Algal Research 5 (2014) 133–139

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Efficient flocculation of microalgae for biomass production using cationic starch

Philip A. Hansel, R. Guy Riefler *, Benjamin J. Stuart

Institute for Sustainable Energy and the Environment, Ohio University, Athens, OH, United States

ARTICLE INFO

Article history:
Received 3 February 2014
Received in revised form 16 May 2014
Accepted 5 July 2014
Available online 15 August 2014

Keywords:
Flocculation
Microalgae
Cationic starch
Biomass production
Scenedesmus dimorphus

ABSTRACT

In order to make algal biomass a suitable feedstock for fuel and bioproducts, a practical means of dewatering and concentrating the algal cells must be devised. An effective algal dewatering scheme will most likely utilize flocculation as an initial concentrating step. Cationic starch could prove to be an effective flocculant for economical production of uncontaminated microalgal biomass. This investigation seeks to ascertain whether or not cationic starch is an effective flocculant for the freshwater green alga *Scenedesmus dimorphus*. Evidence from extensive flocculation tests showed cationic starch to be effective at low dosages, approximately 0.08 g cationic starch to g algal biomass, for both growth phase and stationary phase *S. dimorphus*. Algal cell restabilization occurred at lower starch concentrations in the growth phase than in the stationary phase. The tests also indicated that flocculation was effective with degrees of cationic substitution from 0.14 to 0.64 with no significant change in flocculation efficiency over that range.

1. Introduction

Considerable attention has been paid to microalgae in the recent years due to their possible use as sustainable replacements for petroleum derived fuels and products [13,24,37]. However, a major problem that exists concerning the use of microalgal biomass as a feedstock is an economical and effective means of concentrating the microalgal biomass for use in downstream applications. An effective algal dewatering scheme will most likely utilize algal flocculation as an initial concentrating step.

Traditionally, microalgal research has been concerned with the primary goal of producing a liquid hydrocarbon fuel derived from triacylglycerols (lipids) that have accumulated within the microalgal cells [20,29,37]. Specifically, the concept of microalgal biodiesel has been idealized since the U.S. Department of Energy funded the Aquatic Species Program in response to the 1973 oil crisis—a program designed to support intense research into the collection and characterization of many microalgal species, and then to elucidate whether microalgae are a valuable alternative to petroleum or plant based fuels [10,16,37]. The report concluded that microalgae indeed are potential replacements for petroleum-based fuels, but considering the technology at the time, and oil prices of the early 1990s, the economics of the process simply were not favorable [37].

Currently, with concerns of fossil fuel depletion, record high oil prices, and excessive carbon dioxide atmospheric loads, renewed interest in microalgae-based biofuels has sparked, but the focus of the international community–both academic and industrial–has turned to a more comprehensive approach to the utilization of the harvested microalgal biomass [24,26,36,37]. Published reports [26,37] still conclude that microalgae grown for the sole purpose of lipid production cannot compete with the current p\rices of oil based on the present state of production technologies. However, if the biomass is properly recovered, partitioned, and utilized in several fashions–biodiesel production, high-value products recovery, fermentation, anaerobic digestion, and gasification–additional income streams, which will offset the production costs associated with simple biofuel production, could be created [26,27,29,37].

Concentration of microalgal biomass from growth media is a major problem that contributes between 20 and 30% of the overall costs of a microalgae biofuel production process [6,16,22]. Microalgae are small (5–20 μm), and possess a density similar to water, which makes both filtration and centrifugation difficult [33]. Furthermore, algae cultures are primarily composed of water—well over 90% by mass [6, 15,38]. In order to utilize the biomass, it first must be extracted from the liquid growth media, and concentrated to an extent that renders the downstream processing effective [6,38]. Flocculation–when suspended particulates are destabilized by an added chemical and subsequently agglomerate is a reliable initial concentration technique currently used in water and wastewater treatment processes. It functions to effectively increase particle sizes, so that the larger destabilized particles settle under natural gravitational forces, and generally results in a biomass slurry that is approximately 2% dry weight cellular matter [38].

Regarding flocculation, metallic salts and positively charged polymers have traditionally been used in commercial and municipal flocculation operations [6]. These chemicals, however, are not appropriate for

* Corresponding author.
E-mail addresses: ph257203@ohio.edu (P.A. Hansel), riefler@ohio.edu (R. Guy Riefler), stuart@ohio.edu (B.J. Stuart).

http://dx.doi.org/10.1016/j.algal.2014.07.002
2211-9264/

microalgal biomass production, because they produce a commercially nonviable biomass [15,23]. Metallic salts, such as aluminum sulfate ($Al_2(SO_4)_3$), contaminate the flocculated algae [15], and positively charged polymers, such as cationic polyacrylamides, are known to break down into their monomer substituents, which are noted neurotoxic and carcinogenic compounds [40]. Consequently, the solution to this problem is to find an alternative chemical that is cheaply produced, efficiently flocculates microalgae, and leaves the biomass in an uncontaminated state.

It has been known for some time that starch, a naturally-occurring biodegradable polymer of glucose molecules, can be chemically modified resulting in a compound that possesses very different properties than the native starch [25,33,35]. The abundance and low cost of starch ($US 1–3 per kilogram), make it an attractive polysaccharide for modification, specifically cationization [19,33]. Cationic starches–starches that have been modified by the incorporation of a positively charged functional group–are prepared through etherification reactions involving quaternary ammonium salts such as N-(3-chloro-2-hydroxypropyl) trimethyl ammonium chloride (CHPTAC) and the epoxide equivalent 2,3-epoxypropyl trimethyl ammonium chloride (EPTAC) [7]. These compounds are desirable for starch cationization since they are able to maintain a positive charge regardless of media pH extremes [18,19]. The etherification reaction occurs in alkaline media through the Williamson ether synthesis mechanism. The hydroxyl groups of the anhydroglucose units (AGU) of the starch molecules react in a nucleophilic reaction with the cationic agent, resulting in hydrogen being replaced with the amine functional groups (see Fig. 1, [34]). Though the kinetics are still not completely understood, several factors that influence the reaction are as follows: the cationizing species (CHPTAC or EPTAC), the molar amounts of cationizing agent, the molar amounts of catalyst (NaOH), the reaction duration, and the temperature of the reaction. The charge density of the resultant starches is commonly assayed via a measure of the amount of nitrogen present in the sample, and is reported numerically as the degree of substitution (*DS*) [7,8,11,14]. This degree can vary between 0 and 3, as there are three available hydroxyl sites for cationic substitution on each glucose molecule in the starch polymer chain [17]. These starches have found use as flocculants in both wastewater treatment, and in the papermaking industry [19]. Some work has been done regarding their effectiveness in microalgal flocculation [33]. However, further substantiation in the literature is needed.

This research evaluates the flocculation efficacy of cationic starch using a culture of *Scenedesmus dimorphus*. *S. dimorphus* is a species of microalgae particular to a genus that is common to many freshwater ecosystems [36]. This genera has been identified in several studies as suitable for biomass production because of its rapid growth and high lipid production [2,27,30,32,36,37]. As it is known that flocculation efficiency is directly influenced by the growth state of a microalgal culture [9,31], the clarification tests will be conducted on the *S. dimorphus* culture in growth phase (GP) and in stationary phase (SP). Regarding flocculation activity, correlations will be developed relative to starch *DS* as well as the growth state of the *S. dimorphus* culture.

2. Materials and methods

2.1. Starch etherification using CHPTAC

Potato starch, certified with 75% amylopectin and 25% amylose, (MPBiomedicals, Aurora, OH) was dried at 150 °F for a period of 24 h prior to use. Thirty total cationic starch batches were synthesized using CHPTAC by altering three reaction variables: molar ratio of NaOH:starch, molar ratio of CHPTAC:starch, and reaction time. Molar ratios of both catalyst NaOH and reagent CHPTAC were based on the starch monomer, an anhydroglucose unit (AGU, 162.5 g/mol). Molar ratios for NaOH:AGU were 0.5, 1.0, 1.5, and 1.75 and for CHPTAC:AGU were 0.5, 1.0, and 1.5. Reaction times were 6, 12, and 24 h. For each

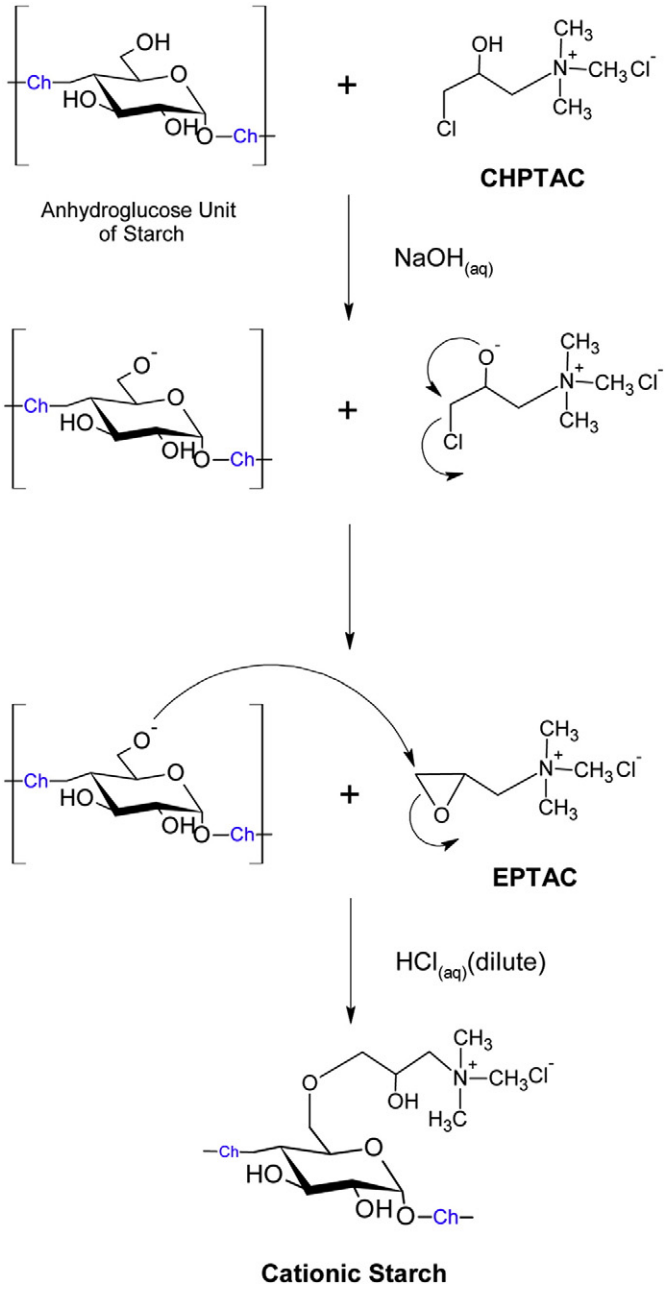

Fig. 1. Proposed cationization reaction (from [19]). Note that CHPTAC in the first step gets transformed to EPTAC in the third step.

batch, dried potato starch (24 g, 0.148 mol) was suspended in 1000 mL of ultrapure water and heated to a temperature of 50 °C over a period of 30 min. The proper molar amount of NaOH was then added to the suspension via a 10 M NaOH stock. A swollen yellow gel formed, and mixing was increased. Following 1 min of mixing, the proper molar portion of CHPTAC solution (60 wt.%) was added dropwise to the mixture. The reaction was then carried out for the specified time period at constant temperature (50 °C). The mixture was cooled to room temperature and 5 M HCl was carefully added to lower the pH below 7, thus effectively stopping the etherification reaction [19]. The product was recovered through precipitation with excess 95% ethanol chilled to a temperature of approximately −15 °C. The product was filtered, dried, and ground into a fine powder. To ensure purity, the starches were then washed with portions of ethanol until a silver nitrate test yielded no sign of residual halide [39]. Following this, they were dried for a period of 24 h at 65 °C prior to use. A control starch batch