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Harvesting microalgae by flocculation–sedimentation

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ABSTRACT

Slurries of five marine and freshwater microalgae (*Chlorella vulgaris*, *Choricystis minor*, *Cylindrotheca fusiformis*, *Neochloris* sp., *Nannochloropsis salina*) were effectively flocculated using aluminum sulfate and ferric chloride as flocculants. The flocculant dose for 95% removal of the algal biomass by sedimentation in a standardized 62 min treatment depended on the following factors: the type of the flocculant; the algal species and cell diameter; the concentration of the biomass in the algal slurry; and the ionic strength of the suspending fluid. For all algae, the flocculant dose for 95% removal of the cells increased linearly with the concentration of the biomass in the slurry. Aluminum sulfate was a generally better flocculant than ferric chloride. Less flocculant was required for flocculation from a high ionic strength medium for the one alga (*C. vulgaris*) that could be grown both in freshwater- and seawater-based media. Quantitative relationships are reported for the flocculant dose dependence on the biomass concentration in suspensions of the five algae.

1. Introduction

Microalgae are attracting much attention as sources of fuel oils and diverse other products [4,8,10,28]. Slurries of microalgae grown via photosynthesis typically have a low amount of the biomass (0.5 to 4 g/L, dry basis [9]) suspended in a large volume of water. The biomass slurry must be dewatered prior to use. Dewatering is expensive [43]. Common methods of dewatering include centrifugation [15,31], various kinds of filtrations [11,15,30], electrical methods such as electrocoagulation [27] and forward osmosis [29]. All these are expensive. The least expensive option for partial dewatering is flocculation followed by gravity sedimentation of the flocs [26,31,45]. Flocculation–sedimentation can remove nearly two-thirds, or more of the water.

Flocculation is a well-established method for removing colloidal suspended solids in water treatment processes [5], but is less well developed for harvesting microalgae. Microalgal cells are typically <15 μm in diameter and have a density only slightly greater than water. As a result the cells do not readily settle out of suspension under gravity. Furthermore, the surface of the cells is negatively charged [15] because of the ionized functional groups on the cell wall [33]. Surface charge is measured in terms of the zeta potential [20]. Electrostatic repulsion between cells prevents them from coming together and spontaneously adhering to each other by van der Waals forces [33]. In a flocculation process, a cationic flocculant is used to neutralize the surface charge on the cells to facilitate spontaneous formation of cell aggregates, or flocs. In addition to charge neutralization, other mechanisms contribute to floc formation [13]. The cationic flocculants include salts of multivalent metal ions [17] such as aluminum sulfate and ferric chloride. Cationic polymers are other widely used and effective flocculants [3,7,11,17,18,37,42,44,46], but are generally much more expensive than metal salts such as aluminum sulfate. Flocculation may be achieved using various acids [36], but a large quantity of acid is generally required. Autoflocculation [16] in which the alga produces the flocculating chemicals at a certain stage of growth and settles out of the water, is a further option that may be useful with specific algae, but is not broadly applicable to the various species. Bioflocculation uses polymeric flocculants produced by various microorganisms to flocculate an algal suspension [7,26,38]. Bioflocculation may be carried out in situ by coculturing the flocculant-producing microorganisms with an alga if the contaminating species is acceptable.

Flocs being much larger than the individual cells, readily settle out under the influence of gravity. The settled flocculated biomass, or sludge, is still quite watery and further dewatering by filtration or centrifugation is generally required. However, the sludge has a much smaller volume compared to the initial algal slurry and this greatly reduces the capacity requirements and the cost of further dewatering. In addition, as flocculation increases the particle size, further dewatering by both filtration and centrifugation becomes easier.

This work reports on flocculation of several marine and freshwater algal species using aluminum sulfate and ferric chloride. Both of these are inexpensive, readily available, safe and have a low environmental impact. Quantitative relationships are developed for the minimum effective flocculant dose for the various algae and the two flocculants. The required dosage is shown to depend on the cell size, the biomass

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concentration in the algal slurry, the algal species, the flocculant and the ionic strength of the culture medium.

2. Materials and methods

2.1. Microalgae and culture conditions

Five microalgae were used: the freshwater microalgae *Chlorella vulgaris*, *Choricystis minor* [26] and *Neochloris* sp.; and the marine microalgae *Nannochloropsis salina* (CCAP849/3) and *Cylindrotheca fusiformis* (CCAP1017/2). The freshwater species were purchased from Landcare Research, Lincoln, New Zealand, and the marine species were from the Culture Collection of Algae and Protozoa (CCAP), Argyll, United Kingdom.

The BG11 medium or its modifications were used to maintain and grow the microalgae [1,40]. The freshwater algae were maintained in BG11 while the marine algae were maintained in the BG11 formulated in seawater. *C. vulgaris* was maintained separately in both BG11 and BG11 seawater media. The medium for the diatom (*C. fusiformis*) always contained silicate (sodium metasilicate nonahydrate, $Na_2SiO_3 \cdot 9H_2O$, at a concentration of 30 mg/L medium). BG11 seawater medium was made using artificial seawater. The artificial seawater was prepared by dissolving 40 g of sea salt (natural unrefined Southern Pacific Ocean salt; Pacific Natural Fine Salt; Dominion Salt Ltd., Marlborough, New Zealand) in 1 L of distilled water and filtering with Whatman GF-C (0.45 μm) 90 mm microfiber filters before use. The artificial seawater had a salinity of approximately 38.5 parts per thousand (ppt) (EcoSense® EC300 conductivity/salinity meter; YSI Inc., Yellow Springs, OH, USA).

Inocula were prepared by aseptically transferring the microalgal stock culture from liquid or solid agar media to 40 mL of BG11 in 250 mL Erlenmeyer flasks. The flasks were held in an incubator shaker at 130–140 rpm, 25 °C, under fluorescent light (~30 μE m^{-2} s^{-1}), for around 20–30 days. This culture (40 mL) was used to inoculate 360 mL of BG11 in a 1 L Duran bottle (borosilicate glass 3.3, LabServ, Biolab, Auckland, New Zealand). These bottles were incubated at room temperature (~25 °C) for approximately 7–14 days. This culture (400 mL) was used to seed 1600 mL of fresh medium in a 2 L Duran bottle and grown for a further 7–14 days. This culture was split into 400 mL lots and transferred to five 2 L Duran bottles each with 1600 mL of BG11, or its modification. All Duran bottle cultures were maintained at room temperature (24–26 °C) under continuous light (~219 μE m^{-2} s^{-1}) from a bank of six tubes of fluorescent lamps (Philips TLD 58w/840, cool white, Thailand). All Duran bottle cultures were continuously bubbled (0.375 L min^{-1}) with humidified air mixed with 5% (vol/vol) carbon dioxide. The inlet and exhaust gas streams were sterile filtered by passing through 0.2 μm Teflon membrane filter (Midisart® 2000; Sartorius AG, Goettingen, Germany). All cultures were grown as three replicate runs.

The cultures were harvested in the stationary phase (30–55 days post inoculation) and kept at 4 °C in the dark. This broth was used in flocculation studies within 7 days of harvest. When necessary, the cultures were diluted with the fresh medium to obtain the desired cell concentration.

2.2. Measurements

2.2.1. Biomass concentration and cell counts

A 20 mL sample of the algal broth was vacuum filtered using a pre-weighed Whatman GF-C (0.45 μm, 90 mm) microfiber disk filter. The filter disk was washed with 2 × 20 mL of distilled water (for the cultures grown in BG11 freshwater medium) or with 2 × 20 mL of 0.5 M ammonium formate (for cultures grown in the BG11 seawater medium). The filtered biomass samples were dried at 80 °C in an oven overnight, cooled in a desiccator and weighed to calculate the dry biomass in 20 mL of the algal broth [25].

A sample of the broth used in the above dry weight measurements was serially diluted with the fresh medium and the optical density was measured at 680 nm in a spectrophotometer (Ultrospec 2000, Pharmacia Biotech, Model 80-2106-00, England). The blank was the fresh medium. The dilutions were such that the maximum measured optical density did not exceed 0.6. The measured optical density was plotted against the precisely known dry weight concentration to obtain a linear calibration plot. Separate calibration plots were made for the different microalgae. Subsequently, the biomass concentration of an unknown sample was determined by comparing the measured absorbance of an appropriately diluted sample with the appropriate calibration curve. The biomass concentration C_b (g L^{-1}) of the sample was calculated using the equation:

$$C_b = \frac{A_{680} \times D}{S} \tag{1}$$

where A_{680} is the measured absorbance, D is the dilution factor and S is the slope of the calibration plot. The S-values (and the regression coefficients, r^2) were: 5.1949 ($r^2 = 0.997$) for *C. vulgaris* grown in freshwater BG11; 7.5673 ($r^2 = 0.999$) for *C. minor*; 3.3285 ($r^2 = 0.997$) for *Neochloris* sp.; 2.1368 ($r^2 = 0.999$) for *C. vulgaris* grown in BG11

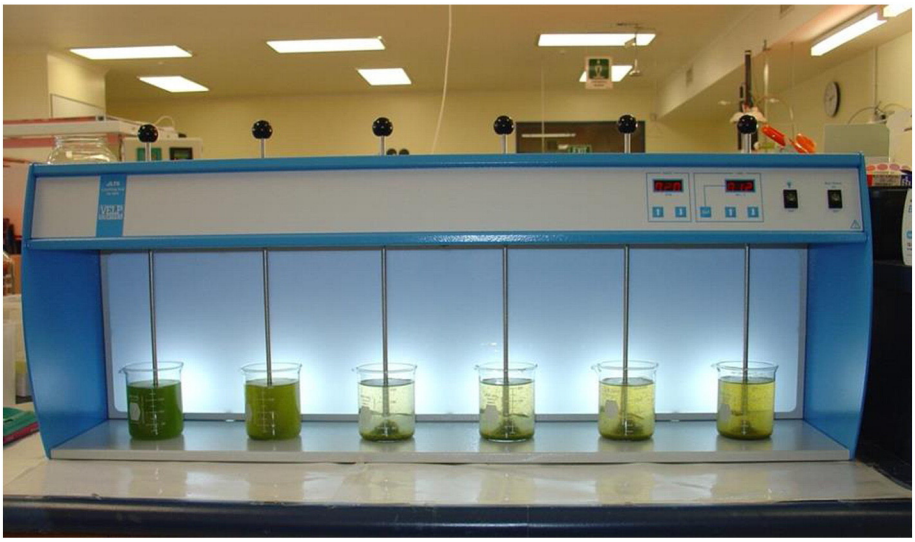

Fig. 1. The jar test apparatus.