



Combined pretreatment using ozonolysis and wet-disk milling to improve enzymatic saccharification of Japanese cedar

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HIGHLIGHTS

- ▶ Japanese cedar was subjected to ozonolysis and subsequent wet-disk milling (DM).
- ▶ Moisture content affected ozone consumption and delignification.
- ▶ Ozone treatment removed mainly lignin, but also a small amount of polysaccharide.
- ▶ Ozonolysis enhanced the ability of DM, resulting in increased sugar yield.

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ABSTRACT

Ozonolysis and subsequent wet-disk milling (DM) were carried out on Japanese cedar (*Cryptomeria japonica*) to improve sugar production by enzymatic saccharification. When the moisture content reached more than 40%, ozone consumption decreased, resulting in less delignification. Ozone treatment removed mainly lignin, but also small amounts of polysaccharides. The application of DM following the ozone treatment further increased glucose and xylose yields, but had no significant effect on mannose yield, due to the loss of mannan in the ozone-treated product and the lack of mannose-releasing activity in the hemicellulase used. Sugar concentration increased with substrate concentration, when a constant ratio of enzyme to substrate was used.

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1. Introduction

The use of lignocellulose to produce ethanol is advantageous because it is abundant and does not rely on food crops to be produced (Alvira et al., 2010). The production process first requires that the cellulose and hemicellulose content of the material is converted into monosaccharides (saccharification), which is often achieved using enzymes (Kumar et al., 2009). Since it is difficult for cellulolytic and hemicellulolytic enzymes to degrade intact lignocellulose, the material needs to be pretreated to increase the amount of sugar produced, and thus obtain a high ethanol yield (Georgieva et al., 2008).

Mechanical pretreatment can be used to increase the sugar yield from lignocellulose via enzymatic saccharification (Agbor et al., 2011). Mechanical treatment which refers to grinding has

the advantage of being environmentally friendly, as it does not depend on chemicals such as acids or alkalis. However, it is also an energy-intensive process (Hendriks and Zeeman, 2009), and so needs to be used in combination with other treatment methods to save energy and reduce costs. It has been reported that hydrothermal treatment improves the ability of mechanical grinding pretreatments such as wet-disk milling (DM) to produce sugar (Hiden et al., 2009, 2012; Lee et al., 2010; Miura et al., 2012; Silva et al., 2010). For example, Lee et al. (2010) reported that hydrothermal treatment of eucalyptus loosened the supramolecular structure of the cell wall by partially removing hemicellulose and lignin, creating nanospaces between the cellulose microfibrils; this facilitated fibrillation by DM, which, in turn, increased the surface area of the product, improving enzymatic saccharification. Similarly, Miura et al. (2012) reported that dilute alkali-catalyzed hydrothermal treatment of sugarcane bagasse allowed the degradation of the hemicellulose and lignin to be regulated by controlling the pH, allowing a high sugar yield to be obtained from the disk-milled product at a high substrate concentration.

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