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Optimisation of simultaneous saccharification and fermentation of wheat straw for ethanol production

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ABSTRACT

With the goal of obtaining a high ethanol yield, wheat straw (WS) was pretreated with a 1.0% (w/w) NaOH solution. Further parameters, including temperature, enzyme loading, yeast concentration and pH that would affect the ethanol yield through simultaneous saccharification and fermentation (SSF), were optimised by means of Box-Behnken design (BBD), a response surface methodology (RSM). All of the experiments were carried out with an initial solid content of 16.0% (w/v) and fermentation time of 120 h. A well fitted regression equation with an R^2 value of 0.9603 was obtained. The predicted maximum response value (ethanol yield) was 69.49% under the optimised conditions for SSF: temperature of 37.5 °C, enzyme loading of 30 FPU/g substrate, yeast concentration of 10 g/L and pH of 4.6. The validation experimental result showed that the maximum ethanol yield was 70.76%, which was in accordance with the predicted value.

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1. Introduction

During the last few years, the energy crisis and environmental problems have become the greatest challenges for society. The intense energy situation, coupled with severe environmental problems such as global warming and air pollution, have forced the world to look for green, non-polluting and sustainable energy resources. Bioethanol made by fermentation from a variety of biomass resources is widely recognised as one of the most unique transportation fuels, with powerful economic, environmental and strategic benefits [1–3]. Today, bioethanol is the most dominant biofuel and its production has increased over the last 25 years, with a sharp increase from 2000 onward [4].

However, the large scale production of first generation bioethanol based on corn or other food grain resources has directly driven up food prices and raised concerns of a food crisis [2,5,6]. In addition, some studies have found that the first generation bioethanol production will actually increase greenhouse gas (GHG) emissions [7]. In contrast, using lignocellulosic agricultural residues such as wheat straw to produce ethanol is advantageous because the resources are abundant, cheap and renewable. Furthermore, ethanol produced in this way can avoid limiting the quantity of food stuff, incur little or no carbon debt and offer immediate and sustained GHG advantages [8–10]. Wheat straw is one of the most abundant lignocellulosic agricultural residues in the world. In 2008, approximately 850 Tg of wheat residues were produced [4]. Hence, bioethanol production from wheat straw cannot only relieve the energy crisis but also generate environmental and economic benefits.

Wheat straw, as a typical lignocellulosic biomass, has a complex and tightly packed structure consisting of three main components: cellulose, hemicellulose and lignin. Pretreatment is currently viewed as a critical step to produce ethanol from lignocellulosic biomass. The process includes four main processes: pretreatment, enzymatic hydrolysis, fermentation and separation. The main purpose of pretreatment is to break the lignin seal and disrupt the crystalline structure of the cellulose [11]. Dilute NaOH pretreatment is one of the most efficient pretreatment methods. The major effect of NaOH pretreatment is the delignification of lignocellulosic biomass while retaining most of the hemicellulose, thus enhancing the reactivity of the remaining carbohydrates. Furthermore, NaOH pretreatment can make the material swell and improve the accessibility of biomass polysaccharides to enzymatic hydrolysis [12].

Simultaneous saccharification and fermentation (SSF) is considered to be an ideal process for ethanol production. It requires less investment and a lower level of enzyme loading because the yeast fermentation step helps reduce end product inhibition from the cellubiose and glucose formed during enzymatic hydrolysis [13–15]. Although SSF technology has many advantages, there are still some difficulties to be solved. The main factors affecting the SSF process are temperature, enzyme loading, yeast concentration, pH, solid content and yeast strain. [16] Temperature is a crucial factor for SSF because of the difference between the optimum

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temperatures for enzymatic hydrolysis (45–50 °C) and fermentation (30–35 °C) [17]. The use of thermotolerant yeast strain which can grow at higher temperature is preferable. Temperature and pH play an important role in affecting the enzymatic activity and cell growth. Moreover, pH determining the fermentation pathway used in anaerobic ethanol production processes [18]. High amounts of yeast concentration can help to obtain a high ethanol yield in a short fermentation time. However, due to the hindered access to nutrients and the assimilation of ethanol which would decrease the final ethanol yield, a much higher yeast concentration is not favourable in the SSF process. Generally, an increase in enzyme loading can result in increased substrate conversion [19], but high enzyme loading is not economic. Apparently, it is a prior necessity to optimise the fermentation parameters in order to make the SSF process running in an economic and efficient way. In addition, since the distillation cost per unit amount of ethanol produced is substantially higher at lower ethanol concentrations, it is important to increase the initial solid content. It has been reported that a solid content of approximately 14–15% (w/v) is desirable to reduce the distillation costs [20]. Obviously, it is necessary to find a solution for the solid content and mixing difficulties.

Response surface methodology (RSM) is a useful statistical technique for the modelling and optimisation of complex reaction process. It can overcome the disadvantages of the traditional "one factor at a time" methodology which cannot interpret and analyse the combined influence of the parameters affecting the fermentation efficiency [21]. RSM has already been successfully applied to the optimisation of medium and enzymatic hydrolysis [5,22].

In this work, the wheat straw was pretreated with dilute NaOH (1.0%, w/w) at a solid content of 5.0% (w/v), temperature of 121 °C and time of 60 min to facilitate enzymatic hydrolysis of the substrate. These parameters used in the pretreatment process were optimised in the previous experiments in our laboratory to obtain relatively high enzymatic hydrolysis yield (data not shown). The surface shape of the substrate before and after the NaOH pretreatment process was observed using scanning electron microscopy (SEM) to investigate the effects of NaOH pretreatment. Because the enzyme and fermenting microorganisms have different optimum temperature and pH requirements, optimisation of the fermentation parameters is vital to the success of the SSF process. The aim of this work was to make use of RSM of Box-Behnken design (BBD) to identify the optimum conditions for ethanol production from NaOH-pretreated wheat straw at high solid contents (up to 16.0% (w/v)) in an SSF process with thermotolerant *Saccharomyces cerevisiae* (*S. cerevisiae*) BY4742. This will be accomplished by analysing the relationships among the parameters that affect the overall process.

2. Methods

2.1. Raw material and enzyme

The wheat straw (WS) used as raw material in the experiments, with cellulose, hemicellulose and lignin contents of 39.31%, 21.51% and 25.73%, was harvested from the rural area around the Tai Hu Lake. The WS was first sliced to a suitable size (4–6 cm), and then be milled to pass through an 80-mesh screen (particle size of approximately 0.18 mm) using a laboratory hammer mill (Retsch GmbH & Co. KG, Haan, Germany). Next, the WS powder was dried in a hot-air oven at 105 °C to a constant weight for at least 24 h and stored in a sealed plastic bag at room temperature in the silica gel drier until its use for subsequent NaOH pretreatment and structural carbohydrate and lignin determination.

Commercial cellulase from *Trichoderma reesi* ATCC 26921 (Celluclast 1.5 L) used in the optimisation of the SSF process was purchased from Sigma–Aldrich, St. Louis, MO, USA. The filter paper activity (FPA) of Celluclast 1.5 L was 82.22 FPU/mL.

2.2. NaOH pretreatment

Dried constant WS powder was pretreated with 1.0% (w/w) NaOH solution with a solid content of 5.0% ((w/v), 100 g of WS power and 2 L of sodium hydroxide solution) in a sealed Erlenmeyer flask. The slurry was pretreated in an autoclave at 121 °C for 60 min. After the mixture cooled to room temperature, it was washed with deionised (DI) water and centrifuged at 4000 rpm for 10 min, and the supernatant was collected. The wash and centrifugation procedures were repeated several times until the supernatant became clear. In the last wash step, the pH was adjusted to 5.0–6.0 with 10 M NaOH. The collected solid residue was dried in a hot-air oven at 105 °C to a constant weight for at least 24 h and stored in a sealed plastic bag at room temperature in the silica gel drier for later use.

2.3. Microorganism and growth conditions

S cerevisiae BY4742 (originally from EUROSCARF, Germany) used in this study was a thermotolerant strain selected and maintained in glycerol vials at −80 °C for use as a working stock in our laboratory (School of Environmental Science and Engineering, Shanghai Jiao Tong University, China). Active cultures for inoculation were obtained in 100 mL Erlenmeyer flasks with 25 mL of growth medium containing 20 g/L glucose, 20 g/L peptone, and 10 g/L yeast extract. The liquid medium was sterilised at 121 °C for 30 min. The pre-culture was performed in a rotatory shaker at 37 °C and 180 rpm for 16 h and then used to inoculate 500 mL baffled shake-flasks containing 250 mL of the above medium with the same conditions. The cells were centrifuged at 4000 rpm for 5 min, the supernatant was decanted, and the cells were washed three times with DI water. The obtained cells were re-suspended in DI water and used as inoculum in the SSF process. The cell count was determined at OD_{600}, and the inoculum volume was determined according to the calibration curve.

2.4. Simultaneous saccharification and fermentation (SSF)

SSF experiments were carried out in duplicate in 50 mL DURAN glass bottles (SCHOTT, Germany) containing a total liquid volume of 10 mL and agitated on a rotatory shaker at 180 rpm for 120 h. Aiming to achieve high ethanol concentration, the initial solid content of all of the samples was set at 16.0% ((w/v), 1.6 g of dry pretreated WS, 10 mL of liquid), and the pH was controlled with 50 mM citrate buffer. The temperature (35–42 °C), enzyme loading (10–50 FPU/g substrate), yeast concentration (1.0–10.0 g/L) and pH (3.6–6.8) were varied based on Box-Behnken designed experiments. At the end of the SSF process, the fermentation liquor was immediately decanted to the centrifugal tub, sealed with parafilm and centrifuged at 4000 rpm for 10 min. The supernatant was filtered through 0.45 μm membrane filters to remove the cells and maintained in 2 mL bottles at −4 °C for later reducing sugar and ethanol determination by HPLC.

2.5. Analytical methods

The cellulose, hemicellulose and lignin contents were determined by quantitative saccharification upon acid hydrolysis and subsequent high performance liquid chromatography (HPLC) and gravimetric analysis, based on the standard NREL procedure [23].

Scanning electron microscopy (SEM) JSM-740F (JEOL LTD, Japan) was used to observe the WS surface shape before and after

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