OVERVIEW ETHANOL PRODUCTION FROM LIGNOCELLULOSIC MATERIALS

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ABSTRACT

Ethanol from lignocellulosic materials attracts interest to fulfill the global demand on decreasing net emission of CO_2, as well as an oxygenate additive to gasoline by sustainable processes. The polymer sugars within lignocelluloses are decomposed to their monomers by either dilute-acid or enzymatic hydrolysis. The lignocellulosic hydrolyzate is then fermented to ethanol using *Saccharomyces cerevisiae* or other fermenting microorganisms. This lignocellulose-to-ethanol process is still considered as newly emerging technology but many scientific papers have been published on various aspects of it. The progress on this conversion technology is briefly reviewed here.

Keywords: ethanol, lignocellulose, chemical hydrolysis, enzymatic hydrolysis, *S. cerevisiae*

1. INTRODUCTION

Ethanol from renewable resources (referred to as bio-ethanol) attracted interest as (additive) fuel for vehicles originally as a consequence of the oil crisis during the seventies. Today, apart from the worries of fossil fuel depletion, the concern regarding the greenhouse effect caused by massive usage of petroleum based fuels makes bio-ethanol even more attractive.

Production and usage of bio-ethanol can help to reduce CO_2-buildup, since biomass utilization can be considered as a closed carbon cycle. According to this principle, the CO_2 from bio-ethanol combustion comes from CO_2 stored in the biomass as the raw materials of the fuel and will be re-absorbed by growing plants through photosynthesis reaction. Thus no net CO_2 will be emitted to the atmosphere.

One of growing interest in bio-ethanol production is producing ethanol from lignocellulosic wastes, which are abundant from various sources of biomass. Lignocellulosic materials can be supplied from a variety of resources at low price. They can be classified in four groups based on type of resource: wood, municipal solid waste, waste paper and crop residue resources (Wiselogel *et al.*, 1996).

Lignocellulose consists of cellulose, hemicellulose, and lignin, plus a small fraction of ash. The amounts of those three major groups of polymers depend on the type of material, but roughly range between 40-45% cellulose, 20-30% hemicellulose, and 20-32% lignin of the dry weight (Sjöström, 1993). The basic steps of producing ethanol from lignocellulosic materials include hydrolysis of the polymers into monomer sugars and fermentation of the sugars into ethanol by fermenting microorganisms. The ethanol is then recovered from the fermentation broth and concentrated by distillation. The hydrolysis and fermentation parts are briefly reviewed here.

2. HYDROLYSIS

Hydrolysis is required prior to fermentation since sugars in lignocellulose are tightly bound in partly crystalline structures and are not accessible to organisms that produce ethanol. Lignocellulose can be hydrolyzed chemically or enzymatically (*e.g.* Galbe and Zacchi, 2002). In chemical hydrolysis, acids (sulphuric, hydrochloric, hydrofluoric, phosphoric, nitric and formic acid) are used to convert cellulose and hemicellulose to monomer sugars. Of the acids, sulphuric acid is the most frequently used because it is cheap and effective. Since concentrated acid causes corrosion and acid-recovery problems, dilute-acid processes are often preferred, although the sugar yields are lower than the yields obtained by concentrated-acid processes. A consequence of the different structures of cellulose and hemicellulose is the need for the process to be carried out in two stages. In the first stage, hemicellulose is hydrolyzed under milder conditions (170-190°C), whereas in the second stage cellulose is hydrolyzed under harsher conditions (200-230°C) (Galbe and Zacchi, 2002).

An alternative to acid hydrolysis is enzymatic hydrolysis. It is applied to decompose cellulose by activity of cellulase enzymes. In enzymatic hydrolysis, lignocellulosic materials are usually pre-treated with *e.g.* dilute sulphuric acid to render the cellulose accessible to the enzymes. While the hemicellulose parts are to a large extent already decomposed during the pretreatment step, the cellulose parts mostly remain unchanged and are decomposed in the subsequent process by the enzymes (Galbe and Zacchi, 2002). The cellulase enzymes are a mixture of three classes of enzymes: exo-1,4-β-D-glucan cellobiohydrolases, endo-1,4-β-D-glucanases, and 1,4-β-D-glucosidases (Jorgensen *et al.*, 2003). Several microorganisms are known to produce these enzymes. Among them,

Trichoderma reesei is until now the most efficient cellulose-hydrolysing organism (*e.g.* Galbe and Zacchi, 2002).

The process involving enzymatic hydrolysis can be carried out as separate hydrolysis and fermentation (SHF) or as simultaneous saccharification and fermentation (SSF). However, glucose released during biomass saccharification strongly inhibits the enzymes, particularly the β-D-glucosidase component that catalyses the hydrolysis of cellobiose to glucose. In SSF, on the other hand, the glucose produced by the hydrolysis is continuously converted to ethanol by a fermenting microorganism. In this way, inhibition of glucose to the enzymes can be minimized (Alfani *et al.*, 2000).

In enzyme-based fermentation processes, the cost of enzymes accounts for a large part of the total cost of producing ethanol from biomass. As long as the enzymes are expensive, dilute-acid hydrolysis would be preferable to the enzymatic hydrolysis of deriving sugars from lignocellulosic materials (Qureshi and Manderson, 1995). However, a major disadvantage of dilute-acid hydrolysis is the poor fermentability of the hydrolyzates caused by the formation of undesirable by-products during the process. These by-products severely inhibit microbial growth and lower ethanol yields in the fermentation process (*e.g.* Larsson, 2000). Potential inhibitors are weak carboxylic acids (acetic acid, levulinic acid, formic acid), furan compounds (furfural, 5-hydroxymethyl furfural (HMF)), and phenolic compounds (vanillin, phenol, vanillic acid, formaldehyde, etc.).

3. FERMENTATION OF THE LIGNOCELLULOSIC HYDROLYZATES

3.1. Fermentation Methodology

Fermentation of lignocellulosic hydrolyzates is not as simple as the fermentation of *e.g.* molasses and starch. Hydrolyzate contains not only sugars but also some compounds that are toxic to the fermenting microorganism. Therefore, it needs a proper fermentation technique that is able to overcome the inhibitory effects during the process. In principle, fermentation of lignocellulosic hydrolyzates can be carried out in three modes of operation, *i.e.* batch, continuous, and fed-batch cultivation. These three fermentation methodology are discussed below.

Batch cultivation

Regarding fermentation of hydrolyzate, batch cultivation does not seem to be suitable, unless the inoculum size is high and the hydrolyzate is detoxified. At the initial stage, the medium has been provided by high concentrations of inhibitors from the hydrolyzate. The inhibitors are actually transformed to other compounds by the cells, provided that the inhibition is not too strong. It was shown that a combination of high concentration of inhibitors and low concentration of cells can inactivate the cells and thus prevent growth (Chung and Lee, 1985). Hence, the capability of *in situ* detoxification, in which bioconversion of inhibitors occurs, is diminished. This results in a long lag phase or even failure of the fermentation (Larsson *et al.*, 1999; Larsson *et al.*, 2000; Taherzadeh *et al.*, 1997). From an economic point of view, the phenomenon of *in situ* detoxification is advantageous because no detoxification process is needed, and this can reduce the overall cost of ethanol production.

Continuous cultivation

In a continuous cultivation, the substrate is continuously fed into the reactor at a certain dilution rate, defined as the feed rate divided by the working volume of the reactor. If everything is working properly, continuous cultivation can go on continuously, and the labor cost is thus lower compared to batch cultivation. A continuous process is relatively easily contaminated, resulting in failure of production in the long term (Nielsen *et al.*, 2003).

In order to achieve a high cell concentration in a continuous cultivation, leading to high productivity, retained cell systems such as immobilization, *e.g.* (Taherzadeh *et al.*, 2001), or cell recirculation, *e.g.* (Brandberg *et al.*, 2005), can be applied. In immobilized continuous cultivation, the bioreactors can be operated at high dilution rates without cell washout. Whereas in a traditional continuous cultivation, the dilution rate is limited by the growth rate of the cells, immobilized cells are retained within the bioreactors, and the dilution rates can be optimized for maximum productivity (Tuite, 1991). Accordingly, the fermentation time is minimized. Furthermore, a high dilution rate potentially reduces the risk of contamination. In a comparison between traditional continuous cultivation and immobilized-cell continuous cultivation, the immobilized culture was more successful (Taherzadeh *et al.*, 2001). When the yeast, *S. cerevisiae* CBS 8066, was immobilized, continuous fermentation of dilute-acid hydrolyzate could be carried out at high dilution rates without any washout of the cells.

Also without immobilization, continuous cultivation has an advantage over batch cultivation for fermentation of dilute-acid hydrolyzates. Unlike the situation in batch cultivation, the concentrations of the convertible inhibitors in the medium are kept low in a continuous cultivation. In view of this, a continuous cultivation gives higher potential for *in situ* detoxification over batch cultivation. However, if the hydrolyzate is inhibiting, the inhibition effects of inhibitors might impair the success of continuous fermentation. The inhibitors decrease the growth rate of cells, which might result in a washout of the fermentor, unless a very low dilution rate is applied, which is not economically attractive. Furthermore, if a low dilution rate is applied, the conversion rate of inhibitors decreases with the decreased growth of cells. This also results in washout of cells. However, in a continuous culture with immobilized cells, washout is prevented. Thus, having immobilized cells in a continuous cultivation can help not only to achieve high productivity but also to overcome the toxicity of the inhibitors. Immobilization demands more costs for the process, but the high productivity achieved by this method can probably decrease the extra cost of production.

Fed-batch cultivation

In fed-batch cultivation, cells are first grown in batch cultivation until the initial substrate is fully utilised. Immediately thereafter, fresh substrate is added and the fed-batch phase begins. During this period no product is withdrawn from the reactor, and the medium volume is successfully increasing. Considering for fermentation of acid hydrolyzates, fed-batch combines some advantages of batch and continuous cultivations; there is no washout of cells, and the concentrations of inhibitors in the medium are lower than the corresponding concentrations in the feed.

Fed-batch technique has been demonstrated as a good method to ferment dilute-acid hydrolyzate. The ethanol productivity is increased in fed-batch fermentation compared to batch fermentation, probably because of high concentrations of inhibitors that can be avoided in fed-batch fermentation (Nilsson *et al.*, 2001; Taherzadeh *et al.*, 2000). Hence, similarly to continuous fermentation, there is a capability of *in situ* detoxification. As the yeast has a limited capacity for the conversion of the inhibitors, the success of this technique strongly depends on the feed rate of hydrolyzate (Taherzadeh *et al.*, 1999). Since there is an inverse correlation between the feed rate and the inhibition effects,

control strategies of the feed rate were developed to optimise dilution rates for different qualities of hydrolyzates (Nilsson *et al.*, 2001; Taherzadeh *et al.*, 2000).

3.2. Microorganisms for Fermentation of Lignocellulosic Hydrolyzates

For fermentation of lignocellulosic hydrolyzates, baker's yeast, *Saccharomyces cerevisiae*, is widely used due to its essential and desirable properties, such as high ethanol yield and productivity, high ethanol tolerance, tolerance of process severity, tolerance of low pH, and GRAS (Generally Regarded As Safe) status (*e.g.* Zaldivar *et al.*, 2001). But *S. cerevisiae* does not have genes encoded for xylose reductase and xylitol dehydrogenase which are needed to be able to take up xylose. Therefore, the use of *S. cerevisiae* is only limited to hexose fermentation. Xylose fermentation is very important when the raw material contains quite significant amount of xylose, so that the ethanol production can be economically accepted. There have been some efforts to insert these two genes into *S. cerevisiae*, but the recombinant one is still not yet satisfactory due to its low ethanol yield and productivity.

The presence of inhibitors in the hydrolyzates, and the use of most sugars, call for a microorganism that is tolerant to inhibitors and able to utilize all sugars in the hydrolyzate, and can produce ethanol as the main product. A number of microorganisms to ferment hydrolyzates from different plant materials have been presented (Millati, 2005) and they are classified into three groups which are bacteria, yeasts, and filamentous fungi. Among the naturally occurring microorganisms from bacteria group, we can name *Escherichia coli* and *Zymomonas mobilis*. The examples from yeast group are *Candida shehatae, Pichia stipitis,* and *S. cerevisiae*. From filamentous group, *Rhizopus oryzae* and *Mucor indicus* have recently been explored for fermenting lignocellulosic hydrolyzate (Millati *et al.*, 2005). The strains investigated showed promising characteristics for ethanol production from lignocellulosic hydrolyzate and outperformed *S. cerevisiae* with regard to xylose utilization. Further comparative studies should be carried out to see whether *Rhizopus oryzae* and *Mucor indicus* are better microorganisms than other known ethanol producers for this purpose.

4. INTEGRATED PLANT DESIGN

While fuel ethanol already contributes to the need of the transportation sector, its use is expected to rise in the market in the future. It is to be stressed that production of bio-ethanol, despite its benefits from an environmental

perspective, is still more sophisticated and expensive than that of the other sources such as sugars or starch. One way to make the process more profitable may be to apply a new concept, called bio-refinery, where ethanol is connected to production of various co-products (Figure 1) (Kamm *et al.*, 2004).

The term green bio-refinery was defined in the year 1997 as follows: green bio-refineries represent complex (to fully integrated) systems of sustainable, environment- and resource-friendly technologies for the comprehensive (holistic) utilization and the exploitation of biological raw materials in the form of green and residue biomass from a targeted sustainable regional land utilization (Kamm *et al.* 1998).

The development of a bio-refinery from biomass is suggested to be "the key for the access to an integrated production of food, feed, chemicals, materials, goods, and fuels of the future". However, Research and development are necessary to: (1) increase the scientific understanding of biomass resources and improve the tailoring of those resources, (2) improve sustainable systems to develop, harvest and process biomass resources, (3) improve efficiency and performance in conversion and distribution processes and technologies for a host of products development biobased products and (4) create the regulatory and market environment necessary for increased development and use of bio-based products (Kamm *et al.*, 2004).

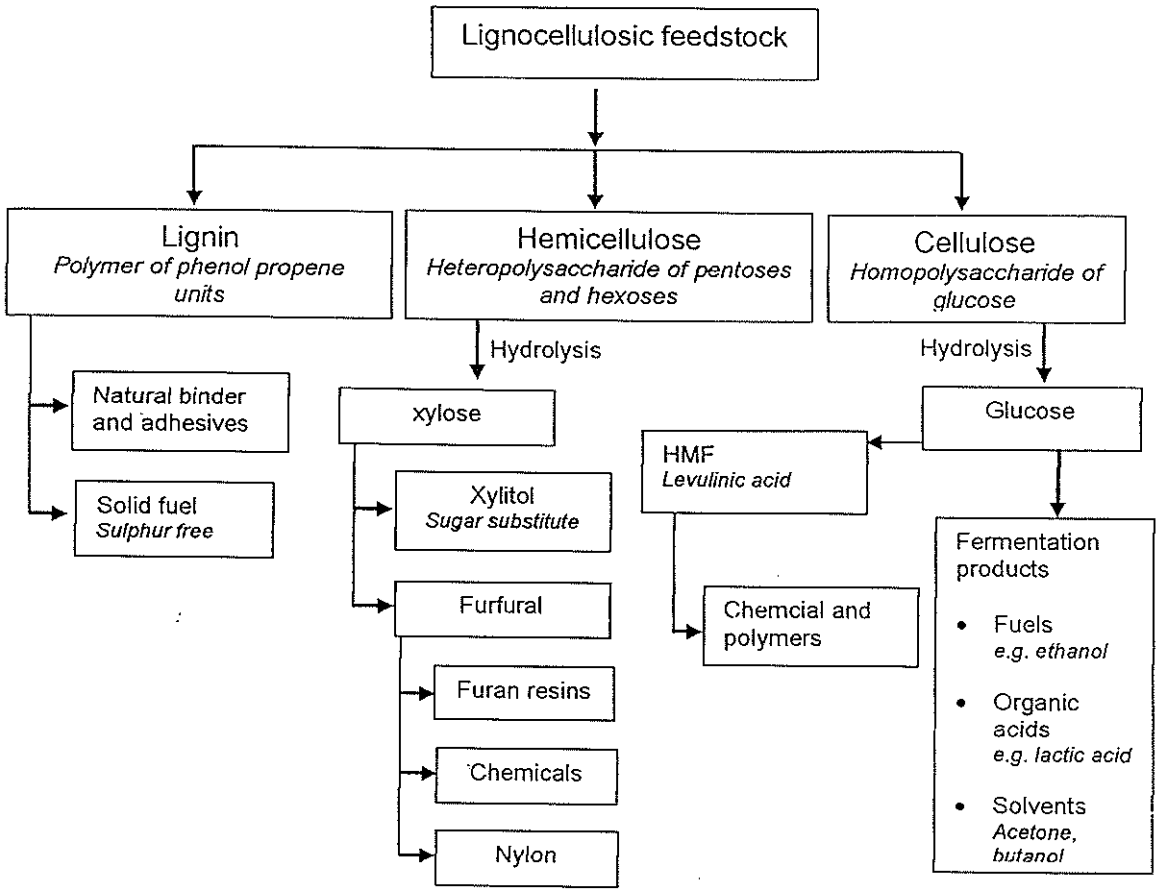

Figure 1. Lignocellulosic feedstock bio-refinery (adapted from Kamm *et al.*, 2004)

5. OUTLOOK

The process of converting lignocellulose to ethanol is still under development partly to reduce the cost of the process to be attractive to industrial interest. Thanks to the progress in the last two decades, bio-ethanol still appears to be able to contribute to future supply of fuels. Furthermore, all the countries worldwide can take the benefit from this progress and then to implement and localize of this growing technology to produce ethanol using the available raw materials from their own country.

REFERENCE

Alfani, F. , Gallifuoco, A. , Saporosi, A. , Spera, A. and Cantarella, M. (2000) Comparison of SHF and SSF processes for the bioconversion of steam-exploded wheat straw. *J Ind Microbiol Biotechnol* 25: 184-192.

Brandberg, T. , Sanandaji, N. , Franzein, C.J. and Gustafsson, L. (2005) Continuous fermentation of undetoxified lignocellulose hydrolyzate with *Saccharomyces cerevisiae* using cell recirculation. *Submitted.*

Chung, I.S. and Lee, Y.Y. (1985) Ethanol fermentation of crude acid hydrolyzate of cellulose using high-level yeast inocula. *Biotechnol Bioeng* 27: 308-315

Galbe, M. and Zacchi, G. (2002) A review of the production of ethanol from softwood. *Appl Microbiol Biotechnol* 59: 618-628.

Kamm, B. and M. Kamm (2004) Principles of biorefineries. Appl Microbiol Biotechnol 64: 137-145.

Kamm B, Kamm M, and Soyez K (1998) The green biorefinery, concept of technology. (First international symposium on green biorefinery) Neuruppin, Society of Ecological Technology and System Analysis, Berlin.

Larsson, S. (2000). Ethanol from lignocellulose- fermentation inhibitors, detoxification and genetic engineering of *Saccharomyces cerevisiae* for enhanced resistance. Lund University of Technology. *PhD thesis.*

Larsson, S. , Palmqvist, E. , Hahn-Hägerdal, B. , Tengborg, C. , Stenberg, K. , Zacchi, G. and Nilvebrant, N. (1999) The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. *Enzyme Microb Technol* 24: 151-159.

Millati, R. Edebo, L. and Taherzadeh, M. J.. (2005) Performance of *Rhizopus, Rhizomucor* and *Mucor* in Ethanol Production from Glucose, Xylose and Wood Hydrolyzates. *Enzyme Microb Technol* 36 (2-3): 294-300.

Millati, R. (2005) Ethanol production from lignocellulosic Materials: Potential of continuous cultivation, immobilization, and zygomycetous fungi. Chalmers University of Technology. *Ph.D Thesis.*

Nielsen, J. , Villadsen, J. and Lidén, G. (2003). *Bioreaction Engineering Principles*, 2 edn. Kluwer Academic/Plenum Publisher, New York.

Nilsson, A. , Taherzadeh, M.J. and Lidén, G. (2001) Use of dynamic step response for control of fed-batch conversion of lignocellulosic hydrolyzates to ethanol. *J Biotechnol* 89: 41-53.

Qureshi, N. and Manderson, G. (1995) Bioconversion of renewable resources into ethanol: an economic evaluation of selected hydrolysis, fermentation, and membrane technologies. *Energy Sources, vol: 17: 241-265* 17: 241-265

Sjöström, E. (1993). *Wood chemistry: fundamentals and applications*, 2nd edn. Academic Press, San Diego.

Taherzadeh, M. J., Millati R., Niklasson C. (2001) Continuous cultivation of dilute-acid hydrolyzates to ethanol by immobilized *Saccharomyces cerevisiae*, *Appl. Biochem. Biotechnol.*, 95(1): 45-57.

Taherzadeh, M.J. , Niklasson, C. and Lidén, G. (2000) On-line control of fed-batch fermentation of dilute-acid hydrolyzates. *Biotech. Bioeng.* 69: 330-338.

Taherzadeh, M.J. , Niklasson, C. and Lidén, G. (1999) Conversion of dilute-acid hydrolyzates of spruce and birch to ethanol by fed-batch fermentation. *Bioresour Technol* 69: 59-66.

Taherzadeh, M.J. , Eklund, R. , Gustafsson, L. , Niklasson, C. and Lidén, G. (1997) Characterization and fermentation of dilute-acid hydrolyzates from wood. *Ind Eng Chem Res* 36: 4659-4665.

Tuite, M.F., Oliver, S.G. (1991). *Biotechnology Handbook 4. Saccharomyces.* Plenum Press, New York.

Turton, R. , Bailie, R.C. , Whiting, W.B. and Shaeiwitz, J.A. (2003). *Analysis, Synthesis, and Design of Chemical Processes*, 2 edn. Prentice Hall PTR, Upper Saddler River, New Jersey 07458.

Wiselogel, A. , Tyson, S. and Johnson, D. (1996). Biomass feedstock resources and composition. In: Wyman, C. E. (ed), *Handbook on bioethanol: production and utilization.* pp. 105-118 Taylor & Francis, Washington DC.

Zaldivar, J. , Nielsen, J. and Olsson, L. (2001) Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration. *Appl Microbiol Biotechnol* 56: 17-34.