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Utilisation of Wood Polysaccharides as Sugars

Uporaba lesnih polisaharidov kot sladkorjev

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Abstract:

In Japan, the forest covers approximately 25 million ha, that is, about two-thirds of total land area, with different forest types from sub-frigid conifer forest in the north to sub-tropical forest in the south. Over a few decades, however, the low-quality hardwoods which had been utilised as firewood and charchoal have not been used except raw materials for pulp and mushroom cultivation. The steaming process of hardwood was studied for utilisation of hardwood polysaccharides as sugars as a part of Biomass Conversion Program by the Japanese Government. The yield of xylan hydrolysate (mixture of xylose and xylooligomers) from various hardwood species on steaming was 14-22 % under optimized conditions. Acidic xylooligomers carrying a 4-O-methylglucuronic acid residue or a galacturonic acid residue comprised approximately 10 % of the xylan hydrolysate. The enzymatic hydrolysis of residual lignocellulose was attemped on a large scale using an apparatus for continuous hydrolysis with a recycle system of cellulases. Glucose was a dominant component of the reducing sugars produced: most of hemicellulose in hardwood could be separated by the procedure of steaming followed by extracting with water. Finally, an economic analysis of hardwood components utilisation process on a plant scale by the Japanese Forestry Agency was described.

Keywords: *hardwood polysaccharides, xylan, cellulose, steaming, enzymatic hydrolysis, economic analysis*

Izvleček:

Na Japonskem gozd pokriva približno 25 mio ha, to je okoli 2/3 celotne površine. Gozd je različen od sub-kontinentalnih iglavcev do sub-tropskega gozda na jugu. V zadnjih desetletjih je bil nekvaliteten les listavcev, ki se je prvotno izkoriščal le za kurjavo in izdelavo oglja, uporaben le kot material za celulozno industrijo in vzgojo gob. Proces parjenja trdih lesov je bil preučevan za izrabo polisaharidov trdega lesa za izdelavo sladkorja kot del "Programa uporabe biomase", ki ga je podpirala japonska vlada. Izplen hidrolizatov ksilana (mešanice ksiloze in ksilooligomerov) iz različnih drevesnih vrst pri parjenju je pod optimiziranimi pogoji znašal od 14-22 %. Kislinski ksilooligomeri, ki so vsebovali 4-O-metilglukronsko kislinski preostanek ali galakturonski kisli preostanek so vsebovali približno 10 % ksilanove hidrolizata. Encimatska hidroliza preostanka lignoceluloze v večjem obsegu je bila izvedena z uporabo aparata za kontinuirano hidrolizo z reciklirnim sistemom celulaz. Glukoza je bila dominantna komponenta preostanka sladkorjev: večino hemiceluloze v lesu lahko ločimo s postopkom parjenja, ki mu sledi ekstrakcija z vodo. Na kraju je opisana ekonomska analiza proizvodnega procesa, ki jo je izvedla Japonska gozdna agencija.

Ključne besede: polisaharidi listavcev, ksilan, celuloza, parjenje, encimatska hidroliza, ekonomska analiza

1. Introduction

In the 1970s, Japan became to have a highly advanced economic society with the growth of the secondary and tertiary industries. But our country has greatly depended on the other countries for energy sources, food, feed, forest products and raw materials for industries. The self-sufficiency rate is only 16 % in energy, 30 % in cereals and 20 % in wood. In agriculture and forestry, only a part of the biological resources is being utilised by simplified processing. As a result, a large amount of biological resources is discarded or left unutilised. This might be causing ecological destruction and exhaustion of the fossil energy.

The total area of broad-leaved forest is 12 million ha and the store of hardwoods reaches about 1.5 billion m^3. Over a few decades, the use of low-quality hardwoods has been limited to raw materials for pulp and mushroom cultivation. Developing the utilisation of low-quality hardwoods is of importance in encouraging the forestry and forest products industry. We studied extensively the steaming process of hardwood as a part of the Biomass Conversion Program of the Ministry of Agriculture, Forestry and Fisheries from 1981 to 1990. In this article, our

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research results on the utilisation of hardwood polysaccharides as sugars are described.

2. Separation and utilisation of hardwood xylan hydrolysate by steam treatment

The steaming of hardwood is one of the effective ways to be capable of fractionating wood major three components, cellulose, hemicellulose and lignin. The hardwood xylan contains acetyl groups at C-2 or C-3 position of xylose residue with a substitution rate of 60-70 %. If hardwood is steamed at elevated temperatures, the acetyl groups are released to form acetic acid which catalyses selective hydrolysis of the hemicellulose. Most of hemicellulose is depolymerised to be water-soluble low molecular weight saccharides, large portion of lignin becomes soluble with organic solvents or dilute alkali, and consequently the residual cellulose is modified into a susceptible form to cellulase attack.

The yield of xylan hydrolysate on steaming was different depending on the hardwood species and steaming conditions (yield: 14-22 % under optimized conditions). Neutral and acidic xylooligomers could be isolated by column chromatography after removal of the extraneous solutes such as furfural, its condensed compounds and phenols from lignin. Aldouronic acids carrying a 4-0-methylglucuronic acid residue or a galacturonic acid residue comprised approximately 10 % of the xylan hydrolysate. The composition of xylose and xylooligomers in the water extracts from shirakamba (*Betula platyphylla*) wood steamed at 180 °C for 20 min is shown in Figure 1 (Ishihara et al., 1996).

Xylose, xylitol and furfural derived from xylan are used as saccharides in transfusion fluids, diabetic and tooth decay-preventive sweeteners, and raw materials for resins, respectively. Xylooligosaccharides, which are commercially utilized as low calorie sweeteners for soft drinks, have a favorable influence upon the intestinal microflora in humans when are ingested (Okazaki et al., 1990). A daily dosage of at least l g of the xylooligomers consisting chiefly of xylobiose brought about a selective increase of *Bifidobacteria* population in 3 weeks. Our research group has shown that the xylooligosaccharides obtained by steaming hardwood exhibited certain physiological activities such as the growth promotion of leguminous tree seedlings and rooting stimulation of conifer cuttings in very small concentration ranges (5-50 mg/L, Ishihara et al., 1995).

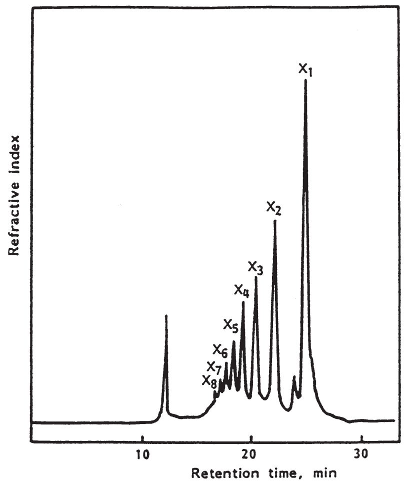

Figure 1. Gel permeation chromatogram of the hemicellulose fraction obtained by water extraction of shirakamba (*Betula platyphylla*) wood steamed at 180 °C for 20 min

Notes: Column, Shodex Ionpak KS-802 (0.8 x 30 cm x 2, in series) ; Temperature, 80 °C ; Eluent, distilled water; Flow rate, 0.8 ml/min; X_1, xylose; X_2-X_8, xylooligomer

Slika 1. Gel permeation kromatogram frakcij hemiceluloze, pridobljene z vodno ekstrakcijo lesa shirakambe (Betula platyphilla), parjenega 20 min pri 180 °C.
Opombe: Stolpec, Shodex Ionpak KS-802 (0.8 x 30 cm x 2, v vrsti); temperatura 80 °C ; topilo destilirana voda; pretok 0,8 ml/min; X_1, ksiloza; X_2-X_8, ksilooligomeri

3. Enzymatic hydrolysis of steamed and water-extracted hardwood

The steamed and water-extracted shirakamba wood was enzymatically hydrolysed at 40 °C for 8 days on a large scale (2 kg of substrate with 20,000 U of commercial cellulase derived from *Trichoderma viride*) using an apparatus for continuous hydrolysis with a recycle system of cellulases (Ishihara and Shimizu, 1991). The schematic diagram of the apparatus is shown in Figure 2. The apparatus is composed of a 10-L reactor, a reservoir tank for the supply of substrate, an ultrafiltration unit which separates hydrolysis products from the reaction mixture through a tubular membrane module, and a suction filter unit which removes the hydrolysis residue from the reaction mixture.

The substrate was added to the reactor at appropriate intervals to keep a concentration of approximately 5 % (w/v). All of the enzyme was added at the beginning and no further addition was done. As a result of operation of

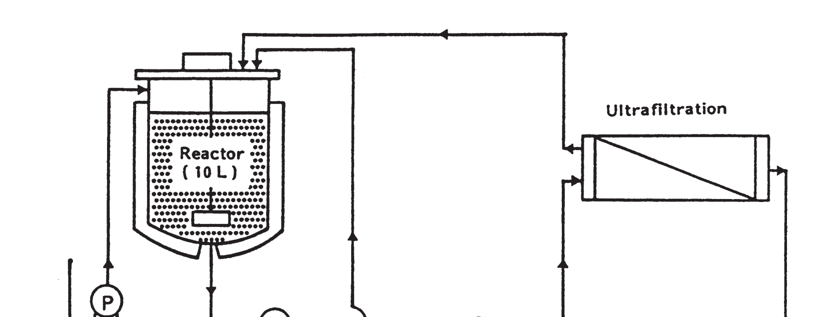

Figure 2. Apparatus for continuous hydrolysis of lignocellulosic materials

Slika 2. Shema naprave za neprekinjeno hidrolizo lignoceluloznih materialov the apparatus at 40 °C for 8 days, 27.3 U of cellulase produced one gram of reducing sugar from steamed and water-extracted shirakamba wood. Compared with a batch hydrolysis, the enzyme requirement was appreciably reduced in this continuous hydrolysis system. Glucose was the dominant component (550g) of the total reducing sugars (630g) produced: most of hemicellulose in shirakamba wood could be separated by the procedure of steaming followed by extracting with water. Small amounts of oligosaccharides (cellobiose and xylobiose) were also present in the hydrolysis products of the steamed and water-extracted shirakamba wood. The addition of ß-glucosidase and ß-xylosidase to the *T. viride* commercial enzyme preparation appeared to be necessary for the complete conversion to monosaccharides.

All of the liberated sugars can he readily fermented to ethanol by organisms such as *Saccharomyces cerevisiae* or *Zymomonas mobilis*. Our research group has also shown that the sugars in the hydrolysate were converted to the single cell protein of Candida utilis in a yield of 46.7 % (the amount of dried yeast mycelium based on the sugars consumed, Shimizu et al., 1983). In addition, economically feasible preparation of glucose as carbon source for production of bacterial cellulose is of increasing importance because *Acetobacter xylinum* forming cellulose is found to be a valuable product (market value in 1998 was ¥7,000~10,000 per kg) with excellent properties such as transpairency, tensile strength, fiber-binding ability and biodegradability. The use of bacterial cellulose is promising in many industrial fields as dietary food, tension-strengthening material for concrete, medical materials and selective permeation-membrane.

4. Economic analysis of hardwood components utilisation process on plant scale

Recently, the Japanese Forestry Agency had an application research project on the development of hardwood components utilization on the basis of the research results of the Biomass Conversion Program with collaboration of the Forestry and Forest Products Research Institute and 13 profit-making companies. The outline of the economic analysis of hardwood components utilisation process on plant scale is shown in Figure 3 (Shimizu et al., 1998). This analysis was done by assuming a plant scale processing 30,000 t of birch wood chips per year (100 t per day) on dry basis. To simplify the cost analysis, the products were specified to be three items, that is, reduced xylooligosaccharides (xylooligosaccharides alcohol) from hemicellulose, carbon fiber from lignin and ethanol from cellulose via glucose. The annual production of the reduced xylooligosaccharides, carbon fiber and ethanol was 4,500 t, 1,542 t and 5,412 t (6,658 kL), respectively.

The capital investment amounted to ¥1,126,000,000 and the facilities for the carbon fiber production, for the steam-explosion and fractionation and for the purification of xylooligosaccharides occupied 36.6 %, 19.2 % and 13.3 %, respectively. As a result of the economic analysis including the costs for labor, utilities, chemicals and hardwood chips, the market value of total products (¥6,910,000,000) was not slightly balanced to the total products costs (¥6,978,000,000). The Forestry Agency concluded that the difference of red figures (¥68,000,000) would be overcome by replacing ethanol with other profitable products and/or by improving production techniques.

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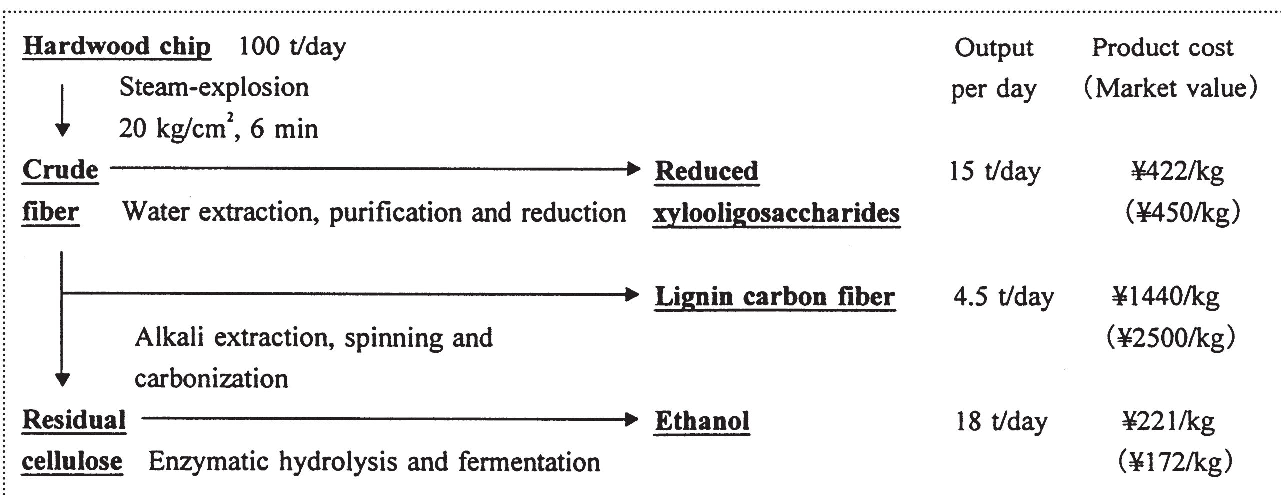

Figure 3. Economic analysis of hardwood components utilization process on plant scale

Slika 3. Ekonomska analiza uporabe komponent lesa listavcev

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POVZETEK

Prispevek japonskega raziskovalca Mitsure Ishihare z naslovom "Uporaba lesnih polisaharidov kot sladkorjev" obravnava trenutno v svetovnem merilu aktualno temo s področja kemične predelave lesa listavcev.

Svoje raziskovalno delo, ki je bilo vključeno v njihov "Program uporabe biomase" utemeljuje s pomanjkanjem večine surovin in energije na Japonskem, zaradi česar je potrebno vsak, čeprav odpadni material, popolnoma izkoristiti.

Komercialno zanimiva je pretvorba ksilanov do ksiloze in ksilooligomerov. Te snovi so uporabne kot sladila v živilski industriji, za izdelavo preparatov za diabetike, sredstev za preprečevanje zobne gnilobe in drugih predvsem farmacevtskih izdelkov.

Najučinkovitejša ločitev glavnih komponent lesa tj. lignina, celuloze in hemiceluloz je s pomočjo hidrotermične obdelave. Poskuse so izvedli na lesnih sekancih breze Betula platyphylla pri 180 °C. Po 20 minutah so pridobili znatne količine ksiloze (X1) in manjše količine oligomerov (X2 - X8) kar je razvidno iz kromatograma na sliki 1.

Parjene in z vodo ekstrahirane sekance brezovega lesa so encimsko hidrolizirali 8 dni pri 40 °C. Uporabljali so komercialno celulazo. V ta namen so izdelali aparat, ki je poleg osnovnega reaktorja vključeval še ultrafiltrator za ločevanje produktov hidrolize od reakcijske mešanice. Na ta način so kot glavni produkt pridobili glukozo, ki je predstavljala surovino za fermentacijo v etanol.

V prispevku je na sliki 3 shematsko podana ekonomska analiza uporabe komponent lesa listavcev, ki jo kaže spodnja slika.

Kemična predelava lesa listavcev v ksilozo, ksilitol, furfural je ekonomsko upravičena. Smiselno je tudi izpopolnjevanje postopkov pridobivanja karbonskih vlaken, med tem ko je fermentacija celuloze v etanol trenutno komercialno še nezanimiva.

Povzetek pripravila dr.
Vesna TIŠLER