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Biocatalytic approach for the utilization of hemicellulose for ethanol production from agricultural residue using thermostable xylanase and thermotolerant yeast

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ABSTRACT

A hydrolysis of 62% and 50% for OSX (Oat spelt xylan) and WBH (Wheat bran hemicellulose) were obtained in 36 h and 48 h using Accellerase™ 1000 at 50 °C wherein thermostable xylanase from alkalothermophilic *Thermomonospora* sp. yielded 67% (OSX) in 3 h and 58% (WBH) in 24 h at 60 °C, favouring a reduction in process time and enzyme dosage. The rate of hydrolysis with thermostable xylanase was increased by 20% with the addition of nonionic surfactant tween 80 or biosurfactant sophorolipid. The simultaneous saccharification and fermentation (SSF) of OSX and WBH using thermostable xylanase and *D. hansenii* in batch cultures produced 9.1 g/L and 9.5 g/L of ethanol, respectively and had a shorter overall process time than the separate hydrolysis and fermentation (SHF). The immobilized yeast cells in Ca-alginate matrix produced ethanol with a yield of 0.46 g/g from hemicellulosic hydrolysates and were reused six times with 100% fermentation efficiency.

1. Introduction

In collation to first generation bioethanol from starch and molasses, the development of second generation bioethanol from lignocellulosic biomass serves many advantages from both energetic and environmental point of views (Zhang et al., 2009). Lignocellulosic biomass in general comprises of 40–50% cellulose, 25–30% hemicellulose and 15–20% lignin (Wiselogel et al., 1996). The effective utilization of all the three components would play a significant role in the economic viability of cellulose to ethanol process. Cellulose bioconversion to sugars/ethanol requires mainly three steps, the first and the most crucial step being pretreatment and the second being hydrolysis by cellulase complex and then fermentation. Most of the effective pretreatment processes involving acid, alkali or solvents, remove lignin and to a large extent hemicellulose is not utilized (Hendriks and Zeeman, 2009). Conventionally the acid hydrolysis pretreatment is used for the removal of hemicellulose for further production of ethanol and value added products. During the process a range of inhibitory compounds are generated which are degradation products of hemicellulose and lignin (Almeida et al., 2007). However enzymatic hydrolysis of hemicellulose to monomeric sugars will minimize the presence of inhibitory compounds enabling better fermentation efficiency and cleaner process.

Xylan is the major constituent of hemicellulose, the second most abundant renewable resource and is a heteropolysaccharides with a homopolymeric backbone of β-D-xylose (Kulkarni et al., 1999). Thermostable xylanases offer advantages in the hydrolysis of hemicellulosic substrates over their mesophilic counterparts, higher specific activity decreases the amount of enzymes, enhanced stability allowing improved hydrolysis performance and increased flexibility with respect to process configuration, all contributing towards the overall improvement of the economy of the process (Viikari et al., 2007). The enzymatic saccharification at higher temperature would potentially reduce the reaction time and the enzyme loading.

Considering the significance of xylose fermentation to ethanol at higher temperature we have isolated thermotolerant yeast from rotten grapes. The yeast is identified to be *Debaromyces hansenii*, grows and ferments xylose at 40 °C producing predominantly ethanol under the experimental conditions.

When enzymatic hydrolysis is performed together with fermentation, it is referred to as simultaneous saccharification and fermentation (SSF) (Takagi et al., 1997). However, the process steps can also be performed sequentially, i.e. separate hydrolysis and fermentation (SHF). The avoidance of end products inhibition and thereby increasing the saccharification rate and the ethanol yield are one of the significant reasons for using SSF; however there are several additional potential advantages as the presence of ethanol in the culture medium causes the mixture to be less vulnerable to invasion by undesired microorganisms. In addition the

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combination of hydrolysis and fermentation decreases the number of vessels needed and thereby reduces the investment costs. The decrease in capital investment has been estimated to be larger than 20% (Olofsson et al., 2008). The disadvantage being the hydrolysis has to be carried out at lower temperature to be compatible with the yeast fermentation system.

From environmental engineering aspects, various waste and under-utilized lignocellulosic agricultural residues can serve as feed stocks for production of biofuel, as the economic value of these by-products as animal feeds is decreasing (Lynd et al., 2005). Wheat bran (WB) a by-product of wheat milling industry, constitutes a significant under-utilized source of sugars and is similar to other high hemicellulosic biomass such as corn fiber, both of which represents a potential low cost feed stock for production of ethanol (Maes and Delcour, 2001; Palmarola-Adrados et al., 2005). Approximately 78.40 million metric tones of wheat is produced in India (world production, 607 million metric tones), from which almost 15.68 million metric tones of bran is generated (www.igc.org.uk).

The current investigations explore the potential of enzymatic hydrolysis of hemicellulose from agricultural by-product, wheat bran using a thermostable hemicellulase from an alkalothermophilic *Thermomonospora* sp. and subsequent fermentation with a thermotolerant pentose fermenting yeast. In addition to WB, oat spelt xylan (OSX) is also studied as a model system under the consonant experimental conditions. The study also focuses for the first time the effect of biosurfactant, sophorolipid on the increased hydrolysis of hemicellulosic substrates and simultaneous saccharification and fermentation (SSF) in which hemicellulose is used together with thermotolerant yeast.

2. Methods

2.1. Raw materials

Oat spelt xylan (OSX) was purchased from Sigma–Aldrich Co., St. Louis, MO USA and Wheat bran (WB) was obtained locally. The proximate composition of OSX is 75% xylose, 10% arabinose and 15% glucose (Sigma) and of WB is hemicellulose 38.50 wt.%, cellulose 13 wt.%, starch 21.95 wt.%, klason lignin 9.35 wt.% and crude protein 17.30 wt.% (Miron et al., 2001). WB was washed and boiled in water at 60 °C for 30 min and was passed through several hot water washes. Subsequently was air dried and used in hydrolysis experiments.

2.2. Isolation of hemicellulose from WB

The washed wheat bran was processed to obtain wheat bran hemicellulose (WBH). The washed bran (2%) was treated with alkaline hydrogen peroxide solution (pH 11.5) at 2% concentration and 60 °C for 4 h (Almeida et al., 2007; Maes and Delcour, 2001). The solution was centrifuged at 4800*g* for 15 min and the supernatant was treated with ethanol in a ratio of 3:1. The precipitated hemicellulose was separated by centrifugation at 9600*g* for 15 min at 4 °C. The precipitate was dried under vacuum to remove traces of ethanol. The dried powder was used for hydrolysis.

2.3. Chemicals

All chemicals were of analytical grade. 3,5-dinitrosalysilic acid (DNS) was obtained from Sigma–Aldrich Co., St. Louis, MO USA. Ethanol was purchased from Les Alcools De Commerce Inc., Brampton, Ontario.

2.4. Microorganisms and cultivation conditions

Alkalothermophilic *Thermomonospora* sp. was maintained on Luria Bertani wheat bran slants at pH 10 and 50 °C according to George et al. (2001a).

2.5. Thermotolerant yeast

Thermotolerant yeast was isolated from rotten grapes by enrichment in a media containing (g/L): xylose, 20.0; malt extract, 3.0; yeast extract, 3.0; peptone, 5.0; agar, 20.0; at pH 6.0 ± 0.2 at 40 °C. After enrichment for 48 h, the sample was used to isolate the yeast. The yeast was further purified by single colony plating. The yeast was identified as *Debaromyces hansenii* by 18*s* rDNA. Inoculum was prepared by growing the organism on a rotary shaker at 172 g for 48 h at 40 °C, in a growth medium containing (g/L): xylose, 40.0; malt extract, 3.0; yeast extract, 3.0; peptone, 5.0; agar, 20.0; at pH 6.0 ± 0.2. After 48 h the inoculum was centrifuged at 4800*g* for 20 min and the supernatant was discarded. The cells were washed twice with sterile 0.9% NaCl solution and centrifuged again. The supernatant was removed and the cells were weighed and used for fermentation studies.

2.6. Enzyme production

Thermomonospora was grown in a modified Reses medium at 50 °C for 96 h on a rotary shaker maintained at 180*g*. At the end of 96 h the fermentation broth was centrifuged at 9600*g* for 15 min at 4 °C. The supernatant was concentrated using ammonium sulphate (0–90%) and was the source of enzyme. The preparation contained 120 U/ml and 0.3 U/ml of xylanase and β-xylosidase, respectively.

The Commercial enzyme Accellerase™ 1000 was a kind gift from Dr. Raj Lad and Dr. Surendra Bade, Danisco US Inc., Genencor Division, USA. The enzyme showed 2250 U/ml of xylanase and 0.6 U/ml of β-xylosidase.

2.7. Enzyme assay

Xylanase and β-xylosidase were measured according to standard procedure recommended by Commission on Biotechnology, IUPAC (Ghose and Bisaria, 1987). One unit of enzyme activity is defined as the amount of enzyme required to liberate one μ mole of reducing sugar per minute under the assay conditions.

2.8. Immobilization of debaryomyces in Ca-alginate beads

D. hansenii cells were harvested after 48 h of growth at 40 °C by centrifugation at 4800*g* for 20 min, washed and 1 g (wet cell weight) was added to 10 ml of 2% (w/v) Na-alginate as described by Kierstan and Bucke (1977). The suspension was then extruded dropwise through a 5 ml syringe into a gently stirred cold solution of $CaCl_2$ and hardened at 4 °C for 1 h in this solution. Particle integrity and absence of microbial contamination were ensured by means of optical microscopy. The beads (mean diameter of 2 mm) were washed with distilled water and used for fermentation.

2.9. Enzymatic hydrolysis

The hydrolysis of oat spelt xylan (OSX), wheat bran (WB) and wheat bran hemicellulose (WBH) were carried out at different substrate concentrations (2.5% and 5%) with two concentrations (150 and 300 U/g) of *Thermomonopsora* xylanase and commercial enzyme from Accellerase™ 1000 at various temperatures (40, 50, 60 and 70 °C). The hydrolysis was carried out in a stoppered flask in 25 ml reaction volume containing appropriate concentrations