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Photosynthetic carbon uptake induces autoflocculation of the marine microalga *Nannochloropsis oculata*

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ABSTRACT

Microalgal biomass has been used to produce biofuels, aquaculture feed, high-value chemicals such as pigments and antioxidants, and even human food. This study addresses one of the key bottlenecks to the commercialisation of microalgal bioproducts: the high energy and environmental cost of harvesting microalgal cells out of suspension. An innovative and sustainable autoflocculation procedure was developed to pre-concentrate microalgal biomass for easier harvesting. Microalgal cell agglomeration by autoflocculation at high pH was induced for the first time, without the addition of a chemical flocculant, in the commercially-relevant microalga *Nannochloropsis oculata*. Photosynthetic inorganic carbon uptake, in the absence of carbon dioxide supply by mass transfer, was used to raise the culture pH. Autoflocculation started at pH 9.5 and reached a maximum flocculation efficiency of 90% at pH 10.4. Microalgal surface charge-neutralisation by calcium cations, and sweep flocculation by calcium carbonate and calcium phosphate precipitates were identified as the dominant flocculation mechanisms. This was also the first study to measure changes in bacterial community composition under autoflocculation. There was a clear shift from free-living bacteria in suspension to attached bacteria during autoflocculation, with *Flavobacteriales* becoming the dominant order of bacteria. This highlights the influential role of attached bacteria and bacteria-produced extracellular polymeric substances in microalgal flocculation. This study shows that regulating carbon dioxide supply is a promising green alternative to traditional microalgal flocculation processes as it alleviates the requirement for costly and harmful chemical flocculants and brings us closer to sustainable microalgal bioproducts.

1. Introduction

Microalgae are a promising biomass feedstock for the production of biofuels, pigments and other bioproducts. The harvesting of microalgal biomass from suspension is energy intensive and unreliable, presenting a critical barrier to commercialisation [1]. Commonly-used mechanical harvesting techniques, such as centrifugation and filtration, are highly dependent on the physical chemistry of a given microalgal species and also on the intended purpose of the biomass harvested [2], though the foremost limitation of these techniques is their high energy demand. The development of effective and sustainable harvesting solutions is needed for microalgae to become a widely-used biotechnological resource [3].

Flocculation of microalgal cells is commonly employed as the first step in the microalgal harvesting process [4]. Flocculation has traditionally been induced by adding chemical flocculants such as activated silica, colloidal clays, metallic hydroxides, polysaccharides or various synthetic polymers [5]. The addition of chemical flocculants leads to the aggregation and settling of microalgal cells, thereby concentrating the suspension for easier harvesting. This approach has been effective at small scale; however, it becomes unsustainable at large scale where the need for large doses of flocculant significantly increases the production costs of commodity products such as biofuels and biopolymers [3]. Furthermore, the use of flocculating agents could be harmful to the microalgal cells, to the environment, or to the end user of the microalgal bioproduct. The need to remove and/or recycle chemical flocculants therefore imposes an additional processing cost and an additional environmental risk [6].

The concept of microalgal autoflocculation was first proposed by Golueke and Oswald when they observed flocculation in an open pond at high pH [7]. There are three principal forms of dissolved inorganic carbon (DIC): carbon dioxide (CO_2), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}), which all exist in an equilibrium that is dependent on pH. During photosynthesis, microalgae take up CO_2 and HCO_3^- from solution, thereby increasing pH as DIC is consumed. DIC is re-supplied by mass transfer of atmospheric CO_2 via the gas phase, which in turn

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reduces pH [8]. Since microalgae can naturally elevate pH through photosynthesis, merely removing the gas phase CO_2 supply should result in a pH increase sufficient to cause autoflocculation [9]. Most commercial microalgal culturing facilities not only rely on CO_2 to increase algal biomass productivity, but they also promote CO_2 sequestration as one of their key business practices. The use of CO_2 supply to also control autoflocculation holds great promise for the microalgal biotechnology industry.

It has been proposed that the aggregation of algal cells through autoflocculation occurs by co-precipitation of calcium and magnesium salts out of the culture medium when the pH increases above pH 9.5 [9,10]. This pH threshold varies with algal species [11]. Some inherent microalgal properties including cell surface characteristics, physiological state, and biomass concentration, can alter the ability of cells to flocculate [12]. However, the most important consideration is the activity of an alga's carbon concentrating mechanism (CCM), which determines how effectively that alga can take up HCO_3^-. Most marine algae have highly-active CCMs and they continue to photosynthesise above pH 10 [13], which could be high enough for autoflocculation to occur. Despite the potential to induce autoflocculation by regulating CO_2 supply, this topic remains largely unexplored. Several studies have examined autoflocculation [9,10,14], though none have used the commercially-important microalga *Nannochloropsis oculata*.

Microalgal cells are negatively charged due to carboxylate ions and other negative functional groups expressed at the cell surface, which results in a very stable microalgal suspension. Overcoming this negative surface charge is therefore considered critical to the autoflocculation mechanism [15]. Three autoflocculation mechanisms have been proposed (Fig. 1): (i) electrostatic interaction between the negative surface charge of the cells and positively charged ions (charge-neutralisation), (ii) the production of salt precipitates and/or extracellular polymeric substances (EPS) and subsequent entrapment of microalgae and/or bacteria in this matrix (sweep flocculation), and (iii) the formation of positively-charged bridges between cells mediated by polymeric flocculants or EPS (bridging) [16].

Autoflocculation at high pH commonly involves the precipitation of some combination of magnesium hydroxide, calcium carbonate and calcium phosphate and subsequent sweep flocculation [9,17]. Both pH and alkalinity control the stability of metal hydroxide and metal carbonate precipitates as well as algal surface charge, but are themselves influenced by algal photosynthesis [18]. For example, pH increases as DIC is consumed by photosynthesis, whereas alkalinity increases as inorganic nitrate (NO_3^-) is consumed. Therefore, it is important to measure changes in water chemistry during autoflocculation.

The aim of this study was to induce, measure and characterise autoflocculation in *Nannochloropsis oculata* by photosynthetic carbon uptake without adjusting pH through NaOH addition. The objective was to determine the pH threshold necessary to induce autoflocculation, to measure the flocculation efficiency, and to determine the mechanisms of autoflocculation. In addition, this study aimed to characterise how the microbial community changed before, during and after autoflocculation. It was hypothesised that high pH would cause precipitation of magnesium and calcium carbonates out of solution, leading to a decrease in alkalinity and autoflocculation. It was also hypothesised that the bacterial community would shift from predominantly free-living bacteria in suspension to predominantly microalgae-attached bacteria under flocculation.

2. Materials and methods

2.1. Algal strain and stock culture

Nannochloropsis oculata (Strain CS-179; Australian National Algae Culture Collection) was cultured in filtered artificial seawater (salinity 32‰) enriched with F medium as described by Guillard and Ryther [19]. Stock cultures of xenic *N. oculata* were maintained at 20 °C in an incubator (Labec) under a 12 h:12 h light:dark cycle with fluorescent illumination at a photon flux density (PFD) of 40 μmol m^{-2} s^{-1}. Xenic cultures of *N. oculata* were used since it is impractical to use bacteria-free cultures, particularly when considering scaling up algal cultivation to an industrial scale.

2.2. Photobioreactor set up

Six photobioreactors (PBRs; Phenometrics) were inoculated with 10% v/v of stock culture in a working volume of 500 mL, with a starting cell density of (6.36 ± 0.88) × 10^4 cells mL^{-1} and culture pH of 7.9. Experiments were performed over a period of 8 days, following a two-day acclimation period, with samples collected daily at 14:00 h, i.e. 1 h before the end of the dark cycle. Within most large-scale microalgal cultivation facilities, it is desirable to maintain and continuously harvest microalgae during the exponential phase to maximise productivity.

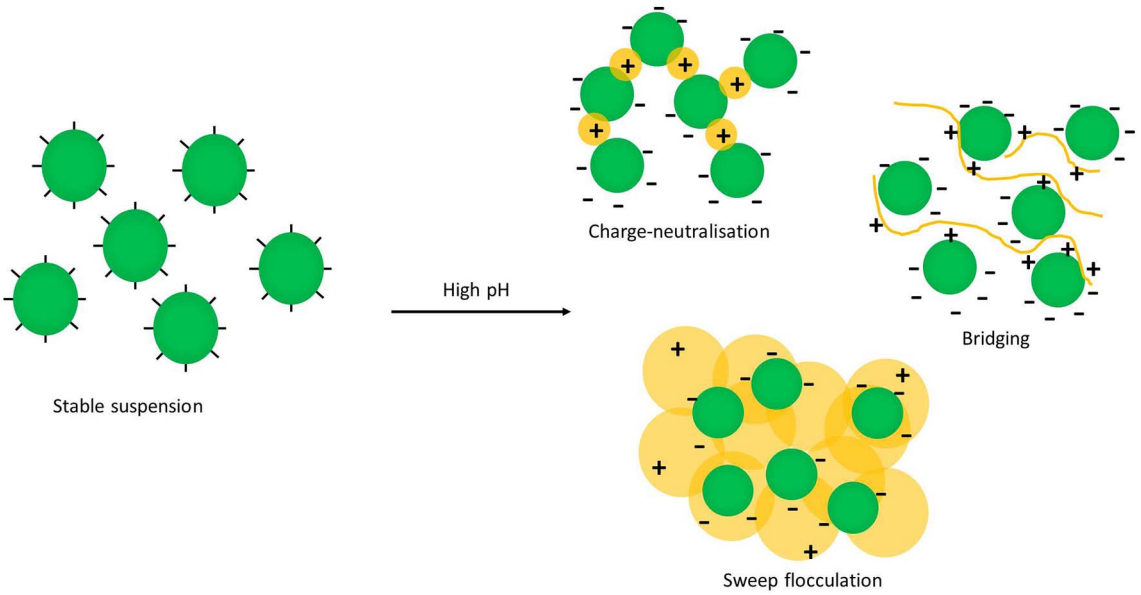

Fig. 1. Microalgal cells have a negative surface charge and they form very stable suspensions. One way to harvest microalgae is to precipitate metal cations at high pH in order to flocculate microalgal cells into larger agglomerates. Three possible autoflocculation mechanisms have been proposed: charge-neutralisation, bridging and sweep flocculation.