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Process Biochemistry 41 (2006) 869–873

PROCESS BIOCHEMISTRY

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Production of ethanol from microwave-assisted alkali pretreated wheat straw

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Received 12 August 2005; received in revised form 8 October 2005; accepted 21 October 2005

Abstract

In order to evaluate the suitability of production of ethanol from the microwave-assisted alkali pretreated wheat straw, the simultaneous saccharification and fermentation (SSF) of the microwave-assisted and conventional alkali pretreated wheat straw to ethanol were optimized and compared using cellulase from *Trichoderma reesei* and *Saccharomyces cerevisiae* YC-097 cells. The SSF optima for the microwave-assisted alkali pretreated wheat straw were 100 g l^{-1} substrate, 40 °C, 15 mg [cellulase] g^{-1} [substrate], initial pH 5.3 and 72 h. Under the above optimum conditions, the ethanol concentration reached 34.3 g l^{-1} and the ethanol yield was 69.3%. The SSF optima for the conventional alkali pretreated wheat straw were 100 g l^{-1} substrate, 40 °C, 20 mg [cellulase] g^{-1} [substrate], initial pH 5.3 and 96 h. Under its optimum conditions, the ethanol concentration and the ethanol yield were 31.1 g l^{-1} and 64.8%, respectively. It was obvious that production of ethanol from microwave-assisted pretreated wheat straw had lower enzyme loading, shorter reaction time and could achieve higher ethanol concentration and yield than from the conventional alkali pretreated wheat straw. The microwave-assisted alkali pretreatment was an efficient pretreatment method of wheat straw for its ethanol production.

Keywords: Simultaneous saccharification and fermentation; Microwave-assisted alkali pretreatment; Alkali pretreatment; Wheat straw; Ethanol

1. Introduction

Energy consumption has increased steadily as the world population has grown and more countries has become industrialized. The fossil fuels, such as crude oil, coal and natural gas have been the major resources to meet the increased energy demand. However, they are gradually being depleted to extinction because they are not renewable. Moreover, serious environmental and ecological problems have been aroused during their exploitation and use. Therefore, there is great interest in exploring alternative energy sources to maintain the sustainable growth of society [1]. Ethanol, a clean and renewable energy source, which can be produced through fermentation from renewable biomass, has drawn much attention from the government and researchers [2]. Apart from an alternative to traditional energy sources, ethanol has been widely used as a solvent or feed stock in industries such as chemicals and pharmaceuticals [3]. However, fermentative production of ethanol has been limited using current maize starch-based technology because of raw materials shortage and high cost. A potential method for low-cost fermentative production of ethanol is to utilize lignocellulosic materials such as agricultural wastes [3]. Production of ethanol from wheat straw, one of the most abundant agricultural wastes, has been extensively studied [4–6]. The conversion of wheat straw to ethanol includes three sub-processes: pretreatment, saccharification and fermentation. Pretreatment of wheat straw greatly affects its saccharification efficiency and ethanol production cost. Among many pretreatment methods of wheat straw, the microwave-assisted alkali pretreatment has proven one of the most efficient pretreatment methods for its saccharification [7]. Besides the pretreatment, the process used for saccharification and fermentation is also an important factor affecting the ethanol production cost from wheat straw. There are many

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doi:10.1016/j.procbio.2005.10.024

reports that the simultaneous saccharification and fermentation (SSF) is superior to the traditional saccharification and subsequent fermentation in the production of ethanol from lignocellulosic materials because the SSF process can improve ethanol yields by removing end-product inhibition of saccharification process and eliminate the need for separate reactors for saccharification and fermentation [3,4,7]. To our knowledge, there are no reports on ethanol production from microwave-assisted alkali pretreated wheat straw. In this study, to evaluate the suitability of production of ethanol from the microwave-assisted alkali pretreated wheat straw, the SSF of the microwave-assisted and conventional alkali pretreated wheat straw to ethanol were optimized and compared using cellulase from *Trichoderma reesei* and *Saccharomyces cerevisiae* YC-097 cells.

2. Materials and methods

All experiments were carried out three times and the given numbers are the mean values. The composition of raw and pretreated wheat straw was expressed on the wet basis throughout this work.

2.1. Materials and chemicals

Raw wheat straw was obtained from local farmers in Badong, Hubei province, PR China. Before any pretreatment it was cut to nominally 1–2 cm length and washed thoroughly with tap water until the washings were clean and colorless and then air dried for further treatment. The main composition of this wheat straw was as follows: moisture, $11.2 \pm 0.2\%$; cellulose, $41.2 \pm 0.5\%$; lignin, $21.3 \pm 0.4\%$; hemicellulose, $25.8 \pm 0.5\%$.

The cellulase enzyme used in this study was a commercial *T. reesei* cellulase (formerly called *Trichoderma viride* cellulase) from Shanghai Boao Biotech. Corporation, Shanghai, PR China. Its CMC-ase activity was 15 IU/mg, measured as the initial rate of reducing sugars formation during hydrolysis of 0.5% CMC at pH 5.0 and 50 °C [8]. Its filter-paper activity was 0.53 FPU/mg, determined following the standard procedure recommended by the Comission on Biotechnology, IUPAC [9]. And its cellobiase activity was 0.19 CBU/mg, measured as the initial rate of hydrolysis of 2 mM cellobise to glucose at pH 5.0 and 50 °C [10].

All other chemicals employed in this study were of reagent grade and purchased from Wuhan Chemicals and Reagent Corporation, Wuhan, PR China.

2.2. Conventional alkali pretreatment

Twenty grams samples of wheat straw were suspended in 160 ml of 1% NaOH aqueous solution after cutting and washing and kept boiling in a 500 ml beaker for 60 min. The residues were collected and washed extensively with tap water until neutral pH, dried at 65 °C for two days. Then they were cut to 10–20 mesh and used as the substrate of the SSF to ethanol. Their cellulose and hemicellulose content was 73.5 ± 0.7 and $11.2 \pm 0.5\%$, respectively.

2.3. Microwave-assisted alkali pretreatment

Twenty grams of wheat straw after cutting and washing was suspended in 160 ml of 1% NaOH aqueous solution in a 500 ml beaker and the beaker was positioned at the centre of a rotating circular glass plate in a domestic microwave oven (LG TIANJIN Model WD700, 2450 MHz) for microwave treatment. The applied microwave power was 700 W for 25 min. The residues were collected and then treated as above conventional alkali pretreatment for washing, drying and cutting and used as the substrate of the SSF to ethanol. Their cellulose and hemicellulose content was 79.6 ± 0.6 and $7.8 \pm 0.5\%$, respectively.

2.4. Preparation of yeast inoculum

The yeast, *S. cerevisiae* YC-097, was used throughout this study. Inoculum was prepared by transferring the organisms maintained on MGYP medium (malt extract, 3 g l^{-1}; glucose, 10 g l^{-1}; yeast extract, 5 g l^{-1}; peptone, 5 g l^{-1}; agar, 24 g l^{-1}) into 250 ml flask with 100 ml basal medium having 36 g l^{-1} glucose. The growth was carried out at 33 °C on an orbital shaker for 12 h. The inoculum concentration was about 1.5×10^8 yeast cells ml^{-1} and the amount of inoculum added was 10% (v/v) of the SSF medium.

2.5. Simultaneous saccharification and fermentation

Simultaneous saccharification and fermentation (SSF) reaction mixtures contained the pretreated wheat straw (conventional or microwave-assisted alkali pretreated), cellulase, 10% (v/v) yeast inoculum and basal medium to make up the volume to 100 ml in 250 ml flasks. The initial medium pH was adjusted to a given value with 1.0 mol l^{-1} HCl or NaOH solution. The reaction mixtures were incubated at a given temperature on an orbital shaker at 160 min^{-1}. Samples were aseptically taken at regular intervals for the analysis of reducing sugar, glucose, xylose and ethanol content. The ethanol yield (Y) was calculated as follows:

$$Y = \frac{E0.9}{S0.51} \times 100 \quad (1)$$

where E is ethanol concentration (g l^{-1}) and S is carbohydrates (cellulose and hemicellulose) concentration in substrate (g l^{-1}).

2.6. Orthogonal experiments

The main affecting factors of the SSF of pretreated wheat straw to ethanol are substrate concentration, initial medium pH, temperature, enzyme loading and reaction time. According to the results of our preliminary study, the suitable scope for substrate concentration, initial medium pH, temperature and enzyme loading during the SSF was 60–100 g l^{-1}, 4.8–5.8, 35–45 °C and 10–20 mg g^{-1} substrate, respectively [7]. The reaction time of the SSF was 72 h for the microwave-assisted alkali pretreated wheat straw and 96 h for the conventional alkali pretreated one. In order to optimize the SSF conditions, the "$L_9(3^4)$" orthogonal table [11] was chosen in this work, factors and their levels were designed in Table 1.

2.7. Analytic methods

The samples taken from the SSF were centrifuged and the supernatants were used to analyze the reducing sugar, glucose, xylose and ethanol concentration. The reducing sugar concentration was estimated using 3,5-dinitrosalicylic acid (DNS) method [12]. The glucose content was determined following the procedure described by McCleary et al. [13]. The xylose content was analyzed according to the method of Ashwell [14]. The ethanol content was determined by gas chromatography as described by Krishna et al. [3]. The moisture was

Table 1
Factors and levels of the orthogonal experiment

Level	Substrate concentration (g l^{-1})	Initial medium (pH)	Temperature (°C)	Enzyme loading (mg g^{-1} substrate)
1	60	4.8	35	10
2	80	5.3	40	15
3	100	5.8	45	20