Journal of Scientific Research
Banaras Hindu University, Varanasi

Vol. 55, 2011 : 57-63
ISSN : 0447-9483

KEY PRETREATMENT TECHNOLOGIES ON CELLULOSIC ETHANOL PRODUCTION

Amrita Verma, Santosh Kumar* and P. K. Jain
Physical Chemistry Division
National Sugar Institute, Kanpur

Conversion of lignocellulosic biomass to fuel ethanol involves pretreatments followed by enzyme-catalyzed hydrolysis to generate fermentable sugars. Efficient pretreatment method can significantly enhance hydrolysis of biomass and thus reduce ethanol production cost. Cellulosic plant materials are mainly composed of cellulose, hemicellulose and lignin, the cheapest source of fermentable sugars. The presence of lignin largely prevents acid and enzymatic hydrolysis of these materials. Optimization of enzyme loading and selection of suitable fermentation techniques based on biomass, may further improve ethanol yield. The present review focuses on various pretreatments based on composition of lignocellulosic biomass and also the simultaneous saccharification and co-fermentation and co-immobilization for cellulosic ethanol production.

Keywords : Bioethanol, Co-immobilization, Enzymatic hydrolysis, Lignocellulosic biomass, Pretreatment, saccharification.

Introduction

Lignocellulosic biomass, the most abundant and low-cost biomass worldover are the raw materials for production of fuel ethanol[1]. For transformation of lignocelluosics into bioethanol, the steps involved are procurement of ignocelluosics, pretreatment, hydrolysis to derive sugars their fermentation bioethanol and its dehydration. In general, ethanol production from lignocellulosic feedstocks grouped as[2]:

i) crop residues (cane bagasse, corn stover, wheat straw, rice straw, rice hulls, barley straw, sweet sorghum bagasse, olive stones and pulp);

ii) hardwood (aspen, poplar);

iii) softwood (pine, spruce);

iv) cellulose wastes (newsprint, waste paper, recycled paper) and

v) municipal solid wastes (MSW).

---

* Bio Chemistry Division National Sugar Institute, Kanpur

Lignocellulosic biomass such as wheat straw and bagasse are inexpensive, and widely available resources containing 75-80% polysaccharides (cellulose and hemicelluloses). These can be hydrolysed to monomeric sugars such as glucose and xylose, That can be used as substrates for fermentative production of useful products. Bioconversion of biomass-derived products like value-added fuels and chemicals offers potential economical, environmental, and strategic advantages over traditional fossil-based products.

Over the last decades, researches have been devoted to converting lignocellulosic materials to bioethanol. Lignocellulosics are composed of heterogeneous complex of carbohydrate polymers (cellulose, hemicelluloses and lignin). Cellulose (40-60% by wt) consists of high molecular weight polymers of glucose rigidly held together as bundles of fibers. Hemicellulose (20-40% by wt) is shorter polymers of various sugars that bind cellulose bundles together. Lignin (10- 30% by wt) consists of a tri-dimensional polymer of propyl-phenol that is imbedded in and bound to hemicellulose to provide rigidity.

Lignocellulosics are resistant to degradation and offer hydrolytic stability and structural robustness, which is mainly due to cross linking between polysaccharides (cellulose and hemicellulose) and lignin via ester and ether linkages. Cellulose and hemicellulose are densely packed by layers of lignin, that offer protect in against enzymatic hydrolysis. So it is open the to break lignin seal and expose cellulose and hemicellulose to enzymatic action.

This presentation discusses pretreatment technologies and the simultaneous saccharification and co-fermentation (SSCF) and co-immobilization for ethanol production.

Pretreatments

Pretreatment of lignocellulosics aims to decrease crystallinity of cellulose, increase biomass surface area, remove hemicellulose, and break the lignin barrior. Pretreatment makes cellulose more accessible to hydrolytic enzymes to facilitate conversion of carbohydrate polymers into fermentable sugars in a rapid way with the concomitant more yield. Pretreatments include physical, chemical and thermal methods, and their combinations. Pretreatment is one of the most expensive processing steps for production of sugars from biomass.

Since many lignocellulosics have different physicochemical characteristics, it is necessary to deploy suitable pretreatment technology based on their properties. Agricultural residues[3,4] and hardwoods[3,5] have low lignin and high pentose content compared to softwoods[3,6], and thus high temperature

treatments are not effective for such biomass type thing to high thermal degradation quality of pentoses.

Agricultural Residues

Agricultural residues (straws, hulls, seeds, linter and other similar byproducts) have high pentose and low lignin content. Dilute acid treatment, low temperature steam treatment with acids, soaking in aqueous ammonia, and microwave-assisted treatment and wet oxidation are used successfully

Straws

Strong crystalline structure of cellulose in rice straw, and the complex structure of lignin, hemicellulose and cellulose limits accessibility of straw to hydrolytic enzymes. Therefore, various pretreatment methods have been developed to open the crystalline complex of cellulose and so also to increase its exposure to hydrolytic enzymes[9].

Hydrothermal pretreatments have proven to be effective in increasing enzymatic digestibility of wheat straw for and conversion into fermentable sugars for bioethanol production[10]. It is reported that hydrothermal pretreatments caused profound lignin re-localisation and major wax and removal of a small fraction of hemicellulose. It is possible to pretreat [10] wheat straw sufficiently without disrupting cell wall. Thus, only a modest pretreatment is necessary in order carbohydrates are digested enzymatically.

Steam explosion[10] seems the best suitable physical pre-treatment of straw as it partially hydrolyses hemicellulose and increases it's enzymatic digestibility in the biomass residue. During steam pretreatments, water in the cells evaporates and as the pressure around straw drops, it explodes to offer increased specific surface area. Part of the hemicellulose decomposes to acids, which catalyse decomposition of hemicellulose and lignin, and release the cellulose.

Steam pre-treatment is affected by steam temperature, residence time in the reactor, particle size, moisture content and the catalyst concentration. Steam pretreatment of sulfuric acid impregnated wheat straw[8] gave reducing sugar yield of 39.6 g/100g dry straw wt. after pretreatment at 190°C (10 mm.) A high yield of xylose (21.6 g) 100g dry straw wt. was also obtained for such pretreatment conditions.

Microwave irradiation is reported[7] to change the ultra structure of cellulose, degrade lignin and hemicellulose in rice straw and increase enzymatic susceptibility[11,12] . Compared to conduction/convection heating, microwave directly interacts between a heated target and the electromagnetic field to generate heat. Therefore, heating is volumetric and rapid when microwave is

used to treat the lignocelluloics. It is hypothesized that this unique heating feature has in an 'explosion effect' among particles, and improves disruption of recalcitrant structure of lignocelluloses.

In addition, the electromagnetic field used in the microwave might cause non- thermal effects that also accelerate destruction of crystal line structures[13]. It is reported that rice following after 30 min of microwave/alkali pretreatment (weight loss 44.6%; cellulose 69.2%, lignin 4.9% and hemicellulose 10.2%) at 700 W, is better than pretreatment alkalis alone (weight loss 41.5%, cellulose 65.4%, lignin 6.0% and hemicelluloses 14.3%) even after 70 min. [11]Thus, microwave/alkali pretreatment could remove more lignin and hemicellulose from rice straw with the shorter pretreatment time compared to alkali-alone.

Soaking the biomass in aqueous ammonia (SAA)[14] at low temperature retains hemicellulose in solids by minimizing interactions with hemicellulose, reported as the feasible approach to increase fermentation yield and simplify bioconversion process. Retained xylan can usually be hydrolyzed to fermentable pentoses by the cellulase and xylanase mixtures[14].

Bagasse

Pretreatment methods for bagasses include steam explosion[15], hot water[16] peracetic acid[17] and with ammonia water[18]. In wet oxidation pretreatments the material is treated with water and air at around 120^0C as reported by Martin et al[19]. Further enzymatic convertibility and fermentability of bagasse pretreated by alkaline wet oxidation[19] at different pH values and at 195°C 15 min. a solid material having 70% cellulose, accompanied by an enzymatic convertibility of cellulose to 75%.

Although acidic wet oxidation (195°C, 15 min) offered good fractionation of bagasse, a significant part of polysaccharides was lost due to degradation and formation of byproducts, mainly carboxylic acids, but the enzymatic convertibility of the pretreated feedstock was poor. Studies[19] have shown that wet oxidation catalyzed transformation of hemicellulose from solid phase to liquid without major hydrolysis of the solubilized hemicellulose molecules accompanied by high production of xylose steam explosion produced more glucose [21]. McGinnis et a1[20] reported that monosaccharides are oxidized to carboxylic acids by wet oxidation, while oligosaccharides are more resistant to oxidation owing to stability of glycosidic linkages with thee resultant more sugar oligomers.

Steam explosion (220°C) for shorter reaction times, followed by acid hydrolysis, proved very effective for recovery of fermentable sugars from pine hemicelluloses[22]. Steam explosion, with SO_2 as acid catalyst, effectively

fractionates softwood carbohydrates[23], releasing soluble hemicellulose (80-90%) and thus enhancing enzymatic hydrolysis of the water-insoluble cellulose fraction.

Enzymatic Hydrolysis

In order that cellulose hydrolysis becomes economically feasible, it is important to identify methods that increase enzyme effectiveness and overcome barriers of enzymatic hydrolysis[4]. Major factors that influence enzymatic conversion of lignocelluloses the fermentable sugars, include accessible surface area of lignocelluloses[24], enzyme loading and presence of inhibitors[25].

External surface area of lignocelluloses can be increased by mechanical milling and grinding. More recently, addition of xylanase serves the same purpose and with cellulase, it proved to be the effective method for enzymatic hydrolysis of lignocelluloses. Ohgren et a1[26] obtained a near theoretical glucose yield (96-104%) from acid catalyzed steam pretreated corn stover, using xylanases as supplement to cellulases during hydrolysis. Since high cost of enzyme limits large-scale lignocellulosic bioethanol production, it is desirable to use low enzyme loading to produce fermentable sugars with high yield. Additives could be promising to improve enzymatic hydrolysis by restricting enzyme activity loss due to nonproductive adsorption[4,27].

Use of surface-active additives (surfactants, proteins and polymers) has been reported to enhance enzymatic hydrolysis of lignocelluloses by preventing unproductive binding of cellulase to lignin. Addition of Tween[25] or polyethylene glycol (PEG)[27] increased efficiency of enzymatic hydrolysis by getting adsorbed on the lignin surface. Ethylene oxide containing surfactants also have the same effect[27].

Addition of PEG to enzymatic hydrolysis medium at 50°C hindered deactivation of enzymes by their exclusion from lignin surfaces and to increased cellulose conversion up to 70%. Surfactants have a more pronounced effect on acid and steam pretreated straw than that of ammonia and hydrogen peroxide treated straws.

Conclusions

To produce economically feasible cellulosic ethanol, ethanol yield and enzyme efficiency have to be improved by optimizing all unit processes (pretreatments, saccharification and ethanol fermentation). Most of the pretreatments focused on low temperature pretreatment to avoid degradation of hemicellulose and to improve the pentose yield. Impregnation by various chemical reagents such as sulfuric acid and application of microwave have been

tried to lower pretreatment temperatures while maintaining high enzymatic digestibility. For saccharification, the accessible surface area and enzyme, reuse were key parameters. With regard to surface area, xylanases addition was effective. To prevent deactivation of cellulase by binding to non-productive sites, the addition of surfactants was the efficient method. Among various reagents, PEG 6000 exhibited best performance. Co-fermentation of glucose and xylose was key in improving ethanol yield. Fed-batch and co-immobilization have been found to be the ideal option for co-fermentation steps.

REFERENCES

1. Hu Z & Wen Z, Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment,Biochem Eng J, 38(2008) 369-378.

2. Oscar J S & Carlos A C, Trends in biotechnological production of fuel ethanol from different feedstocks, Biores Technol, 99(2008) 5270-5295.

3. Mosier N, Wyman C, Dale B, Elander R, Lee Y, Holtzapple M & Ladisch M, Features of promising technologies for pretreatment of lignocellulosic biomass, Biores Technol, 96 (2005) 673-686.

4. Kristensen J B, Boijesson J, Brunn M H, Tjerneld F &Jorgensen H, Use of surface active additives in enzymatic hydrolysis of wheat straw lignocellulose, r:Enz Micro Technol, 40 (2007) 888-895.

5. Sassner P, Martensson C G, Galbe M & Zacchi G, Steam pretreatment of H_2S0_4-impregnated Salix for the production of bioethanol, Biores Technol, 99 (2008a) 137-145.

6. Sassner P, Galbe M & Zacchi G, Techno-economic evaluation of bioethanol production from three different raw Materials, Biomass Bioenergy, 32 (2008b) 422-430.

7. Xiong J, Ye J, Liang WZ & Fan PM, Influence of microwave on the ultrastructure of cellulose I, J Sou Chin Uni Technol, 28 (2000) 84-89.

8. Linde M, Jakobsson E L, Galbe M & Zacchi G, Steam pretreatment of dilute H_2So_4 impregnated wheta straw and SSF with low yeast and enzyme loading for bioethanol production, Biomass Bioenergy, 32 2008) 326-332.

9. Zhu S D, Wu Y X, Yu ZN, Liao J T & Zhang Y, Pretreatment by microwave/alkali of rice straw and its enzymic hydrolysis, Process Biochem, 40 (2005) 3082-3086.

10. Kristensen J B, Lisbeth G T, Claus F, Henning J & Thomas E, Cell-wall structural changes in wheat straw pretreated for bioethanol production, Biotechnol Biotfuels, 1 (2008) 5 doi: 10.1186/1754-6834-1-5

11. Ooshima H, Aso K, Harano Y & Yamamoto I, Microwave treatment of cellulosic materials for their enzymatic-hydrolysis, Biotechnol Lett, 6 (1984) 289-294.

12. Azuma J I, Tanaka F & Koshijima T, Enhancement of enzymatic susceptibility of lignocellulosic wastes by microwave irradiation, J Ferment Technol, 62 (1984) 377-384.

13. De la Hoz A, Diaz-Ortiz A & Moreno A, Microwaves in organic synthesis. Thermal and non-thermal microwave effects, Chem Soc Rev 34 (2005) 164-178.

14. Kim T H & Lee Y Y, Pretreatment of corn stover by soaking in aqueous ammonia at moderate temperatures, Appe. Biochem Biotechnol, 137(2007) 8 1-92.

15. Martin C, Galbe M, Nilvebrant N 0 & Jonsson L J, Comparison of the fermentability of enzymatic hydrolysates of sugarcane bagasse pretreated by steam explosion using different impregnating agents, AppI Biochem Biotechnol, 98 (2002) 699-7 16.

16. Laser M, Schulman D, Allen S G, Lichwa J, Antal M J & Lynd L R, A comparison of liquid hot water and steam pretreatments of sugarcane bagasse for bioconversion to ethanol, Biores Thchnol, 81 (2002) 33-44.

17. Teixeira L C, Linden J C & Schroeder H A, Optimizing peracetic acid pretreatment conditions for improved simultaneous saccharification and cofermentation (SSCF) of sugarcane bagasse to ethanol fuel, Renew Energy, 16 (1999)1070-1073.

18. Kurakake M, Kisaka W, Ouchi K & Komaki T, Pretreatment with ammonia water for enzymatic hydrolysis of corn husk, bagasse and switchgrass,ApplBiochem Biotechnol, 90(2001) 251-259.

19. Martin C, Helene B K & Anne B T, Wet oxidation as a pretreatment method for enhancing the enzymatic convertibility of sugarcane bagasse, Enz Microb Technol, 40 (2007) 426-432.

20. McGinnis G D, Wilson W W & Mullen C E, Biomass pretreated with water and high pressure oxygen: The WO process, Journal : (1983) 352-357.

21. Martin C, Marcet M & Anne B T, Comparison between wet oxidation and steam explosion as pretreatment method for enzymatic hydrolysis of sugarcane bagasse, Bioresources, 3(2008) 670-683.

22. San Martin R, Perez C & Briones R, Simultaneous production of ethanol and kraft from pine (Pinus radiata) using steam explosion, Biores Technol, 53 (1995) 2 17-223.

23. Saddler J N, Ramos L P & Breuil C, Steam pretreatment of lignocellulosic residues. In Bioconversion cf Forest and Agricultural Plant Residues (CAB International, Wallingford, UK) 1993, 73-91.

24. Mooney C, Mansfield 5, Touhy M & Saddler J, The effect of the initial pore volume and lignin content on the enzymatic hydrolysis of softwoods, Biores Technol, 64 (1998) 113-119.

25. Eriksson T, Borjesson J & Tjerneld F, Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose, Enz Microb Thchnol, 31 (2002) 353-364.

26. Ohgren K, Bura R, Lesnicki G, Saddler J & Zacchi G, A comparison between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using steam-pretreated corn stover, Process Biochem, 42 (2007) 834-839.

27. Johan B, Ragna P & Folke T, Enhanced enzymatic conversion of softwood lignocellulose by poly (ethylene glycol) addition, Enz Microb Technol, 40 (2007) 75 4-762.