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Recyclable polyampholyte flocculants for the cost-effective dewatering of microalgae and cyanobacteria

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ABSTRACT

Flocculation provides an attractive route for the primary dewatering of dilute suspensions of microalgae and cyanobacteria, however, economical harvesting and separations remain challenging. In this article, recyclable flocculants are demonstrated in a novel approach for the harvesting of fresh- and saltwater microalgae and cyanobacteria. Polyampholytes, based on model acrylamide polymers, provide reversible electrostatic interactions with negatively-charged cellular microorganisms through changes in pH. The behavior of the polyampholytic flocculants is characterized for the harvesting of *Chlamydomonas reinhardtii* (*Chlamydomonas* Genetics Center CC124), *Synechococcus* PCC 7002, *Aulacoseira ambigua* (Varsity Lake, CU Boulder), *Nannochloropsis gaditana* (CCMP526), and *Chlorella vulgaris* (UTEX 395). The polyampholytic flocculants, with reversible electrostatic interactions, achieve greater than 97% flocculation efficiencies, can be recovered at greater than 90% yields following flocculation, and when recycled retain flocculation efficiencies of at least 95%. Additionally, the recyclable polymer flocculants, in contrast to what is possible with single-use commercial flocculants, are demonstrated to be more robust for the dewatering process, regardless of culture salinity and other differences (e.g., structural, motility, or surface charge) exhibited by the microorganisms. A techno-economic analysis of the dewatering process is also performed, with recyclable polyampholytic flocculants providing an opportunity to reduce flocculant operating costs by ~85% at standard conditions.

1. Introduction

Microalgae and other phototrophic organisms such as cyanobacteria are promising sources of proteins, lipids, chemical compounds, and vitamins [1–3]. These microorganisms have been applied at large scales for wastewater treatment [4,5] and the production of protein-rich food and feed additives [4,1], nutraceuticals [6], and renewable biofuels [7–9]. Although algae-derived products have generated commercial interest, challenges with effective, economical dewatering of dilute microalgal suspensions (0.02–0.5 g dry cell weight biomass/L) [9–12] still remain [1,7,9,10,13–17]. The harvesting of microorganisms is traditionally a multistep process that involves primary and secondary concentration steps that target final biomass concentrations of 10 g/L and 200 g/L, respectively [9,13,17]. Conventional separation approaches for a myriad of reasons are difficult to apply to the dewatering of microalgal biomass; filtration techniques are limited due to blinding and membrane fouling caused by the diversity of microalgal cell sizes (0.1–20 μm) and/or cell-derived macromolecules [3,17,18], and energy intensive processes such as dissolved air flotation and centrifugation are costly [9,17,19]. For example, economic analyses of microalgal biodiesel processes have indicated that the harvesting step accounts for 15–30% of the overall cost per gallon of biodiesel [9,10,17].

For a microalgae-derived biofuels process, in order to reduce the burden on the secondary concentration step and to facilitate a more cost-effective process, a primary dewatering step based on gravitational sedimentation is implemented to concentrate the biomass to ~10 g dry cell weight (dcw)/L [20,21]. The rate and amount of biomass recovered during sedimentation can be enhanced through the use of coagulants or flocculants. By disrupting the electrostatically repulsive interactions between the negatively-charged cells in stable suspensions [22–25], flocculants yield aggregates of cells that separate rapidly from solution [26–30]. The selection of flocculants for microalgal processes depends on factors including cost, toxicity, dosage, and the impact of the flocculant on downstream processes [17]. Multivalent metal salts such as aluminum sulfate, magnesium hydroxide, ferric chloride, and ferric sulfate have been widely used for coagulation in wastewater treatment with microalgae [17,31–33]. Such inorganic salts, however, are known to contaminate waste streams including recycled water streams containing media salts and the spent algal biomass, thereby limiting the use of the recycled water for culture growth or the biomass waste for value-enhancing co-products [13,17,19,34].

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Recently the pH-dependent reversibility of inorganic flocculants such as magnesium upon acidification has even been considered [35].

An alternative to metal salt coagulants is the use of polyelectrolyte flocculants [9,17,36,37]. Cationic polymer flocculants can induce the flocculation of freshwater microalgae (negatively-charged cells) and are most successful in terms of the efficiency of biomass removal [8,11]. However, commercial polymer flocculants can coat cellular microorganisms in a polymer layer that may limit lipid extraction for a biofuels process [9,31] and, simultaneously, foul equipment in further downstream processing [18]. Another limitation in the application of polyelectrolytes for the flocculation of cellular suspensions is the relatively high ionic strength environments required by marine organisms. The production of biodiesel, for example, from marine microalgal species is being pursued because the microalgae can be cultivated in sea water that already contains salts and is not suitable for conventional agriculture, thus limiting the cost of the growth media [8,34] and the impact on food crops [8]. Polyelectrolyte flocculants have been found to be similarly [38] or less [39] effective at flocculating microalgal suspensions with high salinity (up to 36 g/L) and, in general, reducing the salt concentration in the culture improves the flocculation efficiency of cationic polymers [11]. In one case although successful flocculation of the marine microalgae *Chlorococcum* sp. has been achieved with commercial flocculants, the biomass recovery was less than what can be demonstrated with freshwater species and required an elevated operating temperature [8].

Polymer flocculants capable of 1) reversible interactions with microalgae and 2) being recovered and recycled present opportunities to address many of the challenges with current commercial flocculants including cost, contamination, and interference with downstream processing. Successful dewatering has been previously demonstrated on the freshwater microalgal strain, *Chlorella vulgaris*, with the use of such recyclable polyampholyte flocculants [40]. Here we present charge-tunable polyampholyte flocculants for the dewatering of a variety of *freshwater* and *saltwater* microalgae and cyanobacteria: *Chlamydomonas reinhardtii* (*Chlamydomonas* Genetics Center CC124), *Synechococcus* PCC 7002, *Aulacoseira ambigua* (Varsity Lake, CU Boulder), and *Nannochloropsis gaditana* (CCMP526). The application of the recyclable flocculants (Fig. 1) involves initial flocculation of the microorganism suspension, dewatering of the concentrated biomass and pH-induced release of the flocculants, followed by flocculant recovery using secondary concentration and recycling the recovered flocculants. We demonstrate that polyampholyte flocculants effectively dewater cellular suspensions (>95% flocculation efficiencies), but can also be recovered and recycled for fresh- and saltwater microorganisms. The implementation of these recyclable polyampholyte flocculants is also discussed in the context of conventional microalgal biofuel process designs and a techno-economic assessment of the cost effectiveness of such materials is provided.

2. Materials and methods

2.1. Synthesis of copolymer flocculants [40]

The synthesis of polyampholyte flocculants was performed by the free-radical random copolymerization of *N*,*N*-dimethylaminopropyl acrylamide (DMAPAA) and acrylic acid monomers. Example conditions for the polymerization of the polyampholyte (with 75 mol% positive and 25 mol% negative components) are DMAPAA (7.00 g, 44.8 mmol), acrylic acid (0.807 g, 11.2 mmol), and initiator (0.256 g, 1.12 mmol) dissolved in 160 mL DI water, followed by heating to 60 °C and stirring for 48 h. The DMAPAA monomer, free-radical initiator, and solvents were purchased from Fisher Scientific. Acrylic acid was purchased from Sigma Aldrich. Vacuum distillation was used to purify the DMAPAA and acrylic acid monomers prior to polymer synthesis. Following polymerization, the product was precipitated by dropwise addition into acetone and the recovered solids were repeatedly rinsed resulting in a polymer yield of 52.5 wt.% (4.10 g). The polymer composition and molecular weight (5.1×10^5 g mol^{-1}) were characterized by ^{1}H NMR in DMSO-d_6 at room temperature (Bruker Ascend™ 400) and aqueous-phase gel permeation chromatography (GPC) (Waters 2414 refractive index detector), respectively. The GPC results were reported relative to poly(ethylene glycol) standards.

2.2. Preparation of chitosan

Chitosan from shrimp shells was purchased from Sigma Aldrich and a flocculant solution was prepared by dissolving the solid flakes in DI water at 1% w/w. The solution was then adjusted to a pH of 1 with 12 M HCl to ensure complete solubility.

2.3. Culturing of microalgae and cyanobacteria

C. vulgaris (UTEX 395) was grown in modified Bold Basal media (MBBM), *C. reinhardtii* (*Chlamydomonas* Genetics Center CC124) was grown in modified tris–acetate–phosphate (TAP) media, *N. gaditana* (CCMP526) was grown in artificial sea water (ASW) media, *Synechococcus* PCC 7002 was grown in both BG-11 freshwater media and ASW, and *A. ambigua* (Varsity Lake, Boulder, CO) was grown in MBBM with 0.36 g/L sodium metasilicate. All microorganisms were cultured in 7–9 L glass photobioreactors with external illumination from fluorescent bulbs (32 W, 4000 K color). Aeration and agitation was supplied by bubbling with house air (pressurized to 65 psi and filtered by a 0.01 μm coalescing prefilter and a 1 μm particulate final filter) [41]. The concentration and turbidity of the cultures was determined by optical density (OD) measurements at a wavelength of 750 nm using an Agilent

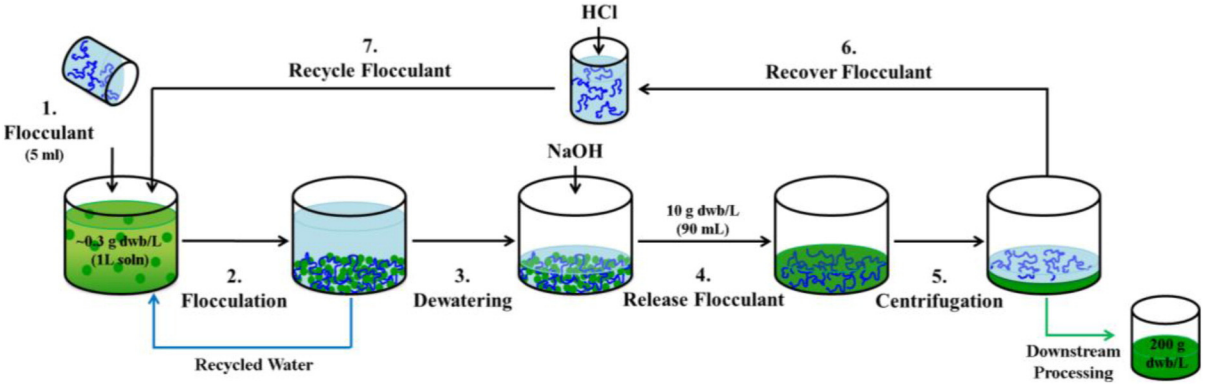

Fig. 1. Process flow diagram of the novel harvesting approach with recyclable polyampholyte flocculants. Key process steps include primary dewatering, pH-induced release and recovery of the flocculants from the dewatered biomass, and recycling of the recovered polyampholytes.