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Effective harvesting of the microalgae *Chlorella prototheocoides* via bioflocculation with cationic starch

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HIGHLIGHTS

- *Chlorella prototheocoides* flocculation efficiency of Greenfloc 120 was evaluated.
- The different biomass densities, pH and flocculant concentrations were tested.
- The optimal flocculation concentration was found to be 40 mg/L.
- Highest flocculation efficiency without flocculant addition was obtained at pH 10.

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ABSTRACT

In the present work, the flocculation efficiency of cationic starch (Greenfloc 120) was tested on the fresh water microalga *Chlorella prototheocoides* under different conditions (pH and flocculant concentrations). Different concentrations of Greenfloc 120 (0, 2.5, 5, 10, 20, 40 mg L^{-1}) were screened against different algal densities (0.44, 0.56 and 0.77 g L^{-1}). Once the optimal flocculation concentration had been established (40 mg L^{-1} for all different biomasses densities) a more detailed analysis was performed in order to investigate if different pH (4.0, 7.7, and 10.0) could increase the flocculation efficiency of cationic starch. Highest flocculation efficiency without addition of Greenfloc 120 was obtained at pH 10, while in the presence of flocculant, the efficiency increased for all the tested pH values, with a maximum of 98% for pH 7.7 and 10. Cationic starch confirmed to be as an easy to use, efficient and cost-effective flocculant for harvesting of microalgae.

1. Introduction

Microalgae can be a promising alternative for the production of biofuels, high value products and feed supplements (Stephens et al., 2010). Microalgae can capture CO_2 from air and bioremediate waters. Moreover, microalgae have the flexibility to be cultivated in areas unsuitable for terrestrial crops (Perez-Garcia et al., 2011; Campbell et al., 2011; Chisti, 2007). Despite these advantages, microalgal cultivation presents a number of challenges making utilization of microalgae as production and/or remediation organisms challenging. One of the major challenges is separation of the microalgae from the liquid medium. This energy consuming process represents a considerable part (20–30%) of the total production costs, being one of the main factors limiting the economic feasibility of microalgal valorization (Chisti, 2007; Salim et al., 2010). The appropriateness of specific harvesting technology depends on the microalgal species and their intrinsic properties as cell size, shape, presence or absence of flagella, oil content and the nature of cultivation media (Chisti, 2007; Lee, 2011; Singh and Sai, 2010; Singh et al., 2011; Chen et al., 2011).

The flocculation process is one of the most promising approaches existing to separate the microalgae from the media. The method has the advantage that allow larger quantities of biomass to be treated, and leaving the cell intact without resulting in lysis (Chen et al., 2011; Linder and Meyer, 2010). In this process a chemical is added for conditioning the suspended microalgae, reducing its negative surface charge causing aggregation into larger particles (flocs) and allowing subsequent precipitation (flocculation). Chemicals such as ferric sulfate, aluminum, zinc and chitosan have been tested for microalgal harvesting. However, these flocculants need to be added at relatively high concentrations for exhibiting a significant effect. At these large dosages several drawbacks are appearing such as pH increases, and residual

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accumulation of salts (aluminum and iron chloride) (Pushparaj et al., 1993; Oh et al., 2001; Vandamme et al., 2010; Morales et al., 1985). On the other hand, biodegradable organic flocculants do not result in residual accumulation, often require lower dosage, do not affect pH, and have relatively low costs (Vandamme et al., 2010; Pal et al., 2005), but they are usually algal species specific. One such commercially available cationic starch, Greenfloc 120, is used for wastewater treatment and paper mill industries (Prakash et al., 2007;Vandamme et al., 2010). Cationic starch is obtained via addition of quaternary ammonium groups to the glucose hydroxyl groups, and has been reported to be an efficient, cost-effective flocculant with no short-term effect on algal viability (Pal et al., 2005; Vandamme et al., 2010). Greenfloc 120 has been extensively tested (Vandamme et al., 2010) on microalgae *Parachlorella, Scenedesmus, Nannochloropsis* and *Pteodactylum*. In the present paper, the analysis of this flocculant is restricted to *Chlorella protothecoides* as representative of a genus, *Chlorella*, which is central to industrial microalgae production. *Chlorella* appears to be the most important microalgae for the production of biofuels and high value added chemicals due to following properties: high lipid content (43–57.8%), relatively high cell densities (up to 51.2 g L^{-1}) and biomass productivities (1.7–4.4 g L^{-1} d^{-1}), ability to grow in a variety of carbon sources (acetate, glucose, other organic compounds and CO_2) under different culture conditions (heterotrophic, phototrophic, mixotrophic) and resistance to contaminations (Xu et al., 2006; Heredia-Arroyo and Bo Hu, 2010; O'Grady and Morgan, 2011; Xiong et al., 2008).

Furthermore, to better reproduce conditions that are closer to industrial applications, the algal concentrations tested in present study were significantly higher (up to 1.1 mg L^{-1}) than the ones found in the literature (up to 0.35 mg L^{-1}) (Vandamme et al., 2010). Finally, a previous work (Vandamme et al., 2010) describes only superficially the effect of pH on Greenfloc 120. In the present investigation, the flocculation efficiencies of Greenfloc 120 are directly compared to the spontaneous flocculation of *C. protothecoides* at different pH values.

2. Methods

2.1. Pre inoculum algal culture

The initial inoculum of the microalgae *C. protothecoides* was obtained from CCAP (The Culture Collection of Algae and Protozoa at the Scottish Marine Institute (SAMS), strain number 221/8D). The microalgae were pre-cultured in flask batch cultures (working volume of 1.8 L) prior to the evaluation. The medium used was based and modified from Xiong et al., 2008. Constant air was supplied by two air pumps with a total capacity of 1.5 L min^{-1} for mixing purposes, 24 h light under two GRO white light tubes (70 μmol photons m^{-2} s^{-1}), and temperature between 26 and 28 °C.

2.2. Experimental design

The flocculating efficiency of Greenfloc 120 was evaluated using a modified jar test methodology as described previously (Vandamme et al., 2010). The effect of flocculant concentration (0, 2.5, 5, 10, 20, 40 mg L^{-1}) on three different dry weight microalgal biomasses (0.44, 0.56 and 0.77 g L^{-1}, all obtained from cultures in late exponential phase) was evaluated. Cationic starch was added to each batch for the coagulation-flocculation phase (time 0) at specific concentration according to the test series and stirred under 500 rpm (intensive) for 5 min. The speed was then reduced to 250 rpm for 25 min (slow stirring). Once the mixing phase was over (total of 30 min), samples were taken from each graduated test tube at different settling times (0, 10, 20 and 30 min) for OD determination (OD_{550}). The experiment was performed at a temperature of 23 °C and at pH 7.7. Once the optimal flocculation concentration had been established, a more detailed analysis was performed in order to investigate if different pH (4.0, 7.7, and 10.0) could increase the flocculation efficiency of cationic starch. The pH levels of 4 and 10 were chosen as several studies (Wu et al., 2012; Vandamme et al., 2012; Liu et al., 2013) reported increased spontaneous flocculation at such values. For this second set of experiments the biomass density was further increased up to 1.1 mg L^{-1} because such density is closer to this achieved in scaled up microalgal systems and therefore our results can justify the suitability of cationic starch in industrial applications. pH values of the medium were adjusted either by HCl or NaOH.

The amount of algal biomass removed was estimated from the ratio of the initial over the final optical density (550 nm using a Jenway 6405 UV/VIS spectrophotometer) from the pipette samples taken from 80% height of the test tubes transferred to 2 mL cuvettes.

The flocculation efficiency was calculated according to equation given by Salim et al., 2010 (Eq. (1)):

$$\text{Recovery}(\%) = \frac{OD_{550}(t_0) - OD_{550}(t)}{OD_{550}(t_0)} * 100 \quad (1)$$

where $OD_{550}(t_0)$: OD measure before flocculant addition; $OD_{550}(t)$: OD measure at different settling times after flocculant addition.

All results were given as averages ± standard deviation, and the statistical analyses were performed with confidence level of 95%. The flocculation efficiency due to different biomass concentrations, flocculant concentrations and the effect of pH, where compared by *F*-test, followed by *t*-test for equal or unequal variances (Snedecor and Cochran, 1976) in Microsoft Office Excel 2007. In addition, a Pearsoń s correlation test (Lee Rodgers and Nicewander, 1988) was performed in order to evaluate any correlation between the flocculant efficiency and the flocculant concentrations.

2.3. Analysis

Analytical determination of total solids (TS) and volatile solids (VS) was made according to Standard Methods (APHA, 1998).

2.4. Chemicals

The commercial cationic starch Greenfloc 120 (Hydra 2002 Research, Developing and Consulting, Hungary), normally used for waste water treatment, was employed for microalgal flocculation experiments. Greenfloc 120 has a 17% concentrated solution of starch ether in water, as ready to use. The flocculant was gradually diluted to the required concentration with distilled water prior the addition to the suspension of microalgae.

3. Results and discussion

3.1. Effect of flocculant concentration onto flocculation efficiencies

The flocculation process reached a stabilization point at a settling time of 20 min (50 min from the beginning of the flocculation experiment). Applying a settling time above this value did not result in a detectable increase in the flocculation efficiency (Fig. 1). An interesting finding was that a flocculation efficiency ranging from 10% to 40% occurred already during the mixing phase.

Following, the results related to a settling time of 30 min (maximum flocculation efficiency reached for each condition) were compared statistically (Table 1). Flocculant addition significantly