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Wastewater to biofuels: Comprehensive evaluation of various flocculants on biochemical composition and yield of microalgae

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ABSTRACT

This study attempted to determine an efficient flocculant that was used for the recovery of microalgal species, namely *Scenedesmus*. The biomass was grown in a pilot-scale raceway pond receiving domestic wastewater discharges. The microalgae achieved treatment efficiencies of 76.5% NH_4^+-N, 83.1% PO_4^{3-}-P, 73.9% biological oxygen demand (BOD), and 42.8% chemical oxygen demand (COD). During microalgal harvesting, the highest biomass recoveries of 99.0%, 97.5%, and 96.5% were obtained by alum 300 mg/L at pH 7, chitosan 100 mg/L at pH 7, and cationic polymer 10 mg/L at pH 10, respectively. The protein, carbohydrate, and lipid yields of polymer-harvested biomass were 28.0%, 12.4%, and 17.4% (dry cell weight), respectively, which were higher than those obtained when applying either alum or chitosan flocculants. Moreover, the cationic polymer provided no detrimental effects on the fractions of fatty acids derived from the harvested microalgal cells. The microalgal biomass subjected to flocculation by either polymer or chitosan were entire and intact, whereas the lysis of cells was noticed during the alum flocculation process.

1. Introduction

Microalgae have recently received much interest as an environmental-friendly source of food and biofuels (Gutiérrez et al., 2015a,b). Microalgal biomass is considered to be a self-sustained cell factory that harnesses and stores energy from sunlight, which can then be converted into metabolites, pigments, and vitamins (Lam and Lee, 2012). Microalgae can grow in areas unsuitable for agricultural activities, accumulate high amounts of lipids, and do not require large land for cultivation (Guldhe et al., 2014). The economic viability of microalgal biofuels involves the utilization of a low-cost medium such as wastewater for growth (Rawat et al., 2011, Gupta et al., 2016). This trend can achieve economic viability and environmental sustainability.

The harvesting of microalgae is considered as a highly energy-intensive process due to the small size of biomass cells that ranges between 3 and 30 μm (Milledge and Heaven, 2013). This step accounts for 20–30% of the total biomass production cost (Rashid et al., 2013). Microalgae are harvested by several methods such as flocculation, sedimentation, flotation, filtration, and centrifugation (Udom et al., 2013). Also, a combination of these mechanisms can be used for microalgal harvesting. Some mechanical harvesting processes can destruct the biomass cells, causing a significant loss of microalgal metabolite contents (Garzon-Sanabria et al., 2012). The optimum harvesting method is selected based on size and density of microalgae and the importance of the desired products (Vandamme et al., 2013).

Flocculation is preferable than other harvesting techniques such as centrifugation and filtration that require high energy inputs (Granados et al., 2012, Gupta et al., 2017). Flocculation is a promising method of de-watering/harvesting of microalgae and it undertakes two successive steps (Teixeira et al., 2012): (a) transportation that causes particles collision due to various settling velocities, random Brownian motion, and mechanical agitation, and (b) attachment that is controlled by van der Waals and electrostatic attractions (Gerde et al., 2014). Several flocculation mechanisms such as charge neutralization, bridging, sweep-out, and precipitation were also observed (Şirin et al., 2012). The mechanism of bridging occurs when a large network of microalgal cells is formed, whereas patching can be detected when the cells are closely attached (Wu et al., 2012).

Various chemical and natural materials have been utilized as flocculants for microalgal harvesting. Although chemical flocculants (e.g.,

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aluminium and ferric salts) are less expensive than other flocculant-types, they can contaminate microalgal biomass (Harith et al., 2009). Natural flocculants such as chitosan and biopolymers have shown promising results regarding the applicability of downstream processes such as lipid extraction from biomass cells (Hansel et al., 2014). Biodegradable organic flocculants do not contaminate the harvested biomass and require lower doses as compared to inorganic metal salts (Gupta et al., 2014). The optimum harvesting process occurs when the dosage of flocculant neutralizes the negative electrostatic repulsive forces of the microalgal cell walls (Teixeira et al., 2012). For example, Hansel et al. (2014) found that a cationic starch dosage of 10 mg/L was efficient for dewatering and concentrating (exceeding 95%) of *Scenedesmus dimorphus*. Moreover, Gutiérrez et al. (2015a,b) reported that the optimum dose of starch, as a flocculant, was 25 mg/L, achieving a biomass recovery of 95.7%.

The obtained biomass after harvesting can be developed for the production of proteins, carbohydrates, and lipids as well as other value-added products (Guldhe et al., 2014). The selected harvesting method should avoid the deteriorating effect of flocculants on the biomass quality (Milledge and Heaven, 2013). Chemical alum flocculant can produce microalgae containing metal constitutes, which restrict the utilization of the protein fraction for animal feed (Vandamme et al., 2013). Moreover, some flocculants adversely affect the biochemical composition of microalgae (Wu et al., 2012). Therefore, further experiments should be conducted to formulate a suitable method of microalgal harvesting, being environmentally friendly and cost-effective.

To the best of our knowledge, not enough information is available about the effects of flocculants on the downstream process of harvested biomass. Hence, this study attempted to evaluate the flocculation efficiency of a synthesized cationic polymer for the harvesting of microalgal species, namely *Scenedesmus*, grown in a wastewater medium. The recovery efficiencies of polymer-harvested biomass were compared to those obtained by other flocculants, viz., alum and chitosan. The effects of different types of flocculants on the metabolites extraction (e.g., proteins, carbohydrates, and lipids) were demonstrated. The profile of fatty acids after microalgal harvesting using different flocculants was also examined.

2. Materials and methods

2.1. Cultivation of microalgae

A microalgae species, namely *Scenedesmus*, was initially cultivated in 500 mL sterilized conical flasks using a domestic wastewater medium at room temperature (22 ± 2 °C). The culture medium was continuously stirred at 120 rpm under illumination using white light (70 µmol photon/m^2/s) and a cycle of 12 h (light)/12 h (dark). The medium pH was adjusted by adding either 1 M H_2SO_4 or 1 M NaOH. After 15 days of cultivation, microalgal seeds were inoculated in a laboratory-scale reactor of 5 L containing wastewater medium and developed for 21 days. The *Scenedesmus* culture was transferred stepwise from the reactor (at the exponential growth phase) to a pilot-scale raceway pond. The pond had an oval shape with a capacity of 300 m^3 and a depth of 0.3–0.6 m. The pond received wastewater discharges in a batch-feed mode and was inoculated with a 20% v/v of microalgae.

2.2. Harvesting of microalgae

Harvesting of microalgae was conducted using the flocculation process. For this purpose, samples were withdrawn from the raceway pond and examined by jar-test. The used flocculants were (a) alum with concentrations of 50, 100, 150, 200, 250, and 300 mg/L, (b) chitosan with dosages of 20, 40, 60, 80, and 100 mg/L, and (c) cationic polymer with concentrations of 2, 4, 6, 8, 10, and 12 mg/L. These ranges were selected based on the rationale that overdosing of flocculants reduces the efficiencies of algae harvesting and contaminates the supernatant with the excess of ions supplied, hence limiting the algae reuse (Borges et al., 2011; Hansel et al., 2014; Granados et al., 2012). The experiments were repeated at different pH values of 7, 8, 9, and 10. The mixture of cell suspension and flocculants was subjected to rapid mixing at 100 rpm for 3 min, followed by slow mixing at 15 rpm for 15 min. After the mixing step, the microalgal flocs were allowed to precipitate for 15 min.

A 10 mL of sample was withdrawn from the center of each beaker to measure optical density (OD). The OD of biomass was measured at 680 nm by a spectrophotometer (Spectroquant Pharo 300, Merck) and the deionized water was used as a blank. Samples having OD higher than 1 were further diluted.

The biomass recovery efficiency was calculated by Eq. (1).

$$R = \frac{OD_o - OD_t}{OD_o} \times 100 \quad (1)$$

where R is biomass recovery (%), OD_o and OD_t are optical density values at time zero and t, respectively.

2.3. Chemicals and reagents

In this study, all chemicals were of analytical grade and purchased from Sigma Aldrich. The inorganic flocculant of alum and natural flocculant of chitosan were procured and used without further purification. A polyamine-based cationic flocculant was synthesized by the polycondensation of ethylenediamine, N,N-Diisopropylamine, and epichlorohydrin. The preparation of the cationic polymer was conducted as described in our previous study (Gupta et al., 2014). Ultrapure water was used for the preparation of polymer solutions throughout the study.

2.4. Biochemical composition analyses

The protein content in microalgae was detected following the method described by Lowry et al. (1951). Briefly, 200 mg of sample and 25 mL of lysis buffer solution were mixed and homogenized for 5 min. The samples were vortexed for 2 min followed by centrifugation at 3000 rpm for 10 min. The supernatant was kept in falcon tubes, and the residual biomass was re-extracted using lysis buffer. The extracts were pooled, and a portion of 0.5 mL was mixed with 0.5 mL of sodium dodecyl sulfate (SDS) solution and vortexed followed by mixing with 5 mL of reagent-C. Further, 0.5 mL of Folin reagent was mixed, and samples were maintained for 30 min. The absorbance was recorded at 750 nm in a UV–visible spectrophotometer (Spectroquant Pharo 300, Merck). Bovine serum albumin (BSA) was used as a standard solution for the calibration purposes. The protein yields (% w/w, based on dry cell weight) was calculated as per the method of López et al. (2010).

The carbohydrate contents were analyzed by phenol–sulfuric acid method (DuBois et al., 1956). In brief, 250 mg of biomass was mixed with 25 mL of 2% H_2SO_4 (v/v) and autoclaved at 121 °C for hydrolysis. Further, the samples were cooled and neutralized to pH 7 with 0.1 M NaOH. The mixture was centrifuged at 1509 × *g* for 10 min, and the supernatant was collected in a separate vessel. The remaining solids were re-extracted twice, and the supernatant was collected in the same vessel. An aliquot of 0.1 mL of supernatant was further diluted to 1 mL and then mixed with 1 mL of phenol (5% w/v) and 5 mL of 96% H_2SO_4. After cooling to 25–30 °C, the absorbance of this solution was measured at 490 nm using a spectrophotometer (Spectroquant Pharo 300, Merck). Total carbohydrates were quantified referring to a calibration curve.

For lipid extraction and conversion, the harvested biomass was decanted and washed three times with deionized water. Further, the microalgal cells were centrifuged at 1790 × *g* for 5 min, and the concentrated biomass was freeze-dried for 16 h. After drying, the biomass flakes were crushed, and the obtained powder was subjected to microwave-assisted lipid extraction with a chloroform: methanol ratio of 2:1 (v/v) (Guldhe et al., 2014). The lipid fractions obtained from the harvested biomass were dissolved in 1 mL of hexane. Transesterification