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Hemicellulose sugar recovery from steam-exploded wheat straw for microbial oil production

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ABSTRACT

There are currently few successful examples of using straw hemicellulose as a carbon source in the fermentation industry. In this paper, hemicellulose hydrolysates were recovered from steam-exploded wheat straw (SEWS) and used to produce microbial oil. The effects of the steam explosion treatment conditions, the elution temperature and the ratio of elution water to SEWS on sugar recovery were examined. A broth with 3.8 g l^{-1} of reducing sugar and 22.3 g l^{-1} of total soluble sugars was obtained with a 10-fold excess (w/w) of water at 40 °C to wash the SEWS treated under steam explosion conditions at 200 °C for 5 min. This broth was used to produce microbial oil by the oleaginous fungus *Microsphaeropsis* sp., which was able to secrete xylanase to degrade oligosaccharides from straw hemicellulose and accumulate microbial oil. Under optimized conditions, the oil concentration was 2.6 g l^{-1}. The yield of oil from sugar consumed was 0.14 g g^{-1}. The microbial oil produced by this research could be used as feedstock for biodiesel production because the microbial oil was primarily composed of neutral lipids. This research establishes a novel protocol for microbial oil production from straw hemicellulose.

1. Introduction

Straw is an abundant source of hexose (C-6) and pentose (C-5) sugars with a potential for use in the production of biofuels, chemicals and biobased materials. The structure of the straw cell wall is tight because the main components of straw, cellulose, hemicellulose and lignin, are held together with different bonds. The main linkage between cellulose and hemicellulose or lignin is hydrogen bond, while hemicellulose and lignin are connected through chemical bonds [1]. Therefore, it is difficult to fractionate and utilize the natural straw efficiently. It is necessary to pretreat straw before its bioconversion. Steam explosion is an efficient and widely used pretreatment method [2,3]. After steam explosion pretreatment, the tight physical structure of the straw is disrupted. As a result, the fractionation of straw becomes easier, and approximately 80% of the hemicellulose is decomposed into soluble sugars such as xylose and oligosaccharides [3].

Production of ethanol from straw has been researched extensively in recent years because ethanol is a renewable and clean fuel and can be used as a substitute for fossil fuels [4]. The main component of straw that can be used for the production of ethanol is cellulose. Although some genetically engineered strains that can produce ethanol from xylose have been developed, the effect of fermentation is not efficient enough [5]. However, the hemicellulose, which accounts for about 30% of the straw, has not been effectively used. Steam explosion pretreatment can induce the degradation of hemicellulose, but at the same time, can produce compounds, such as furfural and phenols, that can inhibit the growth of some microorganisms [6,7]. Therefore, in some cases, the steam-exploded straw was washed to remove inhibitors [6], but this results in a loss of the soluble sugars produced from degradation of hemicellulose. An effective use of the washing solution must be determined to increase the viability of biofuel production from straw. It is necessary to explore suitable approaches to high-value usage of these hemicellulose sugars.

Biodiesel, another important biofuel, is also currently the focus of intense research. However, the limited availability of fat and oil resources curtails its development. The identification of new fat and oil resources is one of the main areas for improvement in the biodiesel industry. Although vegetable oils are currently used as the main biodiesel feedstock, microbial oils, also called single cell oils (SCO), provide a viable potential feedstock for the production of biodiesel. Producing biodiesel from microbial oils has several advantages, including the potential industrialization of microbial oil production, and the fact that microbe cultivation does not require additional land. Recently, increasingly more research [8–11] has focused on microbial oil production for the purpose of making biodiesel.

At present, the high production cost of microbial oil makes it an undesirable feedstock for biodiesel production. One of the main

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costs of microbial oil production is that of the carbon source. To reduce the production cost, cheap carbon sources have to be used. Oleaginous microorganisms have comprehensive carbon sources. Glucose, lactose, starches, oils, corn steep liquor and agricultural produce have been used as carbon sources for production of lipids from fungi [12]. Some oleaginous microorganisms can use xylose as the carbon source to produce microbial oil [13]. The sugars from hemicellulose may be perfect carbon sources for microbial oil production.

In this work, we have evaluated the soluble sugar recovery from steam-exploded wheat straw. The soluble sugars isolated in this fashion were used to produce microbial oil by an oleaginous fungus, *Microsphaeropsis* sp.

2. Materials and methods

2.1. Microorganism

Microsphaeropsis sp., the microorganism used in the present investigation, is an endophytic fungus isolated from fresh stems of *Sabina chinensis* (Lin.) Ant. collected from Handan, Hebei in northern China in February 2005. It could accumulate oil when cultured on a straw-based solid-state medium [11,14]. The stock culture was maintained on a potato dextrose agar (PDA) slant at 4 °C.

2.2. Steam-exploded wheat straw

Steam-exploded wheat straw (SEWS) used in this research was prepared by treating chopped wheat straw (3–4 cm, containing 15% water) in a steam explosion vessel (1 m^3) at 195–211 °C (1.4–1.9 MPa) for 5 min followed by sudden discharge [15]. After this pretreatment, the solid residue was dried at 60 °C for 24 h and used in this study.

2.3. Recovery of the soluble sugars

Dry SEWS was dipped in a confined container containing a 5- to 15-fold excess (w/w) of deionized water at 30–80 °C for 1 h and then filtered with gauze. The supernatant was harvested after centrifugation. The concentrations of sugars in the supernatants and the sugar recovery under different treatment conditions were investigated.

$$\text{Recovery rate of sugars} = \frac{\text{mass of sugars in the supernatant}}{\text{mass of the initial dry SEWS}} \times 100\%$$

2.4. Fermentation

The supernatants obtained from steam explosion were used as culture media for microbial oil production after adding a suitable nitrogen source (yeast extract or peptone) and the following inorganic salts: $K_2HPO_4{\cdot}3H_2O$ 4 g l^{-1}; $CaCl_2{\cdot}2H_2O$ 0.1 g l^{-1}; $MgSO_4{\cdot}7H_2O$ 0.5 g l^{-1}. The pH was adjusted to 5–9 with sodium hydroxide and hydrochloric acid before autoclaving.

The cultures were grown in 300-ml Erlenmeyer flasks with 50 ml of medium. After being autoclaved at 121 °C for 20 min, the cultures were cooled and inoculated with 1 ml spore suspension (10^6 spores per ml) of *Microsphaeropsis* sp. from a 7-day-old culture on a PDA slope. The cultures were incubated at 30 °C on a rotary shaker at 180 rpm.

2.5. Analytical methods

2.5.1. Analysis of sugars

The reducing and soluble sugars were measured by the dinitrosalicylic acid (DNS) method [16] and the phenol sulfuric acid method of Dubois et al. [17], respectively.

2.5.2. Analysis of xylanase

Xylanase activities were assayed by Bailey's methods [18]. The enzyme concentration was expressed in international units (U), which denote the micromoles of xylose released per minute of the reaction.

2.5.3. Analysis of biomass and oil yields

The mycelia were collected after cultivation and washed with running water, then dried at 80 °C for 6 h and weighed. The dry mycelia were refluxed with a solvent containing chloroform and methanol (2:1, v/v) in a Soxlet for 6 h. The oil was collected and weighed after the solvent was evaporated under vacuum. The cell oil content was calculated by the weight of the oil divided by the weight of the dry mycelia.

2.5.4. Analysis of fatty acids

The analysis of fatty acids proceeded as follows. Oil extracts were converted to methyl esters by treatment with a 0.5 M NaOH methanolic solution, followed by heating at 80 °C for 5 min and addition of a BF_3/methanol complex. The mixture was boiled for 2 min and extracted with hexane. Fatty acid methyl esters were detected using a gas chromatograph (Agilent 4890 D) equipped with an HP-INNOWax capillary column (30 m × 0.25 mm × 0.25 μm) and a flame ionization detector. The fatty acids extracted from microbial oil were identified by matching their retention time data with those of the Sigma standards.

2.5.5. Fractionation of microbial oil

Microbial oils were fractionated according to the method of Fakas et al. [19]. A known weight of extracted microbial oil (approximately 500 mg) was dissolved in chloroform (5 ml) and fractionated using a column (25 mm × 150 mm) of silicic acid (5 g), which had been activated by heating overnight at 110 °C. Successive applications of 1,1,1-trichloroethane (500 ml), acetone (500 ml), and methanol (300 ml) produced fractions containing neutral lipids (N), glycolipids plus sphingolipids (G + S), and phospholipids (P), respectively. The weight of each fraction was determined after evaporation of the respective solvent.

3. Results and discussion

3.1. Recovery of sugars from SEWS

3.1.1. Effect of water to SEWS ratio on sugar recovery

Choosing an effective ratio of water to SEWS for the elution of soluble sugars is important because excessive water will lead to a low concentration of sugars that is not conducive to fermentation. A low ratio of water to SEWS, however, will also lead to a low sugar recovery rate because the oligosaccharides in SEWS cannot be sufficiently dissolved in water at such a condition. The effects of water to SEWS ratio (5:1–15:1 w/w) on reducing sugar and total soluble sugar concentrations present in the washing solution, along with the recovery rate of total soluble sugars, were investigated at an elution temperature of 30 °C. The SEWS used in this experiment was pretreated under the steam explosion condition of 200 °C for 5 min.

As can be seen from Fig. 1a, when the ratio of water to SEWS increased, the reducing sugar and total soluble sugar concentrations decreased, while the recovery rate of total soluble sugars increased. When the ratio of water to SEWS was 5:1 (w/w), the concentration of total soluble sugars was somewhat high (30.4 g l^{-1}), but the recovery rate of total soluble sugars was low (15.2%). When the ratio of water to SEWS was 10:1 (w/w), the concentration of total soluble sugars was low (20.3 g l^{-1}), but the recovery rate of total soluble sugars increased to 20.3%. Continuously increasing the amount of water to a 15-fold excess over the SEWS caused the concentration of total soluble sugars to decrease rapidly from 20.3 g l^{-1} to 14.6 g l^{-1}, but the recovery rate of the total soluble sugars only slightly increased from 20.3% to 22.0%. We selected a 10:1 (w/w) ratio of water to SEWS for the following experiments.

3.1.2. Effect of washing temperature on sugar recovery

As the adsorption between the sugars and lignocellulose is affected by temperature, the elution temperature has a certain impact on the sugar concentration of the washing solution and recovery rate of total soluble sugars. Fig. 1b shows that, when the elution temperature increased from 30 °C to 80 °C, the concentrations of total soluble sugars and reducing sugars and the recovery rate of total sugars are all increased. When the elution temperature increased from 30 °C to 40 °C, the recovery rate of total soluble sugars increased from 20.3% to 22.4%. However, when the elution temperature increased from 40 °C to 80 °C, the yield of total soluble sugars increased slowly from 22.9% to 23.5%. Due to energy efficiency considerations, an elution temperature of 40 °C was selected for the following experiments.