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## Chapter 12 Production of ethanol from lignocellulosic biomass

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**Abstract** Ethanol fuel is leading the transition towards a post-petrol era in the transport sector worldwide. Ethanol is produced via sugar fermentation processes by yeasts or bacteria. Although the current industrial production of ethanol mainly involves the use of starch- and sugar-based feedstocks, lignocellulosic biomass is expected to play a key role as renewable, carbohydrate-rich raw material. With the aim of placing lignocellulosic ethanol into the market, the scientific community has made great efforts to develop and implement efficient conversion technologies. Prior to fermentation, lignocellulosic biomass must be pretreated and hydrolysed to obtain the fermentable sugars. Biomass processing is, however, a major limiting step since it is hindered by the native structure of lignocellulose and generates different biomass-derived compounds that are inhibitors of the subsequent microbial conversion. In this context, different pretreatment, delignification and detoxification methods have been investigated to produce less inhibitory pretreated materials. Furthermore, several strategies such as working at high gravity conditions, high temperatures and/or different process configurations, have been shown to maximize ethanol production from lignocellulosic materials. The development of robust microbial strains tolerant to inhibitory compounds and capable of converting sugar mixtures is also needed for cost-effectiveness of the process. This chapter compiles recent advances in lignocellulosic ethanol production processes, from novel raw materials or fermenting microorganisms to new processing technologies addressed to commercialization.

**Keywords** Pretreatment; Lignocellulosic ethanol; Enzymatic hydrolysis; Detoxification; Delignification; Sugar fermentation; Process integration; Microbial robustness.

## 12.1 Introduction

The implementation of a sustainable bio-based economy is considered a priority in today's society. To reach such a goal, lignocellulosic biomass –the major renewable organic matter in nature– has been recognized as a valuable raw material for the production of biofuels and several chemical building blocks within the biorefinery concept. Among lignocellulosic biofuels, bioalcohols are very attractive and promising alternatives for the transport sector, as they can share current fuel distribution systems and are easily stored and handled, in comparison to biogas and biodiesel [1].

With a long history, ethanol is the most widespread alcohol fuel. It has a low boiling point (78 °C), a high research octane number (RON; 107) and its energy content is comparable to that of gasoline (two thirds of the gasoline energy content) [1, 2]. Direct use of ethanol as fuel is possible in neat form (100% pure) or in blends with gasoline (e.g. E85: 85% ethanol and 15% gasoline). Ethanol can be converted to ethyl tert-butyl ether (ETBE) and is used as fuel additive.

Traditionally, bioethanol has been produced from sugar- and starch-based feedstocks such as sugarcane juice and molasses, and corn. Since January 2013, bioethanol also started to be produced from lignocellulosic feedstocks at commercial scale [3]. However, current prices for lignocellulosic ethanol are 0.57-1.20 USD/L, while conventional ethanol cost about 0.40-0.45 USD/L [4, 5]. To ensure a competitive lignocellulosic industry, some challenges both in biomass processing (such as having a good balance between biomass hydrolysability and biomass degradation) and microbial conversion (including the increase of the tolerance of fermenting microorganism to lignocellulose-derived compounds and the conversion of all lignocellulosic sugars into ethanol with high rates and yields) processes still need to be addressed. The present chapter reviews the current advances for a cost-effective lignocellulosic bioethanol production, from the use of novel raw materials and the development of new pretreatment technologies, to the investigation and engineering of fermentative microorganisms.

### *12.1.1 Lignocellulosic bioethanol: A process overview*

Ethanol production is based on sugar fermentation processes. With about 75% carbohydrate content on dry weight basis, lignocellulose represents an interesting feedstock for ethanol production [6]. However, in contrast to conventional bioethanol production technology, lignocellulosic ethanol production is very challenging due to the highly recalcitrant structure of lignocellulose. Lignocellulosic biomass is composed of three polymers: cellulose, hemicelluloses and lignin. The structural polymer, cellulose, is bonded with both hemicelluloses and lignin, forming a difficult to disrupt complex matrix.

Lignocellulosic ethanol production consists of pretreatment, enzymatic hy-

hydrolysis and fermentation steps. Pretreatment is needed to alter the structural characteristics of lignocellulose and increase the accessibility of cellulose and hemicelluloses to hydrolytic enzymes that are responsible for the hydrolysis of polysaccharides into fermentable sugars. Pretreatment is an important process since it has a great impact on final conversion yields and contributes to 30-40% to the overall process costs [7]. As it is further discussed in section 12.3, there is no best pretreatment technology although dilute-acid pretreatment, steam explosion or certain ammonia-based technologies are effective methods that can be applied to a wide range of lignocellulosic feedstocks [8-10]. In pretreatment, high temperatures and pressures and/or the addition of solvents and chemical catalysts are required, which leads to biomass degradation, generating different enzymatic and microbial inhibitors that limit the subsequent saccharification and fermentation steps [11, 12]. Different physical, chemical, and biological detoxification methods have been evaluated with the aim of decreasing the inhibitory power of pretreated materials. Another important limiting factor is the residual lignin, which can unspecifically bind hydrolytic enzymes, decreasing saccharification yields. In a similar way to detoxification methods, different biological and chemical delignification processes have been also studied as complementary steps to enhance saccharification yields.

Considering the steps required for lignocellulosic ethanol production, different process configurations can be proposed as depicted in Figure 12.1. There are three main process configurations, which are explained in detail in section 12.4: separate hydrolysis and fermentation (SHF), simultaneous saccharification and (co)fermentation (SSF/SSCF) and consolidating bioprocessing (CBP) [13]. In these processes, different yeast, bacterial or fungi strains have been used for ethanol production as discussed in section 12.5. However, microbial conversion processes are highly dependent on the composition of pretreated materials. The use and development of more robust fermentative microorganisms is therefore of the utmost importance and represents an interesting alternative to the aforementioned detoxification methods. Several metabolic and evolutionary engineering strategies have been used to obtain fermentative strains with increased capacity to convert and/or tolerate higher concentrations of inhibitory compounds [14-16]. The ability of tolerating high temperatures and osmotic pressures, and converting the full range of sugars present in lignocellulosic feedstocks are important traits to take into consideration [17].