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Influence of organic matter generated by *Chlorella vulgaris* on five different modes of flocculation

[Bioresource Technology: Short Communication]

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Running Title: AOM interferes with flocculation of *Chlorella*

Abstract

Microalgae excrete relatively large amounts of algal organic matter (AOM) that may interfere with flocculation. The influence of AOM on flocculation of *Chlorella vulgaris* was studied using five different flocculation methods: aluminum sulphate, chitosan, cationic starch, pH-induced flocculation and electro-coagulation-flocculation (ECF). The presence of AOM was found to inhibit flocculation for all flocculation methods resulting in an increase of dosage demand. For pH-induced flocculation, the dosage required to achieve 85% flocculation increased only two-fold when AOM was present, while for chitosan, this dosage increased nine-fold. For alum, ECF and cationic starch flocculation, the dosage increased five- to six-fold. Interference by AOM is an important parameter to consider in the assessment of flocculation-based harvesting of microalgae.

Keywords:

extracellular polysaccharides, coagulation, microalgae, biomass, dissolved organic matter

Highlights:

- The importance of algal organic matter (AOM) interference for five different flocculation methods was demonstrated
- The presence of AOM resulted in a 2-9 fold increase of flocculant dose demand
- pH-induced flocculation was to be least affected by AOM

1. Introduction

Due to their high areal productivity and independence of freshwater or arable land, microalgae have received much interest as a source of biofuels (Wijffels and Barbosa, 2010). However, production of microalgae is still expensive and only economically feasible for high-value applications (Savage, 2011). Energy efficient harvesting, especially, is still a major challenge. Due to their small size (5-50 μm) and low concentration in the medium, harvesting using centrifugation is unacceptable from an energetic point of view. Pre-concentration of microalgae prior to final dewatering by centrifugation or filtration can lower the energy needs to one-tenth compared to traditional harvesting by centrifugation (Brentner et al., 2011).

Such a pre-concentration step can be achieved by flocculating the microalgae, a phenomenon which can be induced by the addition of polyvalent metals such as aluminum sulphate (alum) or ferric chloride, or by electrochemical release of metal ions from a sacrificial anode in electro-coagulation-flocculation (ECF) (Uduman et al., 2011; Vandamme et al., 2011). Alternatively, cationic biopolymers, such as chitosan or the cheaper alternative cationic starch (Vandamme et al., 2010), can be used. Finally, flocculation of microalgae can be induced by increasing the pH, which leads to precipitation of magnesium hydroxides which act as a flocculant (Schlesinger et al., 2012; Vandamme et al., 2012). Polyvalent metal ions, cationic polymers and magnesium hydroxides precipitates carry positive charges that interact with the negative surface charge of microalgal cells and induce flocculation by charge neutralisation and/or bridging.

During cultivation, microalgae release significant amounts of organic matter (AOM). In microalgae cultivation systems, microalgae can excrete up to 17% of fixed carbon, which

can amount to 60-80 mg C L⁻¹ in closed photobioreactors (Hulatt and Thomas 2010). This AOM comprises a wide range of compounds such as proteins, neutral and charged polysaccharides, nucleic acids, lipids and small molecules, but polysaccharides comprise the major fraction (Myklestad, 1995). It is well-known that AOM interferes with removal of particulates using metal coagulants in the production of drinking water, where concentrations of AOM are much lower than in algal production systems (0.1 - 1.5 mg C L⁻¹; Bernhardt et al., 1989; Henderson et al., 2010). Little is known about the possible interference of high concentrations of AOM present in dense algal cultures with flocculation-based harvesting of microalgae. It was demonstrated for the cyanobacterium *Aphanotece* (Chen et al., 2008) and for the green alga *Chlorella zofingiensis* (Zhang et al., 2012) that excreted polysaccharides resulted in an increased dose of metal coagulant needed to induce flocculation. No publications have focused on interference with other flocculants. Therefore, in this study, the influence of AOM on flocculation of *Chlorella vulgaris* using five different flocculation technologies was evaluated.

2. Materials and methods

2.1. Cultivation of *Chlorella vulgaris*

Chlorella vulgaris (211-11b SAG, Germany) was used as a model species and was cultured in dechlorinated tap water enriched with inorganic nutrients according to the concentrations of the Wright's cryptophyte (WC) medium (Vandamme et al., 2012). The microalgae were cultured in 30-L bubble column photobioreactors in which the cultures were mixed by sparging with 0.2 µm-filtered air (5 L min⁻¹). Growth of the microalgae was monitored by measuring the absorbance at 550 nm (Griffiths et al., 2011). Microalgal dry weight was determined gravimetrically by filtration using Whatman glass fiber filters

(Sigma-Aldrich) and drying at 105°C until constant weight. All flocculation experiments were performed in early stationary growth phase at a biomass concentration of 0.25 g L⁻¹.

2.2 General setup of flocculation experiments

To investigate the relative importance of AOM in the flocculation process, flocculation of *Chlorella* was compared in medium with and without AOM. To remove AOM, *Chlorella* was separated from the medium using centrifugation and resuspended in fresh medium. AOM concentration in the original medium and in the fresh medium with resuspended microalgae was estimated through measurement of the total carbohydrates, which comprise the major fraction of the AOM (Myklestad, 1995). Carbohydrates were measured using the phenol-sulfuric acid method (Dubois et al., 1956). Carbohydrate content in the original medium was 5 mg C L⁻¹ and this was reduced to only 0.5 mg C L⁻¹ in the fresh medium with resuspended microalgae. Preliminary experiments demonstrated that centrifugation and resuspension of *Chlorella* in the original medium had no influence on flocculation, confirming that these operations themselves did not affect the results.

Five flocculation methods were evaluated and the flocculant demand for *Chlorella* was compared in the presence and absence of AOM: alum, electro-coagulation-flocculation (ECF), chitosan, cationic starch and pH-induced flocculation. For ECF, the setup described by Vandamme et al. (2011) was used. In short, this setup consisted of a 1-L rectangular PVC reactor with an aluminum anode and an inert titanium oxide cathode and a power supply controller (EHQ Power PS3010 DC). Current density in the experiments was set at 1.5 mA cm⁻². Metal release was directly proportional to the operation time, as stated by Faraday's law. The flocculation efficiency η_a was estimated by comparing absorbance at 550 nm between the flocculation treatment and a control. Samples of 3.5 mL were taken every 5 min in the middle

of the clarified zone and optical density was measured after 30 min of sedimentation. The flocculation efficiency η_a was calculated as:

Microalgal flocculation efficiency η_a : $\eta_a = \frac{OD_i - OD_f}{OD_i}$ (Eq. 1)

where OD_i is the optical density of the suspension after 30 min sedimentation without flocculation treatment, and OD_f is the optical density of the suspension after the completed flocculation treatment.

The four other flocculation mechanisms were assessed using jar test experiments. For each method, the flocculation efficiency was estimated at different flocculant doses. For alum, the pH was adjusted to 5.5 prior to addition of the coagulant. A solution of 0.015 M $Al_2(SO_4)_3 \cdot 18H_2O$ (Sigma Aldrich) was added and the pH was immediately re-adjusted to 5.5. For flocculation using chitosan (from crab shells, practical grade, Sigma Aldrich), the pH of the suspension was re-adjusted to 7.5. A solution of 5 g L⁻¹ chitosan in 0.01 M HCl was used. For cationic starch flocculation, a stock solution of 10 g L⁻¹ Greenfloc 120 (Hydra 2002 Research, Hungary) was prepared. The pH was not adjusted, as it has been shown that flocculation efficiency is not pH-dependent (Vandamme et al. 2010). For pH-induced flocculation, 0.5 N NaOH was used to increase the pH. The jar test experiments were carried out in 100-mL beakers whose contents were stirred using a magnetic stirrer. During addition of the flocculant, the microalgal suspension was intensively mixed (500 rpm) for 10 min followed by gentler mixing (250 rpm) for an additional 20 min. Subsequently, the suspension was allowed to settle for 30 min. In order to estimate the flocculation efficiency η_a , samples (3.5 ml) were collected in the middle of the clarified zone and absorbance at 550 nm was measured. The flocculation efficiency η_a was again calculated according to Eq. 1.

3. Results and discussion

The dose response curves of *Chlorella* cells in their original medium containing AOM and resuspended in fresh medium without AOM are presented in Fig. 1. To facilitate comparison between different treatments, the dose required to achieve 85% flocculation efficiency ($D_{85\%}$) was estimated from each dose response curve. For each of the five flocculation technologies tested, 85% flocculation efficiency ($D_{85\%}$) was achieved at a much lower dose in the medium without AOM than in the medium with AOM. For alum, $D_{85\%}$ was only 20 mg L⁻¹ in the absence of AOM and 115 mg L⁻¹ in the presence of AOM. For ECF, flocculation occurred after 5 min in the absence of AOM (electricity demand: 0.2 kWh kg⁻¹ biomass) and after 25 min in the presence of AOM (electricity demand: 1.2 kWh kg⁻¹ biomass). For chitosan, $D_{85\%}$ was 8 mg L⁻¹ in the absence of AOM and 75 mg L⁻¹ in the presence of AOM. For cationic starch, $D_{85\%}$ increased from 20 mg L⁻¹ in the absence of AOM to 90 g L⁻¹ in the presence of AOM. Finally, when pH-induced magnesium hydroxides precipitation was used to flocculate *Chlorella*, flocculation occurred at pH 10.5 in the absence of AOM (requiring 22 mg L⁻¹ NaOH) and at pH 11.5 in the presence of AOM (requiring 49 mg L⁻¹ NaOH). The doses required to induce flocculation in the *Chlorella* cells in their original medium were in the range of the doses mentioned in previous studies (e.g. Vandamme et al., 2010, 2011, 2012), while the doses required after resuspension in the fresh medium were generally much lower.

Flocculation induced by alum required six times higher doses in the presence of AOM. For ECF, in which aluminum ions were released from a sacrificial anode, $D_{85\%}$ was expressed as a function of energy consumption, which is directly proportional to operation time. The five-fold increase in energy consumption needed to achieve flocculation in the presence of AOM using ECF was in accordance to the six-fold increase of the alum dosage for the alum

flocculation method. This outcome is not surprising, as ECF releases aluminum directly proportional to current density and operation time and flocculation by ECF is thus essentially based on the same underlying mechanism as alum flocculation. It is well-known from studies on water treatment that AOM interferes with flocculation using metal coagulants such as alum and ferric chloride (Bernhardt et al., 1985; Henderson 2010). Bernhardt et al. (1989) showed that AOM contains extracellular polysaccharides with negatively charged carboxyl groups that interact with the positively charged metal coagulants making them unavailable for microalgae flocculation and thus resulting in a higher coagulant demand. Both the number and position of these carboxyl groups (Bernhardt et al., 1989) and the size of the polysaccharide polymers (Henderson et al., 2010) influence the interaction with metal coagulants. In addition, the protein fraction of the AOM can form complexes with the metal coagulants, again making them unavailable for flocculation of the microalgae and thus resulting in a higher coagulant demand (Pivokonsky et al., 2006; Takaara et al., 2004). Bernhardt et al. (1989) showed that even low concentrations of AOM (a few mg C L⁻¹) resulted in a strong increase in the coagulant demand. Compared to these low concentrations in surface water systems, AOM of *Chlorella* cultivated in closed photobioreactors are at least an order of magnitude higher (Hulatt and Thomas, 2010). Therefore, it is no surprise that the alum demand and ECF energy consumption increased strongly in the presence of AOM.

The present results showed that AOM also interferes with flocculation using cationic biopolymers such as chitosan and cationic starch. Cationic biopolymer flocculants may interact with oppositely charged polyelectrolytes within the AOM, such as carbohydrates and proteins. Interference by AOM was particularly important for chitosan, where a 9-fold increase in the flocculant dose was observed. For cationic starch, the increase was slightly lower (5-6 fold). Possibly, the difference is related to differences in the three-dimensional

1 structure of both cationic biopolymers (Bratskaya et al., 2005). Overdosing cationic starch
2 lead to a decrease in the flocculation efficiency. This phenomenon of dispersion
3 restabilization is commonly observed with polyelectrolyte flocculants, including cationic
4 starch and is probably the result of steric hindrance and/or electrostatic repulsion (Vandamme
5 et al. 2010).

6
7 The present results demonstrated that AOM also interferes with flocculation by
8 magnesium hydroxide precipitates formed at a high pH. Lee et al. (1998) also showed that
9 flocculation induced by high pH in cultures of *Botryococcus braunii* was affected by growth
10 stage, which is also known to have an influence on the amount of AOM (Hoyer et al. 1985).
11 Compared to the other flocculation technologies, the dose of sodium hydroxide required to
12 induce flocculation was only about 2-fold higher in the presence of AOM. The reason for this
13 is not clear. Possibly, it is due to the fact that sodium hydroxide is not the primary flocculant,
14 but induces precipitation of magnesium hydroxides which act as the flocculant (Vandamme et
15 al., 2012; Wu et al., 2012). As precipitation of magnesium hydroxides increases non-linearly
16 with pH (Leentvaar and Rebhun, 1982), a limited increase in the amount of base added may
17 result in a strong increase in magnesium hydroxides precipitates.

18
19 Our results clearly showed that most of the flocculant dose required to flocculate
20 *Chlorella* in an exponentially growing culture is lost on the AOM rather than being used for
21 actual flocculation of the cells. The degree of inhibition of flocculation by AOM is most
22 likely related to the quantity and the composition of the AOM present in the medium, and
23 these variables will therefore have a major influence on the cost of harvesting microalgal
24 biomass using flocculation. Although all microalgae produce AOM, the quantity and quality
25 of the AOM differs between species and is influenced by culture conditions. For instance,

1 cyanobacteria tend to produce AOM that is relatively rich in proteins compared to eukaryotic
2 microalgae (Takaara et al. 2004; Pivokonsky et al. 2006). The number and position of
3 carboxyl groups on polysaccharides and the size of the polysaccharides differ between species
4 of microalgae and also change with culture age (Hoyer et al. 1985, Pivokonsky et al. 2006).
5 For *Chlorella vulgaris* in stationary growth phase, the composition of AOM was analyzed in
6 detail by Henderson et al. (2010). Chlorella produced about 0.0029 ng AOM per cell. The
7 charge density of AOM was 3.2 meq g⁻¹ and AOM contributed 84% of the total charge of the
8 culture. The AOM had a protein:carbohydrate ratio of 0.4 and 60% of the AOM consisted of
9 > 30 kDa molecules. The current results suggested that some flocculation techniques are more
10 sensitive to inhibition by AOM than others, although the degree of inhibition of the different
11 techniques may be different with other species of microalgae and/or other culture conditions.

12
13 The present findings have important implications for the development of microalgal
14 biofuels. To maximize lipid production in microalgae, cultures are often subjected to nutrient
15 stress (Wijffels and Barbosa, 2010). As nutrient-limited cells generally produce more AOM
16 than exponentially growing cells (Myklestad, 1995), maximizing lipid production will most
17 likely also result in a higher flocculant demand. Furthermore, medium recycling is essential to
18 minimise the water demand and thus the ecological impact and production costs of microalgal
19 biofuels (Brentner et al., 2011). During medium recycling, AOM is likely to accumulate in the
20 medium, probably resulting in a gradual increase in flocculant demand. In wastewater
21 treatment, several technologies, such as chlorine treatment or ozonation, have been proposed
22 to reduce the load of AOM (Henderson et al., 2008). Given the high concentrations of AOM
23 in dense microalgal cultures, it is questionable whether such technologies will be effective in
24 sufficiently reducing AOM in intensive microalgal production systems. Oxidation of AOM

- 1 may also result in lysis of algal cells, which might further increase flocculant demand
- 2 (Henderson et al., 2008).
- 3

4. Conclusions

AOM present in microalgal cultures interferes strongly with harvesting of the biomass using flocculation as a pre-concentration technique. This may have important consequences for the cost of flocculation based harvesting. Some flocculation technologies are more sensitive to interference by AOM than others. The cost (related to the amount of base necessary) of pH-induced magnesium hydroxides flocculation, for instance, increases only 2-fold in the presence of AOM while a 9-fold increase was observed for chitosan. Further research is needed to determine whether these differences in inhibition by AOM between flocculation techniques apply for all species of microalgae and under various culture conditions, and to elucidate the underlying causes for these differences.

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Figures

Fig 1: Dose response curves for different flocculation methods: for *Chlorella vulgaris* in original (AOM+) medium and resuspended (AOM-) in fresh medium: (A) alum, (B) ECF, (C) cationic starch, (D) chitosan, (E) pH-induced flocculation

Figure1

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