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Effect of coagulant/flocculants on bioproducts from microalgae

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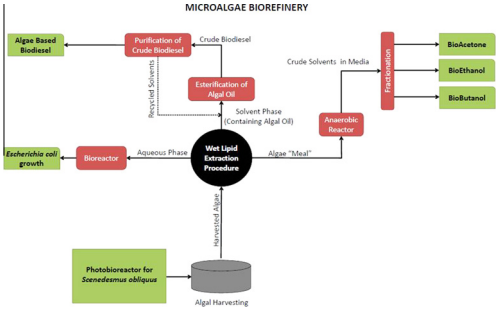
HIGHLIGHTS

- Harvested microalgae using cationic starch, alum, and centrifugation.
- Fractionated into acetone, butanol, ethanol, biodiesel and bacterial growth media.
- Higher yield of acetone, butanol, and ethanol by cationic starches than alum.
- Higher biodiesel produced by cationic starches than alum.
- Higher bacterial growth observed in media obtained from cationic starch harvesting.

GRAPHICAL ABSTRACT



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ABSTRACT

The potential of microalgae as a source of sustainable energy, nutritional supplements and specialized chemicals necessitates a thorough evaluation of the methods of harvesting microalgae with regards to the bioproduct(s) desired. This research assessed the effect of coagulation, flocculation, and centrifugation on the wet lipid extraction procedure, which fractionated microalgae into hydrolyzed biomass for fermentation into acetone, butanol, and ethanol, an aqueous phase as growth media for genetically engineered *Escherichia coli*, and a lipid fraction for the production of biodiesel. Biomass harvested by cationic starches, alum, and centrifugation produced 30, 19, and 22.5 mg/g of dry wt. algae of total combined acetone, butanol, and ethanol, respectively. Higher biodiesel production was also observed for the cationic starches (9.6 mg/g of dry wt. algae) than alum (0.6 mg/g of dry wt. algae) harvested biomass. The results suggested significant effect of the harvesting methods on the yields of bioproducts.

1. Introduction

Microalgae show great potential as a feedstock for a variety of bioproducts such as biosolvents, biodiesel, biogas, and other bioproducts. The flexibility of microalgae as a feedstock, coupled with high growth rates even in brackish water, makes it an excellent candidate for the production of sustainable bioproducts (Chisti 2007). Microalgae can also help remediate wastewater through assimilation of nitrogen and phosphorus as nutrients and accomplish tertiary treatment of wastewater. The cultivated microalgae can be harvested to provide a sustainable supply of biomass for the production of bioproducts (Rahman et al., 2012; Christenson and Sims, 2011).

One of the major hurdles in the processing of microalgae is the harvesting and dewatering steps. The techniques currently employed in microalgae harvesting and recovery include centrifugation, biofilm formation (Christenson and Sims 2012), flocculation, filtration and screening, gravity sedimentation, flotation, and electrophoresis techniques (Uduman et al., 2010). Of all the harvesting methods, chemical precipitation or coagulation/flocculation is shown to be most efficient for large scale harvesting of microalgae (Lee et al., 1998). Chemical precipitation of algae is achieved

Abbreviations: MAPTAC, 3-methacryloyl amino propyl trimethyl ammonium chloride; WLEP, wet lipid extraction procedure; ABE, acetone, butanol, and ethanol; FAMEs, fatty acid methyl esters; CCS, cationic corn starch; CPS, cationic potato starch; DS, degree of substitution; RCM, reduced clostridia media; CFU, colony forming units; DCW, dry cell weight; ANOVA, Analysis of Variance; SAS, Statistical Analysis Software.

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through the addition of inorganic electrolytes such as aluminum sulfate (alum) and ferric chloride (Tadros 2007). The addition of these multivalent electrolyte neutralizes the negative charge on the algae, which then come together to form aggregates by a process known as flocculation. Polyelectrolytes such as magnafloc, which are a combination of a polymer and an electrolyte, have been studied to a certain extent for algae harvesting (Harith et al., 2010).

The use of inorganic coagulants, although effective, is associated with high dosage requirements, excess sludge volumes, biomass with metal hydroxides (Vandamme et al., 2009), additional costs for harvesting and disposal (Zheng et al., 2012), impacts to downstream processes that utilize the biomass as feedstock material (Papazi et al., 2010), and harvested biomass undesirable as animal feed (Bryant et al., 2012). These drawbacks can be addressed by the use of organic flocculants such as cationic starch (Vandamme et al., 2009). Organic flocculants are naturally available, biodegradable, and inexpensive. Cationic starch is prepared by chemically modifying native starch with cationic groups such as ammonium, amines, imines, phosphonium and sulfonium (Pal et al., 2005). For microalgae dewatering, the positive moieties on the cationic starch help facilitate charge neutralization of the algae and the inherent polymer structure of starch exhibits flocculant properties enabling inter-particle bridging of neutralized algae to form flocs. The use of cationic starch for algae harvesting can help reduce negative impacts on downstream processes that use algae as feedstock and aid in higher yield of bioproducts due to the polysaccharide nature of starch.

A variety of bioproducts have been generated from microalgae. Fermentation of algal biomass has shown to produce biosolvents such as bioethanol (Takeda et al., 2011), bioacetone, biobutanol (Ellis et al., 2012), and 1,3-propanediol (Nakas et al., 1983). Lipids from microalgae have been used in biodiesel production (Sathish and Sims 2012), and anaerobic digestion of algal biomass is shown to yield methane as biogas (Yuan et al., 2011). Das et al. (2012) demonstrated antibacterial properties of the organic extracts from microalga *Euglena viridis*. Besides bioproducts for the energy sector, algae have also been used for human and animal nutritional supplements ranging from beta-carotene to proteins (Spolaore et al., 2006). The range of the bioproducts derived from microalgae suggests that the harvesting method chosen should be dependent on the final value of the bioproduct and should not have any undesirable affect on the bioproduct or biomass.

A study by Borges et al. (2011) investigated the effect of anionic and cationic flocculants and concluded that extracts from harvested biomass with anionic flocculants resulted in saturated fatty acids desirable for biodiesel production. However, biomass harvested using cationic flocculants showed higher extractability of unsaturated fatty acids for pharmacy and food industry. In another study, aluminum sulfate and ferric chloride affected the anaerobic digestibility of organic compounds separated from wastewater such as amino acids, proteins, and long chain fatty acids (Dentel and Gossett, 1982). The high aluminum content in alum separated algal biomass was proven to be unpalatable and toxic to animals when used as a component of animal feed (Harith et al., 2010). Although, microalgae have been the topic of discussion for various forms of bioproducts, literature evaluating the effects of different harvesting methods on the quality and yields of bioproducts is limited.

For this research, cationic starch was synthesized with corn and potato starch using 3-methacryloyl amino propyl trimethyl ammonium chloride (MAPTAC) as the cationic moiety. *Scenedesmus obliquus* grown in bioreactors was harvested using various modes of algae separation for processing and bioproduct generation. The harvested biomass was processed by a wet lipid extraction procedure (WLEP) resulting in three product streams (Sathish and Sims, 2012); (1) hydrolyzed biomass, which was fermented by *Clostridium saccharoperbutylacetonicum* N1–4 to produce acetone, butanol, and ethanol (ABE) (Ellis et al., 2012), (2) aqueous phase, which formed the substrate for genetically modified *Escherichia coli* and (3) lipids, which were esterified into fatty acid methyl esters (FAMEs or biodiesel).The objective of this research was to evaluate the effects of cationic starch and aluminum sulfate harvested microalgae on the yields of acetone, butanol, ethanol, biodiesel and *E. coli* growth.

2. Methods

2.1. Synthesis of cationic starch and harvesting of microalgae

Potato starch was obtained from Fisher Scientific (Pittsburgh, PA) and corn starch was obtained locally (Logan, UT). Ceric ammonium nitrate, 3-methacryloyl amino propyl trimethyl ammonium chloride (50% in water) (MAPTAC), aluminum sulfate and nitric acid (trace grade) were procured from Sigma Aldrich (St. Louis, MO). All chemicals were used as received. *S. obliquus* was isolated from the Logan city wastewater lagoons system and grown in a Solar Simulated Bioreactor (SSR) (Dye et al., 2011) (pH = 7.0, temperature = 25 °C) in synthetic wastewater media (McLachan 1964).

Zeta potential measurements on algae were performed using the Brookhaven ZetaPlus zeta meter (Holtsville, NY). For cationic starch synthesis, 5 g/L starch was dissolved in water at 80 °C with 20% ceric ammonium nitrate and heated for 30 min. Following free radical initiation, 15 mL of 3-methacryloylamino-propyl-trimethylammonium chloride was added slowly and adjusted to pH 3 and heated at 80 °C for 2 h after which the mixture was cooled, pH neutralized with NaOH, and the starch precipitated and washed with ethanol. Both cationic corn starch (CCS) and cationic potato starch (CPS) were synthesized by this procedure. Total nitrogen in the cationic starch was measured using Hach Test 'N Tube (Loveland, CO) and the degree of substitution (DS) was calculated using Eq. (1). Degree of substitution is the ratio of the moles of MAPTAC per anhydrous glucose unit of starch:

$$\text{Degree of substitution} = \frac{162 \times N\%}{[1400 - (220.74 \times N\%)]} \quad (1)$$

where, 162 = molecular wt. of one anhydrous glucose unit, 220.74 = molecular wt. of MAPTAC, *N*% = % wt. of nitrogen in starch, and 14 × 100 = molecular weight of nitrogen ×% accommodation. Microalgae were harvested using CCS, CPS, alum, and centrifugation (8000 rpm × 10 min) at pH 7.0. The basis of harvesting was the reduction of the negative zeta potential of algae to 0 mV by the addition of CCS, CPS and alum. Zeta potential is a measure of the average surface charge of the microalgae in suspension, measured in mV. At 0 mV zeta potential, the algal suspension would be destabilized completely and allow the particles to form flocs and be collected by gravity settling. Before adding coagulants, total suspended solids and initial zeta potential of the algal suspension were measured. After adding predetermined concentrations of the coagulants, suspensions were flash mixed for 1 min after which flocs were allowed to form by perikinetic flocculation and settle to the bottom for 1 h. Samples were subsequently analyzed for zeta potential. The algal biomass collected by the four harvesting methods was freeze dried.

The biomass consisted of algae and the associated coagulant. To obtain the mass fraction of algae in the harvested biomass, 0.5 g of the freeze dried biomass collected by the four harvesting methods was washed several times with 0.1 M NaOH. The washed samples were freeze dried again and weighed to determine the mass of algae in the biomass. Washing was carried out in order to provide