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Optimization of wastewater microalgae saccharification using dilute acid hydrolysis for acetone, butanol, and ethanol fermentation

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HIGHLIGHTS

- Optimization of wastewater microalgae acid hydrolysis for ABE fermentation.
- Sugar yield increases as a function of temperature and acid concentration.
- Comparison of retention time and acid concentration affects pretreatment costs.
- Costs associated with acid concentration are higher than costs associated with retention time.

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ABSTRACT

Exploring and developing sustainable and efficient technologies for biofuel production are crucial for averting global consequences associated with fuel shortages and climate change. Optimization of sugar liberation from wastewater algae through acid hydrolysis was determined for subsequent fermentation to acetone, butanol, and ethanol (ABE) by *Clostridium saccharoperbutylacetonicum* N1-4. Acid concentration, retention time, and temperature were evaluated to determine optimal hydrolysis conditions by assessing the sugar and ABE yield as well as the associated costs. Sulfuric acid concentrations ranging from 0 to 1.5 M, retention times of 40–120 min, and temperatures from 23 °C to 90 °C were combined to form a full factorial experiment. Acid hydrolysis pretreatment of 10% dried wastewater microalgae using 1.0 M sulfuric acid for 120 min at 80–90 °C was found to be the optimal parameters, with a sugar yield of 166.1 g for kg of dry algae, concentrations of 5.23 g/L of total ABE, and 3.74 g/L of butanol at a rate of USD $12.54 per kg of butanol.

1. Introduction

While the global consumption of petroleum based products keeps increasing, the demand for alternative, renewable, and efficient energy technologies gains interest. Renewable energy technologies such as solar, wind, hydro, and thermal sources have been successfully developed to mitigate the energy demand; however, renewable approaches to cover liquid fuel consumption, such as biodiesel and bioethanol production, have economic and environmental impacts due to the feedstocks used. The use of corn and soybeans as feedstocks for biofuel production requires fertilizers, pesticides, and seeds, which increases the final cost of the bioproducts [1]. Growing these crops for biofuel production not only affects food prices, but also triggers the contamination of water by the indiscriminate use of pesticides and fertilizers [2–4]. As a result, studies to find alternative, low cost, and environmentally friendly feedstocks for the production of biofuels are being widely established.

The biological production of acetone, butanol, and ethanol (ABE) using wastewater algae as the carbon source is an environmentally sustainable process that could mitigate the demand for petroleum fuel. Butanol, the most abundant solvent produced in ABE fermentation, is an environmentally friendly and competitive drop-in-fuel that can be directly used in vehicles and has a comparable energy density to gasoline [5–7]. In addition, *n*-butanol is a superior transportation fuel over ethanol because of its higher energy content, immiscible properties, lower volatility, lower corrodibility, and lower hygroscopicity [8]. The production of ABE from wastewater algae takes advantage of the substrate source to minimize derived costs of production. Some of the advantages of wastewater algae over other terrestrial biomass include lower nutrient requirements for growth, higher growth rate, higher yield per cultivation area, less land area requirement, non-fresh water required for growth,

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and non-competition with food crop demand [9–12]. In addition, microalgae harvested from municipal wastewater lagoons are considered a bioremediation technique for removing phosphorus and nitrogen to prevent downstream eutrophication [13,14]. Similarly, concentrated CO_2 emissions from industrial sources can be redirected to municipal wastewater lagoons to be used as supplemental CO_2 for algae growth; thus, mitigating CO_2 emissions [9]. ABE fermentation using microalgae from the Logan City Wastewater Lagoon System (LCWLS) by *Clostridium saccharoperbutylacetonicum* was previously demonstrated [15].

The production of ABE from a raw material such as wastewater algae requires pretreatment prior to fermentation to make the sugars bioavailable. The sugar content in algae is reported to be up to 50% dry weight [16]. According to preliminary studies (see Section 2.5), wastewater microalgae yields xylan, maltose, glucose, and xylose after thermal and dilute acid hydrolysis. Glucose, maltose, and xylose have been reported to be fermentable sugars by using various strain of *C. saccharoperbutylacetonicum* [17–21]. Methods used to achieve saccharification of recalcitrant feedstocks are enzyme digestion, thermolysis, dilute acid hydrolysis, and concentrated acid hydrolysis [22,23]. Ellis et al. [15], conducted experiments to compare different pretreatment conditions used to produce ABE from wastewater algae. The ABE fermentation using dilute acid hydrolysis for wastewater microalgae pretreatment produced the lowest ABE yield, 2.74 g/L. The highest ABE yield (9.74 g/L) was obtained with the combination of acid hydrolysis and enzymatic digestion. However, enzymatic digestion is an expensive method that increases the cost of ABE production [24]. In general, the costs associated to pretreatment process of feedstock for biofuel production range from 40% to 70% of the selling prices of biofuel [8]. Thus, the selection of methods for wastewater microalgae saccharification needs to take into account the cost involved.

Acid hydrolysis of wastewater algae as pretreatment for ABE fermentation by *Clostridium spp.* is a potentially effective and low cost method. The negligible content of lignin into microalgae reduces the costs, time, and difficulty of the conversion process [25]. Similarly, because *C. saccharoperbutylacetonicum* is an amylolytic microorganism, the enzymatic hydrolysis step for the conversion of starch into fermentable sugars is not required [26]. Studies focused on acid hydrolysis as pretreatment process to digest cellulose and hemicellulose in algae have been already conducted [1,27–30]. However, there are no studies to date regarding the optimization of wastewater microalgae for ABE fermentation using acid hydrolysis as a saccharification method. The optimization of algae saccharification through acid hydrolysis will result in increased fermentable sugar yields from microalgae while accounting for the cost of the process. Kinetic studies on the dilute acid hydrolysis of cellulosic materials indicate that the acid hydrolysis efficiency depends on substrate, acid concentration, temperature, and retention time [29]. The optimization of microalgae acid hydrolysis through evaluation of these parameters will result in an increased yield of fermentable sugars in the medium. Currently, there are no statistically detailed studies that describes the cost analysis associated with the evaluated parameters for acid saccharification of wastewater microalgae. The aim of this study was to optimize acid hydrolysis using wastewater microalgae for subsequent ABE fermentation by determining the conditions that yields the highest ABE concentration while controlling the costs of the process.

2. Materials and methods

2.1. Algae biomass

Mixed microalgae biomass from the LCWLS was grown in SE media containing 850 mg $NaNO_3$, 350 mg KH_2PO_4, 150 mg $MgSO_4{\cdot}7H_2O$, 150 mg K_2HPO_4, 50 mg $CaCl_2{\cdot}2H_2O$, 50 mg NaCl, and 15 mg $C_6H_8O_7{\cdot}Fe{\cdot}NH_3$ per liter of ddH20. The biomass was freeze dried through sublimation for 48 h. The dry biomass was maintained at 4 °C prior to pretreatment. The mixed microalgae feedstock was primary dominated by *Scenedesmus, Chlorella, Ankistrosdemus, Micromonas*, and *Chlamydomonas*, as previously described [15].

2.2. Reagents

Reagent grade chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA) unless otherwise specified.

2.3. Acid hydrolysis

Algae (3.5 gdw) diluted in 35 mL of ddH_20 was placed in 100 mL serum vials with crimp top, 52 mm diameter, and 95 mm height. The ranges of temperature (25–30 °C, 45–55 °C, and 80–90 °C) were achieved by the use of different hotplates. For room temperature, a magnetic stirrer at stirring level 7 was used; for 45–55 °C, a Fisher Scientific Isotemp Basic stirring hotplate (Cat. 11-100-100SH) at 1200 RPM, and for 80–90 °C a Fisher scientific stirring hotplate (Cat. 11-520-49SH). Stirring bars of 1 inch were used through the experiment. The temperature was monitored using a thermometer and controlled to be maintained within the desired temperature range. The acid concentrations used were 0.0 M, 0.35 M, 0.50 M, 0.70 M, 1.00 M, and 1.50 M. Retention times were 40 min, 80 min, and 120 min. Ca $(OH)_2$ was used to neutralize the hydrolyzed medium. The medium was clarified by means of centrifugation (1500g for 20 min) before and after neutralization. Samples were filtered (0.2 μg) prior to carbohydrate analysis. The experiment was conducted in duplicates. Fig. 1 illustrates the steps of the acid hydrolysis experiment.

2.4. ABE fermentation from fermentable sugars

ABE fermentation was performed as previously described [15] using the optimal acid concentration, temperature, and retention time that yielded the highest amount of liberated sugars.

2.5. Analytical methods

Sugars were quantified by use of High-performance liquid chromatography (HPLC, LC-10AT Shimazdu) along with a CTO-10A Shimazdu column oven equipped with a carbohydrate guard column 802G BP-100H+ and an analytical column 802 BP-100H+, both manufactured by Benson Polymeric. The mobile phase used was 100% ddH20. The samples were injected at a flow rate of 0.4 mL/min by SIL-10A auto injector and detected by an Evaporative Light Scattering Detector (ELSD-LT II), both manufactured by Shimazdu. Standard curves for maltose, glucose, and xylose were generated. The R2 of the regression performed on the standard curves were >0.99. The peak elution time for maltose was 14.3 min, for glucose was 17.1 min, and for xylose was 18.3 min. Other peaks of interest were: galactose, 18.3 min; mannose, 18.2 min; maltotriose, 12.3 min; xylan, 10.5 min; formic acid, 10.4 min; and sulfates 10.6 min. Mannose, galactose, and xylose were essentially inseparable, as well as xylan, formic acid, and sulfates.

Acetone, butanol, and ethanol (ABE) produced in the fermentation was analyzed as previously described by Ellis et al. [15] using gas chromatography (GC).

2.6. Energy calculations

Energy calculations are based on a 10,000 L batch system at 50% capacity. The heat energy and the power of the mixing/stirring were calculated using Eqs. (2.7.1) and (2.7.2), respectively (see