Biochemical Engineering Journal 102 (2015) 38–44

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Current perspectives in enzymatic saccharification of lignocellulosic biomass

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ARTICLE INFO

Article history:
Received 13 January 2015
Received in revised form 21 February 2015
Accepted 25 February 2015
Available online 27 February 2015

Keywords:
Cellulose
Biofuel
Enzymes
Thermophiles
Extremophiles
Ionic liquids

ABSTRACT

With the depletion of fossil fuel reserves, there is an urgent need to search for renewable and cost effective strategies for biofuel production. Lignocellulosic biomass has been perceived as a potential feedstock, wherein effective pretreatment and saccharification is necessary prerequisite for developing viable biofuel processes. Recent approaches in this context are, (i) studying enzymes from extremophilic organisms, particularly thermophiles which are gaining importance in this aspect as they are found to be stable and catalytically more effective under harsh conditions; (ii) usage of ionic liquids for pretreatment is emerging as a greener technology due to their non toxic nature. Developing/screening for ionic liquid tolerant lignocellulosic enzymes in order to attain simultaneous pretreatment and saccharification, offer an interesting option; and (iii) engineering/manipulating the existing lignocellulosic enzymes for desirable traits and viable saccharification and biofuel generation processes. The review encompasses these approaches and the focus on the recent development in the area.

1. Introduction

Considering the fast depleting fossil sources, there has been a major impetus worldwide to develop alternate source of fuels and bio-based chemicals. In this context, lignocellulosic feedstocks have been perceived as most potential and sustainable source for producing alternate fuels and platform chemicals [1,2]. These include agricultural by-products such as wheat and rice straw, husks, corn stover, corn cobs, bagasse, oilseed cakes, wood, grasses and dedicated energycrops such as miscanthus and switchgrass [3,4]. Extensive researches are underway for developing efficient processes to utilize them for viable production for fuels and chemicals.

The basic lignocellulosic utilization strategy involve three essential and inter-dependent steps namely (i) pretreatment of the biomass, necessary for disrupting lignocellulosic interactions to make cellulose and hemicellulose, and other carbohydrate polymers better accessible for enzymatic hydrolysis in the next step commonly called as saccharification; (ii) saccharification of pretreated material by hydrolases such as cellulases, xylanases, and other carbohydrases; and (iii) appropriate fermentation of monosaccharides, generated out of the saccharification, for production of desirable product viz., biofuels or other platform chemicals. All three processes have been extensively reviewed in the recent years [5–7]. It has been generally agreed that effectiveness of pretreatment and saccharification determines the viability and yield of the fuel or other products in the fermentation step. Number of excellent treatment methods such as acid or alkali treatment, hot water washing, steam explosion and ammonia fibre expansion have been developed in the past [8]. Since composition and structure of biomass varies from source to source, the efficacy of the pretreatment method also varies for each biomass and no single method can be said to be suitable in generic manner. Nonetheless, optimum pretreatment have been standardized for most of the commonly available biomass, which can be recommended and used with required variation.

The sacchrification step still remains as one of the critical bottlenecks. An ideal process should generate stoichiometric amount of fermentable monomeric sugars out of the lignocellulosic complex. Yield attainable so far has been less than satisfactory and rather poor. Lower amount of the fermentable sugar makes product yield unviable in next fermentation step [9,10]. The major issue which needs to be resolved are (i) better access and cellulolytic hydrol-

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http://dx.doi.org/10.1016/j.bej.2015.02.033
1369-703X/

ysis, which means search for kinetically more efficient cellulases and carbohydrases; (ii) whether cellulases can sustain highest catalytic activity under the pre-treatment acidic, alkaline or steam heat conditions. Since other aspects of lignocellulosic utilization such as pretreatment and fermentation have been well discussed in the past [11–13]. This paper specifically focuses on the status of available lignocellulosic enzymes, their hydrolytic efficiency and conversion yield with respect to common lignocellulosic biomass. It further encompasses the recent strategies employed to search for new carbohydrases from (i) extremophilic sources; (ii) enzyme engineering efforts; and (iii) medium engineering such as ionic liquid toward achieving simultaneous saccharification.

2. Recalcitrance in lignocellulosic structure and need for pretreatment

Since each biomass has variable composition and structure, the cellulolytic enzymes cannot act uniformly in all the cases. Nigam et al. [14] have excellently compiled the composition of common lignocellulosic feedstocks. It is quite evident that biomass in general consists of 40–50% cellulose, 25–30% hemicellulose and 15–20% lignin and small amount of other extractives [15].

However, the structural alignment of these component make the lignocellulosic biomass quite recalcitrant against hydrolases, and cellulases in particular, affecting enzyme accessibility, activity and resulting into slow kinetics. It is therefore relevant to briefly consider the structural aspect herein.

The main component of lignocellulose is cellulose, with glucose as monomeric units linked to each other by β (1–4) glycosidic bonds. Each of this polysaccharide chain is hydrogen bonded with the other and remain arranged as different layers. This arrangement contributes to the crystallinity in the cellulose, which impedes the hydrolysis. The hemicelluloses, some amorphous cellulose and other polysaccharide especially pectin form cementing layers with core crystalline cellulose. This structure is finally enveloped by lignin. Each unit is referred as microfibril and many such microfibril together constitute the lignocellulose. The hemicelluloses in the biomass are generally heteropolymer of xylose, arabinose, mannose, glucose, and galactose whereas lignin is composed of three major phenolic components, namely p-coumarylalcohol, coniferyl alcohol and sinapyl alcohol [10,16]. The strong covalent bonds between lignin and cellulose and poor access to cellulose core and its crystalline nature together make the enzymatic action kinetically slow with poor yield of fermentable monomeric sugars [17].

This necessitates effective pretreatment to loosen the lignin-cellulose association and making the cellulose accessible during enzymatic hydrolysis. Thus the pretreatment becomes very crucial step in the lignocellulosic biomass utilization. During the past few decades, several approaches have been used for developing low cost pretreatments for generating sugar syrups from cellulose and hemicellulose [18,19]. Pretreatments for lignocellulosic biomass include biological, mechanical, chemical methods and various combinations thereof [20]. The choice of the optimum pretreatment process depends on the feedstocks. There are a number of reports on pretreatment options for various biomass types available with each having its own merit and demerits [21,22].

Menon and Rao [20] provided an excellent overview of different pretreatments. If assessed in terms of sugar yield, minimum inhibitor formation and by- product formation, scalability and generic application to large number of biomass, acid, alkali, liquid hot water, steam explosion, and Ammonia fiber expansion (AFEX) have been considered quite useful to meet most of these criteria. However, acid and liquid hot water pretreatments have individual drawbacks, steam explosion suffer from high inhibitor formation whereas alkali generates less inhibitors but needs longer treatment time with high salt formation. In this context AFEX seems better but success at pilot scale need to be established in more cases.

Nonetheless, methods are available for effective pretreatment to facilitate next step of enzymatic hydrolysis.

3. Enzymatic saccharification of lignocellulosic biomass

Cellulases are primary enzymes for cellulose hydrolysis. These comprises of three predominant activities viz., *exo*-1,4- β-glucanase, *endo*-1, 4-β- glucanase and cellobiase. The balanced and appropriate combination of these activities is what determines the

Table 1
Key cellulases and hemicellulases and saccharification efficiency.

S.No	Enzyme	Biomass treated	Saccharification %	References
1	*Trichoderma reesei* Cellulase	Sugarcane tops	90%	[27]
2.	*T. reesei* cellulases	Rice straw, eucalyptus treated with NaOH and H_2SO_4 and hydrothermal treatment	80%, 100, 100 and 90%, 100, 100 respectively	[28]
3.	*Chrysoporthe cubensis:* *Penicillium pinophilum* 50:50 (v/v)	Alkali treated sugarcane bagasse	Glucan hydrolysis efficiency reached an excess of 60% and xylan conversion exceeded 90%	[29]
4.	Cellulase, hemicellulase and xylanase produced by *Aspergillus* sp. and *A. niger* and *Thermomyces lanuginosus* respectively	Alkali pretreatment of *Miscanthus sacchariflorus* *var*. No. 1	220 mg glucose/g	[30]
5.	*Chrysoporthe cubensis*	Alkaline pretreated sugarcane bagasse	56% and 90% of glucose and xylose, respectively	[31]
6.	White-rot fungi *Pycnoporus sanguineus*	Alkali-treated sugarcane bagasse	60% reducing sugars	[32]
7.	*Aspergillus oryzae* ITCC 4857.01	Alkali treated sugarcane bagasse	9%	[33]
8.	Commercial cellulase	Sugarcane tops treated with dilute acid	0.685 g/g of reducing sugar was produced per gram of pretreated biomass	[34]
9.	Commercial cellulase from Fluka, Onozuka and cellulase from *T. viride* CMIT3.5	Pretreated corn stover, *Miscanthus*, and wheat straw	52%, 59%, and 61% in wheat straw, corn stover, and *Miscanthus* respectively	[35]
10.	Commercial Xylanase XL and Novozyme 188	Steam pretreated wheat straw	Increase from 40 to 50%	[36]