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Utilisation of Pentosans from Sugar Beet Pulp by Different White-Rot Fungi

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Abstract. Finding the alternative energy sources that will efficiently replace fossil fuels has been of tremendous scientific and public interest in recent years. Degradation of lignocellulosic materials, like herbaceous sugar beet waste- a sugar beet industry by-product, and use of its degrading products as substrate for biofuels production may be one of the alternatives. The composition of sugar beet pulp is as follows: 75-80 % carbohydrates, 1-6 % lignin and 10-15 % proteins. Hemicellulose, one of the major constituent of plant material is therefore convertible to bioethanol through hydrolysis and fermentation processes. Hemicellulose is a type of hetero-polysaccharide, containing glucose, xylose, mannose, galactose, arabinose, fucose, glucuronic acid, and galacturonic acid in various amounts depending on the source. Pentosan group is composed of 5C hemicellulose sugars. The aim of this study was to investigate the pentosans utilisation possibility of different white-rot fungi. *Trametes versicolor* exhibited the best pentosans degradation capacity, with only 3.75 % of pentosans in dry matter determined at the end of fermentation.

Keywords: Pentosans, degradation, white-rot fungi

1. Introduction

Rapid industrialization and population growth require environmentally sustainable energy sources. Bioethanol derived from plant biomass (lignocellulosic bioethanol) can contribute to a cleaner environment and help reduce dependency on liquid fossil fuels. A major challenge to full realization of the potential of lignocellulosic biomass is to overcome their high degradation resistance to become fermentable sugars [1]. Removal of hemicellulose and lignin that forms a matrix surrounding cellulose is often the first obstacle for the efficient degradation of cellulose to glucose [2].

Sugar beet pulp (SBP) is a sugar beet industry by-product, produced annually in large quantities. About 107 tons of SBP in dry matter equivalent is left over by the sugar industry every year in Western Europe [3]. SBP as a raw material is mainly used for animal feeding, due to its nutritive value. On a dry weight basis, SBP composition is as follows: 75% – 80% polysaccharides, 1-2 % fat, 10-15 % protein, 3-12 % ash and 1-6 % lignin [4]-[6]. Polysaccharides consists roughly of 22%–30 % cellulose, 25-30% hemicelluloses, mainly arabinans and (arabino) galactans and 25% pectin [4],[5].

The hemicellulose is estimated to account for one third of all components available in plants. Eventhough the potential for utilizing hemicelluloses is vast, it has not yet been applied on an industrial scale [7]. It represents a type of hetero polysaccharide with complex structure containing glucose, xylose, mannose, galactose, arabinose, fucose, glucuronic acid, and galacturonic acid in various amounts depending on the source. Pentosans (known also as arabinoxylans) consist of a backbone of 1-4-linked b-D-xylopyranosyl residues to which a-L-arabinofuranose units are linked as side branches. Although arabinoxylans from various sources share the same basic chemical structure, they differ in the way of substitution of arabinose (Ara) residues to the xylose (Xyl) backbone [8].

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Solid-state fermentation (SSF) is defined as a fermentation process performed on a non-soluble material that acts both as physical support and source of nutrients in the absence of free flowing liquid [9].

SSF is employed in many biotechnological processes such as food production, bioremediation, biodegradation of different toxic compounds or polymers, production of value added products (enzymes, organic acids, amino acids, biologically active secondary metabolites, hormones, antibiotics, flavors, colorants etc.), biobleaching and many more [10], [11]. The research interest in SSF application for utilization of lignocellulosic food and agro waste has increased in recent years due to the ecological problems caused by the large amount of such wastes produced and its possible utilization as renewable energy resources.

In this study five species of white-rot fungi were cultivated in solid-state conditions on SBP as substrate. White-rot fungi are a group of wood-decaying fungi particularly useful for biopulping, biodegradation of recalcitrant materials, and other applications. There are two general white-rot patterns that can be distinguished: simultaneous and selective decay. Simultaneous decay is characterized by the simultaneous degradation of cellulose, hemicelluloses and lignins and the casual agents include among others *Trametes versicolor* and *Phanerochaetae chrysosporiu*m [12]. In the much rarer process of selective lignin degradation, fungal enzymes remove lignin and non-cellulosic polysaccharides without extensive degradation of cellulose. *Ceriporiopsis subvermispora*, as well as *Dichomitus squalens* are the examples of a selective lignin degraders [13].

The aim of this study was to investigate the possibility of SBP pentosans utilisation of five white-rot fungi (*Trametes versicolor* MZKI G-99, *Phanerochaetae chrysosporium* MZKI B-223, *Phanerochaetae chrysosporium* ATTC 24725, *Ceriporiopsis subvermispora* CBS 347.63 and *Dichomitus squalens* CCBAS Reid 750) cultivated in solid-state conditions.

2. Materials and Methods

2.1. Microorganism and fermentation conditions

Five white-rot fungi were screened on their possibility to utilise SBP pentosans: *Trametes versicolor* MZKI G-99, *Phanerochaetae chrysosporium* MZKI B-223 (denoted in the results as *P. chrysosporium* (1)), *Phanerochaetae chrysosporium* ATTC 24725 (denoted as *P. chrysosporium* (2)), *Ceriporiopsis subvermispora* CBS 347.63 and *Dichomitus squalens* CCBAS Reid 750. Microorganisms were cultivated on malt agar medium for 7 days at 28 ℃. Mycelial plugs (diameter 6 mm) were used as inoculum.

SSF was carried in 1000 mL glass flasks. Five mycelial plugs were transferred to flasks containing 50 g of sugar beet waste previously moisturized by addition of 32 mL tap water. In order to provide improved absorption and porosity and to facilitate transport of oxygen as well as nutrients during SSF, the SBP particle size was 5 ± 2.0 mm of length and 2 ± 0.5 mm of width. Incubation was carried out at 27 ℃ for 45 days. To analyze the pentosans utilisation by the above mentioned microorganisms during the fermentation the samples were withdrawn after 10, 20, 30 and 45 days of cultivation. The experiment using each microorganism was performed in triplicate.

2.2. Determination of pentosans

Each withdrawn sample was sterilized after fermentation process in order to stop further microbial activity. Samples were then milled using standard laboratory knife mill with 1 mm screen (MF10 basic, IKA Labortechnik, Germany) to insure the particle size of sample below 1 mm [14].

Dry matter content was determined gravimetrically as residue remaining after drying. Sample of 2 ± 0.0001 g was weighed into aluminium cups (diameter 6 mm) and dried in an oven at 105 ℃ to constant weight. Weighings were made after sample cooling in desiccator.

Pentosans were measured according to TAPPI T223 cm-01 [15]. Pentosans are transformed in boiling 3.85 N hydrochloric acid to furfural, which is collected in the distillate and determined colorimetrically with orcinol-ferric chloride reagent. 0.3 g of previously milled sample was placed in a 250 mL boiling flask, together with 20 g of NaCl, 100 mL of 3.85 N HCl and a few boiling stones. The flask was connected to the distillation apparatus and the acid level in the flask was marked in order to maintain it constant during the

whole distillation by adding HCL dropwise from the separatory funnel. 250 mL of 3.85 N HCl was added to the separatory funnel, the heat was applied and the distillation of the acid was performed at a uniform rate of about 2.5 mL per min. The distillate was collected in a 250 mL volumetric flask immersed in an ice bath. The distillation was performed for 90 min. The temperature of the distillate was brought to about 20 ℃, and 3.85 N HCl was added to the 250 mL mark and mixed thoroughly. 5.0 mL of the distillate was pippeted into a 50 mL volumetric flask and 25.0 mL of orcinol reagent was added. The content was then mixed and placed in a water bath at 25 ±1 ℃. After 60 min, the ethanol was added up to the 50 mL mark, mixed, and returned to the waterbath. After another 60 min, the absorbance of the solution was measured with a spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Japan) at 630 nm. The mass of xylan (anhydroxylose) in the sample was calculated according to the calibration curve previously prepared using xylose as a standard. The mass of xylan is thus calculated according to the formula:

$$\text{xylan [mg]} = \text{xylose [mg]} \times 0.88$$

The percentage of pentosans was calculated according to the formula:

$$\text{pentosans [\%]} = A / 10W$$

where A is amount of xylan in the sample [mg]
W is oven-dry weight of the sample [g].

2.3. Results and Discussion

All of five investigated white-rot fungi were able to grow on sugar beet waste without addition of additional carbon or nitrogen source. The growth of white-rot fungi was observed by visual change of substrate as well as the increase of nitrogen content (data not shown). The results of pentosans content during fermentation process are presented in Fig. 1. The initial amount of pentosans from SBP was calculated to be 21.7 % in dry matter. According to the obtained results it is visible that all five strains of white-rot fungi can successfully utilize pentosans from SBP. The decrease of the pentosans after 10 days was detected only in the experiment performed with *T. versicolor*, while no change or even slightly increase of pentosan content after 10 days was observed in all other experiments. Moreover, the increase in glucose concentration as well as the deliberation of arabinose (data not shown) was also noticed during *T. versicolor* growth which indicates that both of hemicellulose sugar polymers (hexosans and pentosans) were degraded. The highest conversion of pentosans (X = 82.7 %) was obtained by *T. versicolor* (Table 1.) while the lowest conversion (X = 18.8 %) was obtained by *P. chrysosoporum* (2) (Table 1.).

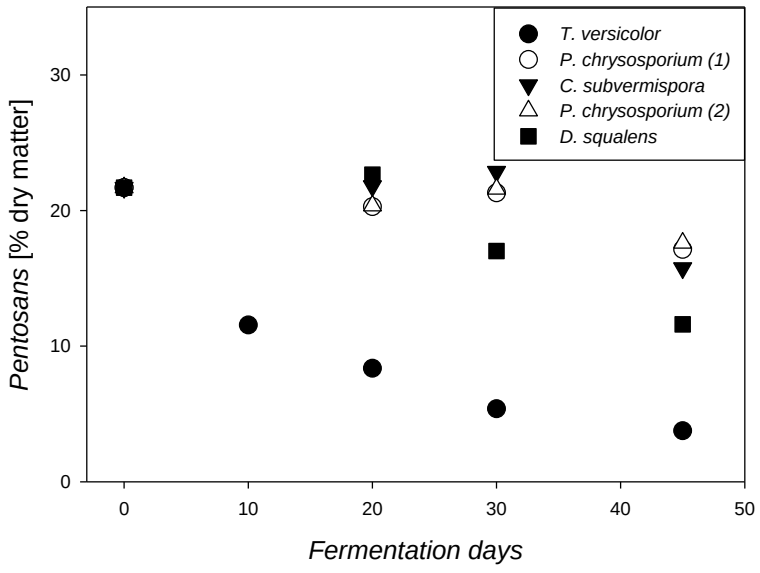

Fig. 1: Content of pentosans in SBP during solid-state fermentation

Table 1. Conversion of pentosans after 45 days of cultivation of different white-rot fungi on SBP

White-rot fungi	*T. versicolor*	*P. chrysosporium* (1)	*P. chrysosporium* (2)	*C. subvermispora*	*D. squalens*
X (% dry matter)	82.7	21.0	18.8	27.3	46.5

According to the literature data, conversion of hemicellulose into value-added useful products by enzymatic and/or fermentation routes holds strong promise for the use of a variety of agricultural residues for practical purposes, as was given in review paper by Saha [16]. Gai-Yun Li et al. [17] published the paper on degradation of pentosans (from 8.58 - 14.95 %) from masson pine wood by *Wolfiporia cocos*. Tanaka et al. [13] reported 17.5 - 21.3 % degradation of pentosans from japanese beech wood by *Ceriporiopsis subvermispora* and 9.3 - 16 % from japanese cedar wood. Furthermore, 21.9 % of hemicellulose degradation was reached by cultivation of *T. versicolor* on corn straw during 21 days by Zhu et al. [18], while up to 18 % of hemicellulose degradation occured after 24 days of solid-state fermentation by *P. chrysosporium* in straw contaminated with different concentration of Pb what indicates that *P. chrysosporium* could also promote lignocellulosic wastes bioconversion and carbon cycle under heavy metal pollution [19].

2.1. Conclusion

All five microorganisms were able to utilize pentosans from sugar beet pulp and showed remarkable growth on this substrate. *T. versicolor* exhibited the best pentosans degradation capacity, with only 3.75 % of pentosans in dry matter determined at the end of fermentation. Further research is needed to determine the development and fate of hemicellulose sugar polymers degradation products. Employing white-rot fungi in solid-state conditions for extensive hemicellulose sugar polymers degradation in order to prepare lignocellulose waste material for further cellulose degradation and use as substrate for bioproducts and bioethanol production could be a good process strategy.

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