Algal Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Algal Research

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

Separation of *Chlorella* biomass from culture medium by flocculation with rice starch

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ARTICLE INFO

Keywords:
Microalgae flocculation
Harvesting
Starches
Residual aluminium
Floc analysis
Cost evaluation

ABSTRACT

Coagulation-flocculation remains as one of the preferred methods for efficient harvesting of *Chlorella* sp. cells. Although the use of established aluminium salts is highly appraised for high harvesting efficiencies, excessive residual aluminium imparted on both the treated supernatant and harvested biomass remained worrisome. Hence, the objective of this present study is to minimize the resulting concentration of aluminium present in the system by evaluating the use of rice starch as an aid to chemical coagulants. The residual aluminium in the starch aided and non-aided treated supernatants and biomass were then determined by using an inductively coupled plasma-optical emission spectroscopy (ICP-OES) and energy-dispersive X-ray (EDX) spectroscopy respectively. At an optimum pH of 6, more than 95% of the initial *Chlorella* biomass was recovered at 72 mg/L of alum or 9 mg/L of PACl. However, high residual aluminium contents in treated supernatants (1.3–1.7 mg/L) and biomass (2.5–4.5% weight distribution) were evident. Through the introduction of autoclaved rice starch by up to 120 mg/L as an aid, the dosage of chemical coagulants applied and the detected residual aluminium concentrations were reduced by up to 54%. Despite the increment in organic loadings for these treated samples, the use of starch which is biodegradable would minimize the resulting toxicity and metal contamination imparted. Thus, rice starch can be considered as a potential alternative to lower the dependence on chemical coagulants which limits the reusability of culture medium. Based on the FE-SEM micrographs obtained, the resulting flocs treated with rice starch were notably filamentous and threadlike; in-line with the coagulation mechanism of adsorption and bridging.

1. Introduction

Photosynthetic microorganisms such as microalgae are prized sources of raw materials with diversified industrial applications [1,2]. At present, the microalgae market is greatly dominated by *Spirulina* and *Chlorella* with combined global annual production of 5000 t; amounting to 50% of the total worldwide algae production [3] with dedicated applications as health food and products [4]. Studies further present estimates close to 30% of the current global algae production being utilized for animal feed [3]. Other commercial uses of *Chlorella* include cosmetics and also aquaculture [5]. The small size (5–50 μm) and low microalgae density in the range of 1030–1140 kg/m^3 [6–8] complicate the harvesting process without the selection of a suitable treatment method. Efficient harvesting of microalgae therefore has been one of the prime focusses in recent years, with emphasis on various methods either of chemical, biological or physical nature [9]. Among these however, coagulation-flocculation is recognized as one of the promising and economical methods, also yielding the removal of intact microalgae cells [10]. Other merits of this method include the convenience handling larger amounts of algae culture for treatment [7,8], easily scalable and caters a wide range of microalgae species [11]. This further prompts the coagulation-flocculation method as a simple solution towards harvesting *Chlorella*.

Depending on the use of harvested biomass, the choice of coagulant should also be cautioned [12]. Inorganic coagulants such as aluminium and ferric salts are among some of the commonly used types [13]

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https://doi.org/10.1016/j.algal.2017.11.012
Received 14 February 2017; Received in revised form 10 November 2017; Accepted 10 November 2017

although alum has been found to be more efficient in most instances [14–16]. At all the studied alum dosages, more than 90% of the initial *Scenedesmus* sp. and *Chlamydomonas reinhardtii* biomass was recovered from culture medium [17]. However, a low alum concentration of 50 mg/L was found effective for the studied microalgae strains in contrast to much higher dosage requirements of at least 3 fold for *Botryococcus* sp. [18]. On the other hand, harvesting marine *Chlorella* cultivated in urea fertilizer mediums showed the required dosage of ferric salts around 6% lower than for alum at respective optimized pH values [19]; further illustrating that the amount of coagulant required would be dependent on the concentration/surface area or the charge of the microalgae of interest. Besides alum as a coagulant, its influence on lipid, protein and carbohydrate contents in the recovered microalgae cells was also evaluated. The alum-harvested cells have lower contents of lipid and protein in contrast to those harvested using natural sedimentation due to inhibitory effects [20]. In spite of high harvesting efficiencies, the use of chemical coagulants in upstream processing of microalgae would raise issues with excessive residual aluminium leading to potential contamination [21] and limitations in the recycling of culture medium with potential to inhibit subsequent microalgae growth [8,22]. The residual aluminium detected in fatty acid methyl esters (FAME) of *Nannochloropsis salina* flocculated with aluminium nitrate sulfate at an initial microalgae concentration of 1000 mg/L was found to have exceeded those stipulated by WHO [23]. Downstream application in microalgae harvested using aluminium-based coagulants is also questionable as aluminium and sulfate have been reported to inhibit specific methanogenic activity in bacteria fed wastewater sludge while land application of treated biomass resulted in increased uptake of heavy metals and caused phosphorus deficiencies in plants [24]. Additionally, the harvested microalgae biomass would be tainted with metal contaminations rendering it to be unfit for further applications particularly for animal feed and human consumptions [13].

On the other hand, materials which are more environmentally friendly such as biopolymers of starches, chitosan, chitin and alginic acid have gained increasing interests from many researchers [25]. Starch which is safe to handle, biodegradable, non-toxic and non-corrosive in nature is an attractive source of flocculant due to its wide availability [26]. Of late, a study conducted on a pilot high rate algal pond has illustrated the successful use of commercial potato starch solution of 1% concentration as the flocculant with microalgae removals above 90% [27]. The performance of starches, specifically rice starch has shown result as comparable with ferric chloride in the harvesting of *Botryococcus* sp. with harvesting efficiency by up to 87% [18]. Besides unmodified starch, algae samples were previously treated solely with cationic starches modified via the esterification of 3-chloro-2-hydroxypropyl trimethyl ammonium chloride (CHPTAC) or 2,3-epoxypropyl trimethyl ammonium chloride (EPTAC) with degree of substitution (DS) ranging from 0.009 to 0.635 [28] and also synthetic polymers of methacrylamidopropyl trimethyl ammonium chloride (MAPTAC) [29]. Apart from yielding plausible harvesting efficiencies, no inhibitory effects on the generation of bio-products [30] and the absence of long-term toxicological effects on the harvested microalgae using cationic starches [31] were observed. Likewise, commercial cationic starches such as Greenfloc 120 and Cargill C*Bond HR have also been used as a primary coagulant for the removal of a variety of freshwater and marine microalgae [25]. In addition to the investigations on coagulation-flocculation of algae cells, other studies focused mainly on the analysis of floc characteristics using metal coagulants [32] and also chitosan-based flocculants [33].

Though literatures highlight various research related to natural coagulants, studies utilizing unmodified and native starches for microalgae harvesting with emphasis for applications in the food and pharmaceutical industries are yet scarce. Among the various types of starches, rice starch has been one of the efficient coagulants for turbidity removal [34] and for harvesting microalgae [18]. The high efficiency of the rice starch and its potential as an environmentally friendly flocculant are the prime motivations in formulating the current research. Hence, the aim of this study is to explore the alternate use of rice starch as a potential flocculant thereby enabling reduced concentration of residual aluminium imparted on the treated supernatants and harvested *Chlorella* cells. For this purpose, the use of chemical coagulants and their implications in relation to rice starch were accessed. Standard jar tests were conducted to optimize pH for the use of chemical coagulants as well as the dosage of rice starch as an aid to harvesting efficiencies was evaluated. The characterization and comparison of the harvested biomass through the use of varying coagulants were also conducted. As the harvested *Chlorella* sp. cells can be utilized as animal feed, it is crucial that the residual aluminium content in the harvested biomass is minimized. An inductively coupled plasma-optical emission spectroscopy (ICP-OES) and energy-dispersive X-ray (EDX) were consequently incorporated to quantify the residual aluminium in both treated supernatants and *Chlorella* flocs respectively. Cost evaluation of the different combinations of coagulants used in the harvesting of *Chlorella* is also performed as part of the economic feasibility study.

2. Materials and methods

2.1. Materials

Chlorella strain (UMACC 283) isolated from a palm oil mill effluent anaerobic treatment pond was subsequently grown in Bold's Basal Medium [35] in this study. The characteristics of the microalga suspension obtained are summarized in Table 1. With 3% solution strength, the conventional chemical coagulants used were aluminium sulfate hydrate ($Al_2(SO_4)_3{\cdot}H_2O$) sourced from Sigma-Aldrich and liquid polyaluminium chloride (PACl) 10% supplied by Holy Mate Sdn. Bhd., Malaysia. Distilled water was used to dilute hydrochloric acid (Merck) to 5 M solution and to dissolve sodium hydroxide pellets (Merck) for the preparation of 1 M solution which was used for pH adjustments. Rice starch procured from Sigma-Aldrich was used as an aid to chemical coagulants after autoclaving with pre-determined amount of water. The detailed information in regards to the preparation of coagulant and rice starch is outlined previously [36]. The multi-element standard solution supplied by Perkin Elmer (Pure IV, Quality Control Standard 23, 10% HNO_3) was used for the preparation of ICP-OES calibration curves.

2.2. Coagulation-flocculation of Chlorella suspensions

Jar tests were performed using conventional 4 jar apparatus (VELP Scientifica JLT4, Italy) in accordance to previous established protocols, see [34]. A control with the same starting conditions as the respective

Table 1
Characterization of *Chlorella* suspension procured and used in jar test experiments.

Parameters	Mean values
Initial pH	7.97 ± 0.28
Initial turbidity, NTU	643 ± 38
Refractive index	1.3329 ± 0.0001
Zeta potential, mV	−23.0 ± 2.8
Total suspended solids, mg/L	1016 ± 107
Elemental contents at pH 6, 167 NTU:	
Zinc (Zn), mg/L	1.048 ± 0.002
Iron (Fe), mg/L	0.242 ± 0.003
Magnesium (Mg), mg/L	2.891 ± 0.013
Calcium (Ca), mg/L	8.338 ± 0.055
Copper (Cu), mg/L	2.027 ± 0.020
Sodium (Na), mg/L	26.703 ± 0.172
Aluminium (Al), mg/L	0.044 ± 0.001
Potassium (K), mg/L	13.625 ± 0.055

The underline in the table is to distinguish the subsequent data on the elemental contents detected in the Chlorella suspension used in the jar test experiments.