Hydrothermal modification of lignocellulosic waste as microbial immobilization carriers for lignocellulosic ethanol fermentation

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Introduction

Lignocellulosic waste from agriculture such as corn stover has great potential for utilization as biofuel. Given its theoretical feasibility, ethanol production from these lignocellulosic waste have been widely concerned. It has been acknowledged that lignocellulosic waste can produce monomers of hexose and pentose for further ethanol fermentation after proper pretreatment and hydrolysis. Besides pretreatment and hydrolysis processes which have been intensively studied, the fermentation for cellulosic ethanol production is another concerned process due to the following reasons: Firstly, free microorganism in fermentation is hardly accumulated in the reaction system as free state; secondly, their activity is easily inhibited by the toxic substances in hydrolysate from lignocellulosic waste (Sittijunda, 2013). This has limited the fermentation efficiency and become one of the main factors that restrict the promotion of ethanol production from lignocellulosic waste.

The microbial immobilization with natural lignocellulosic carrier is an important solution to break through the bottleneck of the bioethanol technology. Immobilization profits from the biomass retainment and the protection from inhibitors. Compared with commercial immobilization carriers, natural lignocellulosic carriers have presented advantages of low cost, high strength, good surface property and no inhibition (Fang, 2011). Some studies reported the application of natural carriers such as rapeseed straw on ethanol fermentation, and the physicochemical properties of different kinds of lignocellulosic carriers (Sittijunda, 2013). These findings reported that the lignocellulosic structure, porosity and surface roughness of the lignocellulosic carriers could be modified by cellulase treatment, so that better performance can be obtained in immobilization (Yan, 2012).

In our previous research, we found that hydrothermal treatment is an effective approach to dissolve and hydrolyse the components in lignocellulosic waste including cellulose, hemicellulose and lignin in different extent with different temperatures, pressures and reaction time (Zhao et al., 2015). Therefore, it is promising to utilize hydrothermal process for physicochemical modification of lignocellulosic waste to provide better carrier properties for microbial immobilization. With corn stover as typical lignocellulosic carrier, this study thus investigated the hydrothermal modification and its impacts on specific surface area and porosity property under different temperatures, reaction times and sizes. The results showed that the modified lignocellulosic carriers achieved much better physicochemical properties after proper hydrothermal modification than the untreated ones, and thus offered better performance on yeast immobilization for potential ethanol fermentation.

Materials and Methods

Corn stover was used as raw materials after cut into chosen sizes and dried. *Saccharomyces cerevisiae* was used for immobilization testing. The hydrothermal modification was performed in a 600 mL customized reactor made from stainless steel. During the hydrothermal modification experiments, prepared corn stover with chosen sizes (side length 0.5, 1.0 and 2.0 cm as cube) were placed into the reactor with 300 mL deionized water. After sealed, the reactor was immersed by a preheated salt bath to reach chosen temperatures (190, 200, 210 and 220 ºC) and lasted till chosen times (5, 10, 20, 30 and 40 min). The reactor was then cooled down rapidly by ice water and the modified lignocellulosic carriers were taken out for further analysis and immobilization test after drying.

The specific surface area and porosity property of untreated and modified corn stover were analysed by Specific surface area and porosity analyser (Autosorb-1MP, USA). The immobilization test was performed by mixing the modified carriers and *Saccharomyces cerevisiae* at certain amount and analysing the microbial concentration of suspension to determine the biomass absorbed at the carriers.

Results and discussion

The results showed that corn stover could be solved effectively by hydrothermal treatment in the range of 180 to 220 ºC at around 30 min, with dissolution ratios of 50-60% in mass. The physicochemical properties of the lignocellulosic carriers changed significantly after modification under different hydrothermal conditions. Table 1 lists the specific surface area, porosity and pore diameter of the modified carriers at different temperatures. When the reaction time and size were fixed at 20 min and 2.0 cm respectively, increase in temperature resulted in significant increase in porosity compared to that of untreated corn stover (0.005 cm^3/g). This is mainly because of the change of lignocellulosic structure by dissolving the major components such as hemicellulose and lignin in corn stover. By contrast, the specific surface area generally increased with temperature rise and achieved a peak value of 2.873 m^2/g at 210 ºC, which was twice as that of untreated corn stover (1.421 m^2/g).

Higher temperature at 220 ºC did not result in higher specific surface area due to the larger pore volume after intensive dissolution. This was proved by the sudden increases in porosity and pore diameter at 220 ºC.

Table 1. Physicochemical properties of the modified carriers at different temperatures (20 min and 2.0 cm)

Temperature (ºC)	Specific surface area (m^2/g)	Porosity (cm^3/g)	Pore diameter (nm)
190	1.572	0.009	3.057
200	1.916	0.009	3.416
210	2.873	0.011	3.414
220	2.652	0.018	6.548

The reaction time impacts on the carrier properties were shown in Table 2. When the temperature was fixed at 190 ºC, the time extension caused significant increase in specific surface area and porosity. The highest values were both obtained at 40 min, with 0.9 and 2.2 times higher than those of untreated corn stover, respectively. However, both specific surface area and porosity presented low points at 10 min, which were even lower than those of untreated corn stover. This was attributed to the swelling effect at the beginning of hydrothermal reaction. The absorption of water resulted in the volume expansion of lignocellulosic components and thus compressed the structural pore. This effect gradually weakened with the progress of hydrothermal reaction.

Table 2. Physicochemical properties of the modified carriers at different reaction times (190 ºC, and 2.0 cm)

Reaction time (min)	Specific surface area (m^2/g)	Porosity (cm^3/g)	Pore diameter (nm)
5	1.542	0.004	9.565
10	0.647	0.002	3.057
20	1.572	0.009	3.057
30	2.256	0.013	4.896
40	2.688	0.016	5.608

Carrier size is another parameter influencing the modification performance. Figure 2 shows the properties of the modified carriers at different size. It can be seen that both specific surface area and porosity reached maximum at 1 cm carriers, with 2.440 m^2/g and 0.011 cm^3/g, respectively. Larger carriers led to lower specific surface area and porosity given the difficulty of accessing the inner structure for water. Proper carrier sizes also benefit to the capacity utilization of potential fermentation reactors. Further immobilization test with the chosen modified carriers (200 ºC, 20 min, 1.0 cm) showed that their absorption biomass of *Saccharomyces cerevisiae* reached $1.67\times10^9/cm^3$ in volume, 49% higher than that with untreated carriers (1.12×10^9/cm), and 80% higher in the absorption biomass in unit mass.

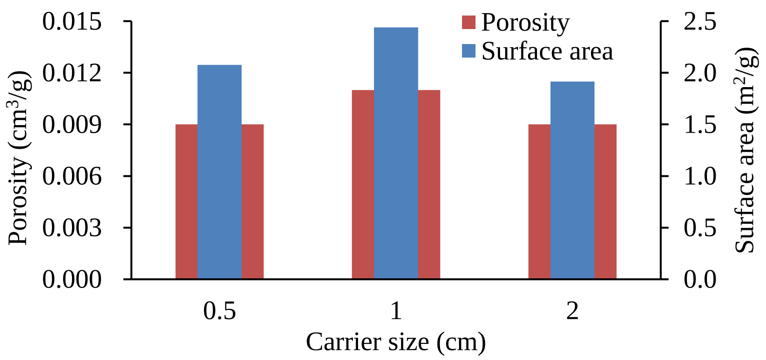

Figure 1. Physicochemical properties of the modified carriers at different sizes (200 ºC, and 20 min)

This study addressed a promising approach to modify the physicochemical properties of lignocellulosic waste as potential immobilization carriers for microorganisms in ethanol fermentation. The modified carriers presented better performance in specific surface area, porosity and also in microbial absorption. The results provide important support to the immobilization technology with natural lignocellulosic waste and thus benefit the potential bioethanol fermentation process.

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