Available online at www.sciencedirect.com

SciVerse ScienceDirect

http://www.elsevier.com/locate/biombioe

Lignocellulosic ethanol: From science to industry

L. Viikari [a], J. Vehmaanperä [b], A. Koivula [c,*]

[a] University of Helsinki, Department of Applied Chemistry and Microbiology, P.O. Box 27, FI-00014 Helsinki, Finland
[b] ROAL Oy, P.O. Box 57, FI-05201 Rajamäki, Finland
[c] VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 VTT, Espoo, Finland

ARTICLE INFO

Article history:
Received 12 December 2011
Received in revised form
9 May 2012
Accepted 14 May 2012
Available online 6 June 2012

Keywords:
Ethanol
Lignocelluloses
Trichoderma reesei
Enzymes
Cellulases
Hydrolysis

ABSTRACT

Lignocellulosic raw materials, not competing with food production, can provide environmental, economic, and strategic benefits for the production of biofuels. The cost of biomass-based biotechnical ethanol production has been recently reduced significantly, mainly due to advances in the conversion techniques; *i.e.* by improved enzymes and new yeast strains. Conversion of the cellulosic components into fermentable sugars is, however, still the major technological and economical bottleneck in the production of fuels or other high-volume commodity products from cellulosic biomass. Especially, the enzymatic hydrolysis still forms a major cost factor. The targets for reducing the costs of biotechnical conversion processes of lignocelluloses to ethanol can be divided into three categories: the costs of enzymes, the costs of produced sugars and the costs of ethanol production. The efficiencies of individual enzymes can be improved by designing enzymes with optimal domain structures and binding properties, and with higher specific activity, lower end-product inhibition and higher thermal stability, as well as by optimizing the production processes. The cost of the enzymatic hydrolysis is dependent on the efficiency, yield and costs of the pretreatment, synergistic action of cellulases and accessory enzymes, as well as on the needed amount of externally added enzymes. The costs of ethanol production are further affected by the yield, concentration and production rate of ethanol. This work reviews the major bottlenecks in the conversion process, as well as highlights recent approaches to overcome these problems.

1. Introduction

Among potential alternative bioenergy resources, lignocellulosics have been identified as the prime source of biofuels and other value-added products. Lignocelluloses from agricultural, industrial and forest sources account for the majority of the total biomass present in the world and compose a vast renewable resource. Lignocellulosic raw materials, not competing with food production, can provide environmental, economic, and strategic benefits for the production of fuels. Ambitious goals have been set for biofuels in most parts of the world, including those of the U.S. Department of Energy Office with a scenario for supplying 30% of the gasoline demand with biofuels by the year 2030, and the European Union with a vision of 10% of the EU's transportation fuels deriving from biofuels by 2020. Today, the main aim is not only to replace fossil fuels by any biofuel but to review the greenhouse gas emission saving thresholds. Thus, the political timetables concerning the use of lignocellulosic raw materials for production of ethanol and other biofuels bring challenges to

* *Corresponding author*. Tel.: +358 40 720 7158.
E-mail address: Anu.Koivula@vtt.fi (A. Koivula).
0961-9534/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biombioe.2012.05.008

the development of novel science-based technologies. To speed up the implementation of second generation biofuels in Europe, the contribution of biofuels produced from wastes, residues, non-food cellulosic and lignocellulosic materials will be considered as counting twice in national obligations [1].

During the last decades, the cost of biomass-based biotechnical ethanol production has been reduced dramatically, mainly due to advances in enzymatic hydrolysis techniques. Cellulolytic enzymes have provided a key opportunity to reduce costs and improve the conversion of biomass, thanks to the modern tools of biotechnology. Bioconversion of the cellulosic components into fermentable sugars is, however, still the major technological and economical bottleneck in the production of fuels or other high-volume commodity products from cellulosic biomass.

The goal of this paper is to give a review on the developments of lignocellulosic hydrolysis, including the main bottlenecks and open issues, as well as recent achievements in enzyme hydrolysis and various process concepts.

2. History of enzymatic biomass conversion technologies

The study of plant cell wall degradation, especially of cellulose hydrolysis by the aerobic fungus *Trichoderma reesei* (an asexual, clonal derivative of *Hypocrea jecorina*) and other ascomycetes, began in the early 1950s. The cellulase complex was first described to contain the C_1 decrystallizing enzyme (exo-β-1,4-glucanases or cellobiohydrolases, CBH) yielding cellobiose, followed by the C_x-cellulases (endo-β-1,4-glucanases, EG) which break down cellulose to glucose. Since then, the concept of cellulose-degrading enzymes has been complemented and the field of cellulase research has developed significantly, enabled by advances in molecular biology, protein engineering, structural biology and computational methods. Obviously, the fungus *Trichoderma reesei* is still the most extensively studied cellulase producing organism.

The energy crisis in 1973 first time seriously raised the question of the availability and cost of fossil fuels and stimulated research on the development of biotechnical conversion processes, as reviewed by Reese [2]. These initial attempts did, however, not lead to commercialization of cellulose hydrolysis processes in spite of extensive research and early piloting experiments. It became clear that the cost of cellulases was the major obstacle for commercial-scale hydrolysis processes. The thorough research driven by the energy crisis resulted, however, in significant achievements in the basic mechanisms of cellulose and hemicellulose hydrolysis. Instead of a total hydrolysis of lignocellulosic substrates, the scope was moved to a partial hydrolysis, *i.e.* modification of fibre-based carbohydrates, leading to applications for various industrial sectors, especially for textile, feed, detergent and pulp and paper sectors (Fig. 1). While cellulases and hemicellulases are today widely used in several industries, the major future application of these biocatalysts is, however, again expected to be in the conversion of plant biomass into sugars for further fermentation to ethanol and other energy carriers as well as to useful platform chemicals.

3. 2G ethanol bottlenecks: why is lignocellulose conversion so difficult?

The recalcitrance of lignocellulosic raw materials is due to the resistant characteristics of the terrestrial plant cell wall, evolved to form a barrier against external intrusion and degradation. The structural integrity of cellulose is believed to play the most important role in the recalcitrance of lignocellulosic biomass. The strong intra- and intermolecular hydrogen bonds make crystalline cellulose (cellulose I) resistant to enzymatic hydrolysis, whereas hemicellulose and amorphous cellulose are more readily digestible. The enzyme accessibility is known to be affected by the crystallinity but, on the other, discrepant results have been published on changes of the crystallinity during the hydrolysis [3,4] Many studies indicate that only minor changes in crystallinity take place during the hydrolysis; first a decrease followed by an increase. Enzymatic cellulose hydrolysis is a complex process, and the crystallinity index alone may not adequately explain differences in observed hydrolysis rates. On the other hand, taking into account the tightly packed microfibrillar structure, it can be expected that hydrolysis takes place at the outermost fibrils, leaving the bulk of the cellulose unhydrolysed, which would explain the measured minor changes in the crystallinity index.

The highly organised cell wall structures in plants also contribute to biomass recalcitrance [5]. Crystalline cellulose is coated by hemicelluloses in the microfibrils, limiting the access of cellulases, and explaining the reasons for improvement of cellulose hydrolysis by the removal of hemicellulose [6]. The structure, composition and location of hemicelluloses vary among different plant genera and cell types. The function of hemicelluloses is to maintain flexibility in the cell wall by avoiding cellulose fibrils to adhere to each other and to cross-link the cell wall components together [7].

Furthermore, the polysaccharides in the secondary cell wall are embedded in lignin. Lignin is reported to be covalently bound to some of the carbohydrates and, depending on the pretreatment, cover some of the carbohydrates, thus hindering the enzymatic accessibility. In addition, lignin and its phenolic degradation products have been observed to inhibit the hydrolysis in various ways [8,9]. The secondary cell wall in lignocellulosic materials can thus be described as a composite matrix, further complicated by the chemical and physical modifications taking place in various pretreatment methods. The complex heterogeneous nature of biomass creates mass-transport limitations especially for the high molecular weight biocatalysts.

In order to increase the accessibility and porosity of lignocelluloses, pretreatment is needed. The major pretreatment techniques can be differentiated on the basis of modifications produced in the structures and location of lignin and hemicelluloses. Dilute acid and steam explosion or autohydrolytic hydrothermal methods hydrolyse and partially solubilise hemicelluloses whereas the alkaline methods attack either lignin alone or also partially hemicelluloses. Both approaches have been proven effective in increasing the hydrolysis of cellulose [10–12]. The removal of hemicelluloses seems to be relatively more important to the improvement of