Continuous culture for the production of ethanol from wood hydrolysate was carried out in an internal membrane-filtration bioreactor. The hydrolysate medium was sterilized at a relatively low temperature of 60°C with the intention of reducing the formation of inhibitory compounds during the sterilization. The maximum ethanol concentration and productivity obtained in this study were 76.9 g/L and 16.9 g/L-h, respectively, which were much higher than those (57.2–67 g/L and 0.3–1.0 g/L-h) obtained in batch cultures using hydrolysate media sterilized at 60°C. The productivity was also found to be much higher than that (6.7 g/L-h) obtained in a continuous cell retention culture using a wood hydrolysate sterilized at 121°C. These results show that the internal membrane-filtration bioreactor in combination with low-temperature sterilization could be very effective for ethanol production from wood hydrolysate.

Introduction

Cellulosic material, such as wood and municipal solid wastes, have been targeted as a potential substrate for fuel ethanol production because of its low cost (Lynd et al., 1991; Ballerini et al., 1994; Olsson and Hahn-Hägerdal, 1996; von Sivers and Zacchi, 1996). Considering ethanol separation cost, it is necessary to have a high ethanol concentration in the fermentation broth. However, a high ethanol concentration in the fermentor is usually limited by the presence in wood hydrolysate of various inhibitory compounds such as p-hydroxybenzoic aldehyde, vanillin, and furfural. A number of studies were carried out to overcome the toxicity of the hydrolysates using high cell density (Chung and Lee, 1985) and detoxification processes (Parajo et al., 1996; Rivard et al., 1996; Palmqvist et al., 1997), but no significant progress has been made yet. We have proposed an effective method to obtain a high ethanol concentration above 70 g/L using low-temperature sterilization (Lee et al., 1999). However, a long fermentation time was required.

Therefore, it is also necessary to reduce fermentation time and thus to improve the productivity, because achieving a high productivity plays a crucial role in the economics of fuel ethanol. Cell recycle using an external membrane module has been very popular in obtaining a high productivity (Lee and Chang, 1987; Chang et al., 1994). However, this process has limitations that need to be overcome prior to its industrial applications: industrial substrates contain various types of particles that make pumping through an external membrane module difficult; oxygen supply and carbon dioxide removal may not be properly done while the broth is in the recycle loop; sterilization of the external membrane device is difficult; and recirculation of the broth requires pumps and additional energy. To overcome these problems, an internal membrane-filtration bioreactor system that allowed microbial separation to be carried out inside the fermentor was developed (Chang et al., 1993). This reactor system has been successfully employed for ethanol production from glucose and starch hydrolysate (Lee et al., 1994; Park et al., 1999).

In this study, we attempted to obtain a high ethanol concentration and productivity from highly concentrated wood hydrolysates by sterilizing the medium at a low temperature and by using an internal membrane-filtration bioreactor.

Materials and Methods

Microorganism. The yeast strain used in this study was an industrial Saccharomyces cerevisiae kindly provided by SeoYoung Ethanol Ind. Korea. This strain was maintained on agar slants containing 0.3% yeast extract, 0.3% malt extract, 0.5% bacto peptone, 2% glucose, and 2% agar at 4°C.

Preparation of Wood Hydrolysate. Chips of oak wood of size 2 mm × 4 mm were made by a chipper designed by the Biomass Research Team at Korea Institute of Energy Research. The composition of oak wood is as follows: cellulose, 49.3%; hemicellulose, 25.9%; Klasson lignin, 21.7%. Steam explosion of the oak wood chips was conducted for 3 min at 215°C in an 8-L exploder designed by the Biomass Research team. The yields of sugars after steam explosion (g after treatment/g before treatment) were as follows: glucan, 0.88; xyllose–maltose–galactose (XMG), 0.2. However, the concentration of individual sugars (xyllose, maltose, galactose) was not determined. Five kilograms (dry weight) of residue

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after the explosion and washing was enzymatically hydrolyzed with Celluclast (Novo Co., Denmark) and Novozym (Novo Co., Denmark) in a reactor with a 30-L working volume (Korea Fermentor Co., Korea) for 3 days at 50 °C. Cellulose contained endoglucanase and cellobiohydrolase activities and had 78 filter paper units (FPU)/mL of cellulase activity. Novozym contained 800 IU/mL β-glucosidase activity. The enzyme loading was 20 FPU and 30 IU/g-residue for filter paper and β-glucosidase activities, respectively. Cellulase activities were measured according to the method suggested by IUPAC (Ghose, 1987). The hydrolysate was centrifuged, and the supernatant contained about 40 g/L of glucose. The concentration of reducing sugars including glucose was measured according to the method suggested by IUPAC (Lee et al., 1999). The hydrolysate medium was sterilized for 120 min at 121 °C to reduce the formation of toxic materials during sterilization (Lee et al., 1999). Fresh hydrolysate was obtained.

### Inoculum Preparation

The growth medium consisted of 0.3% yeast extract, 0.3% malt extract, 0.5% bacto peptone, and 10% glucose. Inoculum was prepared by growing cells aerobically in a 500-mL flask containing 100 mL of the growth medium in a rotary shaker incubator for 24 h at 30 °C. Inoculation volume was 10% (v/v) of the fermentor working volume.

### Experimental Setup

The filter module used in this study consisted of 13 vertical cylindrical ceramic tubes with an inner diameter, an outer diameter, and a height of 8, 11, and 80 mm, respectively. More details of the filter module are given elsewhere (Park et al., 1997). The filter module was installed inside a 3-L jar fermentor with a working volume of 1.5 L (Korea Fermentor Co., Korea). The fermentor and filter module were sterilized assembled at 121 °C for 40 min.

### Continuous Culture

Initially, the culture was carried out batchwise using the growth medium until the substrate was depleted and was then switched to a continuous mode with the feeding of wood hydrolysate. The hydrolysate medium was sterilized for 120 min at 60 °C to reduce the formation of toxic materials during the sterilization (Lee et al., 1999). Fresh hydrolysate medium was continuously supplied to the fermentor by a peristaltic pump, and at the same time cell-free liquid was removed from the bioreactor through the filter module. The cake layer accumulated on the filter membrane surface was periodically removed by backflushing with filtrate. As a whole, the volume of broth in the fermentor was controlled at 1.5 L by making the total filtrate flow rate counterbalance the total feed flow rate. Fermentation was carried out at 30 °C, and the pH was not controlled. To maintain good cell viability, filter-sterilized air was supplied to the fermentor at the rate of 0.75 L/min.

### Analytical Methods

Ethanol concentration was measured by a gas chromatograph with a flame ionization detector (Young Lin, M600D, Korea) using 2-butanol as the internal standard. The packing material was Chromosorb W coated with Carbowax 20M. Glucose was analyzed by spectrophotometer (Beckman DU-65, U.S.) at 550 nm using the glucose oxidase/peroxidase (GOD/POD) enzyme method (Glucose E-kit, Yeoungdong Pharm. Inc., Korea). Cell number was counted by a hemocytometer (American Opticals Inc., U.S.).

### Results and Discussion

The culture was started in a batch mode as mentioned earlier (Figure 1). At the end of the batch culture when glucose in the growth medium was depleted, continuous operation was started with the feeding of a wood hydrolysate containing 137 g/L glucose at a dilution rate of 0.22 h⁻¹. In the beginning of the continuous fermentation, the glucose concentration increased to 25 g/L as a result of the low concentration of yeast cells. The glucose concentration gradually decreased and became nil in about 10 h. The cell concentration increased up to about $1.4 \times 10^6$ cells/mL, and the concentration of ethanol was 58.8 g/L at 37 h when the feed glucose concentration was changed to 180 g/L for a higher ethanol concentration and productivity. The maximum concentrations of cells and ethanol obtained in this condition were around $1.5 \times 10^6$ cells/mL and 76.9 g/L, respectively. The residual glucose concentration was almost zero. The ethanol yield was 0.43 g-ethanol/g-glucose, which was comparable to those in other fermentation systems (Table 1). The

### Table 1. Comparison of Ethanol Concentrations and Productivities of Different Fermentation Systems Using Wood Hydrolysate

<table>
<thead>
<tr>
<th>Batch (Lee et al., 1999)</th>
<th>Chemostat (Lee et al., 1996)</th>
<th>Chung and Lee, 1985</th>
<th>Lee et al., 1996</th>
<th>this work</th>
</tr>
</thead>
<tbody>
<tr>
<td>initial (feeding) glucose concn (g/L)</td>
<td>80</td>
<td>140</td>
<td>170</td>
<td>65</td>
</tr>
<tr>
<td>residual glucose concn (g/L)</td>
<td>0</td>
<td>0</td>
<td>34.7</td>
<td>13.5</td>
</tr>
<tr>
<td>ethanol concn (g/L)</td>
<td>36.8</td>
<td>67</td>
<td>57.2</td>
<td>21.1</td>
</tr>
<tr>
<td>ethanol yield (g-ethanol/g-glucose)</td>
<td>0.46</td>
<td>0.48</td>
<td>0.42</td>
<td>0.41</td>
</tr>
<tr>
<td>maximum cell concn (cell/mL)</td>
<td>1.6 $\times 10^7$</td>
<td>1.6 $\times 10^7$</td>
<td>1.6 $\times 10^7$</td>
<td>1.6 $\times 10^7$</td>
</tr>
<tr>
<td>fermentation time (h)/dilution rate (h⁻¹)</td>
<td>41.5</td>
<td>68</td>
<td>210</td>
<td>0.18 h⁻¹</td>
</tr>
<tr>
<td>productivity (g/L-h)</td>
<td>0.9</td>
<td>1.0</td>
<td>0.3</td>
<td>3.8</td>
</tr>
</tbody>
</table>

- Sterilization was carried out at 121 °C.Sterilization was carried out at 60 °C. Sterilization temperature was not specified in the paper. Saccharomyces cerevisiae H1-7 was used. Saccharomyces cerevisiae (ATCC 26603) was used.
productivity of about 16.9 g/L-h in this work, however, was at least 16.9 times higher than those of batch fermentations previously carried out by our team with wood hydrolysates sterilized at 60 °C (Lee et al., 1999). The productivity was also much higher than that when the wood hydrolysate medium was sterilized at 121 °C and the same internal membrane-filtration bioreactor system was used (Lee et al., 1996). A cell recycle culture using cell settling carried out by another group with a wood hydrolysate gave much lower productivity than ours, although their sterilization temperature was not specified (Chung and Lee, 1985). In addition to high productivity, higher ethanol concentration was also obtained than those in the other fermentation systems.

Contamination might have been a serious problem in the sterilization at a low temperature, but it did not occur probably because of the high toxicity of the wood hydrolysate. From these results, it is evident that the low-temperature sterilization of wood hydrolysate effectively reduced the formation of inhibitory compounds during the sterilization step (Lee et al., 1999) and made it possible to obtain higher ethanol concentrations and productivities.

In conclusion, the internal membrane-filtration bioreactor in combination with low-temperature sterilization was a very effective system for ethanol production in terms of ethanol concentration and productivity as compared to other bioreactor systems reported to date.

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**References and Notes**


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