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Evaluation of biochemical algal floc properties using Reflectance Fourier-Transform Infrared Imaging

Gonzalez-Torres A.[a], Rich A.M.[b], Marjo C.E.[b], Henderson R.K.[a,*]

[a] bioMASS lab, School of Chemical Engineering, University of New South Wales, Sydney 2052, Australia
[b] Mark Wainwright Analytical Centre, University of New South Wales, Sydney 2052, Australia

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ABSTRACT

Coagulation and flocculation (C-F) followed by a separation process such as sedimentation or dissolved air flotation is a major barrier to algal or cyanobacterial cells in water treatment plants and a common harvesting technique for algae cultured for high value products. Tailoring algal physical floc properties (size, strength, and density) has been shown to be important to optimise separation, however, the chemical properties of the flocs that lead to the physical properties are not yet well understood. This study investigated the biochemical composition of algal (*Chlorella vulgaris*) and cyanobacterial (*Microcystis aeruginosa*) flocs produced under sweep flocculation using aluminium sulphate as a coagulant. These flocs had very different physical properties. A new approach was developed to undertake biochemical characterisation of associated flocs using reflectance Fourier transform infrared (FTIR) imaging. Analysing the biochemical composition of the algal and cyanobacterial flocs revealed that the distribution of proteins and carbohydrates in the flocs are most likely responsible for the unique physical floc characteristics observed. The large flocs of *C. vulgaris* were characterised by a homogenous distribution of proteins and polysaccharides, and a high glycoprotein and biopolymer content. In contrast, the smaller but stronger flocs of *M. aeruginosa* had more concentrated protein regions within the flocs that tended to associate at the edge of regions which were absent of biomolecules, potentially comprising coagulant. Overall, the technique shows great potential for analysing algal flocs to gain a better understanding of the underlying biochemical composition and distribution of these biomolecules that leads to the varying physical floc properties.

1. Introduction

One of the most widely applied techniques for algal treatment in drinking water production [1–3], and more recently in microalgal harvesting for biomass production [4,5] is coagulation and flocculation (C-F), followed by separation by a unit operation such as sedimentation or flotation [5–9]. Floc properties are usually studied by the measurement of physical parameters, such as size, strength and fractal dimension [10,11], which give some indication of the structure of the flocs and the suitability of the flocs for downstream separation. For example, strong and porous flocs aid the filtration process due to their high permeability [12]; large and compact flocs are desirable in sedimentation due to their high settling rate [13]; while small pinpoint particles (approx. 30 μm) are beneficial if dissolved air flotation (DAF) is used for separation [14]. The observed physical floc structure is dictated by the underlying chemical structure; hence, the fundamental study of the chemical composition of the resultant algal flocs, specifically the identification and mapping of macromolecules within algal floc structures, will assist in gaining a better understanding of associated physical floc properties, leading to improved algae removal efficiency by downstream solid-liquid separation.

Algal floc structure is complex as it is affected by different factors including the coagulation conditions applied, cell morphology and surface chemistry and algal organic matter (AOM). AOM is released during exponential and stationary growth regions due to the cyanobacterial or algal metabolic processes or during cell autolysis. It can act as a polymer aid, depending on its concentration, as it frequently contains biopolymers which can bridge the cells and/or with hydroxide precipitates to form large flocs [15]. On the other hand, AOM can also interfere in the C-F process as it can form complexes with the coagulant [15–18], removing its ability to coagulate and thereby increasing the required coagulant dose. However, the mechanism by which AOM positively or negatively impacts on algal and cyanobacterial floc properties is still not well understood as floc studies have focused on the effects of natural organic matter (NOM) [19,20] and only a few limited investigations focused on the role of AOM in C-F process [21].

* Corresponding author.
E-mail address: r.henderson@unsw.edu.au (R.K. Henderson).

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In addition to the cell system biochemistry, floc structure is dependent on the coagulation mechanism. Usually, either charge neutralisation or sweep flocculation (or a combination of these) governs when metal salts are utilised as coagulants. A low pH and a low dose of coagulant are used to drive charge neutralisation (CN) as highly charged dissolved metal hydroxides are generated, whereas high doses of coagulant at neutral to alkaline pH values produce amorphous hydroxide precipitates in which algal cells are incorporated, driving sweep flocculation (SF) conditions [22,23]. Flocs formed under SF conditions typically produce relatively strong flocs, in comparison with those formed by CN, that have low capacity for size recovery after exposure to high shear [20,24].

There is no universally adopted approach to chemically characterise algal or cyanobacterial flocs to investigate the biochemical floc structure and gain insight into the chemical interactions that drive physical floc properties. A range of methods have been used to quantify lipid, protein and carbohydrate composition of biological samples, typically involving chemical extraction followed by gravimetric determination or mass spectrometry [25,26]. However, these methods can be time consuming and involve sample destruction, therefore they are not suitable for floc analysis and may not be sensitive enough to determine the macromolecular composition of a single cell or cell aggregates. A rapid, label-free, direct and non-destructive method for the study of the biochemical composition of algal flocs is required.

Fourier-transform infrared spectroscopy (FTIR) is a common technique used to identify functional groups in organic molecules based on their vibrational modes at different wavenumbers [27,28]. The functional group vibrations are representative of cellular constituents such as lipids, proteins, polysaccharides and nucleic acid compounds and has been used to assess quantitative changes in the biochemical composition in algae under nutrient stress [29–33]. More recently, it was used to characterise bioflocculants that algae produce during the C-F process [28]. When used as an imaging technique, FTIR can provide information pertaining to the distribution and intensity profiles of biomolecular analytes without the requirement of a label as would be the case for fluorescence techniques. FTIR spectroscopy may therefore offer a powerful tool to simultaneously characterise biochemical algal cells and algal floc components with minimal disturbance to the cellular components and floc structures.

FTIR imaging can be performed using transmission, reflectance or attenuated total reflectance (ATR) mode. In transmission FTIR imaging, the transmission of infrared light through the sample is affected by the sample thickness [34] that can be very difficult to control in algal flocs due to their three dimensional structure [35]. ATR-FTIR imaging offers the advantage that the sample can be measured at relatively high spatial resolution of 1.56 μm (note that cell size may range from 3 to 20 μm) [36,37]. However, it requires the application of pressure to ensure contact between the sample and the surface of the ATR-FTIR crystal, significantly disrupting the floc structure. By comparison, reflectance FTIR imaging on laboratory-based instruments offers poorer lateral spatial resolution (~6.25 μm); however, it is a non-contact technique so there is no disruption to a delicate floc during imaging. Hence, reflectance FTIR imaging may be a promising technique for algal and cyanobacterial floc analysis. Another consideration for FTIR imaging is the sensitivity to the presence of water, so careful sample preparation is required to minimise the water in the sample without compromising the floc structure or integrity of the algal cell membrane.

This study therefore develops a novel protocol using reflectance FTIR imaging to examine the biochemical composition of algal and cyanobacterial flocs. The physical floc properties of flocs formed from cells and associated AOM of both the cyanobacteria, *M. aeruginosa,* and the green algal species, *C. vulgaris*, both commonly found in freshwater sources [38] and known to have very different AOM compositions [39], were examined and compared against the biochemical floc composition determined using the new method. The outcomes of this study therefore demonstrate that it is possible to determine the biochemical floc properties of algal flocs and that it is possible to relate these to physical floc properties. By improving our understanding of the underlying biochemical conditions that lead to specific floc properties there is opportunity to better tailor floc characteristics for more efficient separation.

2. Materials and methods

2.1. Materials

2.1.1. Cell cultures

The freshwater cyanobacterial culture, *M. aeruginosa* (strain CS-555/01) and the green algal culture, *C. vulgaris* (CS-42/7), were obtained from the CSIRO Australian National Algae Culture Collection (ANACC), Hobart, Australia, and cultured in MLA media and Jaworski media respectively. The cyanobacteria and algae were subjected to a 16/8 h light/dark cycle, with temperature controlled to 21 °C, in a 500 L PG50 incubator with a photosynthetic photon flux output of 600 ± 60 μmol $m^{-2} s^{-1}$ (Labec, Australia). All samples were taken using the cells in the late exponential region of 10–13 days. Samples were diluted to normalise the cell concentration to an environmentally-relevant concentration of approximately 8×10^5 cells mL^{-1} (average for all tests: 8 (± 4) × 10^5 cell mL^{-1}) using deionised water to which 0.5 mM $NaHCO_3$ was added. Cell counting was performed using a haemocytometer or Sedgwick Rafter counting chamber and a light microscope (Leica DM750 Microscope, Germany). To characterise the cell samples, the following parameters were analysed: cell size was measured using a Mastersizer 3000 (Malvern, Australia), zeta potential (ZP) was measured (in triplicate) after each experiment using a ZetaSizer Nano, Malvern, Australia) and DTS1060 plastic folded capillary cells. Charge density was measured using a Mütek PCD-04 Travel Particle Charge Analyser (BTG, Eclépens, Switzerland) of the cell system.

2.1.2. Algal organic matter preparation

Extraction of AOM from the cell suspension was undertaken following Henderson et al. [39]. Cell and AOM suspensions were centrifuged at 10,000 × *g* for 15 min with an Allegra® X-15R Centrifuge (Beckman Coulter, Australia). The suspension was then filtered by applying vacuum-filtration through a Whatman 0.45 μm glass microfiber filter paper. The sample was then diluted with deionised water as required to obtain a final DOC concentration between 0 and 5 mg L^{-1}. The AOM thus comprises a mixture of both dissolved and loosely bound extracellular organic matter (EOM), as well as any intracellular organic matter (IOM) that may have been released due to cell lysis, although IOM concentration is expected to be minimal due to the growth phase selected.

2.1.3. Chemicals

A solution of 8% paraformaldehyde (PFA) (ProsSciTech, Australia) was used as a fixation agent to prevent possible biochemical changes that could occur during the subsequent drying step. A sample volume of 0.5 mL was diluted in 1 mL of 8% PFA. Aluminium sulphate (aluminium sulphate hexadecahydrate, "alum", Sigma Aldrich, Australia) was used as a coagulant. The coagulant was added at a range of doses to determine the optimum coagulation dose using a stock solution of alum (10 g L^{-1} was prepared). Either 0.1 M NaOH or 0.1 M HCl was used to adjust the pH to 7 as required. The coagulant dose used for the AOM flocs was determined according to Henderson et al. [39].

2.2. Methods

2.2.1. Floc preparation and physical characterisation

Floc preparation and physical characterisation was carried out only for *C. vulgaris* cells as that for *M. aeruginosa* physical floc characterisation results were obtained from a previous study published by the

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