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Flocculation properties of several microalgae and a cyanobacterium species during ferric chloride, chitosan and alkaline flocculation

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HIGHLIGHTS

- High variation in optimal dosages between species and flocculation methods.
- Chitosan was ineffective for harvesting marine species.
- Species selection for low-cost separation is important.

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ABSTRACT

Flocculation holds great potential as a low-cost harvesting method for microalgae biomass production. Three flocculation methods (ferric chloride, chitosan, and alkaline flocculation) were compared in this study for the harvesting of 9 different freshwater and marine microalgae and one cyanobacterium species. Ferric chloride resulted in a separation efficiency greater than 90% with a concentration factor (CF) higher than 10 for all species. Chitosan flocculation worked generally very well for freshwater microalgae, but not for marine species. Alkaline flocculation was most efficient for harvesting of *Nannochloropsis*, *Chlamydomonas* and *Chlorella* sp. The concentration factor was highly variable between microalgae species. Generally, minimum flocculant dosages were highly variable across species, which shows that flocculation may be a good harvesting method for some species but not for others. This study shows that microalgae and cyanobacteria species should not be selected solely based on their productivity but also on their potential for low-cost separation.

1. Introduction

Microalgae and cyanobacteria attract a lot of interest as new biomass feedstocks for the production of food, feed, fuels, and chemical building blocks (Greenwell et al., 2010; Pienkos and Darzins, 2009; Savage, 2011). However, global production is still very limited (10–20,000 tonnes year^{-1}) and microalgae applications are restricted to niche markets for high-value products (Gerardo et al., 2015; Vanthoor-Koopmans et al., 2013). Upscaling of production is limited by the high cost and energy requirements of different technologies along the entire production chain. Harvesting the microalgal biomass is particularly challenging given the small size of the cells (5–20 μm) and the relatively low biomass concentration in the culture medium (0.5–5 g L^{-1}) (Barros et al., 2015; Wijffels and Barbosa, 2010). Flocculation is widely considered as a promising approach for large-scale and low-cost harvesting of microalgal biomass (Coons et al., 2014; Molina Grima et al., 2003; Vandamme et al., 2013). Using flocculation, small individual microalgal cells are aggregated into large flocs, which can be separated relatively easily from the culture medium using either filtration-based (e.g. membrane filtration) or gravity-based (e.g. sedimentation, centrifugation, flotation) technologies.

Flocculation is generally induced by addition of chemicals that interact with the negatively charged microalgal cell surface (Molina Grima et al., 2003). These chemicals can induce flocculation through different mechanisms: by neutralizing the negative surface charge of the cells (charge neutralization), by connecting

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individual cells (bridging), or by forming a precipitate that binds and enmeshes the cells (sweeping mechanism) (Vandamme et al., 2013). In the past years, several studies have evaluated the potential of different flocculation methods for harvesting microalgae. However, these studies generally focused on a single microalgal or cyanobacterial model species such as *Chlorella* sp., *Scenedesmus*, or *Nannochloropsis* sp. (e.g. 't Lam et al., 2014; Delrue et al., 2015; García-Pérez et al., 2014; Garzon-Sanabria et al., 2012; Vandamme et al., 2012; Xu et al., 2012). Thus, it is currently unknown whether the results can be extrapolated to other economically interesting but less studied species, such as *Pseudanabaena* or *Diacronema*. Microalgae and cyanobacteria are a highly diverse group of aquatic photosynthetic microorganisms, belonging to divergent evolutionary lineages and differing strongly in size, shape, and cell surface properties (Georgianna and Mayfield, 2012; Henderson et al., 2008). Therefore, a flocculation method that is effective for one species may not necessarily be successful for other species of microalgae or cyanobacteria. Comparison between different studies is complicated because experimental conditions are often different (e.g. biomass concentration and cultivation stage of the culture, parameters of flocculation experiments). A study of the flocculation properties for various species using standard cultivation and evaluation protocols is needed to allow systematic comparison of the flocculation behavior of different microalgae species.

When evaluating the feasibility of a flocculation as a low-cost method for harvesting microalgae, the dosage of flocculant required to induce flocculation is a critical parameter as the quantity of these chemicals will be the main determinant of the harvesting costs. Other parameters are important as well. Flocculation-mediated separation should enable the removal of a large proportion of the cells, i.e. the separation efficiency should be high. The size of the flocs that are formed should also be sufficiently high to obtain flocs that settle easily (Vandamme et al., 2014). Finally, the biomass concentration factor after settling should be maximized to ensure a sufficiently concentrated biomass fraction after settling. Such parameters have never been reported for little-studied but promising species such as *Pseudanabaena*, *Chlamydomonas*, or *Diacronema*. Moreover, the correlation between each of these different parameters has not been analyzed before.

The aim of this study was to systematically compare the flocculation properties of 10 economically interesting microalgal and cyanobacterial species, belonging to different phylogenetic groups and differing in shape, size, and surface charge. For each species, three flocculation methods were tested that differ in the main flocculation mechanism: the metal salt coagulant ferric chloride (charge neutralization), the biopolymer chitosan (bridging), and alkaline flocculation induced by magnesium hydroxide precipitation (sweeping mechanism). The specific objectives of this study were to determine to what extent the flocculant dosage, floc size, and concentration factor differ between species and the impact of these parameters on the cost of harvesting with the respective flocculant.

2. Materials and methods

2.1. Cultivation of microalgae

Nine species of microalgae and one cyanobacterium belonging to different evolutionary groups were selected for this study. They differ strongly in size, shape, and zeta potential (ZP) (Table 1). Cell surface area and volume were calculated using the corresponding formulas for idealized shapes as described by Hillebrand et al. (1999) (Suppl. Table 1). ZP can be used as an indicator of the electrostatic repulsion between the microalgal cells. ZP was estimated from electrophoretic mobility measurements obtained via the phase analysis light scattering (PALS) technique as previously described by Vandamme et al. (2015b).

Four freshwater species (*Chlorella*, *Pseudanabaena*, *Chlamydomonas*, and *Scenedesmus*) were cultivated in Wright's Cryptophyte medium prepared in deionized water. Because alkaline flocculation is caused by precipitation of magnesium hydroxide at high pH and requires a sufficient concentration of magnesium in the medium, the magnesium concentration in this medium was raised to 2 mM (Vandamme et al., 2015a). Six marine species were cultivated in Wright's Cryptophyte medium prepared in artificial seawater (deionized water with 30 g L^{-1} synthetic sea salt; Homarsel, Zoutman, Belgium). Since seawater contains a high concentration of magnesium, no additional magnesium was required to induce alkaline flocculation. The microalgae were cultivated in 30-L bubble column photobioreactors (1 m height, 20 cm diameter). The cultures were mixed by sparging with 0.2-μm-filtered air (5 L min^{-1}) and the pH was maintained at 8.5 by addition of 2–3% CO_2 using a pH-stat system. The culture was irradiated on two sides with daylight fluorescent tubes to reach a light intensity of 60 μEinst m^{-2} s^{-1} at the surface of the reactor. Microalgal growth was monitored spectrophotometrically by measuring optical density at 750 nm. Absorbance was calibrated against microalgal dry-weight concentration (determined gravimetrically by filtration on Whatman GF-C filters and dried until constant weight at 105 °C (Moheimani et al., 2013)). Flocculation experiments were carried out after 12 days when cultures had reached stationary phase. At that stage, the biomass concentration was between 0.35 and 0.45 g L^{-1}, except for *Chlamydomonas* and *T-Isochrysis* cultures that had a lower biomass concentration (0.20–0.25 g L^{-1}) (Table 1).

2.2. Flocculation experiments

Three flocculation methods: ferric chloride, chitosan, and alkaline flocculation, were tested for each species. These three methods were selected because they are commonly used in studies on microalgae flocculation and they also differ with respect to the flocculation mechanism: the metal salt ferric chloride (Iron(III) chloride, Merck, analytical grade) induces flocculation predominantly through charge neutralization (Wyatt et al., 2012), the cationic polymer chitosan (from crab shells, Sigma-Aldrich) induces flocculation through a bridging mechanism, and alkaline flocculation causes flocculation predominantly through a sweeping mechanism (Brady et al., 2014; Vandamme et al., 2015a). Alkaline flocculation was induced by addition of sodium hydroxide (Sigma-Aldrich). Since phosphate was depleted in the stationary phase cultures, alkaline flocculation was induced by precipitation of magnesium hydroxide (Brady et al., 2014; Huo et al., 2016; Vandamme et al., 2012). Stock solutions of 0.5 M sodium hydroxide and 10 g L^{-1} ferric chloride were prepared in deionized water. For chitosan, 5 g L^{-1} of stock solution was prepared in 0.01 M HCl. A series of 10–15 jar test experiments were carried out to determine the minimum dosage of flocculant required for induction of flocculation (Suppl. Fig 1). Jar test experiments were carried out in a volume of 100 mL. During addition of the flocculant, the microalgae suspensions were intensively mixed (350 rpm) for 10 min, followed by gentle mixing (250 rpm) for 20 min (Vandamme et al., 2012). The suspensions were subsequently allowed to settle for 30 min. The supernatant was sampled in the middle of the clarified zone and absorbance was measured at 750 nm. The separation efficiency η_a was calculated as:

$$\eta_a = \frac{OD_i - OD_f}{OD_i} \times 100$$