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Review

Feasibilities of consolidated bioprocessing microbes: From pretreatment to biofuel production

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ABSTRACT

Lignocelluloses are rich sugar treasures, which can be converted to useful commodities such as biofuel with the help of efficient combination of enzymes and microbes. Although several bioprocessing approaches have been proposed, biofuel production from lignocelluloses is limited because of economically infeasible technologies for pretreatment, saccharification and fermentation. Use of consolidated bioprocessing (CBP) microbes is the most promising method for the cost-effective production of biofuels. However, lignocelluloses are obtained from highly diverse environment and hence are heterogeneous in nature. Therefore, it is necessary to develop and integrate tailor-designed pretreatment processes and efficient microbes that can thrive on many different kinds of biomass. In this review, the progress towards the construction of consolidated bioprocessing microbes, which can efficiently convert heterogeneous lignocellulosic biomass to bioenergy, has been discussed; in addition, the potential and constraints of current bioprocessing technologies for cellulosic biofuel production have been discussed.

1. Introduction

Fuels are essential because human society relies extensively on machines to perform even simple tasks. The global primary energy demand was 302 EJ/year in 1980 (1 exajoule = 1018 J), which was increased to 420 EJ/year in 2000 and 491 EJ/year in 2006, and is expected to increase to 826 EJ/year by 2050 (Waldron, 2010). The rapid increase in global energy demand and the availability of current energy sources argues to forgo fossil fuels and search for alternative, cleaner, renewable and sustainable energy sources such as solar energy, hydroelectric energy, wind energy, and bioenergy (Chu and Majumdar, 2012). In recent days, the production of bioenergy (especially biofuels) from lignocellulosic biomass has a significant global interest because biofuels are considered carbon–neutral and have high similarity, both in property and energy content, to petroleum-based transportation fuels (Lee et al., 2008). The NREL (National Renewable Energy Laboratory, Golden City, CO, USA) defined the biorefinery concept as an analog to present chemical refineries, which produce multiple fuels and products from petroleum (Hasunuma et al., 2013).

In general, lignocellulosic biomass can be obtained from 4 major sources; (1) agricultural residues, such as corn stover, rice straw, etc.; (2) forest residues (woody biomass), such as woods, branches, foliage, etc.; left behind in the forest following clearance, (3) energy crops, such as switchgrass, yellow poplar, etc. that are specifically bred and cultivated to produce bioenergy, and (4) cellulosic waste, such as municipal solid waste, food waste, etc. The availability and composition of lignocellulosic biomass in each country or region depends primarily on the climate and on the types of vegetation in those areas. For example, the agricultural residues produced in each country is directly associated with its total farming productivity due to different technologies employed for cultivating, harvesting, transportation, storage, and processing (Table 1). Thus, composition of lignocellulosic biomass varies from place to place and over time.

Hence, it is necessary to either develop a region/season specific lignocellulosic biofuel plant or to develop an efficient process that could tackle the heterogeneity of the lignocellulosic substrates without a compromise in the yield and productivity. This review addresses the heterogeneity of lignocellulosic substrate and various bioprocessing options available for efficient lignocellulose-based biofuel production (Hereafter referred to as, LCB). In addition, the potential of recombinant cellulolytic microbes to thrive

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on the heterogeneous and recalcitrant lignocellulosic substrate is reviewed.

2. Bioprocessing options for LCB production

The composition of lignocellulosic biomass is typically cellulose (30–45% of biomass weight), hemicellulose (15–30% of biomass weight), and lignin (12–25% of biomass weight). The major challenge with lignocellulosic biomass (when compared to the hydrolysis of grains and sugar crops) is the presence of lignin and the recalcitrant structure of cellulose. Hence, the first step in the bioconversion of lignocellulosic biomass is the removal of lignin and breakdown of the rigid structures of cellulose by chemical pretreatment, and then the enzyme saccharification step converts them into their component sugars in recurring reactions (Table 1) (Kim, 2013). These sugars can be converted to many valuable products such as ethanol, butanol, biodiesel, numerous organic acids, and many other products using microbial catalysts. A typical biofuel production process is comprised of the following unit operations: (1) biomass collection and storage, (2) solid handling, (3) pretreatment, (4) enzyme production, (5) saccharification, (6) fermentation, and (7) product recovery. Of these operations, pretreatment, saccharification, and fermentation are considered the rate-limiting steps that determine the overall efficiency of the conversion process. Various bioconversion process schemes have been suggested in order to overcome critical issues in pretreatment, enzymatic saccharification, and fermentation of biomass-derived sugars.

2.1. Pretreatment of lignocellulosic biomass

Typically, the primary effects of pretreatment process include the following; (1) decrease of lignin, hemicellulose, and extraneous components; (2) increase of surface area and porosity; and (3) reduction of crystallinity. In nature, microbes could thrive efficiently upon lignocellulosic materials that are subjected to simple pretreatment process like mastication in the mouth of grazing animals (Weimer et al., 2009). However, in industrial process design, pretreatment is a critical step to overcome the recalcitrance of lignocellulose, which in turn enhances enzymatic saccharification and the yield of final products (Himmel et al., 2007; Lynd et al., 2008). Developing more effective and economical pretreatment process would help remove the lignin portion of lignocellulosic biomass and would provide greater accessibility to cellulose. Improving the pretreatment process may be one of the most feasible ways for efficient LCB production because it can reduce the enzyme cost significantly (Kim, 2013).

Several pretreatment options have been suggested for the cost-effective processing of lignocellulosic biomass, including grinding/milling, steam/steam explosion, hot water/autohydrolysis, acid treatment (sulfuric acid, hydrochloric acid, etc.), alkali treatment (ammonia, lime, NaOH, etc.), and biological treatment (da Costa Sousa et al., 2009; Garrote et al., 2002; Jacobsen and Wyman, 2000; Kim et al., 2003; Kim and Lee, 2006, 2007; Mosier et al., 2005; Yoo et al., 2011a; Zheng et al., 2008). Widely used pretreatment technologies include dilute sulfuric acid, lime, hot water, ammonia fiber explosion (AFEX) or ammonia recycle percolation (ARP) methods (Mosier et al., 2005; Wyman et al., 2005a,b).

The net effect of pretreatment also varies widely in terms of their physical and chemical characteristics. For example, the alkaline pretreatment effectively solubilizes lignin whereas the acid pretreatment is highly selective for hemicellulose hydrolysis. The early removal of lignin would eliminate the interaction between lignin and cellulases making enzymatic hydrolysis more efficient. Alkali solubilizes only one-third to one-half of hemicellulose to oligomeric sugars (mainly pentoses), whereas acids generally solubilize most of the hemicellulose to oligomeric sugars. An efficient pretreatment process should thus be determined based on the chemical composition and characteristics of the biomass and the microbe. Even for model bioenergy crops like switchgrass, transgenic variants with less lignin content were constructed to improve pretreatment and saccharification (Fu et al., 2011). Pretreatment is therefore a critical step in determining the downstream process for the efficient saccharification and fermentation of lignocellulosic biomass.

2.2. Enzymatic saccharification and fermentation

Both enzymatic saccharification and fermentation processes are the key determinants of the sustainability of the LCB production. Extensive research identifies 4 important process configurations for efficient LCB production depending on the changes in saccharification/fermentation conditions (Fig. 1): SHF (separate hydrolysis and fermentation), SSF (simultaneous saccharification and fermentation), SSCF (simultaneous saccharification and co-fermentation), and CBP (consolidated bioprocessing). Of these 4 process configurations, CBP is still in its early stage of establishment and hence is the main focus of this review. It should be noted that bioprocessing technologies for biofuel production show a trend towards consolidation over time (Waldron, 2010) and recent research support the notion that this highly integrated CBP configuration, may be feasible in the near future.

CBP combines all 3 steps of LCB production (enzyme production, enzyme saccharification, and sugar fermentation) within a single recombinant microbe. This process results in reduced cost because only 1 vessel is required. It also helps avoid the costs associated with enzyme production (Lynd et al., 2005). The main disadvantage with CBP process is that saccharification and fermentation

Table 1
Agricultural residues from different agricultural practices and their composition.

Crops	Residue	Glucan (%)	Xylan (%)	Galactan (%)	Arabinan (%)	Mannan (%)	Lignin (%)	Ash (%)	References
Maize	Stover	38.7	22.0	1.5	3.1	0.7	17.9	5.1	Yoo et al. (2011b)
	Cob	35.4	28.7	1.1	3.1	0.6	12.1	2.6	Wang et al. (2011)
Wheat	Straw	36.7	22.2	0.7	2.1	0.5	21.3	8.6	Kootstra et al. (2009)
	Bran	39.4	10.5	1.2	6.2	NA	4.2	NA	Choteborská et al. (2004)
Rye	Straw	33.1	19.5	0.3	2.5	NA	19.8	6.2	Sun and Cheng (2005)
Barley	Straw	41.0	22.4	1.2	3.0	NA	21.3	4.1	Yoo et al. (2013)
	Hull/husk	33.6	30.5	0.6	6.1	NA	19.3	3.6	Kim et al. (2008)
Rice	Straw	34.9	19.7	2.5	3.3	3.7	16.0	16.1	Ko et al. (2009)
Sugar cane	Bagasse	43.2	21.0	0.5	1.9	0.3	23.9	2.8	Travaini et al. (2013)

All numbers are average values. N/A, Not applicable.

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