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Producing bioethanol from pretreated-wood dust by simultaneous saccharification and co-fermentation process

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ABSTRACT

The aim of this study was to utilize a new and highly effective bioreactor system, *i.e.*, simultaneous saccharification and fermentation (SSCF), for bioethanol production by the cocultivation of *Trichoderma reesei*, *Aspergillus niger*, and *Zymomonas mobilis* by using a direct conversion process of pretreated-wood dust medium. Wood dust has been effectively used to obtain reducing sugars (glucose, xylose, and other byproducts) through the use of either a supercritical fluid extraction (SFE) or steam explosion (SE) pretreatment step. In addition, experimental results showed that polyurethane as a porous carrier could enhance total saccharification enzyme activity at an inoculum proportion of 1/1 of *T. reesei*, and *A. niger* and at a total inoculum concentration of 6.5×10^6 spores/ml. Furthermore, the concentration of alginate beads (3%) and immobilized proportion of *Z. mobilis* to alginate beads (1:4) were also examined. In accordance with previous reports, bioethanol production was carried out in a SSCF bioreactor by the cocultivation of *T. reesei* and *A. niger* in the polyurethane carrier and *Z. mobilis* immobilized in alginate beads using pretreated-wood dust medium. Experimental results revealed that, after 24 h of cultivation, the yield of bioethanol produced using pretreated-wood dust medium (1%) were 0.069 g/g and 0.049 g/g, for SFE and SE, respectively. Meanwhile, the sugar conversion rate reached 20.72% and 24.39% for SFE and SE, respectively. Thus, the results of this study show that pretreated-wood dust medium has significant potential for use in bioethanol production.

1. Introduction

Continued industrialization and population growth have increased annual energy consumption [1]. Increasing fossil fuel prices and greenhouse gases have motivated the research and development of renewable resources in many countries. The principal substitute for petrol in road vehicles is bioethanol. One of the advantages of using bioethanol is that doing so reduces greenhouse gas emissions. Also, blending bioethanol with petrol, as in E85, helps to extend diminishing oil supplies, increase fuel security, and eliminate heavy reliance on oil producing nations. Bioethanol is seen as the most promising prospective renewable energy source, which can be produced from microbial fermentation by converting sugars from cellulosic materials such as wood dust [2]. Lignocellulose is an abundant natural carbohydrate that can be transformed into a substitute renewable energy resource by microbial conversion [3]. The advantages of using cellulosic materials for bioethanol production are their low cost, ready availability, lack of conflict with use for food, and the potential production of fuel from lignin [4].

A pretreatment step is necessary for modifying structural characteristics of lignocellulose and increasing the availability of glucan and xylan for enzymatic saccharification [5]. The pretreatment step can destroy the lignin structure to allow for easier break down of the cellulose by the saccharification enzyme [4]. Traditional pretreatments of biomass for producing bioethanol include physical methods and chemical methods, involving liquid hot water and alkali (or acid), for example [6]. However, several disadvantages for all the different pretreatment options exist, and it is necessary to adapt suitable pretreatments based on the properties of the raw material. Physical and chemical pretreatments inhibit the

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hydrolysis of cellulose and hemicellulose and lignin fraction owing to the close among the components of lignocellulosic biomass. Therefore, in this study, steam explosion (SE) and supercritical fluid extraction (SFE) were used in this work to pretreat wood dust to avoid the aforementioned problems.

Steam explosion (SE) technology has become one of the most common and widely employed physicochemical pretreatments for lignocellulosic biomass. The process of SE, developed by William H. Mason in 1925, is used for the pretreatment of agricultural wastes, *i.e.*, wood dust in this study [5]. SE technology is a hydrothermal pretreatment that consists of three main steps: (1) the treatment step, (2) the explosion step, and (3) the impact step [5]. The first step is a process wherein lignocellulosic biomass is treated with pressurized steam for a certain period. The SFE rapid release of pressure causes the explosion of lignocellulosic biomass. Finally, the impact of the lignocellulosic biomass mixture is done to form a raw material used for enzyme hydrolysis.

Supercritical fluid extraction is also one of the pretreatment methods for disrupting the crystalline structure of lignocellulose under certain conditions such as temperature and pressure above its critical point [7]. SFE is becoming necessary to reduce the environmental hazards of common chemicals and solvents used in traditional methods. SFE shows excellent potential as a method for lignocellulosic biomass pretreatment [8]. Reports have shown that SFE has been successfully used for the pretreatment of commercial cellulosic materials, recycled paper mix, and sugarcane bagasse [7,8]. In addition, the use of SFE, as a green solvent for biomass pretreatment in a biorefinery concept, is increasing and it is expected to continue growing in the future.

Saccharification enzymes can be produced by fungi such as *Trichoderma reesei* and *Aspergillus niger*, and agricultural waste can be converted into bioethanol by *Zymomonas mobilis*. Conversion of agricultural waste reduces its environmental impacts by efficiently using it to produce secondary energy. The conversion of cellulose into monosaccharides by microorganisms has been proved and does not cause secondary environmental pollution. A modified bioreactor that supports simultaneous saccharification and cofermentation (SSCF) was used for the effective conversion of agricultural waste into bioethanol. Previous studies have reported the conversion of cellulose into monosaccharides using microorganisms [9,10]. The SSCF has recently been used to produce bioethanol according to its properties, *i.e.*, reduced inhibition of cellulase by fermented hydrolyzed sugars, higher product yield, lower cellulase requirements, lower requirements for sterile conditions, shorter process time, and reduced reactor volume. Additionally, SSCF has many advantages over bioethanol production such as a higher yield, shorter processing time, and lower reactor volume.

This work has two parts. First, wood dust is pretreated by SFE and SE to form substrates on which *Z. mobilis* can produce bioethanol. The respective amounts of bioethanol produced are compared. Second, the activities of saccharification enzymes were evaluated by cocultivating *T. reesei* and *A. niger*. Pre-treated wood dust is applied as a medium in the SSCF bioreactor to evaluate its potential for bioethanol production. The whole experimental procedures were shown into the Fig. 1A.

2. Materials and methods

2.1. Microorganisms and maintenance media

T. reesei BCRC 31,863 and *A. niger* BCRC 31,130 were obtained from the Bioresource Collection and Research Centre (BCRC) of Taiwan. The stock culture was maintained aseptically on potato-dextrose-agar (PDA) Petri plates (BD, New Jersey, USA). The PDA plates were incubated at 30 °C for 7 days until good sporulation was reached and then stored at 4 °C. *Z. mobilis* BCRC 10,809 was obtained from the BCRC of Taiwan. Plate stock culture medium consisted of yeast extract (5.0 g/L, BD), glucose 20 (g/l, Sigma-aldrich, Maryland, USA), Bacto peptone (5 g/l, BD), $MgSO_4 \cdot 7H_2O$ (1.5 g/l, Sigma), KH_2PO_4 (2 g/l, Sigma), and $(NH_4)_2HPO_4$ (1.5 g/l, Sigma) at pH 6.8, with cultivation at 30 °C for 2 days. Bushnell-Haas selection D medium (BHSD) was used for cultivation of *T. reesei* and *A. niger* spores, and *Z. mobilis*, and consisted of carboxymethylcellulose (CMC, 10 g/l, Sigma), $MgSO_4 \cdot 7H_2O$ (0.4 g/l, Sigma), KH_2PO_4 (1 g/l, Sigma), $(NH_4)_2HPO_4$ (1 g/l, Sigma), $CaCl_2$ (0.02 g/l, Sigma), and $FeCl_3$ (0.04 g/l, Sigma).

For ethanol production Bushnell–Haas selection W medium (BHSW), consisting of wood dust (dried weight, 10 g/l, Far Eastern New Century, Taoyuan City, Taiwan), $MgSO_4 \cdot 7H_2O$ (0.4 g/l, Sigma), KH_2PO_4 (1 g/l, Sigma), $(NH_4)_2HPO_4$ (1 g/l, Sigma), KNO_3 (1 g/l, Sigma), $CaCl_2$ (0.02 g/l, Sigma), and $FeCl_3$ (0.04 g/l, Sigma). The pH was adjusted to 5.5–6 with NaOH or H_2SO_4.

2.2. Pretreatment by steam explosion

Wood dusts (Chang Chun Plastics Co., LTD, Taipei City, Taiwan) was immersed in 4% sulfuric acid solution for 2 h After removing out the residual water, 10 kg mass samples of the water-riched wood dusts were treated with high-pressure steam in a 250-l steam-explosion reactor. The final temperature in the reactor was 120 °C for 5 min. Then the process temperature varied to 180 °C for steam treatment around 3 min. After cooling, the solids from the steam-explosion process were filtrated with water. The resulting aqueous hydrolyzate solutions were rich in hemicellulose-derived sugars and acetic acid. The hydrolyzates were first subjected to an over-liming process with $Ca(OH)_2$ to remove the lignin-derived phenolics and final pH adjusted to near 7.0. Next, 12–15 (FPU)/g of acid-treated residue was further processed with glucan CTec 2 enzyme (Sigma) at 50 °C for 72 h After filtration, the glucose was removed to harvest 1.8 kg of lingocellulose.

2.3. Pretreatment by supercritical fluid extraction

The experiments were performed using ten kilograms of wood dusts (moisture content of 80%) that was placed inside a 50-ml stainless steel reactor vessel (Thar - Pittsburgh, USA). Pressurized CO_2 was fed into the reactor until 20.6 MPa was reached. The temperature was raised to 120 °C, 150 °C, or 180 °C for 1 h to create the supercritical state. At the end of each experiment, the supercritical CO_2 was suddenly released into the atmosphere via an expansion valve. The residues were collected and washed extensively with deionized water. After supercritical CO_2 pretreatment, the samples were stored in sealed bags at 4 °C to prevent contamination until needed for composition analysis after enzymatic hydrolysis.

2.4. Evaluation of enzyme activities of endo-1,4-β-glucanase, exo-1,4-β-glucanase, and 1,4-β-glucosidase

The activities of endo-1,4-β-glucanase (EC 3.2.1.4, Sigma), exo-β−1,4-glucanase (EC 3.2.1.155, Sigma), and 1,4-β-glucosidase (EC 3.2.1.21, Sigma) were determined using 1.0 mL of the following substrate preparations in 50 mol/l acetate buffer pH 4.8, respectively [11]: 1 wt% of Avicel type 50, 2 wt% of CMC, and 15 mmol/l of cellobiose. These substrates were incubated with 1.0 ml of the enzyme preparation for 60 min (Avicel type 60) or 30 min (CMC and cellobiose), depending on the assay. For total cellulase activity, the reaction was stopped by the addition of 3.0 ml of 3,5-dinitrosalicylic acid (DNS) solution, including 3,4-dinitrosalicyclic acid (10 g/l), potassium sodium tartrate tetrahydrate (403 g/l) and NaOH (anhydrous, 16 g/l). One unit (U) of each enzyme activity of endo-1,4-β-glucanase, exo-β−1,4-glucanase and 1,4-β-glucosidase