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Integrated utilization of algal biomass and corn stover for biofuel production

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HIGHLIGHTS

- Algal hydrolysate can be used as reaction medium to enhance ethanol production.
- Soluble protein and Ca^{2+} were the active components for the enzymatic process.
- Algal residue was potential nitrogen source for methane production from corn stover.
- Algal biomass could be integrated into the ethanol process of corn stover.

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ABSTRACT

This paper describes a novel route for the utilization of algal and lignocellulosic biomass for biofuel production. Dilute sulfuric acid treatment was employed to destroy the rigid algal cells and generate algal hydrolysate. Algal hydrolysate was used as the reaction medium for the enzymatic hydrolysis of lignocellulose from corn stover. The acid treatment parameters were optimized based on the enzymatic efficiency of corn stover digestion and the promotion mechanism is discussed. The algal residues from the acid treatment process were co-digested with corn stover to generate methane. This study showed that algal hydrolysate can be used as a supplemental feedstock and reaction medium to enhance ethanol production. Furthermore, the residues could be utilized as the nitrogen source during the anaerobic digestion of corn stover.

1. Introduction

Industrial and academic researchers worldwide are showing an increasing interest in renewable energy sources, especially energy production based on renewable and waste biomass [1]. Currently, third-generation biofuel feedstocks are represented by micro- and macro-algae, which have advantages over first- and second-generation biofuels [2,3]. Algal biomass has a high photosynthetic yield and does not compete with land needed for food production. It also improves control of greenhouse gas emissions. Biomass applications during wastewater treatment, lipid production, and for CO_2 sequestration have been extensively investigated [4]. Algal biomass collected from refloatation and eutrophic water systems has become a major algal resource that complements biomass harvested from industrial application processes [5,6].

Due to the enriched nutrient conditions, algae grown in wastewater, waste streams, and eutrophic waste systems tend to accumulate more proteins and carbohydrates than lipids [7,8] and finding valuable uses for these proteins and carbohydrates is critical to enhancing the efficiency of algal biomass utilization. A hydrolysis step is needed to break down rigid algal cells. Dilute acid methods have been widely adopted by numerous studies to hydrolyze lignocellulosic biomass. However, to date, there have only been a few reports on using dilute acid hydrolysis to generate carbohydrates and proteins [9,10].

During typical biofuel production from second-generation biofuel feedstocks composed of lignocellulosic biomass, enzymatic hydrolysis is the critical step that releases mono-sugars. It is hindered, in part, by the high lignin contents in the feedstocks. Reducing the effects of lignin on the enzymatic hydrolysis of cellulose remains one of the major challenges in improving the hydrolysis of lignocellulosic biomass. It has been reported that adding bovine serum albumin (BSA) and exogenous protein prior to cellulase addition was particularly effective in increasing the hydrolysis rate [11,12]. Furthermore, anaerobic digestion has also been employed during biodiesel production to recover more energy when algal biomass has been used as the feedstock in the bio-refinery process [13]. All these methods provide a potential pathway to convert

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algal biomass to ethanol and methane, and the protein in the algal hydrolysate might improve enzymatic efficiency at the same time.

In this study, an overall utilization route for algal biomass, based on the dilute acid treatment, was constructed. Firstly, the dilute acid treatment was used to hydrolyze the algal biomass which would release soluble carbohydrate and protein. Secondly, the released hydrolysate was added to the enzyme system for corn stover breakdown to improve the enzymatic hydrolysis efficiency. Thirdly, the algal residue was used as nitrogen source to improve the methane yields from corn stover.

2. Material and methods

2.1. Algal biomass and corn stover

The algal biomass used in this work was collected from Lake Chaohu in Hefei (Anhui, China). The volatile solid (VS) content was 94.0 ± 7.3% based on the total solid (TS). The carbohydrate and protein contents of the biomass, based on the TS, were determined to be 32.7 ± 0.5% and 34.7 ± 2.1%, respectively. A relatively small amount of lipid (3.4 ± 0.3%) was also detected. Elemental analysis result indicated that it was composed of 27.5 wt.% C, 8.6 wt.% H, 48.7 wt.% O, 5.5 wt.% N, and 0.47 wt.% S, based on TS.

Corn stover was collected from a private farm located in Hefei (Anhui, China). The sun-dried corn stover was crushed into pieces and used as the fermentation substrate. The VS content was 95.0 ± 0.3%. The chemical compositions were determined using previously reported methods [14]. Cellulose, hemicelluloses, and lignin accounted for 43.4 ± 1.5, 24.6 ± 0.7 and 7.6 ± 0.5% of the TS, respectively. Elemental analysis result indicated that corn stover contained 43.4% C, 6.0% H, 44.9% O, and 0.5 wt.% N, based on TS.

Diluted sulfuric acid pretreated corn stover was achieved as follows. Corn stover (20 g TS) were mixed with 200 mL of 0.75% sulfuric acid and the mixture was heated to 105 °C for 1.5 h in an autoclave. A pretreated sample was adjusted to pH 5 using 30% sodium hydroxide (NaOH) solution, then centrifuged and rinsed three times using 500 mL deionized water. Wet solid sample was stored at −20 °C and used as the feedstock for the enzymatic process.

2.2. Optimization of the dilute sulfuric acid pretreatment for algal biomass

Aliquots (0.5 g) of dried algae were mixed with 10 mL aliquots of sulfuric acid at three concentrations (2%, 3%, 4%, and 5% w/w), and autoclaved in flasks at three temperatures (105, 115, and 125 °C), and for three reaction time lengths (0.5, 1.0, and 1.5 h) using a completely randomized design with two replications. The hydrolyzed mixture was adjusted to pH 5 using calcium carbonate ($CaCO_3$). Solid residues were completely removed by centrifugation (2846g, 10 min) and the liquid hydrolysate was saved. Acid pretreated corn stover samples (1.0 g TS) were mixed with liquid hydrolysate to a total mass of 100 g, which makes the solid concentrations of 1% (w/w). All mixed samples were autoclaved before cellulase was added at loading of 10 filter paper unit (FPU) g^{-1} TS. The flasks were placed on a shaker table (150 rpm) in an incubator at 50 °C for 72 h. The optimal temperature, time, and sulfuric acid concentration were determined based on the enzymatic efficiency of cellulose breakdown in the acid treated corn stover. The algal residues produced under the optimal conditions were stored at was stored at −20 °C used as the feedstock for the following anaerobic reactor experiment.

2.3. Enzymatic hydrolysis of corn stover with algal hydrolysate

The algal hydrolysate produced under the optimum conditions was fractionated using a series of dialysis membranes with a molecular weight cut-off (MWCO) of 1000, 3500, 7000, 10,000 and 15,000 Da. The solutions were labeled as AH-1, AH-2, AH-3, AH-4 and AH-5. The raw algal hydrolysate was labeled AH-0. The effects of algal hydrolysates on enzymatic hydrolysis of corn stover were compared based on the conversion efficiency of the cellulose. Effects of BSA and Ca^{2+} on the enzymatic process of corn stover were conducted. BSA solution (0–5.0 g/L) was prepared using bovine serum albumin and 50 mM sodium citrate buffer. $CaCl_2$ was added into the citrate buffer solution to prepare a series concentration of 0–2.0 g/L. Acid treated corn stover samples (1.0 g TS) were mixed with the medium to a total mass of 100 g, which made the solid concentrations of 1% (w/w). All mixed samples were autoclaved before cellulase was added at loading of 10 FPU g^{-1} TS to initiate the enzymatic hydrolysis. The flasks were placed on a shaker table (150 rpm) in an incubator at 50 °C for 72 h. All batches were replicated three times. Samples were taken at the initial and final phase to determine the reducing sugar levels.

2.4. Anaerobic digestion of corn stover and algal residues

Digester sludge was collected from anaerobic reactors at China Resources Snow Breweries and was passed through a sieve of 40 mm. The filtered digester sludge was used as the inoculum. Anaerobic digestion was performed in 250 mL serum bottles with a working volume of 150 mL. The initial corn stover was fixed at 10 g volatile solid (VS) L^{-1} and the inoculum sludge was set as 1.0 g VS L^{-1}. 5 mL microelements and vitamin solutions were added. Constituent of microelement solution is listed as follows (mg/L): $CoCl_2{\cdot}6H_2O$, 130; $ZnCl_2$, 70; $CaCl_2{\cdot}2H_2O$, 2; $NiCl_2{\cdot}6H_2O$, 24; $H_3BO_3{\cdot}6H_2O$, 6 and $Na_2MoO_4{\cdot}2H_2O$, 36. Vitamin solution (mg/L): D(+)-biotin, 2; folic acid, 3; niacin, 10; Ca-D(+)-pantothenic acid, 5; vitamin B1, 10; VB6, 15 and 4-aminobenzoic acid, 4. The algal residues were introduced at dosages of 0, 1, 2, 5, or 10 g VS L^{-1}. For endogenous methane production determination, blanks containing only digester sludge were also run. All batches were replicated three times. The initial pH was adjusted to 7.0 ± 0.1 and distilled water was to make a working volume of 150 mL. The reactors were purged with a mixture of N_2 and CO_2 gases (40:60) for 30 s and then sealed with aluminum bungs. The reactors were placed in an air bath shaker at 120 rpm and the temperature was maintained at 35 ± 1 °C.

2.5. Analytical methods

The reducing sugar levels in each hydrolyzed algal solution and in the enzymatic hydrolysis systems were measured using the dinitrosalicylic acid (DNS) method and the protein content was measured using by the modified Lowry method [15,16]. Lipid in dried algal samples was extracted using a chloroform–methanol–water solution (2:1:0.8, v/v) [17]. The gas production was measured by glass syringes. Methane content was determined by gas chromatography (GC-2010, Shimadzu Co., Ltd) equipped with a RTX-1 column (30 m × 0.25 mm × 0.25 μm). Elemental analysis of the algal biomass and corn stover was performed using a Vario EL cube (Elementar, Hanau, Germany). Ca^{2+} in the algal hydrolysate was determined using TAS-986 atomic absorption spectrometer (Beijing Purkinje General Instrument Limited Liability Company). Significant differences were determined by a *t* test which was performed using the SPSS program 16.

3. Results and discussion

3.1. Optimization of acid treatment conditions

The enzymatic efficiency of corn stover digestion after using the different algal hydrolysates as reaction media is illustrated in