Renewable Energy 86 (2016) 858–865

Contents lists available at ScienceDirect

Renewable Energy

journal homepage: www.elsevier.com/locate/renene

Effect of doping pretreated corn stover conditions on yield of bioethanol in immobilized cell systems

Shuang-Qi Tian [a, b, *], Xin-Wei Wang [a, b], Ren-Yong Zhao [a], Sen Ma [a, b]

[a] College of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, China
[b] National Engineering Laboratory for Wheat and Corn Further Processing, Zhengzhou 450001, China

ARTICLE INFO

Article history:
Received 10 March 2015
Received in revised form
10 August 2015
Accepted 10 September 2015
Available online xxx

Keywords:
Immobilized yeast
Corn stover
Doping
Bioethanol

ABSTRACT

The surface characteristics of immobilized yeast before and after adding CO_2-laser pretreated corn stover (LPCS) substrates were investigated using bioethanol production. Response surface methodology (RSM), based on the Box–Behnken design (BBD) for experiments, was used to optimize the doping condition. An optimum experimental condition was obtained at pH 4.5, 2.08% yeast concentration, and 0.20% LPCS substrates. Under this condition, doping LPCS increased the yield of bioethanol from 53% to 84%, which matched the predicted value. After doping LPCS, the results of inverted microscope (IM) and atomic force microscopy (AFM) illustrated that the immobilized gel beads changed from rod-like in shape with a smooth surface to a larger rod-like ultrastructure with a rougher surface. The yield was relatively stable within 28 d, with a downward trend subsequently appearing.

1. Introduction

Rapid depletion of global fossil fuels (coal and oil), combined with concerns about greenhouse gas emission, has resulted in increased interest in transportation fuels that can serve as alternatives to crude oil-based fuels [1]. A majority of bioethanol is made from terrestrial biomass which is essentially food, such as corn, sweet potato, and sugarcanes [2]. However, food crop-based bioethanol production has brought up issues of food security, the usage of pesticides, arable land, and fresh water throughout their growth process [3]. Corn stover is a fermentation lignocellulosic feedstock for bioethanol production because it is an abundant agricultural residue, produced annually in China [4,5]. In fact, corn stover is an effective feedstock for cellulosic bioethanol production because of its high cellulose content [6,7]. Current scientific research on bioethanol is driven by reducing the cost of bioethanol production and has focused on improving raw materials pretreatment methods, enzymes utilization, and fermentation [8,9]. The efficient conversion of lignocellulose into bioethanol requires optimum release of total reducing sugar [10].

Currently, most bioethanol production is prepared by batch fermentation. Because batch operations have the advantages of low capital and operational costs, simple controls, and entire processes that do not require specialized labor, total sterilization and the supply of raw materials are easier to obtain than in other processes [11]. Another advantage of batch operations using immobilized cells is the ability to isolate immobilized yeast from the bioethanol product, allowing the immobilized cells to be reused for further bioethanol fermentation [12]. In the conversion process of fermentable sugars to bioethanol, immobilized cell systems could offer advantages over cell suspension systems in terms of bioethanol productivity and stability of yeast cell activity [13]. In addition, immobilized yeast cell technology in bioethanol fermentation could possess a higher cells concentration, higher mass transfer, high bioethanol fermentation rate, and recycling utilization of the yeast, lower product inhibition in the process of fermentation [14,15].

It is clear that the function of such biocatalytic structures composed of microorganisms entrapped in gel beads leads to the appearance of cell-growth gradients that induce a heterogeneous development of the biomass inside the structure [16]. The maximum cell concentration is located near the gel–solution interface [17]. Nevertheless, there are very few reports in literature concerning both the theoretical analysis of this phenomenon and

Abbreviations: LPCS, CO_2-laser pretreated corn stover; RSM, Response surface methodology; BBD, Box–Behnken design; AFM, atomic force microscopy; IM, inverted microscope; YPG, yeast extract peptone dextrose medium; DDW, double distilled water; DNS, 3,5-Dinitrosalicylic acid; PEG, polyethylene glycol.

* Corresponding author. College of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, China.
E-mail address: tianshuangqi2002@126.com (S.-Q. Tian).

http://dx.doi.org/10.1016/j.renene.2015.09.029
0960-1481/

the influence of the various parameters and process conditions of function on the characteristics of the immobilized cell systems [18]. These phenomena are often interpreted as being a consequence of mass transfer phenomena inside the structure [19].

The aim of the present work was to obtain maximum bioethanol with immobilized *Saccharomyces cerevisiae* using CO_2-laser pretreated corn stover (LPCS) hydrolysates [9,20]. To obtain the maximum cell concentration during the yeast immobilization process, doping LPCS technology was used in immobilizing the yeast gel beads. The process of immobilizing the yeast gel beads was conducted using the embedding method with sodium alginate and manganese alginate. The reducing sugars in LPCS hydrolysates were subsequently fermented using immobilized *S. cerevisiae*. This study focused on the doping LPCS concentration, yeast concentration, and pH. The optimal fermentation conditions and the bioethanol conversion rates were identified at various fermentation conditions.

2. Methods

2.1. Materials and yeast strain

Corn stover was harvested from Taiping Farm in Harbin, China. It was milled and sized through a 2-mm sieve shaker. The corn stover was catalyzed with a CO_2 laser for 68 min at 265 W and a water to solid ratio of 21:1 (mL/g) [9]. The wave-length of the CO_2-laser was in a infra-red region, and its irradiation produces prominent increase in heating temperature within a micro sec to milli sec order enough for evaporating out the irradiated samples. The reaction mixture was stirred by magnetic stirrer. The catalyzed residues were washed three times by double distilled water and dried at room temperature. After through the 60 meshes sieve, it was hydrolyzed as substrate by crude cellulase preparation, supported by Gansu Hualing Biological Technology Co., Ltd., China. The filter paper activity was mensurate at 64.5 filter paper unit (FPU)/g, as the measurement method described by the NREL Laboratory Analytical Procedure [21].

An inhibitor tolerant strain of *S. cerevisiae*, AS 2.607, which was presented as a gift by China General Microbiological Culture Collection Center, was used in the bioethanol fermentation experiments. Bioethanol fermentation experiments were conducted in LPCS hydrolysates, without external nutrient addition.

2.2. Preparation of culture medium

Yeast strains were cultured on agar plates which contained 20 g/L peptone, 20 g/L glucose, 10 g/L yeast extract, and 20 g/L agar. Dry yeast activity was carried out in sterilized YPG medium that contained 20 g/L peptone, 20 g/L glucose, and 10 g/L yeast extract at 30 °C and 200 rpm.

Immobilization proliferation medium, used for seed cultures in bioethanol fermentation experiments, contained the following: 10 g/L peptone, 40 g/L glucose, 10 g/L yeast extract, 2.5 g/L $CaCl_2$, 2.5 g/L KH_2PO_4, and 0.25 g/L $MgSO_4$ at 30 °C and 200 rpm [22]. The fermentation was carried out in the hydrolysates of LPCS, with 2.5 g/L $CaCl_2$, 2.5 g/L KH_2PO_4, and 0.25 g/L $MgSO_4$ at 30 °C and 200 rpm. The pretreated corn stover was hydrolyzed by crude cellulose and then the hydrolysates of LPCS were concentrated before the bioethanol fermentation experiments [9,20]. All mediums were adjusted to pH 5.0 before cultivation [23].

2.3. Preparation and proliferation of immobilized yeast cell

(1) Preparation of calcium alginate beads

A 48-h culture, rooted in slant culture-medium, was collected and blended with the sodium alginate solution. To prepare the calcium alginate beads, 3 mL of yeast seed culture and 0.05–0.3% LPCS substrates were added to 2% sodium alginate in 100 mL of double-distilled water (DDW). An alginate solution (2%) was sprayed through a thin inner nozzle (2–3 mm diameter at exit) into a 150 mM $CaCl_2$ solution. The calcium alginate beads were stored after being washed three times with DDW to remove residual $CaCl_2$. The beads were packed and stored in DDW at 4 °C for 3 days for the preparation of manganese alginate beads.

(2) Preparation of manganese alginate beads

Elemental analysis and stability studies of manganese alginate beads revealed marked differences in ion binding capacity, rendering manganese alginate beads with high guluronic acid content the most stable [24]. According to Mørch methods, the calcium alginate beads were added to 1% $MnSO_4$ solution and placed in the refrigerator for 24 h (4 °C) to solidify. The manganese alginate beads were then ready for proliferation of immobilized cells.

(3) Proliferation of immobilized cells

The manganese alginate beads of immobilized yeast cells were placed in the immobilization proliferation medium at 30 °C and 200 rpm. The proliferation mediums were replaced with fresh medium every 12 h, a total of four times during the proliferation. The proliferated immobilized cells were then ready for bioethanol fermentation.

2.4. Concentration of saccharification liquid

Concentrated hydrolysates of LPCS were prepared using vacuum evaporation. Evaporation was carried out under low vacuum at 0.5 bar and 55 °C for approximately 30 min. After evaporation, the concentrated hydrolysates were sterilized at 120 °C for 15 min and then used for bioethanol fermentation. Before evaporation, there was 2.62 mg/mL glucose and 0.30 mg/mL xylose in the hydrolysates. After evaporation, the concentrations of glucose and xylose increased to 12.07 mg/mL and 1.28 mg/mL, respectively.

2.5. Glucose and ethanol concentration

Reducing sugar concentration was measured using the DNS method, and the concentrations of glucose and xylose were detected using a Dionex CarboPac PA1 column. Bioethanol concentrations were detected using a gas chromatography (GC-8A Shimadzu, Tokyo, Japan) equipped with a 20% PEG column. Isopropyl alcohol was used for an internal standard to detect bioethanol assay from each bioethanol fermented sample. The column temperatures at the detector and injector were 110 °C and 130 °C, respectively. The yield of bioethanol was calculated using the following equation:

$$X = \frac{C_1}{C_2 \times 0.51} \times 100\% \quad (1)$$

where X is the yield of bioethanol (%), C_1 is the concentration of bioethanol in fermentation broth, and C_2 is the concentration of reducing sugar (glucose and xylose) before fermentation.

2.6. Determination of immobilized yeast embedding rate

In the process of preparing immobilized yeast cells for bioethanol fermentation, the embedding rate of calcium alginate gel