学位論文内容の要旨

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学位論文題名

Production of high concentration bioethanol by fed-batch type simultaneous saccharification and fermentation of lignocellulosics with amphipathic lignin derivatives

（両親媒性リグニン誘導体を用いたリグノセルロースの同時糖化発酵による高濃度バイオエタノールの製造）

Bioethanol production is one of candidates to solve energy crisis caused by depletion of fossil resources. Lignocellulosics, a second-generation feedstock for bioethanol production, are collecting much attention because of its abundance and no competition with food production. Bioethanol production from lignocellulosics mainly consists of pretreatment, saccharification, fermentation and distillation. For the saccharification of polysaccharides in lignocellulosics, there are two main methods, acid hydrolysis and enzymatic hydrolysis. Nowadays, the enzymatic hydrolysis seems to be a more promising saccharification method, because of its mild reaction conditions and no requirement for special reaction vessels. To avoid the end-product inhibition of enzymatic saccharification process, a combined process of enzymatic saccharification and fermentation, termed as simultaneous saccharification and fermentation (SSF), was investigated in my study for production of high concentration bioethanol.

Forest Chemistry Lab. in Hokkaido University has already developed amphipathic lignin derivatives (A-LDs) from various isolated lignins and epoxylated poly(ethylene glycols). A-LDs were shown to have significant surface activity like non-ionic surfactants, and the ability to accelerate enzymatic hydrolysis of lignocellulosic materials. The latter performance was brought about by the direct associations between A-LDs and cellobiohydrolase among the cellulase components, which suppressed the non-productive adsorption of cellulase onto substrate. However, it is unclear that A-LDs positively affect the fermentation and the SSF process. Therefore, an aim of this thesis is to clarify the performance of A-LDs in SSF process.

In this study, I prepared A-LDs from cedar soda lignin (CSL). Firstly, an effect of the A-LDs on yeast fermentation of glucose was investigated. Secondly, A-LDs performance to fed-batch type SSF process (FB-SSF) in a 100-mL scale was examined as a preliminary experiment, where FB-SSF meant that lignocellulosics as a substrate was successively fed into SSF media. As a result, A-LDs were found to be useful as an additive to improve the bioethanol productivity, resulting in higher concentration bioethanol than that without A-LDs. Therefore, as a third target, optimization of the fed-batch SSF conditions such as temperatures, stirring programs and A-LDs loadings were investigated to further increase bioethanol production efficiency.

1. Preliminary fed-batch type SSF with A-LDs

Two A-LDs were prepared by the reaction of CSL with dodecyloxy poly (ethylene glycol) glycidyl ether (DOPEG) and ethoxy (2-hydroxy) propoxy poly (ethylene glycol) glycidyl ether (EPEG) under alkaline conditions followed by

purification with an ultrafiltration with polysulfone membrane (cut-off molecular mass, 1000 Da, Advantec, Japan) to give DOPEG-SL and EPEG-SL, respectively.

The A-LDs was found to accelerate ethanol fermentation of glucose with Japanese sake yeast in addition to improvement of enzymatic saccharification of softwood unbleached soda pulp and kraft pulp (NUKP), just like previously reported A-LDs prepared from different isolated lignins. As expected, these A-LDs at a loading amount of 2.5 g/L enhanced bioethanol concentration from 37.8 g/L to 49.4 g/L in a 100-mL scale FB-SSF process with 180 FPU of cellulase for 18 g of NUKP. Such concentrations were corresponded to 49% and 64% of ethanol yield on the theoretical ethanol yield.

2. Optimization of fed-batch type SSF conditions to produce highly concentrated bioethanol

The temperature in SSF and A-LDs loading were optimized in 100-mL scale FB-SSF. As a result, the optimized temperature at 38^{o}C in SSF after 12-h prehydrolysis at 50^{o}C gave the highest ethanol yield and concentration. The optimized loading of A-LDs was found to be 3.0 g/L, although I anticipated that the ethanol yield would depend on charged amount of A-LDs. Under these optimized conditions, the ethanol concentration and yield was increased to 63.3 g/L and 70.7%, respectively, which were 1.3 and 1.1 times larger than those of above preliminary experiment.

Conditions for substrate agitation were optimized by using a 3-L jar fermenter equipped with a powerful mechanical stirrer to achieve rigorous agitation at high consistencies of substrate. Four stirring conditions were tested without A-LDs. As a result, the following conditions gave the highest ethanol concentration of 64.5 g/L; continuous stirring at 40 rpm in the pre-hydrolysis process, continuous stirring at 20 rpm for the beginning 3 days of SSF, and then periodic stirring at 40 rpm for 5 min in every hour until 6 days. When A-LDs were added to the medium, the concentration was reached 83.0 g/L with a substrate loading of 24%(w/v). By using this jar-fermenter, FB-SSF with higher substrate loading of 30%(w/v) was carried out, resulting in concentrated ethanol of 87.9 g/L. Thus, bioethanol concentration in FB-SSF was remarkably improved by such optimizations.

Conclusions

In this study, the A-LDs derived from CSL were shown to be useful not only for improving the enzymatic saccharification efficiency, but also for enhancing the bioethanol concentration in FB-SSF. Besides, the custom-made jar fermenter equipped with strong stirring motor significantly enabled FB-SSF at higher consistency of substrate, resulting in highly concentrated bioethanol. The concentrated bioethanol will significantly reduce the distillation cost. Thus, my results would probably help to produce low-cost liquid fuel to establish sustainable society with effective energy recycle. Furthermore, this research will contribute to a novel, value-added utilization of isolated lignin and low quality lignocellulosics.