学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Enzymatic Saccharification of Cedar and Sago Waste Pulps with Amphipathic Lignin Derivatives as Cellulase-Aid agent

(両親媒性リグニン誘導体を助剤としたスギ及びサゴ廃棄物パルプの酵素糖化)

Lignocelluloses are considered to be one of the most promising, alternative renewable materials to fossil resources. Japanese cedar (*Cryptomeria japonica*) is a main plantation wood that occupies around 60% of softwood plantation in Japan. A significant amount of Japanese cedar wood is exhausted by tree thinning, and left unused in forest every year. On the other hand, sago palm (*Metroxylon* sp.) is considered to be the most important plant species for starch production in Indonesia. Its fibrous waste is also produced and disposed as a byproduct in 4.75 million tons per year by starch mills. To utilize such unused lignocellulosics, it is attempted to convert cedar wood and fibrous sago waste to fermentable sugar as a feedstock for bioethanol production through enzymatic saccharification. A dominant obstacle for the saccharification process is the cost of enzyme; cellulase cost accounts for 25-50% of the total cost for bioethanol production. Several cellulase-aid agents and systems, such as surfactants and water-soluble immobilized enzyme system, are proposed to reduce the high enzyme cost.

Three types of amphipathic lignin derivatives as a surfactant have been developed by the reaction of several isolated lignins with epoxylated polyethylene glycol, poly(ethylene glycol) diglycidyl ether (PEGDE), ethoxy-(2-hydroxy)-propoxy-poly(ethylene glycol) glycidyl ether (EPEG), and dodecyloxy-poly(ethylene glycol) glycidyl ether (DAEO). The aims of this study are to evaluate the ability of the lignin derivatives to improve enzymatic saccharification and to propose an effective saccharification process for producing a feedstock for bioethanol.

1. Improvement of enzymatic saccharification of unbleached cedar pulp with amphipathic lignin derivatives

Unbleached cedar pulp as a substrate for enzymatic saccharification was prepared by soda pulping of Japanese cedar wood chips, and amphipathic lignin derivatives were prepared by the reaction of acetic acid lignin, which was obtained by an organosolv pulping process of birch chips with acetic acid, with the epoxylated PEG derivatives mentioned above. The enzymatic saccharification of unbleached cedar pulp together with the lignin derivatives was attempted with two types of cellulases commercially available, Meicelase and Genencor

GC220. The GC220 showed higher saccharification efficiency (SE) than Meicelase, suggesting that the GC220 was a suitable cellulase for the saccharification of cedar pulp. SE was improved by the addition of amphipathic lignin derivatives as well as PEG4000 that was reported to be an effective cellulase-aid agent. Although PEG4000 showed the highest SE at low enzyme charge (10 FPU/g of substrate), such performance was not observed at high enzyme charge (20 FPU/g of substrate). The lignin derivatives exhibited a notable ability to maintain the residual cellulase activity at a higher level than PEG4000 after one-time saccharification. Especially, EPEG- and PEGDE-derivatives showed complete recovery of cellulase activity at 20 FPU/g of substrate. It was proved that the different behavior of lignin derivatives from that of PEG4000 during saccharification was caused by the direct interaction between cellulase and lignin derivatives.

2. Enzymatic saccharification of sago pulp from starch extraction waste using sago lignin-based amphipathic derivatives

Fibrous sago waste disposed from sago mills was pulped with aqueous NaOH in the presence of anthraquinone, after removing residual starch in the waste using 4% of aqueous HCl. Soda sago lignin (SSL) was isolated from black liquor of the pulping. SSL was also converted to amphipathic SSL derivatives by the reaction of the epoxylated PEG derivatives. The unbleached sago pulp was enzymatically hydrolyzed with GC220 (10FPU/g of substrate) in the presence of amphipathic lignin derivatives. After the hydrolysis, the cellulase was recovered by using ultrafiltration, and then the next hydrolysis was performed with the recovered enzyme. This process was repeated 4 times. Without lignin derivatives, SE by the repeatedly used cellulase was remarkably decreased after 2nd saccharification. By contrast, SE was improved by the addition of amphipathic SSL derivatives, DAEO-SSL in particular, as well as amphipathic lignin derivatives prepared from acetic acid lignin, and was maintained at a high level until 4th saccharification. In addition, DAEO-SSL also showed the highest residual activity after 4th saccharification. This high activity of DAEO-SSL might bring about high SE throughout 4-times saccharification. These results suggest that amphipathic lignin derivatives enable re-use of cellulase for enzymatic saccharification of lignocellulosics.

General discussion: I have developed amphipathic lignin derivatives from Japanese wood and sago palm wastes as cellulase-aid agent, which enables re-use of cellulase. This means that the saccharification cost will be remarkably reduced by the use of such lignin derivatives. Therefore, this research will provide a useful method to produce feedstock for 2nd generation bioethanol and an effective utilization of unused lignocellulosics.