicellulose, all of which are bound to scaffoldin protein (CipA cellulosome integrating protein), which is multidomain protein and one of them is CBD, (cellulose binding domain). Beside this there are 9 repeating domain called as cohesin which interacts with cellulosome enzyme. This is the feature reported in *C. thermocellum*. Thus many CBM has been identified (around 39) based on amino acid composition (see http://afmb.cnrs-mrs.fr/CAZY/index.html). There are around 20 CBM studied in detail [1] CBMs can recognize varieties of substrate such as crystalline cellulose, or even non-crystalline cellulose, chitin, β-1,3-glucans and β-1,3-1,4-mixed linkage glucans, xylan, mannan, galactan and starch. In general, CBM in case of other carbohydrate appended to glycoside hydrolases that degrade insoluble polysaccharides.

Efficient Cellulase is constantly required to work at higher temperature and pH for various industrial purposes. Therefore, various existing enzyme have been modified to work at higher pH (10.7) [2]. Recently A "K" protease have been developed recently that have been isolated from Bacillus species KSM K16 [3]. These strains are actually prepared after the mutations in gene E ad G in *Bacillus subtilis* (364 aa). Some of cellulases that work at alkaline condition is called as CMCase (corboxymethylcellulase enzyme) which is often identified by making hallow / clear zone in the CMC media around colonies [4].

Bacillus KSM635 have been found to grow on various substrates beside carboxymethyl cellulose, such as glycerol (5.5), mannitol (4.4), sucrose (5.3) and maltose (4.2) but they have best production of cellulase (3.64 units/ml) at maltose as carbon substrate [4]. Some of the noted amino acid reported to take part in acid catalysis are Asp/Glu/His while alkaline cellulase is reported to have 1 Asp, 3 Glu, and 2 Trp by site directed mutagenesis. His331 has a possible role in alkaline cellulase which has been proved by diethylpyrocarbonate which stops CMCase activity.

One such strain have been isolated from *Bacillusli cheniformiss* mp1 (NCBI accession no GenBank KF522027-KF522028 after a 16 s RNA analysis) giving alkaline cellulase production which has been optimized for higher activity (4.5 IU/ml) with a broad alkaline range (9-10). In addition to improving the enzyme, use of Agri-waste is the cheap and sustainable input for such commercial enzyme which could be processed at commercial level.

Few scanned literature is illustrating the alkaline cellulase enzyme and its commercial significances, in high quality laundry detergent as additive, and for stone washing in denim industry, but it has not been explored fully in Biofuel sector in form of "field Bioprocessing" against "Consolidated bioprocessing" (CBP) which has not proved to be worth for Biofuel industry.

Field Bioprocessing or local processing of Biomass facilitates its conversion to glucose for production of fuel ethanol at fermentation facilities at a distance from the growing fields [5,6]. Advantages include enhanced transportation feasibility and optimization of fermentation operations, both of which are considered necessary for the cost-effective production of bioethanol [7,8]. Alkali-cellulase (Alkcell) processing [9] involves pretreatment of biomass with strong bases followed by enzymatic hydrolysis that has been identified as one of the possible approaches to cellulose saccharification [10,11]. However, the Alkcell Process for practical local implementation requires better characterization. Alkcell processing utilizes NaOH or other strong alkalis to pretreat biomass under mild conditions. This is followed by cellulase adsorption to the pretreated cellulose which followed by release of glucose using a volume expansion technique [9]. Glucose and other products of the enzymatic hydrolysis have been shown to inhibit cellulase activity [12,13] but volume expansion, technique reported to be very effective for enhanced release of glucose. In due course of

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industrial expansion (bio-fuel, laundry, animal feed, leather and textile sectors) such pH and temperature supporting enzyme becomes the universal need.

The major bottleneck in the conversion of lignocellulosic biomass into ethanol is the high cost associated with the hydrolytic enzymes. Therefore, focus of the research should be to find cheaper ways of producing the enzymes and to find more active enzymes with high thermal tolerance and high pH endurance.

Alkaline cellulase is also called as subtilisin (alkaline serine protease) enzyme and 50% of this enzyme is produced alone by Bacillus species. According to Outtrup H et al. the cellulase market has been estimated in the United States to be as high as US $ 400 million per year [14,15]. The current cost of enzyme is 7,615.14 INR for 50 ml enzyme (Sigma Aldrich for alkalase). Cellulases are currently regarded as the third largest volume of industrial enzyme [16]. The Bacillus cells have proven capability to produce gram enzyme per liter and thus considered as ideal candidate for enzyme production in any industry. Most of the industrially formulated strains such as Alkalase, Esperase are known to be derived from bacillus species. Of the three well known species such as *Bacillus licheniformis*, *B. subtilis* and *B. pumilus* are alone estimated to produce around 500 metric ton of pure enzyme [17].

Potentialalkaline cellulase producers reported to date are from B. amyloliquefaciens, *B. licheniformis*, *B. mojavensis*, and *B. subtilis* [17] with pH optima 7-9 and good thermal stability and have been applied successfully in detergent, abating of enzymes, and de-haring of leather [18]. Bacillus has been improved genetically using gene technology where deletion of unnecessary enzyme resulted in many fold production of enzyme. Beside these Protein-engineered variants of the *B. liquefaciens* and *B. halodurans* for α-amylases were commercialized for its improved alkali tolerance.

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