Chapter 14
Microbial Cellulase in the Production of Second Generation Biofuels:
State-of-the-Art and Beyond

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ABSTRACT

Bioethanol from inedible cellulose-rich biomass is the most promising candidate to replace fast depleting, environmentally polluting fossil fuels. Hydrolysis of cellulose to glucose is the crucial step in its biotransformation to bioethanol. Enzymatic hydrolysis is favored over acid hydrolysis, as enzymes are eco-friendly biocatalysts with high substrate specificity and superior catalytic efficiency in mild reaction conditions. Complete hydrolysis of cellulose is achieved by cellulase. Higher cellulase production yield, stability, and catalytic efficiency are the main attentive points for the successful implementation in industrial production of bioethanol. This chapter will highlight general characteristics of microbial cellulases and their role in the bioconversion of cellulose to biofuels, economic sustainability of cellulose-based biofuels, and the latest innovations in cellulase immobilization as the most comprehensive strategy for improvement of enzyme stability, activity, and reusability for cost-effective large-scale application.

INTRODUCTION

In 2016 the use of bioethanol-gasoline blends in transportation reduced the emission of carbon dioxide by 43.5 million tons, the equivalent of removing 9.3 million cars from the road for the entire year (Robak & Balcerek, 2018). Lignocellulose biomass is the most abundant and renewable source of carbohydrates in the biosphere. It is obtained from inedible energy crops, agricultural and industrial waste (Kricka et al., 2015; Verma & Kumar, 2021). Bioethanol from lignocellulose biomass is a second-generation biofuel, whose production does not compete against food supplies, in contrast to the first-generation bioethanol,

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produced from edible seed-derived starch. Accordingly, second-generation bioethanol is the most promising candidate to replace conventional gasoline in the near future (Hoffman et al., 2021; Horn et al., 2012). Enzymes are green biocatalysts with remarkable substrate specificity and catalytic efficiency in mild reaction conditions. They can be genetically or chemically modified to meet the specific requests of the biotechnological process in which they are employed (Ejaz et al., 2021; Trbojević et al., 2013). Enzymatic hydrolysis of cellulose to glucose is the critical step in production of the second-generation bioethanol. Complete hydrolysis of cellulose is achieved by concerted activity of endoglucanase, exoglucanase (cellobiohydrolase) and β-glucosidase, which form a multi-enzyme cellulase complex. Mesophilic and thermophilic bacteria and fungi are the major industrial cellulase producers (Acharya & Chaudhary, 2012; Bhardwaj et al., 2021; Contreras et al., 2020; Horn et al., 2012; Menendez et al., 2015; Srivastava et al., 2018; Verma & Kumar, 2021). Commercial cellulase cocktails have contributed to the significant cost-reduction of cellulose bioconversion to bioethanol (Bhardwaj et al., 2021; Contreras et al., 2020; Lamichhane et al., 2021). However, further decrease in cellulase price is necessary by improving cellulase production yield, stability, specific activity and catalytic efficiency for economically viable bioethanol production.

Immobilization is the strategy of choice for improving catalytic performance of industrial enzymes, since it encompasses significant biocatalyst stabilization and reusability. Enzyme reusability significantly reduces the cost of the biocatalyst application at industrial scale and can be achieved exclusively by immobilization (Milosavic et al., 2016; Trbojević et al., 2013). With myriad of possibilities regarding immobilization methods and the choice of immobilization support, cellulases can be tailored for cost-effective and optimum catalytic performance. This chapter is a comprehensive overview of current state-of-the-art in the industrial application of cellulases in biofuel production. It will provide detailed fundamental understanding of microbial cellulases and their critical role in bioconversion of cellulose to bioethanol. Furthermore, the techno-economical aspect of the cellulose-derived biofuels will be discussed, with additional analysis of COVID-19 impact. The separate section of the chapter will be dedicated to cellulase immobilization and its effect on enzyme stability, activity and reusability. It will specifically deal with nanomaterials as innovative immobilization carriers, having in mind their increasing popularity in this area of research and application.

BACKGROUND

Lignocellulose biomass is a complex mixture of carbohydrate and non-carbohydrate compounds. Cellulose is the dominant component (40-60% of the total biomass weight), followed by hemicellulose (20-40%) and lignin (20–30%). It also contains proteins, lipids, pectin, minerals and soluble sugars (Adegboye et al., 2021; Horn et al., 2012; Kricka et al., 2015). Structure of major constituents of lignocellulose biomass is represented in Figure 1.

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