4

Present Status On Enzymatic Hydrolysis of Lignocellulosic Biomass for Bioethanol Production

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Abstract

Bioethanol is becoming a better alternative to fossil fuels. Production of ethanol by using edible feedstock such as grains, sugarcane etc., became a point of concern in terms of the food supply and demand. In such a scenario lignocellulosic biomass that includes nonedible feedstocks opened up a new avenue for the second-generation bioethanol production. Lignocellulosic bioethanol production is composed of three major steps: pretreatment, enzymatic hydrolysis and fermentation. The main factor restraining the commercialization of bioethanol lies in the development of the enzymatic hydrolysis step. During the enzymatic hydrolysis step carbohydrates (cellulose and hemicelluloses) polymers get converted into free monomeric sugars. The major problems associated with enzymatic hydrolysis are cost of the enzyme, higher incubation time for complete degradation of carbohydrates, inhibition of enzyme activity in the presence of phenolic compounds and thermal inactivation of cellulase enzyme. The present article discusses recent trends and development of the enzymatic hydrolysis process for cost-effective bioethanol production. In this review the authors cover the following points: development of cellulase-producing organisms, the enzyme production process, the

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improvement or enhancement of enzymatic hydrolysis and its future prospects for commercial lignocellulosic bioethanol production.

***Keywords*:** second-generation bioethanol, lignocellulosic biomass, cellulase enzyme, enzymatic hydrolysis

4.1 Introduction

The steady increase in energy demand and the limiting of fossil fuels are creating an energy gap which poses a serious need for alternative energy sources. The best way to fill this energy gap is the use of sustainable sources of energy, i.e, renewable. Bioethanol is one such promising renewable energy source which is capable of replacing fossil fuels usage partly because of its higher energy density, greater air-fuel ratio, more specific energy and heat of vaporization [1].

Bioethanol is differentiated as first- and second-generation ethanol based on the raw material used. First-generation bioethanol is derived from food crops such as corn and sugar cane while second-generation converts lignocellulosic biomass. But due to controversy of food versus energy, ethanol production from lignocellulosic substrates has gained significant interest as a wide variety of feedstocks can be used as materials with no significant competition with the food chain. The majority of the process cost of ethanol production is dependent on the cost of raw material and in such a scenario, lignocellulosic biomass has made the process commercially feasible.

Lignocellulosic bioethanol production highly depends on two promising steps, which are pretreatment and saccharification. Pretreatment is the critical step of removing the lignin because the extent to which the biomass becomes accessible to the enzyme for saccharification highly depends on the type of pretreatment employed. Apart from the pretreatment process, another significant step is the efficient hydrolysis during saccharification of lignocellulosic substrates as it is the rate limiting step towards techno-economical feasibility of lignocellulosic bioethanol. Enzyme cellulase catalyzes the hydrolysis of cellulose by breaking the 1, 4-β-glycosidic bonds in between the cellulose chain of biomass. Complete use of carbohydrate components in lignocellulosic biomass is reliant on the improvement or development of cost-effective/cheaper technologies for cellulase production, and also on the development of enzymatic hydrolysis of carbohydrate components to monomeric sugars (hexoses and pentoses). A previous study revealed that enzyme production is the most expensive step in lignocellulosic ethanol production [2]. It covers approximately 40% of the total cost. So, for commercial lignocellulosic bioethanol production

development of cost-effective cellulase production technology is needed. Therefore, the present chapter discusses the current status of enzymatic hydrolysis to provide insight into the hydrolysis/saccharification process.

4.2 Hydrolysis/Saccharification

The saccharification process, i.e., the hydrolysis of cellulose and hemicelluloses, can be carried out mainly in two ways, i.e., biological (enzymatic) and chemical (acidic). The acidic reaction is done by using either dilute or concentrated acid. The enzymatic process has several benefits such as low toxic compound generation, high product yield, less chemical requirements, etc. (Figure 4.1).

4.2.1 Cellulase

The cellulases enzyme system is a mixture of endo-β-glucanase (EC 3.2.1.4), exo-β-glucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21). Cellulase acts on cellulose in the following manner: endo-β-glucanase acts randomly inside the cellulose chain, exo-β-glucanase acts on the external end of the cellulose chain and β-glucosidase degrade cellobiose into glucose or free monomeric sugar (Figure 4.2).

Individual enzymes are not capable of degrading the cellulose chain to a monomeric unit, hence synergistic action leads to a proper saccharification.

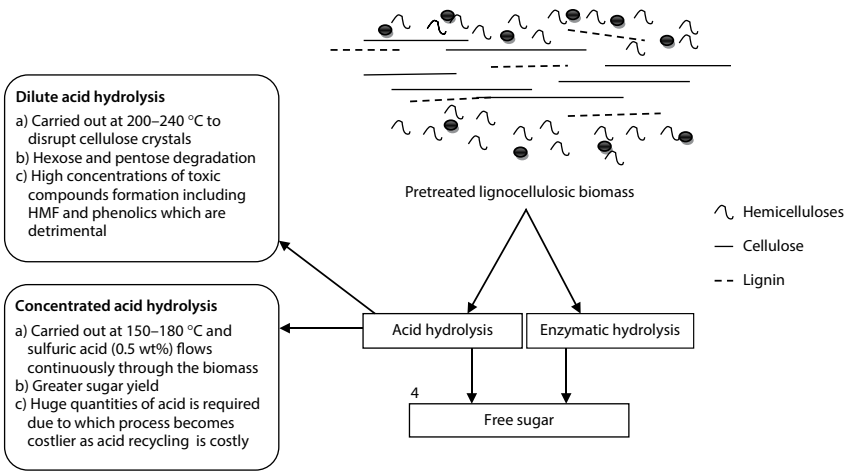

Figure 4.1 Saccharification process for lignocelluosic material.

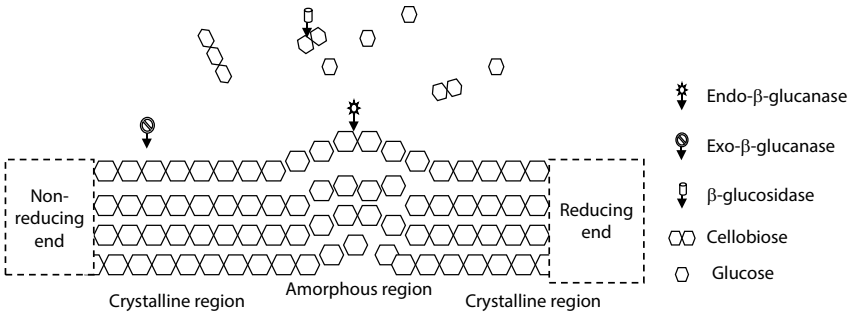

Figure 4.2 Schematic representation of cellulase mediated hydrolysis.

Major synergism has been noticed firstly between endo and exo-β-glucanase and secondly between exo-β-glucanases which act from both reducing and nonreducing end. β-glucosidase overcomes catabolic repression by preventing accumulation of cellobiose.

4.2.2 Screening of Cellulase-producing Microorganisms

There are several bacteria and fungi microorganisms capable of producing cellulase for the saccharification. Bacteria have a very low growth rate and require anaerobic growth conditions, therefore fungal cellulase have been mostly used for the given purpose (Table 4.1). The fungal cellulases production system works on the repressor/inducer phenomena where cellulose or other oligosaccharide act as inducers while glucose or other easily metabolized carbon sources act as repressors.

Trichoderma and *Aspergillus* are the most studied microorganisms for cellulase production. The crude enzyme extract of these microorganism are available for commercial use. *Trichoderma* produce endo-β-glucanase and exo-β-glucanase in higher quantity and β-glucosidase in lower quantity. In the case of *Aspergillus*, it produces endo-β-glucanase and β-glucosidase in higher quantity and exo-β-glucanase in lower quantity. It was reported that *Trichoderma reesei* QM-9414 is one of the best cellulase producers [13]. Later it was subjected to mono-colony isolation to obtain *Trichoderma reesei* KY-746. The mutated version gave higher cellulase activity [13].

Aspergillus niger is an important fungal strain for higher cellulase production. *Aspergillus niger* is a group of nine genera, and among them some possess higher potential of cellulase production. Different scientists have reported various media for cellulase production by using *Aspergillus niger* [14–16]. Abostate *et al.* [17] reported isolation of five potential *Aspergillus* sp. for cellulase production. They reported maximum

Table 4.1 Reducing sugar yield from different types of biomass.

Lignocellulosic biomass	Source of cellulase	Reducing sugar yield (mg/g dry substrate)	Incubation time (h)	References
Wheat straw	*Trichoderma reesei* NCIM 1186	371.44	24	[3]
Parthenium sp.	Commercial cellulase	574	48	[4]
Wheat straw	*Trichoderma reesei*	270	48	[5]
Wheat straw	*Trichoderma longibrachiatum*	294	72	[6]
Sorghum straw	*Coriolus versicolor*	440	168	[7]
Sargassum sp.	Commercial cellulase	326.89	72	[8]
Rice straw	Commercial cellulase	567	48	[9]
Rice straw	Commercial cellulase	414.16	72	[10]
Rice straw	*Aspergillus niger*	623.90	24	[11]
Pinus roxburghii	Locally isolated microorganism	334.00	24	[12]

endo-β-glucanase production in the case of *Aspergillus* MAM-F23. A previous study reported use of sorghum straw as a potential substrate for cellulase production [18]. Using *A. niger* under submerged fermentation, maximum cellulase production (0.77 IU/mL) was reported using sorghum straw as substrate, and lowest cellulose production (0.28 IU/mL) was reported using wheat straw as substrate. Maurya *et al*. 2012 [19] used *Trichoderma reesei* NCIM 992 for cellulase production under solid state fermentation. Kurup *et al.* (2005) compared cellulase production by different bacteria using water hyacinth as substrate. They found maximum cellulase production of 216 FPU/gds. Amira *et al*. 2012 [20] found higher xylanase activity (14.41 FPU/mg) using *Aspergillus niger* under solid state fermentation. Kumar *et al*. 2012 [21] reported maximum CMCase production (7.814 U/mg) from *Paenibacillus polymyxa*. Nair *et al*. 2008 [22] isolated 34 fungal strain strains for cellulase and xylanase production and they reported maximum cellulase production using *Trichoderma* sp. SBS60 and maximum xylanase production using *Aspergillus sydowii* SBS45. Ali and El-Dien 2008 [23] reported use of two different strains (*Aspergillus niger* and *Aspergillus nidulans*) for fungal cellulae production on water hyacinth.

4.2.3 Cellulase Production

Initial cellulase production was attempted on liquid culture but due to accumulation of free sugar catabolic repression took place, which hampered the cellulase synthesis during the microbial growth. Fed batch or continuous mode culturing can overcome the issue but adds to the overall cost.

Cellulase production on the agro industrial residues through solid state fermentation (SSF) is one of the promising technologies in terms of reduced processing cost. Carbohydrate moieties present in these cheap residues act as a carbon source for fungal growth. For cellulase production different substrates, such as wheat bran, rice straw, corn cob, sorghum straw, groundnut shell, cotton flower, saw dust, eater hyacinth, etc., have been reported [17, 24]. Table 4.2 shows the cellulase production under solid state fermentation by different fungal strains.

4.2.4 Factors Affecting the Cellulase Mediated Hydrolysis

Cellulase mediated hydrolysis consists of primarily three steps:

Adsorption of cellulase enzymes onto the surface of the cellulose

1. Bioconversion of cellulose to fermentable sugars
2. Desorption of cellulase

Table 4.2 Cellulase (CMCase) production by different fungal strains under SSF condition.

Microorganisms	CMCase activity (IU/gds)	Reference
Aspergillus niger	25.20	[25]
Fungal strains CG-10	29.04	[26]
Bacillus licheniformis	2.11	[27]
A. niger NRRL 567	425.3	[28]
Trichoderma atroviride	90.43	[29]
Aspergillus niger HN-1	416.3	[30]
Aspergillus awamori	19.00	[31]
Aspergillus fumigatus Z5	526.30	[32]
Trichoderma sp.	172.31	[33]
Humicola insolens TAS-13	18.98	[34]

3. The governing factors for these steps are mainly substrate concentration, enzyme dosage and reaction conditions.

At low substrate concentration the reducing sugar yield and reaction rates are increased but at high substrate concentration the reducing sugar yield and reaction rates are decreased. At high substrate concentration the decrease in the reducing sugar yield and reaction rates are due to end product inhibition of cellulase enzyme. Mojovic *et al*. 2006 [35] reported lower substrate concentrations were more suitable in order to avoid substrate inhibition. The authors found that at 16% suspension of corn flour the glucose yield was 76%, while when a 40% suspension was hydrolyzed the yield was only 50.2%.

High enzyme dosage enhances the reducing sugar yield but at the same time significantly increases the processing cost. Therefore, selection of optimum parameters such as temperature, pH, and incubation time at low enzyme dosage can be one approach to overcome the issues. Mahamud and Gomes [36] reported use of crude *Trichoderma* cellulase for enzymatic saccharification of alkali pretreated sugarcane bagasse. They reported maximum degree of hydrolysis (37.29%) at 50 °C. Ahmed *et al.* [37] reported that enzymatic saccharification of alkali treated bagasse rapidly increased up to 8 h and the rate of this increase was

Table 4.3 Effect of additive on cellulase mediated hydrolysis.

Additives	References
Addition of Ca(II) and Mg(II) results in lignin- metal complex formation	[42]
poly(ethylene oxide) polymer (PEO) and poly(ethylene glycol) (PEG)	[45]
Surfactants and bovine serum albumin (Tween 20, Tween 80, Triton X-100, Agrimul and SDS	[46]
Ammoniationand various N compounds	[47]

substantially reduced at later stages. Han *et al.* [38] reported maximum reducing sugar yield (341.87 mg/g dry substrate) from alkali pretreated wheat straw at 55 °C using cellulase produced from *Penicillium waksmanii*. The variation in temperature was due to different species used for cellulase production. Moreover, the hydrolysis rate was influenced by the duration of the hydrolysis process [39]. Saha *et al.* [40] achieved maximum reducing sugar yield (554 mg/g dry substrate) after 72 h of saccharification of dilute acid pretreated wheat straw at 45 °C. In the case of alkali pretreated wheat straw maximum reducing sugar yield (343.95 mg/g dry substrate) was obtained after 30 h of enzymatic saccharification [38].

Jeya *et al*. 2009 [41] reported optimization of enzymatic saccharification of alkali-treated rice straw by using CCD based RSM. The authors found a maximum saccharification rate of 88% at an enzyme concentration of 37.5 FPU/g-substrate after optimization of the hydrolysis parameters. Liu *et al*. 2010 [42] used CCD based RSM for optimization of enzymatic hydrolysis of recycled pulp. Phuengjayaem and Teeradakorn, 2011 [43] reported that maximum yield of glucose was 0.366 g/g dry substrate at the optimal condition: 1.0–2.5% of the acid pretreated sweet sorghum straw, 30 FPU/g-substrate of cellulase, pH 3–5, at 30–50 °C in 96 h. Higher reducing sugar yield in short incubation time is required for improved process economics of bioethanol production [44].

Lignin has also an adverse effect on cellulases. It affects the whole process by nonproductive adsorption and irreversible binding of enzymes which limits the accessibility of cellulose to cellulase. Various methods have been used to eliminate lignin inhibition (Table 4.3).

4.3 Future prospects of enzymatic hydrolysis

The saccharification process, though it seems similar, faces various bottlenecks which are both technical and economical. Technical problems associated with the process are inefficient cellulase adsorption and efficacy due to limited accessible substrate surface, end product inhibition and lignin, while economic issues are related to cost of raw material, cellulase enzyme, etc. Hence, the current cellulase mediated hydrolysis problem needs to be taken care of for further advancement of lignocellulosic-bioethanol technology. Use of genetically modified cellulolytic organisms by cloning cellulase coding sequences into bacteria, fungi and plants is recommended to increase the cellulase yield and productivity under stress conditions. Even genetically engineered raw materials with higher carbohydrate content and low lignin content could reduce the cost. Simultaneous saccharification and fermentation (SSF) is also considered to be cost-effective by overcoming the end product inhibition. There is a serious need to understand the mode of action of the critical factors that control interactions between biomass, cellulase and inhibitory compounds. This knowledge will provide a new avenue to identify better pretreatment and saccharification strategizes as per industrial needs.

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