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学位論文題目	Microbial Xylitol Production from Various Hemicellulose Hydrolyzates by the Yeast *Candida Magnoliae* （酵母 *Candida magnoliae* を用いた様々なヘミセルロース加水分解からのキシリトール発酵生産）
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学位論文内容の要旨

Xylitol, a naturally occurring sugar alcohol, is of interest to the food and oral care industries because of its high sweetening power equivalent to sucrose, great negative heat of dissolution, and anticariogenic properties. It absorbs slowly from human digestive tract and entries into metabolic pathway independently of insulin. The glycemic index of xylitol (GI = 13) compares favorably with that of sucrose (GI = 65). Therefore, xylitol is used clinically as a sucrose substitute for diabetics and for patients deficient of glucose-6-phosphate dehydrogenase (G6PD).

Xylitol has been widely found in fruits and vegetables. However the small quantities present in nature make its extraction impractical. It is currently produced by a catalytic reduction of $_{D}$-xylose present in the spent sulfite cooking liquors of hardwood chips or corn cob hemicellulose hydrolyzates. Because the hemicelluloses of hardwoods or gramineous plants contain other monosaccharides, such as $_{L}$-arabinose and $_{D}$-glucose, extensive separation and purification steps are necessary to remove these contaminants before chemical reduction. The inefficiency of current xylose preparation techniques seriously affects the recovery of xylose from lignocellulosic raw materials. The yields of xylitol correspond to only 50% to 60% of xylan present in the raw materials. An alternative method for the xylitol production is microbial conversion of $_{D}$-xylose in the hemicellulose hydrolyzates. Among the microorganisms that can assimilate xylose, the yeasts belonging to the genus *Candida* are the best xylitol producers. Although the microbial production of xylitol requires additional costs for the detoxification of hemicellulose hydrolyzates and purification of xylitol in the fermentation media, the additional costs for the environmentally friendly process may be accepted by ecology-minded consumers.

This study was performed to establish the microbial xylitol production from locally available lignocellulosic wastes by the yeaste, *Candida magnoliae*. Hemicellulose hydrolyzates were prepared from different raw materials, *i.e.*, the culms of bamboo (*Phyllostachy pubescens*) and bamboo grasses (*Sasa* genus), and Japanese white birch wood (*Betula platyphylla* var. *japonica*). These raw materials were subjected to hydrolysis with dilute sulfuric acid to give sugar solutions rich in $_{D}$-xylose. First, the effects of hydrolysis

conditions on the sugar composition of the hydrolyzate were examined, in order to establish the optimal hydrolysis conditions for preparing the fermentation substrate. It is known that acid hydrolyzates of lignocelluloses usually contain not only fermentable sugars but also some undesirable byproducts, such as acetic acid, furan derivatives and solubilized lignin fragments. They act as an inhibitor of the microbial metabolism. Direct use of the hydrolyzates reduces the efficiency of the growth of microorganisms and the product formation. Therefore, detoxification of the hydrolyzates before the fermentation is necessary for the successful bioconversion of the solubilized sugars. Detoxification of the hydrolyzates with carbonaceous sorbents and an anion exchange resin was evaluated. When the hydrolyzate was treated with commercially available activated chars, the concentrations of furfural and 5-hydroxymethylfurfural remarkably decreased. However, the concentration of acetic acid originating from hemicelluloses stayed almost constant after treatment with the activated chars. When the birch wood hydrolyzate detoxified with a steam-activated char was further treated with an anion exchange resin (Amberlite IRA 67), a large portion of the acetic acid (68%) present in the hydrolyzate detoxified with the activated char could be eliminated.

Yeast cultures adapted to the hemicellulose hydrolyzates by repeated recycling has been used to overcome the inhibition of microbial metabolism caused by the toxic compounds present in the hydrolyzates. In this study, adaptation of the strain of *Candida magnoliae* was performed by sequentially transferring and growing cells in media containing increasing concentrations of the hydrolyzate. Under the aerobic condition used, the acetic acid was almost completely consumed by the adapted cells grown on hemicellulose hydrolyzates.

The microbial production of xylitol depends on the fermentation conditions employed. In this study, two phase aeration process was employed. The first step was carried out under aerobic conditions to improve glucose consumption through cell proliferation. The second step under limited oxygen conditions is intended to increase the xylitol accumulation. Together with initial substrate concentration, the aeration greatly affects the microbial production of xylitol. Strict control of aeration is, therefore, required for the successful xylitol production.

論文審査結果の要旨

要　　旨：キシリトールはショ糖と同程度の甘味、冷涼感、抗う蝕性、インスリン非要求性の特性を持つためオーラルケア商品や糖尿病患者などに用いられている。キシリトールの需要は日本で約15000トンあり、全て輸入により賄っているにも関わらず、その需要量は年々増加傾向にあるため日本での生産が求められる。ササ類やタケは日本に広く分布しているが、これらの成熟稈の利用はほとんどされていない。従って、イネ科植物資源の新たな用途開発として、ヘミセルロースの希酸による加水分解と微生物変換の組み合わせによる有価糖質の生産を行った。モウソウチクの加水分解は3%硫酸、120°C、1時間、固液比1:4の条件で比較的高いキシロース溶液を得ることができた。水解液中に含まれる発酵阻害物質は、水蒸気賦活炭により単糖を損なうことなく選択的に除去することがわかった。酢酸は活性炭では除去することができなかったが、イオン交換樹脂を用いることで大幅に除去することができた。しかしながら、交換樹脂は高価であるためキシリトール生産においては採算が合わない。そこで、菌株を水解液にアダプテートさせることで酢酸への耐性を向上させた。結果、交換樹脂を用いることなく好気条件において酢酸を完全に代謝させることに成功した。発酵では二段階通気プロセスを運用した。培地中に存在するグルコースはカタボライト抑制を持つためにキシリトール生産を阻害する。従って、一段階目では槽内を好気条件にし、グルコースを優先的に代謝させた。二段階目で微好気条件にすることで、菌体増殖を抑え、キシリトール生産を増加させた。よって、高い効率でキシリトールを得るには、酸素供給量の精密な調整が求められることを見出した。

以上の結果より、申請者は北見工業大学博士(工学)の学位を授与される資格があると認定した。