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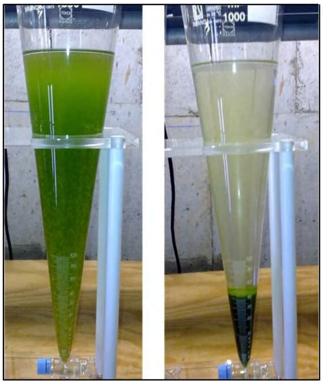
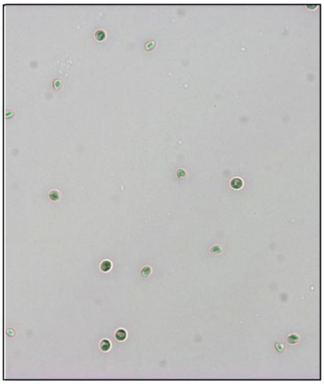
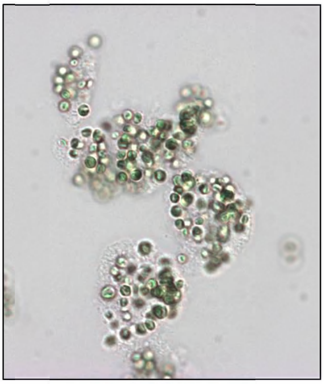
ARENBERG DOCTORAL SCHOOL
Faculty of Bioscience Engineering

Flocculation based harvesting processes for microalgae biomass production







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Dissertation presented in partial fulfillment of the requirements for the degree of Doctor in Bioscience Engineering

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Preface

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Abstract

The global demand for biomass for food, feed, biofuels, and chemical production is expected to increase in the coming decades. Microalgae are a promising new source of biomass that may complement agricultural crops. Production of microalgae has so far however been limited to high-value applications. In order to realize large-scale production of microalgae biomass for low-value applications, new low-cost technologies are needed to produce and process microalgae. A major challenge lies in the harvesting of the microalgae, which requires the separation of a low amount of biomass consisting of small individual cells from a large volume of culture medium. Flocculation is seen as a promising low-cost harvesting method for primary concentration. In this study, we overview the challenges and possible solutions for flocculating microalgae focussing on three flocculation modes: flocculation using cationic starch, electro-coagulation and flocculation induced by high pH. Secondly, those modes were compared to two reference modes (flocculation using aluminum sulphate and chitosan) in function of their interaction with algal organic matter and floc properties.

We evaluated the potential of cationic starch as a flocculant for harvesting microalgae using jar test experiments. Cationic starch was an efficient flocculant for freshwater (*Parachlorella, Scenedesmus*) but not for marine microalgae (*Phaeodactylum, Nannochloropsis*). At high cationic starch doses, dispersion restabilization was observed. The required cationic starch dose to induce flocculation increased linearly with the initial algal biomass concentration. Of the two commercial cationic starch flocculants tested, Greenfloc 120 (used in wastewater treatment) was more efficient than Cargill C*Bond HR 35.849 (used in paper manufacturing). For flocculation of *Parachlorella* using Greenfloc 120, the cationic starch to algal biomass ratio required to flocculate 80% of algal biomass was 0.1. For *Scenedesmus*, a lower dose was required (ratio 0.03). Flocculation of *Parachlorella* using Greenfloc 120 was independent of pH in the pH range of 5 to 10. Measurements of the maximum quantum yield of PSII suggest that Greenfloc 120 cationic starch was not toxic to *Parachlorella*. It could thus be concluded that cationic starch may be used as an efficient, nontoxic, cost-effective, and widely available flocculant for harvesting microalgal biomass.

Secondly, the use of electro-coagulation flocculation (ECF) as a method for harvesting a freshwater (*Chlorella vulgaris*) and a marine (*Phaeodactylum tricornutum*) microalgal species is evaluated. ECF was shown to be more efficient using an aluminum anode than using an iron anode. Furthermore, it could be concluded that the efficiency of the ECF process can be substantially improved by reducing the initial pH and by increasing the turbulence in the microalgal suspension. Although higher current densities resulted in a more rapid flocculation of the microalgal suspension, power consumption, expressed per kg of microalgae harvested, and release of aluminum were lower when a lower current density was used. The aluminum content of the harvested microalgal biomass was less than 1% while the aluminum concentration in the process water was below 2 mg L^{-1}. Under optimal conditions, power consumption of the ECF process was around 2 kWh kg^{-1} of microalgal biomass harvested for *Chlorella vulgaris* and 0.3 kWh kg^{-1} for *Phaeodactylum tricornutum*. Compared to centrifugation, ECF is thus more energy efficient. Because of the lower power consumption of ECF in seawater, it is a particularly attractive method for harvesting marine microalgae.

We explored the potential of flocculation induced by high pH for harvesting *Chlorella vulgaris*. Our results demonstrated that flocculation can be induced by increasing medium pH to 11. Although both calcium and magnesium precipitated when pH was increased, only magnesium (0.15 mM) by the formation of magnesium hydroxide proved to be essential to induce flocculation. The costs of four different bases (sodium hydroxide, potassium hydroxide, calcium hydroxide, magnesium hydroxide) were calculated and evaluated and the use of calcium hydroxide appeared to be the most cost-efficient. Flocculation induced by high pH was thus shown to be a potentially useful method to preconcentrate freshwater microalgal biomass during harvesting.

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 sulfate and chitosan as reference modes in comparison with cationic starch, electro-coagulation–flocculation (ECF) and pH-induced flocculation. 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 2-fold when AOM was present, while for chitosan, this dosage increased 9-fold. For alum, ECF and cationic starch flocculation, the dosage increased 5 to 6-fold. Interference by AOM is an important parameter to consider in the assessment of flocculation-based harvesting of microalgae.

Flocculation of microalgae should not only be effective in terms of flocculation efficiency, but also in terms of settling rate and concentration of the biomass. Floc characteristics such as settling velocity, concentration factor and floc size were therefore studied for the five previously mentioned flocculation modes for *Chlorella vulgaris*. These floc characteristics were influenced by the flocculation mode, which depends on the coagulation mechanism: adsorption – charge neutralization, sweeping or bridging. Secondly, the influence of the presence of AOM was evaluated. This resulted in a decrease of the concentration factor. The floc characteristics upon flocculation using cationic starch were least affected by the presence of AOM, while flocculation using chitosan was most affected. The impact on floc characteristics is an important parameter to consider next to flocculation efficiency in the assessment of flocculation-based harvesting of microalgae.

Samenvatting

In de nabije toekomst zal de vraag naar biomassa als basisgrondstof voor de aanmaak van voedingsproducten, voeders of biobrandstoffen alsmaar toenemen. Biomassa afkomstig van micro-algen is een veelbelovende nieuwe bron van biomassa en kan een aanvulling kan zijn op traditionele biobrandstofgewassen. Momenteel heeft de productie van microalgen enkel hoogwaardige toepassingen. Om ook op grote schaal microalgen te kunnen gebruiken voor laagwaardige producten, zijn er nieuwe en goedkopere technologieën nodig om microalgen te produceren en te verwerken. In het bijzonder vormt het efficiënt en goedkoop oogsten van microalgen momenteel nog steeds een uitdaging. Het gebruik van flocculatie is veelbelovend om de totale productiekost een grootteorde te doen dalen. In deze studie worden de mogelijkheden van drie flocculatie technieken van nabij bestudeerd: flocculatie via het biopolymeer kationisch zetmeel, electrocoagulatie flocculatie en flocculatie bij hoge pH. Deze drie technieken werden daarenboven vergeleken met twee referentietechnieken om de interactie van organisch materiaal geproduceerd door microalgen met flocculatie beter te begrijpen. Daarbij werd eveneens de invloed op de vlokeigenschappen na flocculatie bestudeerd.

Kationisch zetmeel is een interessant biopolymeer dat geregeld gebruikt wordt bij het zuiveren van afvalwater. Tijdens onze experimenten bleek dat dit biopolymeer enkel een efficient flocculant was voor zoetwater maar niet voor zoutwater micro-algen. Bij toevoeging van hoge hoeveelheden van het flocculant trad herstabilitatie op waardoor de flocculatie efficiëntie daalde. De benodigde dosis kationisch zetmeel bleek lineair toe te nemen met de initiële biomassa concentratie. Er werd een duidelijk verschil in flocculatie efficiëntie waargenomen tussen de twee geteste kationische zetmelen. Greenfloc 120, gebruikt in waterzuiveringstoepassingen, was beduidend efficiënter dan het zetmeeltype dat gebruikt wordt tijdens papierproductie (Cargill C*Bond HR 35.849). Om de zoetwatermicro-alg *Parachlorella* optimaal te doen flocculeren, was 0.1 g g^{-1} biomassa noodzakelijk. Maar voor een andere zoetwatersoort (*Scenedesmus*) was dit slechts 0.03 g g^{-1} biomassa nodig. Er kon dus besloten worden dat

kationisch zetmeel is dus een efficiënt en mogelijk goedkoper alternatief is om zoetwater microalgen te oogsten.

Electrocoagulatie flocculatie werd geëvalueerd voor zowel zoetwater (*Chlorella vulgaris*) als zoutwater (*Phaeodactylum tricornutum*) micro-algen. Een eerste reeks experimenten toonde aan dat het gebruik van aluminium als kathode materiaal efficiënter was dan ijzer. Daarenboven werd duidelijk dat lagere initiële pH waarden en het creëren van turbulentie in het medium de flocculatie efficiëntie ten goede kwamen. In het algemeen leidde een hogere stroomsterkte tot een hogere aluminium vrijgave aan de kathode. In optimale omstandigheden was het aluminium gehalte in de biomassa onder 1% en in het medium onder 2 mg L^{-1} na flocculatie. De benodigde elektrische energie was 2 kWh kg^{-1} biomassa voor het oogsten van *Chlorella* terwijl dit slechts 0.3 kWh kg^{-1} was voor *Phaeodactylum.* Juist omwille van het lage energieverbruik in zoutwater, is deze techniek dus uitermate interessant voor het oogsten van zoutwater micro-algen.

Flocculatie via verhoogde pH werd bestudeerd voor *Chlorella vulgaris* en we toonden aan dat flocculatie geïnduceerd kon worden vanaf een pH van 11. Niettegenstaande het feit dat zowel calcium als magnesium zouten precipiteerden, werd aangetoond dat magnesium door middel van magnesium hydroxide vorming een belangrijke rol speelde tijdens het coagulatie proces. De kost van vier verschillende basen (natrium hydroxide, kalium hydroxide, calcium hydroxide en magnesium hydroxide) werd berekend, waarbij calcium hydroxide het meest potentieel had. Er kon besloten worden dat flocculatie via verhoogde pH een interessante methode vormt om micro-algen op te concentreren.

Micro-algen staan bekend om het feit dat ze in bepaalde omstandigheden grote hoeveelheden organisch materiaal afscheiden, wat voor mogelijke interferentie tijdens het flocculeren kan zorgen. De invloed van de aanwezigheid van organisch materiaal op flocculatie werd onderzocht voor *Chlorella vulgaris.* De drie eerder vermelde flocculatie methoden werden geëvalueerd in vergelijking met 2 referentie methoden: flocculatie via aluminium sulfaat en via chitosan. De aanwezigheid van organisch materiaal had een inhiberend effect op de flocculatie en dit voor alle geteste methoden. Om een flocculatie efficientie van 85% te verkrijgen, was de dosering bij flocculatie via verhoogde pH in aanwezigheid van organisch materiaal dubbel zo hoog. Maar voor chitosan verhoogde de dosis negen maal in aanwezigheid van organisch materiaal. Voor aluminium sulfaat en electrocoagulatie flocculatie verhoogde de dosis vijf tot zes keer. Deze studie toonde aan dat mogelijke interferentie door aanwezigheid van organisch materiaal een belangrijke bijkomende parameter is tijdens de evaluatie van flocculatie van micro-algen.

Naast flocculatie efficientie werden ook vlokeigenschappen zoals sedimentatie snelheid, concentratiefactor en vlokgrootte geëvalueerd voor *Chlorella vulgaris* en dit voor de vijf eerder vernoemde flocculatie methoden. Deze vlokeigenschappen werden beïnvloed door de flocculatie methoden, en meer bepaald door het coagulatie mechanisme van deze methoden. Bijkomend werd de invloed van de aanwezigheid van organisch materiaal getest. Daarbij werd het duidelijk dit vooral de concentratie factor beïnvloedde. Aanwezigheid van organisch materiaal zorgde vooral voor een afname van de vlokcompactheid, en dit in het bijzonder voor flocculatie via chitosan. De vlokeigenschappen bekomen na toevoeging van kationisch zetmeel werden daarentegen het minst beïnvloed door de aanwezigheid van organisch materiaal. De impact op vlokeigenschappen is naast flocculatie efficientie een belangrijke parameter om te gebruiken tijdens de evaluatie van flocculatie gebaseerde oogstmethoden voor micro-algen.

Abbreviations

ALA	alpha-linolenic acid
alum	aluminum sulphate
AL	flocculation using aluminum sulphate
AVI	aggregated volume index
AOM	algal organic matter
ASP	aquatic species program
CAPEX	capital expenditures
CF	concentration factor
CH	flocculation using chitosan
CS	flocculation using cationic starch
DHA	docosahexaenoic acid
DW	dry weight
ECF	electro-coagulation flocculation
EPA	eicosapentaenoic acid
HRAP	high rate algal ponds
OPEX	operating expenditures
PUFA	polyunsaturated fatty acid
ROS	reactive oxygen species
SI	saturation index

List of symbols

ζ	zeta potential [mV]
I_z	ionic strength [mol m^{-3}]
κ^{-1}	Debeye length [m]
c_i	molar concentration [mol m^{-3}]
z_i	number of charges [-]
ϵ	electrical permittivity [C^2 J^{-1} m^{-1}]
k	Boltzmann constant (1.380 10^{-23}) [J K^{-1}]
N_a	Avogadro number (6.022 10^{23}) [mol^{-1}]
e	elementary charge (1.602 10^{-19}) [C]
T	temperature [K]
v	particle settling velocity [m s^{-1}]
r	cell radius [m]
g	gravitational acceleration (9.81) [m s^{-2}]
ρ_p	mass density particle [kg m^{-3}]
ρ_f	mass density fluid [kg m^{-3}]
η	dynamic viscosity [N s m^{-2}]
η_a	microalgae flocculation efficiency [%]

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Context and aim

About 12% of the world's ice-free land area is currently being used to produce biomass from agricultural crops. This corresponds to more than 1.5 billion hectares. This biomass is mainly used for food (65%) and animal feed (35%). Due to an increase in the world's population and improving living standards in developing and transitional economies, global demand for biomass for food or feed production is expected to increase with more than 50% in the next two decades [61]. On the other hand, to reduce emissions of carbon dioxide threatening to disrupt the climate, developed economies are taking initiatives to move from a fossil fuel economy to a biobased economy. In such an economy, biomass replaces petroleum as a source of transport fuel and as a feedstock for the chemical industry [84]. As a result, in developed economies, an increasing proportion of agricultural crops is converted to biofuels or chemicals [130]. This growing demand for biomass for food, feed, fuel and chemicals is already resulting in a rise in food prices, with dramatic consequences for the world's poorest people [101]. Therefore, there is a need for additional sources of biomass that can complement agricultural biomass production.

Microalgae are currently considered to be the most promising new source of biomass [41]. They do not require arable land and thus do not compete for land with agricultural crops. Many microalgae can be cultivated in seawater and thus do not depend on the world's limited freshwater supplies. But even for microalgae that grow in freshwater, water demand is lower than for terrestrial crops because microalgae do not actively transpire water [145]. Furthermore, microalgae can produce more biomass per unit land area than agricultural crops. They are capable of producing between 40 and 90 tonnes dry biomass ha^{-1} year^{-1}, depending on the technology used and the local climate [194]. Therefore, microalgae are attractive for biomass production in a crowded world. Because they do not require roots and shoots for water uptake or support, microalgal biomass contains less structural compounds such as lignin and cellulose and therefore produces less waste products and more useful products such as lipids and proteins than agricultural crops.

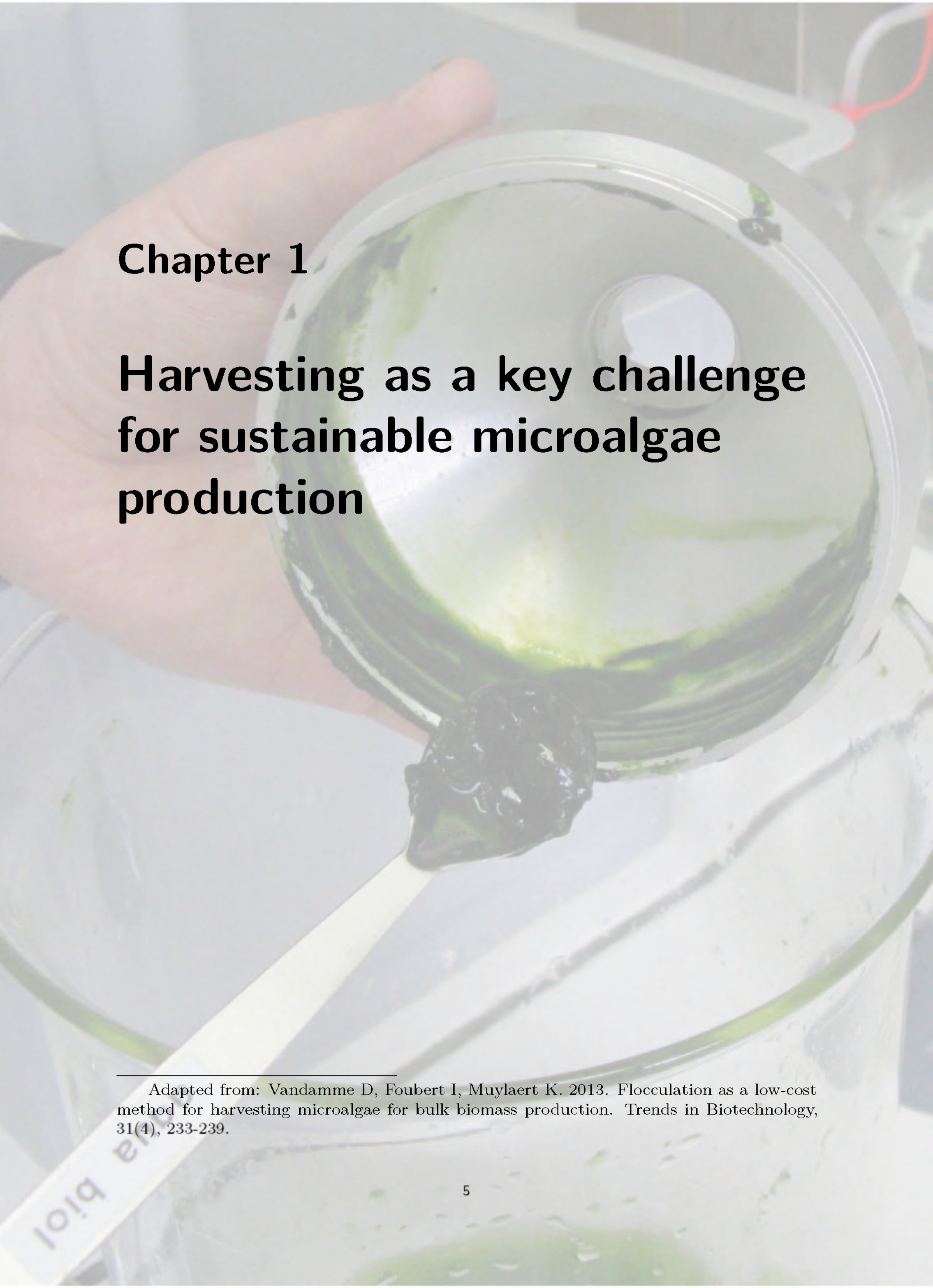
However, for microalgal biomass to become a commodity like most agricultural crops, the cost of production has to be reduced. The past years have seen an explosion in research and development on microalgal biomass production, both in academia and industry [42]. Much progress has been made in increasing the yield through photobioreactor design [125], selection of strains [102] and genetic engineering of metabolic pathways [67]. Much less progress has been made on research and innovation in downstream processing, although this is essential to reduce the cost of the production process [38, 72]. Today, microalgal production is rapidly moving from lab- and pilot scale to full-scale installations [67], prompting the need for cost- and energy efficient downstream processing technologies.

A major challenge in downstream processing of microalgae lies in separating the microalgae from their growth medium, i.e. the harvesting process. Because a high biomass concentration leads to mutual shading of the microalgal cells and thus a reduction in productivity, biomass concentrations in microalgal cultures are usually low: from 0.5 g L^{-1} in open pond reactors to about 5 g L^{-1} in photobioreactors. This means that a large volume of water has to be removed to harvest the biomass. Due to the small size of the microalgal cells (2 – 20 μm), harvesting by means of sedimentation or simple screening is not feasible, except perhaps for larger species such as *Arthrospira*. Centrifugation is a proven technology for fast and effective harvesting of most microalgae, but is currently not feasible as single step harvesting method for low-value applications of microalgae because of its high capital and operational costs [23]. If the microalgae could be primary concentrated by coagulation-flocculation and gravity sedimentation prior to further dewatering steps, the energy demand for harvesting could be strongly reduced.

The aim of the present study was to investigate and evaluate several flocculation based harvesting processes as primary concentration step for microalgae biomass production. Our research was focussed on defining the variables that influence flocculation, understanding the coagulation mechanism and specifying the implications involved in the integration of flocculation into microalgal processing.

In this study, *Chlorella vulgaris* was selected as model species. *Chlorella vulgaris*, a freshwater green algae belonging to the phylum Chlorophyta, is one of the most studied microalgae and it is known for its high productivity under various cultivation conditions [102, 140]. To evaluate the effect of medium salinity, *Phaeodactylum tricornutum* was used. This microalgae species is a well-studied marine diatom, belonging to the phylum Heterokontophyta, that can be rich in long chain omega-3 polyunsaturated fatty acids (PUFAs, Section 1.1.3) [202]. Additionally, other species were also tested in particular parts of this study for comparison (*Parachlorella kessleri*, *Scenedesmus obliquus.* and *Nannochloropsis salina*).

Chapter 1 gives a brief overview of current literature on some general aspects of microalgae biotechnology concerning applications, production process and the challenges of harvesting microalgae. Additionally, the recent evolutions of several approaches for microalgae flocculation are discussed. In Chapter 2 to 4, we evaluate novel approaches for flocculation, including flocculation using cationic starch (Chapter 2), electrocoagulation (Chapter 3) and pH induced flocculation (Chapter 4). Especially for the latter approach a lot of attention is paid to the coagulation mechanism. Furthermore, we compare different flocculation modes in function of their interaction with algal organic matter and floc properties. In Chapter 5, the influence of organic matter generated by *Chlorella vulgaris* on the flocculation efficiency is investigated for the three flocculation modes presented in Chapter 2-4 in comparison with two reference modes: flocculation using aluminum sulphate (alum) and chitosan. Additionally, the link between floc properties, coagulation mechanism and presence of organic matter is investigated in Chapter 6. Finally, the outcomes are integrated in a last Chapter 7, where conclusions are drawn and future research avenues are explored.



Chapter 1

Harvesting as a key challenge for sustainable microalgae production

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1.1 Microalgae: from biology to biotechnology

1.1.1 What are microalgae?

Any organism with chlorophyll a and a thallus which is not differentiated into roots, stem and leaves is regarded by phycologists to be an alga [105]. Therefore, the term microalgae refers to the microscopic algae *sensu stricto* and the oxygenic photosynthetic bacteria, i.e. the cyanobacteria. Microalgae are mostly unicellular and can be found in marine, brackish or freshwater environments. They are found all over the world, mainly distributed in the waters, but also on the surface of all type of soils. Besides water, they need CO_2, phosphate, nitrate and specific trace elements such as zinc and copper. Due to their simple structures, microalgae are able to achieve high growth rates and photosynthetic efficiencies. The photosynthetic mechanisms of microalgae are similar to that of land plants, but microalgae are able to capture nutrients very efficiently out of their aquatic environment. In addition to this, microalgae are able to grow exponentially when the conditions are optimal. Because of these unique characteristics, microalgae have the potential to become one of the world's most efficient organisms to transform light into biomass.

The industrial value of microalgae lies primarily in their potential utilization for food, feed and fine chemicals, using solar energy (Section 1.1.3). Only recently, they gained additional interest as feedstock for biofuels [40]. The origins of applied phycology most probably date back to the establishment of a culture of *Chlorella* by Beijerinck in 1890 [9]. Even today, *Chlorella* takes up an important place in the commercial use of these micro-organisms mainly for human nutrition.

Generally, the chemical composition of microalgae is comparable with soya. They can contain high protein and lipid levels. Table 1 shows a comparison of the global chemical composition of different microalgae versus traditional human food sources. Variations in carbohydrates, lipids and soluble proteins are species and even strain specific. Additionally, the cultivation conditions (Section 1.1.2) can affect the chemical composition.

1.1.2 Biological principles of mass cultivation

Microalgae have a typical sigmoid growth response over time with a distinct difference between the growth phases: lag, exponential, linear, stationary and declining growth phase. In the lag phase, the microalgae population is adapting to the environmental conditions. Depending on several conditions such as

Table 1.1: Global chemical composition of different microalgal species compared with human food sources (% of dry matter) [8, 180, 31, 150].

Commodity	Protein	Carbohydrates	Lipids
Rice	8	77	1
Egg	47	4	41
Soya	37	30	20
Chlorella vulgaris	55	15	18
Phaeodactylum tricornutum	34	26	18
Nannochloropsis salina	17	23	26
Arthrospira platensis	65	18	8

nutrient availability, light intensity, temperature and contamination, the growth can achieve its maximum rate during the exponential phase. When nutrients are depleted or light is limited, a transition to linear and stationary growth will occur. Under these conditions, competition with other organisms such as different algae species, zooplankton, bacteria and fungi can lead to culture contamination resulting in a declining growth rate.

One of the major studied factors influencing growth and productivity is light [152, 77, 97, 4]. The conversion of light into chemical energy is covered by the photosynthetic efficiency. Only a small fraction of the photon flux can be absorbed and used by the photosynthetic complexes within the cells. Moreover, light intensity decreases exponentially with depth in water surfaces, resulting in light and dark zones. This is especially important for the design of microalgae photobioreactors (Section 1.1.4). Depending on the light intensity and the microalgae species, there is no increase in biomass yield beyond a certain amount of solar radiation. In other words, light saturation will occur and this will affect the photosynthetic rate. In addition, high light intensities can lead to photoinhibition and causes damage to the photosynthetic complexes. Additionally, increasing cell density causes a mutual shading effect and affects the light penetration depth. The photosystems of those cells present in the dark zones will adapt to the new light conditions, a process known as photoacclimatisation. Studies have shown that the reintroduction of those cells by for example mixing, will result in stronger photoinhibition [82]. All these factors contribute to the fact that the photosynthetic efficiency in existing cultivation systems is only around 1 %, while theoretically 4.5% is possible [191].

Besides light, nutrient availability is a key factor for the growth of microalgae. The availability of carbon, nitrogen and phosphorous is essential. Next to these nutrients, a series of trace elements such as zinc, iron, magnesium, calcium, potassium, cobalt, manganese and boron are necessary. The minimal amount and ratio of carbon, nitrogen and phosphorous is determined by the Redfield ratio: 106C:16N:1P. This means that for each mole of C, 0.11 mole N and 0.01 mole P needs to be available. It must be noted that this ratio depends on species and cultivation conditions [66]. Carbon is commonly supplied by CO_2 and HCO_3^-. Contrary to land plants, atmospheric CO_2 alone cannot satisfy the C-requirements of high yielding autotrophic algal production systems. Diffusion rates for CO_2 from the atmosphere into open ponds can at most sustain algal productivities around 10 g m^{-2} d^{-1} [152]. As a consequence, it is not uncommon to measure pH's as high as 11 in high algal density production systems where no additional CO_2 has been supplied. Secondly, CO_2 supply is important with respect to respiration activity, which is determined by the ratio of dissolved O_2 and CO_2 concentrations. The transfer of these molecules is catalysed by ribulose-1,5-bisphosphate carboxylase oxygenase, commonly known as the enzyme Rubisco. Although Rubisco has a 60 times higher affinity for CO_2 than for O_2, high dissolved O_2 concentrations in combination with depleted CO_2 can result in a high photorespiration activity. This consequently leads to a temporal decrease in biomass productivity. Prevention of CO_2 depletion can therefore limit respiration activity.

After carbon, nitrogen is the most important nutrient contributing to the biomass production. Nitrogen content can range from 1% to more than 10% and it not only varies between different groups (e.g. low in diatoms) but also within a particular species, depending on the supply and availability. Typical responses to nitrogen limitation are discolouration of the cells (decrease in chlorophylls and increase in carotenoids) and accumulation of organic carbon compounds such as polysaccharides and lipids [76]. Nitrate and also urea are mostly used for nitrogen supply, with similar growth rates recorded [202].

Phosphorus is essential not only for growth but also for many cellular processes such as energy transfer, biosynthesis of nucleic acids, etc. Orthophosphate (PO_4^{3-}) is the preferred form to be supplied to microalgae. Although less then 1% phosphorus contributes to the algal biomass composition, it is often one of the most important growth limiting factors in algal cultivation. Phosphorus is easily bound to other ions (e.g. calcium or iron) resulting in precipitation and consequently rendering it unavailable for algal uptake. Interestingly, microalgae are known to be able to store excess phosphorus in polyphosphate bodies during the so-called luxury uptake [152].

1.1.3 Applications: from high value to bulk products

History of microalgal mass cultivation

The cultivation of microalgae in the laboratory only started 140 years ago, while commercial production was only initiated less than 60 years ago. It is thus one of the modern biotechnologies in comparison to the thousands of years of experience in production of conventional crops. The first unialgal cultures were achieved by Beijerinck in 1890 with *Chlorella vulgaris* [9], and the use of such cultures for studying plant physiology was developed by Warburg in the early 1900's. Mass culture of microalgae really began to be a focus of research after 1948 at Stanford (USA), Essen (Germany) and Tokyo and the classic book edited by Burlew (1953) summarises many of these early studies [32]. Interest in applied algal culture continued, especially with studies on the use of algae as photosynthetic gas exchangers for space travel and as microbial protein sources.

Commercial large-scale production of microalgae started in the early 1960s in Japan with the culture of *Chlorella*, followed in the early 1970's with the establishment of an *Arthrospira* (formerly known as *Spirulina*) culturing and harvesting facility in Lake Texcoco, Mexico by Sosa Texcoco S.A. In 1977 Dai Nippon Ink and Chemicals Inc. established a commercial *Arthrospira* plant in Thailand, and by 1980 there were 46 large-scale factories in Asia producing approximately 5,000 kg of microalgae (mainly *Chlorella*) per month. In 1996 about 2,000 tonnes *Chlorella* was traded in Japan alone. Other *Arthrospira* plants were established in the USA (e.g. Earthrise Nutritional LCC in California and Cyanotech Corp in Hawaii). In the late nineties, *Arthrospira* production became established in China and rapidly grew to a yearly production of 5,000 tonnes. Commercial production of *Dunaliella salina*, as a source of ß-carotene, became the third major microalgae industry when production facilities were established by Western Biotechnology Ltd and Betatene Ltd (now Cognis Nutrition and Health) in Australia in 1986. These were soon followed by other commercial plants in Israel and the USA with an estimated yearly production of >1,000 tonnes. More recently several plants producing *Haematococcus pluvialis* as a source of astaxanthin have been established in Israel, the USA and India. In a short period of about 30 years the industry of microalgal biotechnology has grown and diversified significantly.

Simultaneously with the developments in Japan in the sixties, William Oswald and colleagues at the University of California did pioneering research on the large-scale microalgae production for wastewater treatment using high rate algal ponds (HRAP) [135]. In their study the fermentation of microalgae was proposed to produce methane as a source of energy. This work was the basis for a new critical assessment of algae for energy by Oswald en Benemann towards the end of the 1970s [134]. In 1980, the US Department of Energy initiated the "Aquatic Species Program" (ASP) aimed to develop algae as sources of liquid oil fuels which would be able to compete with fossil fuels. The ASP programme demonstrated the technical feasibility of the large scale cultivation of some species of microalgae for relatively long periods in open pond systems (Section 1.1.4). However, the application of biofuel production from these microalgae was evaluated as not economically viable. Moreover, the report stated that the only possible near- to mid-term application of microalgae biofuels needs integration with wastewater treatment [166]. Recent increase in fossil energy prices boosted new interest for microalgal biofuels research and applications.

Present state of microalgal production and applications

Today, only a few species are available on the market in commodity scale, comprising a total annual microalgae production of less than 10,000 tonnes (Table 1.2). Basically, all established species are extremophiles and therefore easier to control during cultivation. *Chlorella* favours high nutrient concentrations, while *Arthrospira* is cultivated at high alkalinity and *Dunaliella* in highly saline waters. Other species cultivated on small scale with niche applications are *Nannochloropsis, Porphyridium, Haematococcus, Tetraselmis, Phaeodactylum, Pavlova, Skeletonema, Thalassiosira* and *Chaetoceros*. The main products are high in value and related to human and animal nutrition, aquaculture and cosmetics. Examples are tablets of dried *Arthrospira* or *Chlorella* sold as nutritional supplements, food enriched products with *Arthrospira* or *Chlorella*, or purified phycocyanin (blue pigment) from *Arthrospira* used in candy and sweets. In animal nutrition, both species are also used for chicken or fish feed. Specific species are used as food for larval fish in aquaculture (*Nannochloropsis, Tetraselmis, Thalassiorisa* and *Chaetoceros*) [128]. For cosmetics, mostly *Chlorella* and *Arthrospira* are used in creams and soaps while astaxanthin extracts are often used in skin-care products.

Novel applications of microalgae

Currently, new promising high-value applications based on lipids are under development. The lipid content of microalgae can be up to 30% (Table 1.1). Especially marine species can be rich in long chain omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA). Those PUFAs are known to have beneficial effects for cardiovascular diseases and for neuropsychiatric disorders including depression and dementia. Several beneficial effects of PUFAs are legally accepted claims [62]. Secondly, short chain omega-3 fatty acids such as alpha-linolenic acid (ALA) can be found

Table 1.2: Present state of established microalgal production on commodity scale [8, 152, 23, 173].

Species	Annual production (ton dw)	Producer country	Application	Price order (US$ kg^{-1})
Arthrospira	>5000	China, India, USA, Japan	human and animal nutrition, cosmetics	8-15
Chlorella	5000	Taiwan, Germany, Japan	human and animal nutrition, aquaculture, cosmetics	20-30
Dunaliella salina	>1000	Australia, Israël, USA, China	β-carotene	600-3,000^1
Haematococcus pluvialis	300	USA, India, Israël	astaxanthin	10,000^2

^{1}: price β-carotene
^{2}: price astaxanthin

in a wide variety of microalgal species. This type of PUFAs can have interesting applications as biobased bulk chemicals in industrial applications such as paints, lubricants or bioplastics [84].

Microalgae contain antioxidants to protect the cells against reactive oxygen species (ROS) which are continuously produced when exposed to light. Next to the carotenoids that can be found in plants (e.g. β-carotene, lutein, zeaxanthin), microalgae contain additional carotenoids such as astaxanthin and fucoxanthin [49]. They also have phycobiliproteins such as phycocyanin (*Arthrospira*) and phycoerytrin (*Porphyridium*) which are interesting pigments that can be used as natural colorants. Other important antioxidants found in microalgae are the vitamins C (ascorbic acid) and E (tocopherols) and glutathion [148, 25]. Recent studies have shown the potential of microalgae as source of bioactive compounds with pharmaceutical potential such as halogenated fatty acids and sulphated polysaccharides [170, 25, 78]. Currently the focus of this research is still on screening of species and strains.

Next to these high value applications, the use of microalgae in the nutrient recovery process in wastewater treatment holds great potential. Recent studies revealed successful treatment of municipal, agricultural and industrial wastewaters [35]. In contrast to conventional methods, the use of microalgae leads to an efficient removal of both N, P and toxic metals. They can therefore play an important remediation role in the future particularly during the final (tertiary) treatment of wastewater [142].

Biofuels from microalgae

Recent increases in fossil oil prices have worldwide boosted the search for renewable energy resources. The production of microalgae has gained increasing interest for bioenergy production to improve fuel security and reduce CO_2 emissions. Microalgae are particularly interesting compared to other crops because they can be produced on non-arable land, utilizing saline and wastewater streams [102]. Moreover, lipid accumulation can be boosted by applying stress conditions resulting in 'fat' microalgae enriched in triacylglycerols (TAGs). These TAGs can then be used directly or converted to biodiesel by transesterification. Alternatively, hydrogen can be produced by certain microalgae (e.g. *Chlamydomonas reinhardtii*) under specific conditions such as an anaerobic environment or sulfur deprivation [170]. To date, published studies concerning biofuels from microalgae have focused on less than 20 species taken from culture collections. Of these publications, 70% refer to only one of the genera and are thus not comparative studies (Fig 1.1). A significant number of scientific papers have published optimistic numbers of oil yield derived from microalgae biomass which strengthened the promise of biofuels from microalgae, while other studies posed concerns about economical viability and sustainability [72, 40, 161, 191, 141, 115, 194, 182].

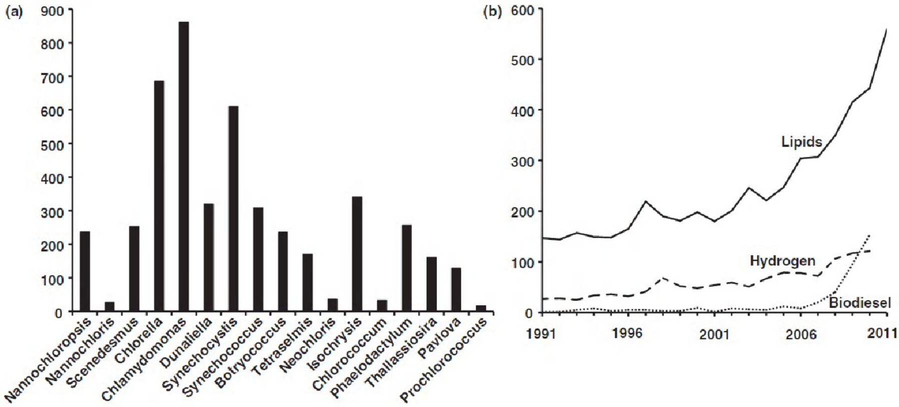


Figure 1.1: (a) Microalgae strain-specific publications related to biofuels published in Web of Science since 1991. The references presented capture 70% of all microalgal biofuel publications. (b) Number of publications by year for microalgae biofuel publications referring to biodiesel, hydrogen and lipids [102].

In 2009, research funding and private initiatives were boosted by the $600M initiative by Exxon Mobil Corporation to research and develop next-generation biofuels produced by photosynthetic organisms. Today, new initiatives are taken worldwide to bring that promise into reality. A recent example is the construction of a 120 ha green crude farm in New Mexico, USA by Sapphire Energy to yearly produce 3.8 million liter of jet fuels and biodiesel. Recently, numerous studies have proposed the concept of a biorefinery in order to meet the economical challenges of biofuels production from microalgae. This approach integrates nutrient recovery processes within a concept of a sequential production of various co-products, including biofuels [189, 133, 52]. Unlike for mature biorefineries such as sugar, starch, vegetable oil or lignocellulosic pulp, the proof-of-concept for microalgal biorefineries remains to be established [153].

1.1.4 General production process

Several production steps are needed in order to obtain dry microalgal biomass. First, microalgae are cultivated in specific open or closed systems. Secondly, the microalgae are harvested and subsequently dried. Additionally, further downstream processing like cell disruption and extraction is necessary to produce specific products derived from microalgae (Fig 1.2).

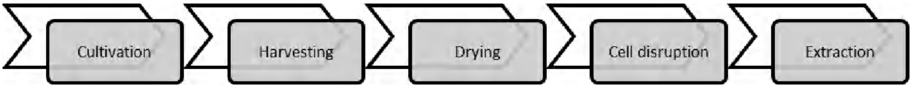


Figure 1.2: General production process of microalgae and derived products

Cultivation

Microalgae can be cultivated using two main types of system: open pond culture systems or closed photobioreactors (Fig 1.3). Open ponds are currently the main system used to commercially produce microalgae, while most research and development is done on the improvement of closed photobioreactor systems.

Open pond systems can broadly be classified into three types: circular central-pivot ponds, raceway ponds and shallow lagoons and ponds (Fig 1.4). Circular ponds are mixed using a rotating arm and are the oldest type of ponds. They are still used for commercial cultivation of *Chlorella* in Taiwan (Section 1.1.3). Raceway ponds are the most widely used culture system for commercial microalgae cultivation as well as in wastewater treatment [23]. Typically, water

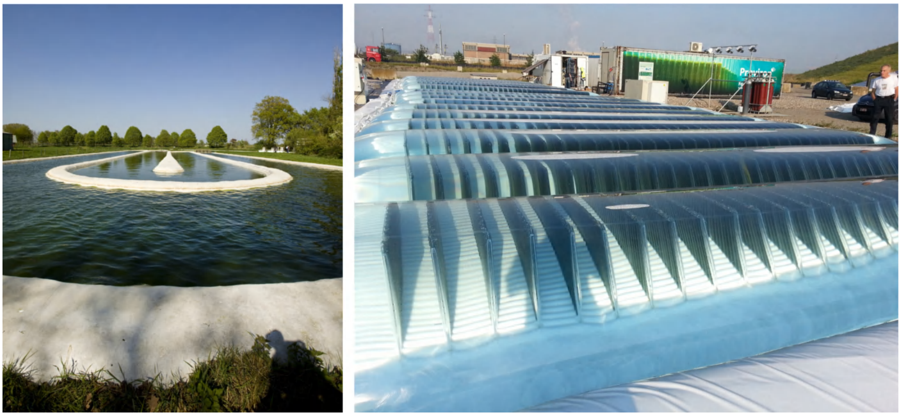


Figure 1.3: Example of open raceway pond reactors (Ingrepro, The Netherlands) and a novel design of a low-cost photobioreactor (Proviron, Belgium) [187].

flow rates of between 20 and 30 cm s^{-1} are required to achieve reliable high productivities and are mainly obtained by using paddlewheels. Shallow pond systems are similar to raceway ponds, but have the additional feature of being sloped. This results in the need of pumping the culture back to the highest point of the system. Such a system does not continuously require agitation power and allows the implementation of a pumping strategy dependent on environmental conditions such as temperature and light intensity.

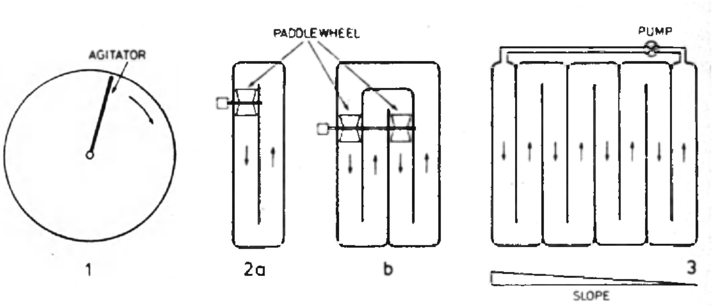


Figure 1.4: Main types of open pond cultivation systems. 1 Circular central-pivot ponds. 2a Single raceway pond. 2b joined raceway ponds. 3 Shallow pond with circulating pump [8].

Photobioreactors are closed systems to cultivate microalgae and can be divided into two main types: flat panel and tubular photobioreactors [125]. For both

Table 1.3: Advantages and drawbacks of design approaches for algae production [42].

Design	Culture density[1]	Productivity[2]	Gas exchange	Scalability	Culture control
Raceway pond	0.25-1	10-20	Low	High	Low
Tubular system	1.5-5	20-30	Very low	Medium	High
Biofilm system	50-70[3]	5-10	High	High	Low

[1]: expressed in g L^{-1}
[2]: expressed in g m^{-2} d^{-1}
[3]: bacteria are included

types, productivities are generally higher when compared to open pond systems because light is provided more efficiently to the system. At this moment, only a few hundred tonnes of microalgae are being produced in closed photobioreactors, especially for high value products such as astaxanthin production (Section 1.1.3). The development of new types of photobioreactors is mainly focused on decreasing capital costs and improving temperature control of the system. A good example is the ProviAPT system developed by the Proviron Holding in Belgium [154] (Fig 1.3).

An alternative system is cultivation using biofilms. In this case, microalgae are grown attached to supportive material and as such the challenge of harvesting is overcome (Section 1.2). Biofilm formation benefits from the presence of bacteria and therefore this approach is mainly used in wastewater treatment applications.

Both open and closed systems have their advantages and drawbacks (Table 1.3). Among scientists, there is an ongoing debate about which system is most suitable for large scale production of microalgae. In most cases, the choice for a particular system will depend on the application. Generally, open systems have a lower capital and operational cost than closed systems while closed systems can deliver higher culture densities and productivities. Temperature control and gas exchange needs special attention during photobioreactor design while open system face evaporation. Since closed systems allow better process control, culture management is easier in closed systems compared to open systems.

Harvesting, dewatering and drying

After cultivation, microalgae are harvested and subsequently dewatered and dried. A huge amount of water needs to be removed in order to obtain a dry concentrate (>90%) since cultivation densities can be lower than 0.05%. In a first step, microalgae are harvested as a wet paste with up to 15-25 % dry solids. The fundamental properties of microalgae such as particle shape, particle size, presence of a cell wall, specific weight and surface charge will influence their recovery. The challenge of harvesting is discussed in further detail in Section 1.2. Subsequently, the wet paste is further dewatered using solar drying, spray-drying, drum-drying or freeze-drying. The choice of a drying method can have consequences with respect to stability of the biomass content. A recent study showed that spray-dried *Phaeodactylum tricornutum* was more susceptible to oxidation than freeze-dried microalgae, possibly due to breakdown of protecting carotenoids upon spray-drying [157].

1.1.5 Challenges for microalgae production

For commodity applications of microalgae, production cost is the main driver for process development. Currently, production costs need to be reduced at least an order of magnitude to become competitive for energy markets. A recent study stated that production costs need to be less than US\$ 1 kg^{-1} biomass in order to become cost-competitive on the energy market. But even then, a biorefinery approach (1.1.3) is needed to valorise all co-products [195].

Improving cost efficiency can be done by reducing the cost of each process step. For example, the harvesting process can be improved (Section 1.2). The challenge of harvesting is discussed in detail in Section 1.2. Additionally, drying is also a very energy intensive process and extra energy is also needed to unlock microalgal compounds from the cells in order to achieve satisfactory extraction yields. Current research is focused on the development of wet extraction routes in order to exclude the drying process and energy-efficient disruption methods to improve extraction yield [190, 160].

Alternatively, a lot of research has focussed on the improvement of microalgal productivities. Theoretical productivities can be calculated based on light energy conversion using the photosynthetic efficiency as important parameter. As stated in Section 1.1.2, numerous factors influence this parameter resulting in a disagreement in literature about a realistic target. Conservative calculations propose 4.5% as a potential future target [191] resulting in a theoretical productivity of 130 ton ha^{-1} yr^{-1}. Based on current productivities in pond systems, actual photosynthetic efficiencies can be estimated to be around 1%. Currently, research is focusing on improving the biology of photosystems by reduction of the cellular antenna size as well as on improvement of photobioreactor design with respect to light dilution, gas exchange and temperature control [125].

Next to cost and productivity, new challenges will appear in the near future. A lot of species and strain selection is ongoing with respect to screening for bioactive compounds. This will trigger the need for research on novel cultivation methodologies since current cultivation processes are only established for a few - mostly extremophilic - species. The development of new process tools for monitoring and controlling large scale cultivation systems is boosted by recent developments in low cost and miniaturised sensor technology. Besides this, culture management is still a challenge. There is a need for the development of long term cultivation control strategies preventing contamination and predation.

1.2 The challenge of harvesting microalgae

A major challenge in downstream processing of microalgae lies in separating the microalgae from their growth medium, i.e. the harvesting process. Because a high biomass concentration leads to mutual shading of the microalgal cells and thus a reduction in productivity, biomass concentrations in microalgal cultures are usually low: from 0.5 g L^{-1} in open pond reactors to about 5 g L^{-1} in photobioreactors. This means that a large volume of water has to be removed to harvest the biomass. Due to the small size of the microalgal cells (2 – 20 μm) and their colloidal stability in suspension (Section 1.3.1), harvesting by means of sedimentation or simple screening is not feasible, except perhaps for larger species such as *Arthrospira*.

When microalgae are produced for high-value products, harvesting is done in one step by centrifugation. Centrifugation is however too expensive and energy-intensive if biomass is to be used for low-value products such as biofuels due to the large volumes of culture medium that need to be removed (Section 1.2.1). Finding an alternative technology that is capable of processing large volumes of culture medium at a minimal cost is thus essential to reduce the cost and increase the scale of microalgal biomass production to a level that will make commercial application of low-value products feasible [121, 181, 28, 162]. Furthermore, alternative harvesting approaches should minimize contamination of the biomass and extracted compounds and avoid interference during cell disruption or extraction processes. Finally, the harvesting process may not inhibit the recycle of the cultivation medium after harvesting by interfering with the growth of the microalgae.

Microalgal biomass can be spoiled in hours if the moisture content remains higher than 85%. Therefore, the residence time during harvesting is also important in the evaluation of harvesting methods because it will additionally determine cost and reliability of methods for further processing [117].

1.2.1 State of the art harvesting technology

Centrifugation

Centrifugation is based on the generation of a centrifugal force which acts radially and accelerates the separation of particles based on their density difference. Since the density of microalgae is similar to that of water (density *Chlorella* = 1.070 kg m^{-3}), high centrifugal forces and thereby a high energy input is needed to achieve separation. On the other hand, centrifugation can be operated continuously and can be used for almost all types of microalgae. Generally, three types of centrifuges are used for harvesting microalgae: disc stack, decanter, and spiral plate centrifuges.

Disc stack centrifuges are the most commonly used in commercial plants for high value algal products [121]. This type of centrifuge operates with a rotating, relatively shallow cylindrical bowl containing a number of closely spaced metal discs. They are ideally suited for separating particles of the size (3-30μm) and concentration (0.02-0.05%) of microalgae cultures up to 15 % solids while consuming 0.7-1.3 kWh m^{-3} [120].

The decanter or scroll centrifuge is one of the most promising centrifugal devices for recovery of microalgae. They can operate continuously, have a high capacity and low maintenance requirements. However, the high capital cost and energy demand often limits their use to high value products. Essentially, this type of centrifuge contains two concentric rotating elements surrounded by a stationary cover and can deliver microalgae biomass concentrates up to 22% while consuming 8 kWh m^{-3} [120].

Recently, a centrifuge system based on spiral plate technology was designed specifically for microalgae harvesting by Evodos™. The system contains rotating curved plates inside a sliding cylindrical drum to reduce the particle settling distance and the system is operated at low rotation speed. It is claimed to be able to concentrate *Nannochloropsis* from 0.025% up to 31.5% with an energy usage of 0.95 kWh m^{-3}. Current systems only allow a discrete discharge of harvested microalgae limited to 4 m^3 h^{-1} and long term reliability has yet to be proven [120].

Filtration

Numerous types of filtration systems such as micro-stainers, vibrating screens, filter presses, belt filters and vacuum drums have been used to harvest microalgae. Generally, filtration can be classified by the pore size of the membrane; macro-filtration (> 10 μm), micro-filtration (0.1-10 μm), ultra-filtration (0.02-2 μm) and reverse osmosis ($<$0.001 μm). Pressure is needed to force the liquid through the membrane. Generally, the required energy for this increases with reducing membrane pore size.

Macro-filtration is widely used for larger microalgae species like *Arthrospira*. Belt filters are able to filter up to 20% with an energy consumption of 0.5 kWh m^{-3} if the feed is pre-concentrated at 4%.

Micro-filtration is seen to have the most appropriate pore size to retain the majority of common species. While it has been stated that micro-filtration could be even less economic than centrifugation for the recovery of microalgal cells on a large scale [121], recent studies showed that harvesting using submerged filtration in combination with centrifugation could achieve concentration up to 22% and reduce energy needs under 1 kWh m^{-3} [16].

Ultra-filtration is a possible alternative in particular for very fragile cells, but has not generally been used for recovery of microalgae since operating and maintenance costs are high [155, 120]. Energy consumption is believed to be between 1 and 3 kWh m^{-3}.

Flotation

Flotation is a separation process based on the adhesion of particles to air or gas bubbles, which carry the particles to the liquid surface, allowing further separation usually via skimming. Classification of flotation processes is based on the method of bubble production: dispersed air flotation, dissolved air flotation and suspended air flotation [196].

During dispersed air flotation, air is continuously pumped into a flotation cell and foam is created using a surface-active chemical. Hydrophobic solids adsorb to the air bubbles and are separated from the suspension. While concentration factors between 50 and 200 have been reported in the past, this method is not widely used for recovery of microalgae. A recent study however claims that this method has potential as primary concentration method. Initial concentration at a very low cost can improve efficiency and costs of secondary or tertiary dewatering methods. The energy requirement concentrating *Chlorella* up to 2.5% was 0.015 kWh m^{-3} [45].

Higher flotation efficiencies can be obtained if air-supersaturated water is injected under pressure in the flotation cell, a process known as dissolved air flotation. In addition, the solid concentration that can be achieved in dissolved flotation systems is higher (7%). Unfortunately, operational costs of such systems is high due to the high energy cost of supersaturating the water with air under pressure [83].

Suspended air flotation eliminates the need for a compressor and saturator for the creation of small air bubbles. However, this system utilises chemicals such as cationic surfactant to create small bubbles. In addition, the bubbles can be electrically-charged to increase float stability. Bench-scale results claim an energy usage of 0.003 kWh m^{-3} to concentrate microalgae up to 5% [196]. However, this technology is rather new and upscale reliability is yet to be proven.

Sedimentation

Solid-liquid separation by sedimentation is one of the simplest ways to harvest microalgae. The separation is caused by gravitational forces and the sedimentation rate is determined by Stokes' Law, which states that the sedimentation velocity is proportional to the radius of the cells and the difference in density between the microalgae and the medium (Eq 1.1). This method acquires low design costs and low requirements for skilled operators. For a spherical shaped microalgae, *Chlorella*, the settling velocity was calculated to be 0.1 m day^{-1} [120]. But Stokes' law holds only for spheroid shapes, while microalgae are most often not spherical. In a study on 24 autotrophic microalgae ranging in size from 10 - 1,000 μm it was found that the sedimentation rate varied between 0.4 to 2.2 m day^{-1} [138]. Especially motile species are not forced to settle.

$$v = \frac{2}{9} r^2 g \frac{\rho_p - \rho_f}{\eta} \tag{1.1}$$

v	particle settling velocity [m s^{-1}]
r	cell radius [m]
g	gravitational acceleration (9.81) [m s^{-2}]
ρ_p	mass density particle [kg m^{-3}]
ρ_f	mass density fluid [kg m^{-3}]
η	dynamic viscosity [N s m^{-2}]

Cell recovery using sedimentation is generally low : 60-65% with a solid concentration up to 1.5%. Energy consumption of sedimentation using lamella separators is up to 0.1 kWh m^{-3}. Settling velocity, cell recovery as well as solid concentration can be improved by inducing flocculation prior to sedimentation. The different approaches for microalgae flocculation are discussed in Section 1.3.

Table 1.5: Comparison of state of the art microalgal harvesting methods [120, 121].

Method	Advantages	Drawbacks	DS[1] (%)	ER[2] (kWh m^{-3})
Centrifugation	rapid, efficient, suitable for most microalgae species	high CAPEX[3] & OPEX[4]	10-22	0.7-8
Filtration	high system variety	species specific, fouling	2-27	0.5-3
Flotation	faster than sedimentation	species specific, high CAPEX	2.5-7	0.015-1.5
Sedimentation	low CAPEX & OPEX	species specific, low final concentration	0.5-3	0.1-0.3

[1] DS = dry solids output concentration
[2] ER = energy requirement
[3] CAPEX = capital expenditures
[4] OPEX = operating expenditures

Comparison of methods

A recent review stated that there is no superior method for harvesting and dewatering of microalgae [181]. An overview of advantages and drawbacks are given in Table 1.5. Centrifugation can rapidly and efficiently handle most algal species, but capital and operational costs remain high. Filtration is best suited for microalgae species with large cells but struggles from time to time with fouling issues. Flotation can be more rapid than sedimentation, but is very algae specific and costly. Sedimentation is promising because of its low cost, but is limited to specific non motile species.

1.3 Approaches for microalgae flocculation

1.3.1 Colloidal stability of microalgal suspensions

Surface charge

Microalgae exhibit a slightly negative charge at neutral pH, due to the presence of proton-active carboxylic, phosphoric, phosphodiester, hydroxyl and amine functional groups [81, 121]. To maintain electrical neutrality, opposite charged ions (counter-ions) will be attracted in the surrounding solution, while negative ions (co-ions) will be repelled. Close to the particle surface, the counter-ions form a dense layer that is inaccessible to other counter ions, which is called the Stern layer. A dynamic equilibrium of charges attributed by counter-ions and co-ions is established and is free to move around the Stern layer to form a diffuse layer. This layer extends from the edge of the Stern layer to a certain distance in the surrounding solution until the concentration of counter-ions and co-ions are identical and there is a zero charge. This results in the development of a surface potential around the particle. The total system of the particle surface charge and associated counter ions in the surrounding solution is called the electrical double layer (Fig 1.5a).

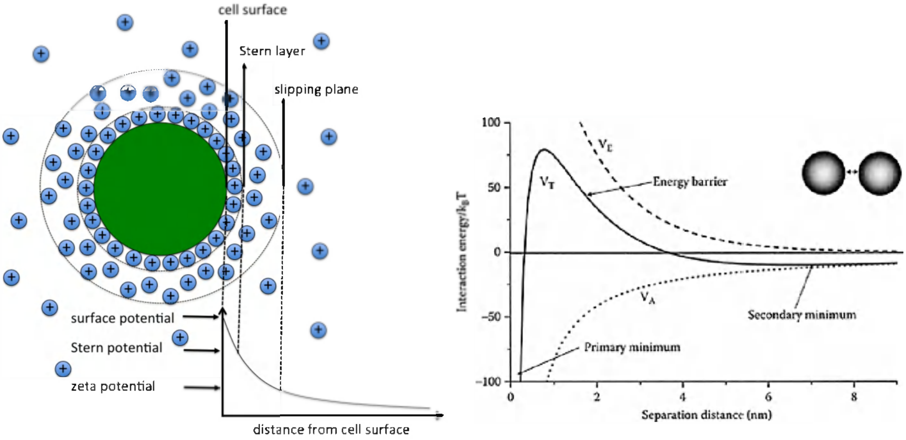
The surface potential is difficult to measure but can be estimated by the surface charge, which is determined by pH titration [81]. Another frequently used parameter of any colloid is the potential at the end of the diffuse layer (slipping plane), known as the zeta (ζ) potential (Fig 1.5a). This parameter is related, but not identical to the surface potential. In contrast to the surface potential, the zeta potential is however easy to measure by determining the mobility of charged particles in an electric field. For microalgae, the zeta potential is typically negative and is usually within the range of -10 to -35 mV [85].

Interaction between charged particles

The interaction between colloidal charged particles is described by the DLVO theory, which is named after its developers: Derjaguin, Landau, Verwey and Overbeek. This quantitative theory describes the interaction between colloids as a competition model between attraction by van der Waals forces and electrostatic repulsion in terms of energy as function of the distance between particles (Fig 1.5b).

The zeta potential is an important parameter to evaluate the colloidal stability of a system. When the zeta potential is high ($>$ 25 mV, positive or negative), electrical repulsion between particles is strong and the suspension is said to be stable. When the zeta potential is close to zero, particles can approach each other to a point where they will be attracted by Van der Waals forces. When that happens, particles will aggregate and flocculation or coagulation will occur.

Next to the zeta potential, the size of the double layer is also important as it will determine the relation between attraction and repulsion and is mainly dependent on the ionic strength (I_z), which is a measure for all ions present in the solution (Eq 1.2). Increasing the I_z results in a decreased size of the double layer, which will promote attraction and finally aggregation. This phenomenon is known as double layer compression and is highly relevant to the stability of colloidal particles. The thickness of the double layer can be quantified as the Debye length κ^{-1} according to Equation (1.3). For typical sea waters and natural waters, values of the Debye length κ^{-1} can range from less than 1 nm to around 100 nm or more. For completely deionized water at 25 °C, the concentrations of H^+ and OH^- are each 10^{-7} M and κ^{-1} is 960 nm.


cell surface
Stern layer
slipping plane
surface potential
Stern potential
zeta potential
distance from cell surface
100
50
0
−50
−100
Interaction energy/k_BT
V_E
V_T
Energy barrier
V_A
Secondary minimum
Primary minimum
0
2
4
6
8
Separation distance (nm)

(a) (b)

Figure 1.5: (a) Structure of the electrical double layer of charged ions in solution surrounding a negatively charged microalgal cell and the potential difference between the particle and the bulk fluid as a function of the distance from the particle surface [187] (b) Potential energy diagram for the interaction of equal spheres. The curves show the electrical (V$_E$), van der Waals (V$_A$), and total (V$_T$) interaction energy [73].

$$I_z = \frac{1}{2}\sum_i c_i z_i^2 \tag{1.2}$$

I_z	ionic strength [mol m^{-3}]
c_i	molar concentration [mol m^{-3}]
z_i	number of charges [-]

$$\kappa^{-1} = \sqrt{\frac{\epsilon k T}{2e^2 N_a I_z}} \tag{1.3}$$

κ^{-1}	Debye length [m]
ϵ	electrical permittivity [C^2 J^{-1} m^{-1}]
k	Boltzmann constant (1.380 10^{-23}) [J K^{-1}]
N_a	Avogadro number (6.022 10^{23}) [mol^{-1}]
e	elementary charge (1.602 10^{-19}) [C]
T	temperature [K]
I_z	ionic strength [mol m^{-3}]

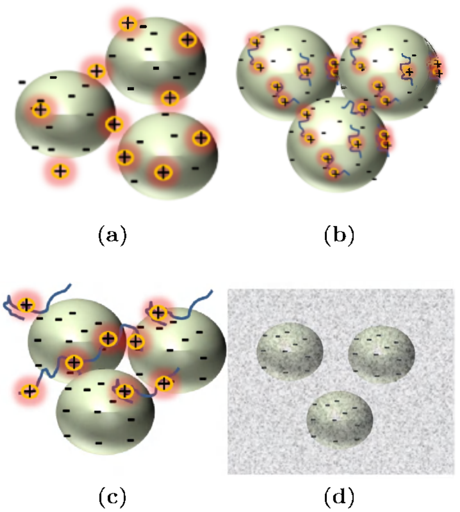


Figure 1.6: Overview of different coagulation mechanisms (a) charge neutralization (b) electrostatic patch mechanism (c) bridging mechanism (d) sweeping flocculation.

1.3.2 What is flocculation?

Flocculation is the process whereby destabilized particles are induced to coagulate, make contact, and thereby form larger agglomerates [26]. Flocculation of particle suspensions can often be attributed to four coagulation mechanisms, acting alone or in combination. (1) Charge neutralization is the phenomenon in which charged ions, polymers or colloids strongly absorb on the opposite charged surface of a particle, followed by destabilization, coagulation and flocculation (Fig 1.6a). (2) The electrostatic patch mechanism is the phenomenon in which a charged polymer binds to a particle with opposite charge. The polymer locally reverses the charge of the particle surface, resulting in patches of opposite charge on the particle surface. Particles subsequently connect with each other through patches of opposite charge, causing flocculation (Fig 1.6b). (3) Bridging is the phenomenon in which polymers or charged colloids simultaneously bind to the surface of two different particles to form a bridge between them. This bridge brings the particles together and causes flocculation (Fig 1.6c). (4) Sweeping flocculation is the process in which particles are entrapped in a massive precipitation of a mineral which causes their flocculation (Fig 1.6d).

1.3.3 Chemical flocculation

Metal salts such as alum and ferric chloride are widely used for flocculation in industries such as water treatment and mining. Although metal salts are being applied for harvesting microalgae (e.g. *Dunaliella* [11]), their use results in high concentrations of metals in the harvested biomass. These metals remain in the biomass residue after extraction of lipids or carotenoids [156], but may interfere with the use of the protein fraction in this residue. Despite this shortcoming, metal coagulants provide a good model system to study the interaction between flocculants and microalgal cells because their properties are well understood [198, 204].

Other commonly used chemical flocculants in other industries are synthetic polyacrylamide polymers. These may however contain traces of toxic acrylamide and thus also contaminate the microalgal biomass [26]. Flocculants based on natural biopolymers are therefore a safer alternative. To be able to interact with the negative surface charge of microalgal cells, these biopolymers should be positively charged, which is rare in nature. A well-known positively charged biopolymer is chitosan, which is derived from chitin, a waste product from shellfish production. Chitosan is a very efficient flocculant but it works only at low pH, while the pH in microalgal cultures is relatively high (Fig 1.7a) [113, 51, 200]. An alternative to chitosan is cationic starch, which is prepared from starch by addition of quaternary ammonium groups (Fig 1.7b; Chapter 2). Other examples of biopolymers that can be used to flocculate microalgae are poly-γ-glutamic acid (an extracellular polymer produced by *Bacillus subtilis*) [205] or polymers present in flour from *Moringa oleifera* seeds [179]. A general problem of polymer flocculants is that they undergo coiling at high ionic strengths and become ineffective [181]. Therefore, they are less suitable for harvesting microalgae cultivated in seawater. Two very recent studies showed that the usage of cationic aluminum and magnesium backboned organoclays has potential to be used for microalgae flocculation [107, 58].

1.3.4 Autoflocculation

Flocculation often occurs spontaneously in microalgal cultures when pH increases above 9 [172]. This type of flocculation is often referred to as autoflocculation because it occurs spontaneously in microalgal cultures as a result of a pH increase due to photosynthetic CO_2 depletion. Autoflocculation is associated with the formation of calcium or magnesium precipitates. Depending on the conditions, these precipitates carry positive surface charges and can induce flocculation (Chapter 4).

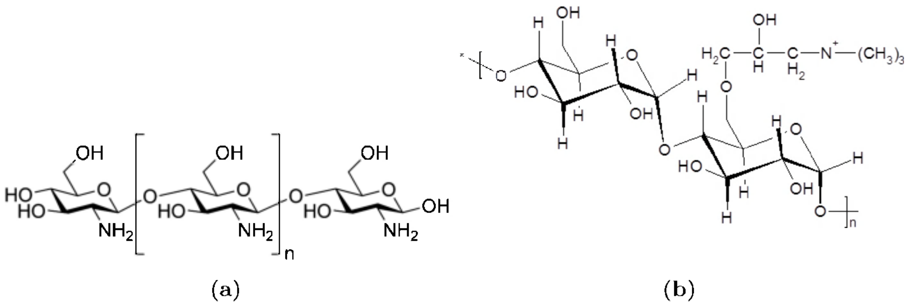


Figure 1.7: Structure of (a) chitosan and (b) cationic amino starch.

Calcium phosphate precipitates are positively charged when calcium ions are in excess of phosphate ions and interact with the negative surface charge of microalgal cells [42, 162]. High phosphate concentrations are required for this type of flocculation to occur. As a result of the declining phosphate resources and increasing prices of phosphate, flocculation by calcium phosphate precipitation is unsustainable, except perhaps in applications where microalgae are used for wastewater treatment and excess phosphate needs to be removed [114]. Magnesium hydroxide or brucite also precipitates at high pH (Chapter 4).

1.3.5 Physical flocculation methods

Contamination of the biomass could be avoided if it were possible to induce flocculation by applying only physical forces. For instance, flocculation of microalgae can be accomplished by applying a field of standing ultrasound waves. Although this method works well in the laboratory, it is difficult to apply on larger scales [24]. In electrocoagulation flocculation, flocculation is induced through electrolytic release of metal ions from a sacrificial anode and avoids contaminations of anions (Chapter 3). OriginOil™ claims to have developed a system that is similar to this principle. Their method uses only electromagnetic pulses to neutralize the surface charge of microalgal cells and induce flocculation [69].

Recently, several studies have explored the use of magnetic nanoparticles to harvest microalgae [147]. Magnetite (Fe_2O_3) nanoparticles may adsorb directly on the microalgal cells, upon which the cells can be separated from the medium by applying a magnetic field. This method thus combines flocculation and separation in a single process step [37]. Magnetite nanoparticles seem to adsorb more easily on some microalgal species than on others [199]. Adsorption can be improved by coating the nanoparticles with cationic polymers [110, 112]. An advantage of using magnetite nanoparticles is that they can be recovered after harvesting and reused later [37].

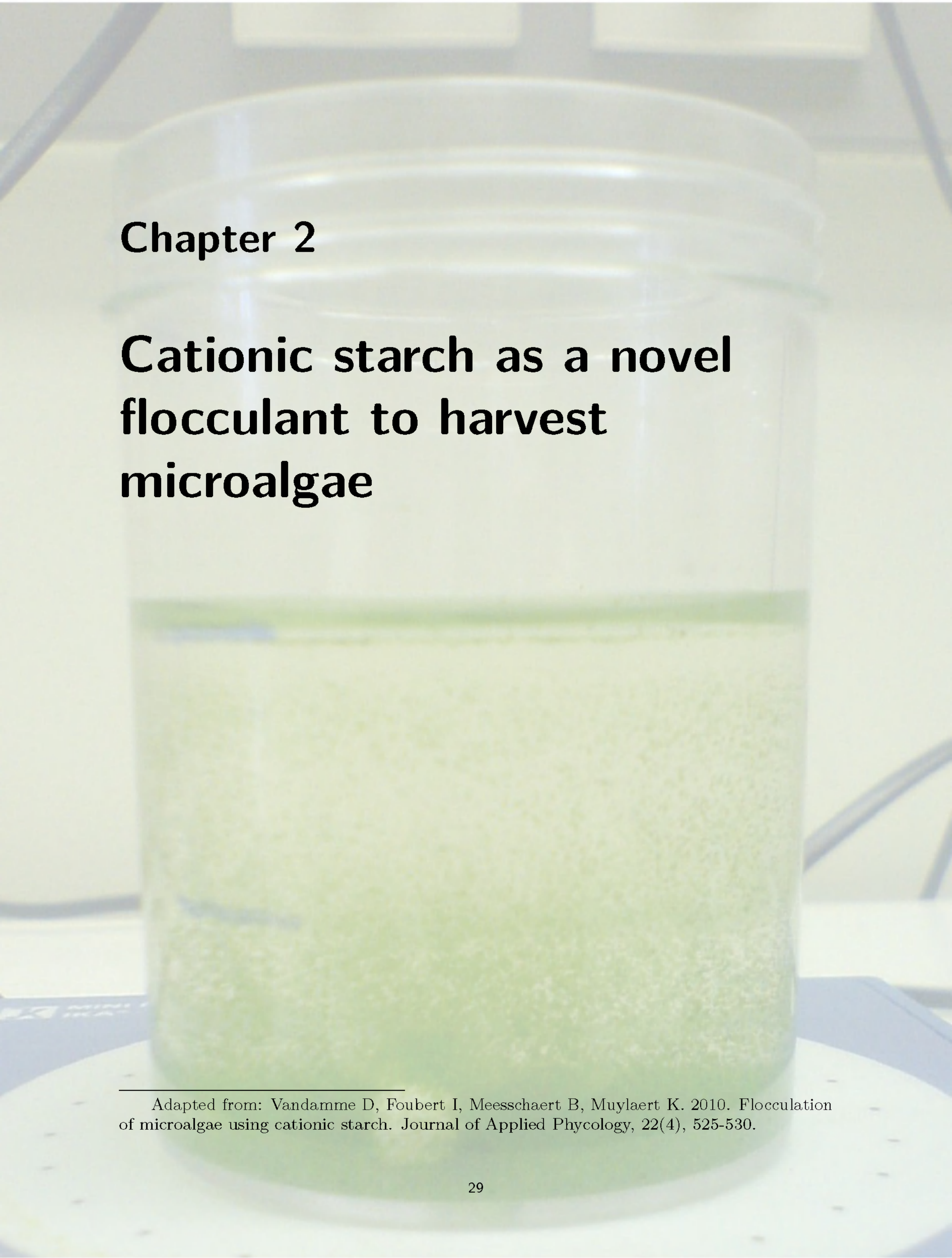
1.3.6 Biological flocculation

In natural blooms of microalgae in lakes or rivers, flocculation sometimes occurs spontaneously. This spontaneous flocculation is assumed to be caused by extracellular polymer substances in the medium and is called bioflocculation [102]. Bioflocculation is often used successfully for harvesting microalgae in facilities where microalgae are employed for wastewater treatment [46]. The underlying mechanism, however, is poorly understood and deserves further research as it may lead to a chemical-free method for flocculating microalgae. Some microalgal species flocculate more readily than others and such naturally bioflocculating microalgae can be mixed with other species to induce flocculation [158, 159]. There are indications that bioflocculation may be initiated by infochemicals. Recently, an infochemical isolated from a senescent and flocculating culture of a *Skeletonema* species was found to be capable of inducing flocculation in a culture of another species of microalgae [178].

Bacteria or fungi can also induce bioflocculation of microalgae. Some fungi, for instance, have positively charged hyphae that can interact with the negatively charged microalgal cell surface and cause flocculation [203, 206, 207]. Specific consortia of bacteria can also induce flocculation of microalgae [80, 104]. These flocculating fungi or bacteria can be cultivated separately or in combination with the microalgae. In the latter case, a carbon source is required in the medium. In wastewater, a carbon source is usually present which allows co-cultivation of microalgae and bacteria. This results in a culture of mixed algal–bacterial flocs that can easily be harvested [175, 183]. The use of bacteria or fungi as a flocculating agent avoids chemical contamination of the biomass but results in microbiological contamination, which may also interfere with food or feed applications of the microalgal biomass.

1.3.7 Flocculation induced by genetic modification

Many research efforts are currently directed towards genetic modification of microalgae. Most recently published studies and granted patents in this field are aimed at increasing biomass productivity or increasing production of specific metabolites, most often lipids [67, 102]. However, genetic modification may also be a promising way to harvest microalgae [42, 67]. Here, achievements in genetic modification of yeast may be used as an example. In yeast, genetically modified strains have been developed that express flocculin proteins in their cell walls, causing the cells to aggregate [70]. The expression of these proteins can be induced by an environmental trigger or during a specific growth stage. Sapphire Energy™ has described a method for flocculating microalgae in which ligand–receptor pairs can be expressed in different strains that are mixed to induce flocculation, or that are expressed sequentially in the same strain [119]. Genetic modification or selection may also be aimed at facilitating flocculation by other methods. For instance, a cell wall-deficient mutant of *Chlamydomonas* has been found to flocculate much more easily under alkaline conditions than the wild type strain [163]. This indicates that minor genetic modifications may greatly facilitate flocculation.



Chapter 2

Cationic starch as a novel flocculant to harvest microalgae

Adapted from: Vandamme D, Foubert I, Meesschaert B, Muylaert K. 2010. Flocculation of microalgae using cationic starch. Journal of Applied Phycology, 22(4), 525-530.

2.1 Introduction

Harvesting microalgae by means of flocculation has a lot of potential to decrease microalgal biomass production costs. However, the applied flocculants need to be cheap, efficient and avoid contamination of biomass and minimize toxicity of residual medium after cultivation. Inorganic flocculants such as alum and iron chloride are efficient but are required in high doses and result in contamination of the biomass with aluminum or iron [8]. Biodegradable organic flocculants do not contaminate the algal biomass and are often required in lower doses [169]. They are based on biopolymers like chitin, guar gum, alginic acid, or starch. Of these, chitosan has been shown to be an effective flocculant for microalgae [51]. It has no apparent toxic effects on fish feeding on the harvested algae [99]. It is, however, a high-value product with a market value of about $US 10 per kilogram [8, 149].

Starch consists of a mixture of amylose and amylopectin and is one of the most abundant natural polymers. Chemically modified starches have properties very different from the parent starch and have many applications in industrial processes [146]. Cationic starch is prepared by addition of quaternary ammonium groups to the glucose hydroxyl groups. Because of its low cost (about $US 1–3 per kilogram), cationic starch is increasingly used as an alternative for inorganic and synthetic organic flocculants in liquid–solid separation processes, more specifically in wastewater treatment and paper mill industries [136]. As polymer flocculants are often specific, flocculants that are effective for clay dispersions or cellulose are not necessarily applicable to algal cells. The goal of this chapter is to evaluate the potential of cationic starch for flocculation of microalgae.

2.2 Materials and methods

Four microalgal species were obtained from culture collections: *Parachlorella kessleri* (SAG 27.87), *Scenedesmus obliquus* (CCAP 276j3A), *Phaeodactylum tricornutum* (CCAP 1055/1), and *Nannochloropsis salina* (SAG 40.85). The microalgae were cultured in Wright's Cryptophyte medium [79], which was prepared from pure salts and deionized water. The concentration of the medium was increased five times to allow the microalgae to attain a biomass concentration comparable to commercial culture systems (up to 0.5 g dry weight per liter). For the marine species, synthetic sea salt (Ultramarine Synthetica, Waterlife Research, UK) was added at a concentration of 30 g L^{-1}. The medium was adjusted to pH 8 and autoclaved. An inoculum was added under a sterile hood at a 1:10 ratio. The microalgae were cultured in five parallel 2 L bottles incubated in a temperature controlled room (20°C). The bottles were irradiated with daylight fluorescent tubes (light intensity = 100 μmol photons m^{-2} s^{-1}) and were bubbled with sterile-filtered air at a rate of approximately 200 mL min^{-1} to create turbulence and avoid CO_2 limitation. Flocculation experiments were carried out when algae were in their exponential growth phase. The algal biomass concentration in the reactors at that moment varied between 0.15 and 0.5 g dry weight per liter. Algal biomass was estimated from optical density measurements at 550 nm using a spectrophotometer (Hach Lange DR 2800). Optical density was calibrated against dry weight measured gravimetrically on preweighed GF/F glass fiber filters ($R^2 > 0.98$).

Two commercial cationic starches were used in the experiments. Greenfloc 120 (Hydra 2002 Research, Development and Consult, Hungary) is a cationic starch with a degree of substitution of 0.15 that is mainly used in wastewater treatment. It was supplied as a concentrated solution in water (16%) that was ready for use. Cargill C*bond HR 35.849 (Cargill Deutschland, Germany) is a cationic starch with a degree of substitution of 0.11 that is used in the paper manufacturing industry. It was supplied as a dry product that was dissolved in water and heated to 80°C for 20 min before use.

Flocculation of microalgae after addition of cationic starch was evaluated using jar tests [44]. The algal suspensions were divided into replicate 100 mL beakers. The initial algal biomass concentration in the beakers was estimated from the optical density at 550 nm. Cationic starch was added at a specific dose under intensive stirring (1,000 rpm) using a magnetic stirrer. After 5 min, the stirring speed was reduced to 250 rpm. Stirring was stopped 30 min after addition of the cationic starch. After another 30 min, the optical density of the supernatant was measured at half the height of the clarified layer. The percentage of algal biomass removed was estimated from the ratio of the initial over the final optical density. To evaluate the influence of pH on flocculation, pH was adjusted using 0.5 N HCl or 0.5 N NaOH. Results were statistically evaluated using one-way analysis of variance (ANOVA) and a Tukey's test (Sigmaplot 11, Systat Software, Inc.). The potential toxicity of cationic starch on the microalgae was evaluated using measurements of the maximum quantum yield of photosynthetic efficiency of photosystem II, measured using an AquaPen-C fluorometer (Photon Systems Instruments, Czech Republic). This parameter is a sensitive indicator of stress experienced by microalgae and is often used for evaluating toxicity of substances towards microalgae [43]. The quantum yield of photosynthetic efficiency of photosystem II was measured 3 h after addition of cationic starch and after 20 min of dark adaptation of the microalgae. Statistical analysis was performed using one-way ANOVA (Sigmaplot 11, Systat Software, Inc.).

2.3 Results and discussion

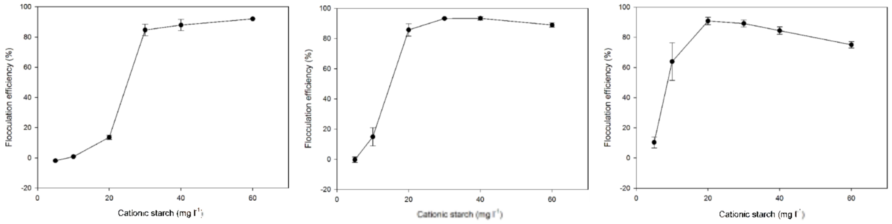
Our results indicate that cationic starch is an efficient flocculant for the freshwater microalgae *Scenedesmus* and *Parachlorella*. Suspensions of unicellular microalgae are stabilized by the negative surface charge of the algal cells. Cationic starch can induce flocculation of negatively charged particles through bridging and/or patch charge neutralization [165, 26]. In jar tests using *Parachlorella* and the cationic starch Greenfloc 120, the flocculation efficiency increased strongly over a relatively narrow range of cationic starch concentration (about 10-20 mg L^{-1}; Fig 2.1). At the optimal dose, more than 90% of the biomass was removed by the flocculant. The Greenfloc 120 cationic starch dose required to flocculate 80% of *Parachlorella* biomass was above a certain concentration linearly related to the algal biomass concentration (Fig 2.2). A linear relation between flocculant dose and particle concentration is often observed in cationic polyelectrolyte flocculants [19].

The ratio of cationic starch over *Parachlorella* biomass required to achieve 80% flocculation was approximately 0.1. For *Scenedesmus*, a lower dose of Greenfloc 120 cationic starch was required to induce flocculation. The ratio of cationic starch over algal biomass required to achieve 80% flocculation for *Scenedesmus* was 0.03 or less (Fig 2.3). As *Parachlorella* and *Scenedesmus* have a comparable charge density [86], this difference can probably be ascribed to the larger size of *Scenedesmus*. Larger particles often require a lower polymer dose for flocculation than smaller particles [26].

In the experiments with low biomass concentrations of *Parachlorella* as well as in the experiment with *Scenedesmus*, it was clear that overdosing of cationic starch resulted in dispersion restabilization. This phenomenon is commonly observed with polyelectrolyte flocculants, including cationic starch [59, 27, 112] and is probably the result of steric hindrance and/or electrostatic repulsion.

For the marine microalgae *Nannochloropsis* and *Phaeodactylum*, the ratio of Greenfloc 120 cationic starch over algal biomass required to induce flocculation was around 1 (results not shown). Therefore, it appears that cationic starch is inefficient for flocculating marine microalgae. This is probably due to high NaCl concentrations. In experiments with kaolin dispersions, a decrease in flocculation efficiency of cationic starch was observed at high NaCl concentrations [18]. Like cationic starch, chitosan is also ineffective for flocculating microalgae in seawater [17, 51, 112, 86].

In dense microalgal cultures, pH is often highly variable: it may increase to 10 due to intensive primary production or decrease to 6 during CO_2 addition or as a result of respiration. As pH affects the zeta potential of charged particles, it may interfere with flocculation. Flocculation of *Parachlorella* using Greenfloc



(a) (b) (c)

Figure 2.1: Effect of cationic starch (Greenfloc 120) dose on the percentage of algal biomass (*Parachlorella*) removed by flocculation for three different initial algal biomass concentrations: (a) 0.30 g L^{-1} (b) 0.15 g L^{-1} and (c) 0.075 g L^{-1}.

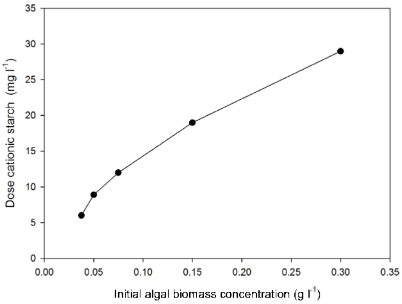


Figure 2.2: Relation between the initial algal biomass (*Parachlorella*) concentration and the cationic starch dose (Greenfloc 120) required to achieve 80% flocculation.

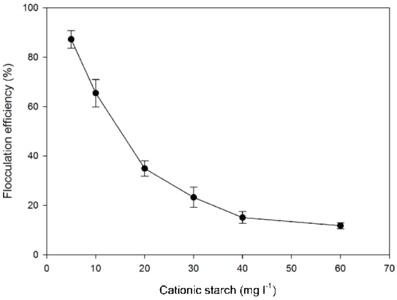


Figure 2.3: Effect of cationic starch (Greenfloc 120) dose on the percentage of algal biomass (*Scenedesmus*) removed by flocculation. Initial algal biomass was 0.15 g L^{-1}.

120 cationic starch increased slightly but significantly with pH over a pH range of 5 to 10 (Fig 2.4, ANOVA, $p < 0.001$).

This is in contrast to flocculation of microalgae using chitosan, which is only efficient at a pH below 8 [51, 112]. In cationic starch, the positive charge is due to quaternary ammonium salts, which maintain their positive charge even at relatively high pH. The increase in flocculation efficiency at high pH is probably due to some autoflocculation of *Parachlorella*, which occurs at a pH of 10 or higher (unpublished results). The Greenfloc 120 cationic starch had no significant (ANOVA, $p = 0.330$) effect on the maximum quantum yield of photosynthetic efficiency of photosystem II in *Parachlorella* (Fig 2.5). Therefore, it appears that cationic starch has no short-term effects on the viability of the algae.

For *Parachlorella*, we compared flocculation by two commercial cationic starch polyelectrolytes, both with a relatively low degree of substitution. Greenfloc 120 is a flocculant designed for wastewater treatment while C*bond HR 35.849 is designed for applications in the paper industry. Using C*bond HR 35.849, a

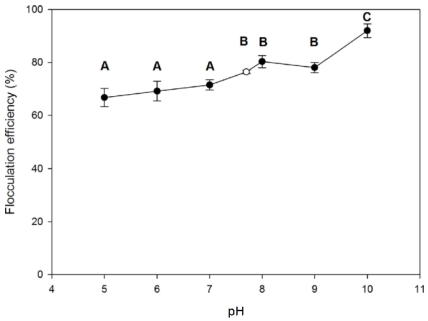


Figure 2.4: Effect of pH on the flocculation of the alga *Parachlorella* using cationic starch (Greenfloc 120) at an algal biomass concentration of 0.43 g L^{-1} and a cationic starch dose of 70 mg L^{-1}. The white point corresponds to the control in which pH was not adjusted. A, B, and C indicate whether pH has a significant influence on flocculation efficiency; means with the same letter are not significantly different ($\alpha = 0.1$).

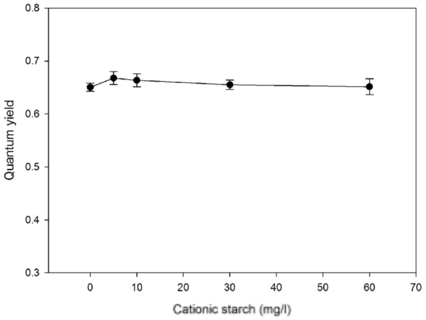


Figure 2.5: Effect of different cationic starch (Greenfloc 120) concentrations on the maximum quantum yield of photosynthetic efficiency of photosystem II in *Parachlorella*.

higher dose was required compared to the Greenfloc 120 cationic starch (Fig 2.6). The ratio of C*bond HR 35.849 cationic starch over algal biomass required to achieve 80% flocculation was approximately 0.3. Moreover, the flocculation increased more slowly with increasing cationic starch concentration. The lower flocculation efficiency of C*bond HR 35.849 may be due to the lower degree of substitution (0.11 versus 0.15). It is well-known that the flocculation efficiency of polyelectrolytic flocculants in general and cationic starch in particular is related to the degree of substitution [27]. However, the flocculation efficiency generally increases linearly with the degree of substitution, especially at a low degree of substitution [100]. As the degree of substitution of Greenfloc 120 is only 1.4 times that of C*bond HR 35.849, while the optimal dose for flocculation was at least three times lower, other factors probably contributed to the difference in flocculation efficiency. The location of the substitutions [168], the molecular weight of the polymers [100], the steric configuration [59], and the amylose to amylopectin ratio have been shown to influence the flocculation efficiency of cationic starch [136].

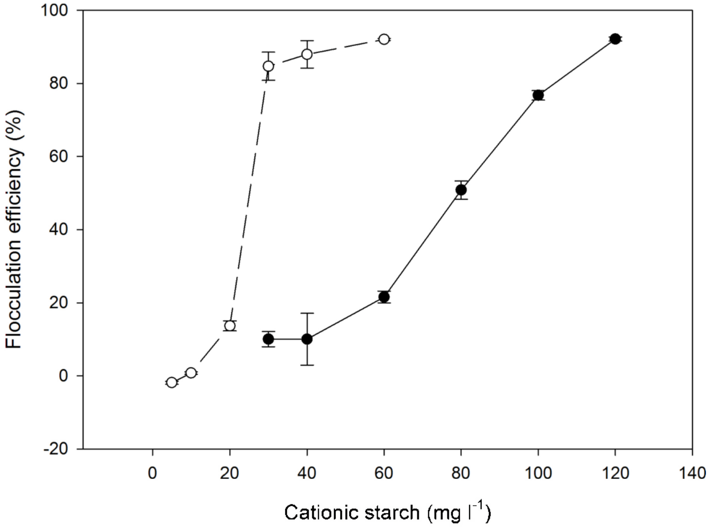
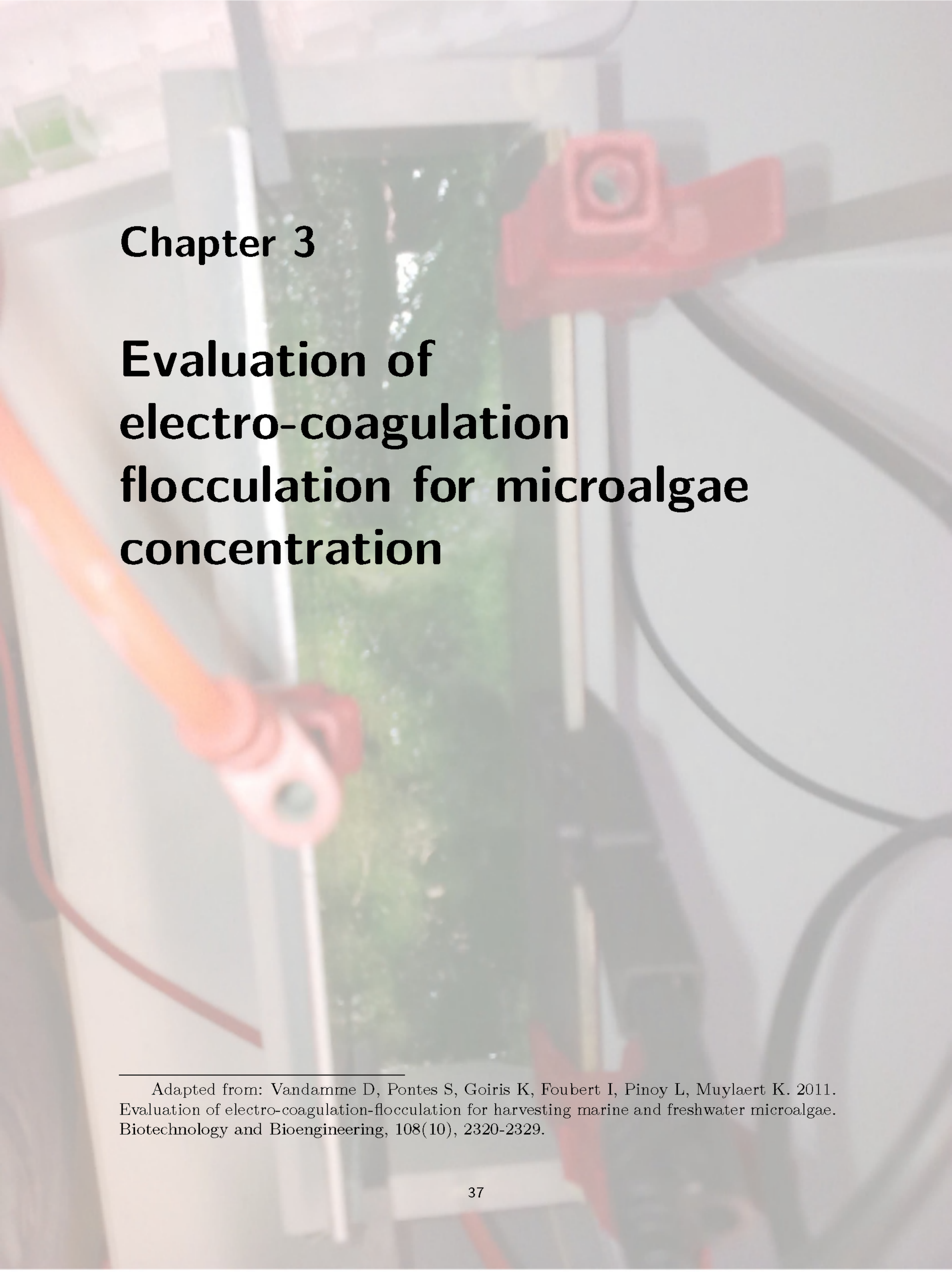


Figure 2.6: Comparison of flocculation of *Parachlorella* using two types of cationic starch (white points correspond to Greenfloc 120 and black points correspond to Cargill C*Bond HR 35.849). Initial algal biomass was 0.3 g L^{-1}.

2.4 Conclusions

Our results show that cationic starch is a potentially useful flocculant for harvesting freshwater microalgae. Compared to inorganic flocculants, cationic starch requires a lower dose. Moreover, it is approved for food contact and for use in treatment of drinking water [100]. In these aspects, cationic starch is similar to chitosan. Due to the lower number of functional groups, the dose required for cationic starch is higher than that for chitosan. On the other hand, chitosan is more expensive than cationic starch; it is not available in very large volumes and is more difficult to apply due to its pH-dependence.

The cationic starches used in this study were not designed for harvesting algae. The large difference between the two cationic starches tested suggests that there is room for improvement of the efficiency of cationic starches for flocculating algae. The flocculation efficiency might be improved by increasing the degree of substitution. It should be noted, however, that the production cost of cationic starch increases with the degree of substitution. Other options to improve the flocculation efficiency include modification of the amylose to amylopectin ratio or modification of the polymer chain lengths.



Chapter 3

Evaluation of electro-coagulation flocculation for microalgae concentration

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Adapted from: Vandamme D, Pontes S, Goiris K, Foubert I, Pinoy L, Muylaert K. 2011. Evaluation of electro-coagulation-flocculation for harvesting marine and freshwater microalgae. Biotechnology and Bioengineering, 108(10), 2320-2329.

3.1 Introduction

Microalgae can easily be flocculated using metal coagulants such as Fe^{3+} or Al^{3+} salts [1, 13, 137]. In wastewater treatment, electro-coagulation flocculation (ECF) has been proposed as an alternative for chemical coagulants [123, 122]. In ECF, iron or aluminum ions are released from a sacrificial anode through electrolytic oxidation. Compared to coagulation–flocculation with Fe^{3+} or Al^{3+} salts, ECF has the advantage that no anions such as chlorine and sulphate are introduced in the process water. The electrolytic oxidation of the sacrificial anode, however, requires electricity.

During ECF, the following reactions occur at the anode.
Using an aluminum anode:

$$\mathrm{Al} \rightarrow \mathrm{Al}^{3+} + 3\,\mathrm{e}^{-} \tag{3.1}$$

$$\mathrm{xAl}^{3+} + \mathrm{yOH}^{-} \rightarrow \mathrm{Al_x(OH)_y^{z+}} \tag{3.2}$$

The speciation of the aluminum hydroxides formed during ECF is highly variable and is strongly influenced by pH [126].

Using an iron anode:

$$\mathrm{Fe} \rightarrow \mathrm{Fe}^{2+} + 2\,\mathrm{e}^{-} \tag{3.3}$$

$$\mathrm{Fe}^{2+} + 2\,\mathrm{OH}^{-} \rightarrow \mathrm{Fe(OH)_2} \tag{3.4}$$

or

$$\mathrm{Fe} \rightarrow \mathrm{Fe}^{3+} + 3\,\mathrm{e}^{-} \tag{3.5}$$

$$\mathrm{Fe}^{3+} + 3\,\mathrm{OH}^{-} \rightarrow \mathrm{Fe(OH)_3} \tag{3.6}$$

It is not clear whether ferrous or ferric ions are formed during ECF [12]. Moreover, Fe^{2+} can be rapidly oxidized in solution to Fe^{3+} in the presence of oxygen. Release of Fe^{2+} during ECF leads to green hydroxide precipitates, while Fe^{3+} ions result in yellow hydroxide precipitates.

At both the Al and Fe anodes, water is oxidized as a side reaction and oxygen is produced:

$$2\,\mathrm{H_2O} \rightarrow \mathrm{O_2} + 4\,\mathrm{H}^{+} + 4\,\mathrm{e}^{-} \tag{3.7}$$

The main reaction at the cathode is the reduction of water and the formation of hydrogen gas:

$$2\,H_2O + 2\,e^- \rightarrow H_2 + 2\,OH^- \tag{3.8}$$

So far, the use of ECF for harvesting microalgal biomass has not been thoroughly evaluated. Some studies have investigated the use of ECF for removal of microalgae from drinking or wastewater [3, 5, 63, 64, 144, 174]. In these studies, however, microalgal densities were much lower than those typically occurring in microalgal production systems. Moreover, these studies all focused on freshwater and not on marine microalgae. The chemical composition and conductivity of freshwater and seawater differ strongly and this may have a strong effect on the efficiency of the ECF process. It is relevant to evaluate the use of ECF as a harvesting method for marine microalgae because marine microalgae are attractive as a source of biofuels due to their limited dependence on freshwater resources.

The general aim of this chapter is to demonstrate the proof of principle for harvesting of microalgae using electro-coagulation flocculation (ECF) in both a freshwater and a marine environment. Specific goals are (1) to study the influence of several important variables on the efficiency of the ECF process, (2) to evaluate contamination of the microalgal biomass and process water with metals released from the sacrificial anode, and (3) to estimate the electricity demand of the ECF process.

3.2 Materials and methods

3.2.1 Cultivation of microalgae

Because we expected large differences in the efficiency of ECF for harvesting microalgae from marine and freshwater medium, all experiments were carried out with the freshwater chlorophyte *Chlorella vulgaris* (SAG, Germany, 211-11B) and the marine diatom *Phaeodactylum tricornutum* (UGent, Belgium, Pt 86). Both *Chlorella* and *Phaeodactylum* are promising species for the production of microalgal biomass for food, feed, or fuel, and are currently intensively studied. *Chlorella vulgaris* was cultured in Wright's cryptophytes medium prepared from pure chemicals dissolved in disinfected tap water [79].

Phaeodactylum tricornutum was cultured in WC medium prepared in deionized water to which 30 g L^{-1} synthetic sea salt (Homarsel, Zoutman, Belgium) was added. Table 3.1 illustrates the differences in chemical composition and

Table 3.1: Main differences in chemical composition of freshwater and marine cultivation medium.

	Freshwater (mM)	Marine water (mM)
Cl	1.7	442.1
Na	1.9	338.6
Mg	1.0	80.5
Ca	2.7	9.1
K	0.3	6.4
SO_4	1.3	40.2
Conductivity (mS cm^{-1})	0.8	43.0

conductivity between both media. Both species were grown in 30 L plexiglas bubble column photobioreactors (diameter 20 cm). Degassing was carried out with humidified and filtered air at a rate of 5 L min^{-1}. The pH was controlled at 8.5 by addition of CO_2 (2-3%) using a pH-stat system. The ECF experiments were carried out at the beginning of the stationary phase, corresponding to a microalgal density of 0.3-0.6 g dry weight per liter.

3.2.2 ECF experiments

All the ECF experiments were carried out at room temperature in a PVC batch reactor of 20 cm (length) × 5 cm (width) × 15 cm (height) filled with 1 L of microalgal broth. The electrodes consisted of two parallel flat metal plates with a surface area of 200 cm^2, placed 4.4 cm apart near the walls of the reactor. Aluminum or iron plates were compared as anodes while an inert net of IrO_2/TiO_2 was used as the cathode. The anode and cathode were connected to the positive and negative outlets of a DC power supply (EHQ Power PS3010), respectively. The current density was controlled by changing the current of the DC power supply, which was operated in the constant current mode. The microalgal broth in the vessel was stirred using an overhead stirrer (IKA Labortechnik Eurostar digital Model RW-16).

To determine the microalgal recovery efficiency η_a of microalgal biomass, samples were collected at different time points during the ECF process. 10 mL samples were collected at 5 cm below the water surface in the ECF reactor. In the samples, flocs of microalgae either settled to the bottom or floated to the surface of the sample tube. Flotation of the flocs was caused by the formation of H_2 at the cathode and O_2 at the anode. The microalgal recovery efficiency η_a was determined based on the decrease in optical density of the microalgal suspension (measured at 550 nm with an UV-VIS spectrometer Thermo Scientific Nicolet Evolution 100). The recovery efficiency η_a was subsequently calculated as:

$$\eta_a = \frac{OD_i - OD_f}{OD_i} \tag{3.9}$$

where OD_i is the optical density of the suspension prior to the start of the ECF process, and OD_f is the optical density of the suspension at time t.

3.2.3 Influence of variables on the ECF process

The influence of several important variables on the ECF process was studied using a one-variable-at-a-time approach. Consecutively, the influence of the anode material (Fe or Al), the sedimentation time after finishing the ECF-treatment, the current density, the (initial) pH, and the stirring speed were investigated. The influence of a specific variable was studied using the best values found for the variables that were already investigated.

3.2.4 Calculation of the power consumption

The power consumption E (in kWh kg^{-1} of recovered microalgae) was calculated as

$$E = \frac{UIt}{1000V\eta_a c_i} \tag{3.10}$$

where U is the voltage (V), I the current (A), t the time of the ECF treatment (h), V the volume of the microalgal solution treated (m^3), η_a the microalgae recovery efficiency, and c_i the initial microalgae biomass concentration (kg m^{-3}).

3.2.5 Al, Ca, and Mg analyses in the harvested algal biomass and the process water

To determine the degree of contamination of the microalgal biomass and the process water, the Al, Ca, and Mg content of the microalgal biomass recovered during the ECF process as well as of the supernatant remaining after the ECF treatment was determined. Al, Ca, and Mg in solution were determined by atomic absorption spectroscopy (AAS, Solaar UNICAM 989). For measurements on the microalgal biomass, calcination was done in a furnace at 550°C during 4 h and then the ashes were dissolved in 37% fuming hydrochloric acid. The total amount of metals released during ECF was estimated by assuming that the electrical efficiency for the release of metal was 100%. This is in reality an overestimation, as the formation of O_2 competes with Al^{3+} formation at the anode.

3.3 Results and Discussion

3.3.1 Influence of variables

In all ECF experiments, η_a increased with time following a sigmoid pattern. This observation is in accordance with a model in which metal ions such as Al^{3+} or Fe^{2+}/Fe^{3+} are continuously released from the anode during the ECF treatment. These aluminum and iron ions react with water to form metal hydroxides [53]. Positively charged soluble metal hydroxides bind to the negative surface of the microalgal cells and destabilize the microalgal suspension by charge neutralization. Insoluble metal hydroxides can destabilize the microalgal suspension through a mechanism known as sweeping flocculation, resulting in enmeshment of microalgae and insoluble precipitates [54]. For both mechanisms, the inflection point of the sigmoidal curve corresponds to the time required to produce a sufficient amount of aluminum or iron hydroxides to destabilize the microalgal dispersion [123, 122].

Visual observation of the solution during the ECF process revealed the formation of insoluble metal hydroxides, either as brown-green precipitates when using an iron anode, or as a milky precipitate when using an aluminum anode. The brown-green color of the precipitates, formed when an iron anode was used, suggests that Fe^{2+} rather than Fe^{3+} was released from the anode during ECF. The metal hydroxide precipitates interfered to some extent with the spectrophotometric quantification of microalgal biomass. On the one hand, they may have caused a residual turbidity in the solution after η_a reached a plateau and therefore may have caused a slight underestimation of the maximum η_a. These insoluble metal hydroxides also explain why in some cases negative recovery efficiencies were measured prior to the destabilization of the microalgal suspension.

In Figure 3.1, the performance of aluminum and iron electrodes is compared. For both *Chlorella vulgaris* and *Phaeodactylum tricornutum*, dispersion destabilization of the microalgal suspension occurred much faster with aluminum electrodes than with iron electrodes. The lower efficiency of the iron electrodes is probably due to the lower current efficiency generated by iron electrodes when compared to aluminum electrodes [33, 209]. Also, iron hydroxides are relatively inefficient coagulants compared to aluminum hydroxides [57]. In a study on the use of ECF for removal of microalgae from eutrophic surface waters, Gao et al.

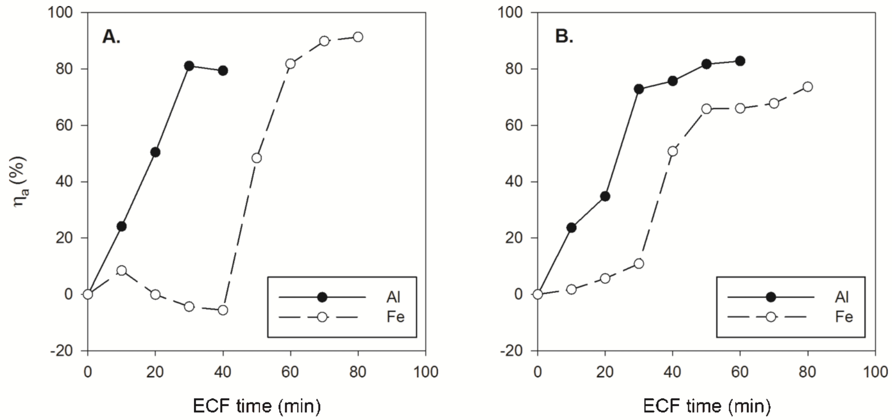


Figure 3.1: Microalgae recovery efficiency η_a as function of ECF time using different electrodes. Conditions: (A) *Chlorella vulgaris*, (B) *Phaeodactylum tricornutum*, 3mA cm^{-2}, pH = 8, no stirring and no sedimentation time.

(2010) also noted a higher efficiency of aluminum compared to iron electrodes [63]. Because of this higher efficiency, aluminum electrodes were selected as the anode material in further experiments.

When samples were taken from the ECF reactor, destabilization of the microalgal suspension continued after sampling. This is illustrated for *Chlorella vulgaris* and *Phaeodactylum tricornutum* in Tables 3.2 and 3.3, respectively. Particularly for samples collected at time points close to the inflection point of the sigmoidal curve, this continued coagulation flocculation sedimentation of microalgae after sampling resulted in a substantial increase of η_a, up to 25% over a period of 30 min. This can be ascribed to continued reaction between dissolved metal hydroxides and microalgal cells and to the fact that some time is needed for sedimentation of the flocs. Because of this continued coagulation–flocculation–sedimentation after sampling, η_a was determined in further experiments 30 min after sampling.

As electricity is the driving force for the reactions occurring at the anode, current density is an important variable in the ECF process (Fig 3.2). For *Chlorella vulgaris*, current densities between 1.5-12 mA cm^{-2} were evaluated. It was not possible to maintain a lower current density in a stable way in the freshwater medium. For *Phaeodactylum tricornutum*, current densities between 0.6-3 mA cm^{-2} were used. The use of higher current densities in the salt water medium resulted in the electrolytic formation of NaClO or bleach, which visually

Table 3.2: Microalgae recovery efficiency η_a (%) as function of additional sedimentation time ST for different ECF times. Conditions: *Chlorella vulgaris*, 3 mA cm^{-2}, pH = 8, no stirring.

	ST (min)			
ECF (min)	0	10	20	30
10	16	19	19	32
20	87	88	91	91
30	88	89	92	91

Table 3.3: Microalgae recovery efficiency η_a (%) as function of additional sedimentation time ST for different ECF times. Conditions: *Phaeodactylum tricornutum*, 3 mA cm^{-2}, pH = 8, no stirring.

	ST (min)			
ECF (min)	0	10	20	30
10	58	56	58	60
20	53	72	78	77
30	54	76	72	78

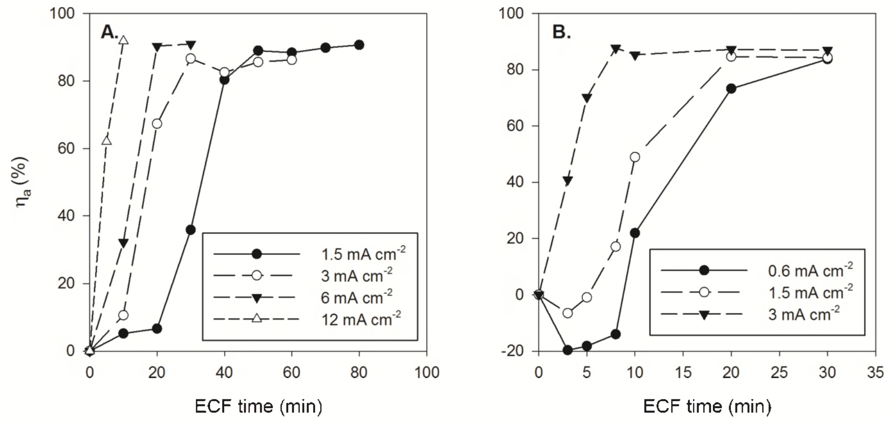


Figure 3.2: Microalgae recovery efficiency η_a in function of ECF time using different current intensities. Conditions: (A) *Chlorella vulgaris*, (B) *Phaeodactylum tricornutum*, pH = 8, no stirring, sedimentation time = 30 min.

led to the disappearance of microalgae flocs. This bleach formation was also reported by Gao et al. (2010) and should be avoided [64]. For both *Chlorella vulgaris* and *Phaeodactylum tricornutum*, the time required to destabilize the microalgal suspension decreased with increasing current density. To reach an η_a of 95% for *Chlorella vulgaris*, 50 min ECF was required using 1.5 mA cm^{-2}, while only 10 min ECF was required using 12 mA cm^{-2}. For *Phaeodactylum tricornutum*, an η_a of 80% was reached after 30 min using a current density of 0.6 mA cm^{-2}, while only 10 min were required using 3 mA cm^{-2}.

In Figure 3.3, the influence of the initial pH on the ECF process is shown. For both *Chlorella vulgaris* and *Phaeodactylum tricornutum*, the efficiency of the

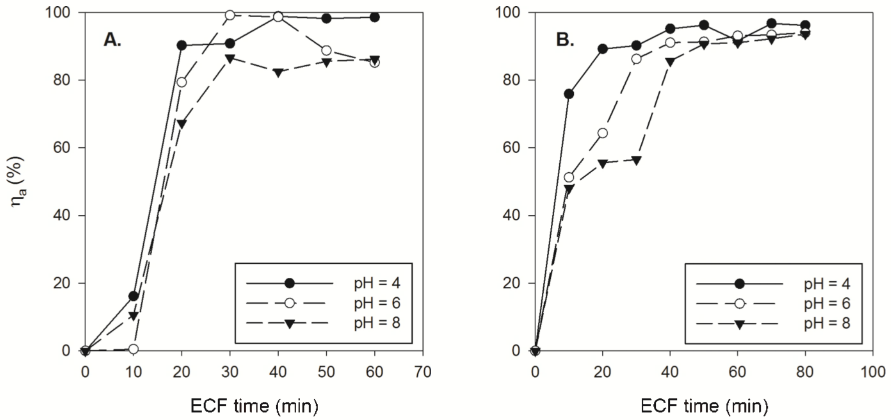


Figure 3.3: Microalgae recovery efficiency η_a as function of ECF time using different pH levels. Conditions: (A) *Chlorella vulgaris*, (B) *Phaeodactylum tricornutum*, 3 mA cm^{-2}, no stirring, sedimentation time = 30 min.

process decreased with increasing pH. This influence of pH was more pronounced for *Phaeodactylum tricornutum* than for *Chlorella vulgaris*. It is well known that pH is an important variable in ECF [126], as it determines speciation of aluminum hydroxides in the solution [54, 73]. Under acidic conditions, the formation of positively charged monomeric aluminum hydroxides such as $Al(OH)^{2+}$, or polymeric aluminum hydroxide cations such as $Al_6(OH)_{15}^{3+}$, $Al_7(OH)_{17}^{4+}$, $Al_8(OH)_{20}^{4+}$,$Al_{13}O_4(OH)_{34}^{7+}$, and $Al_{13}(OH)_{34}^{5+}$ is promoted [33, 123]. These react with the negatively charged surface of the microalgal cells and are able to destabilize the microalgal suspension by charge neutralization. At more alkaline pH levels, the formation of the negatively charged aluminum hydroxide $Al(OH)_4^-$ is promoted, which will not react with the negatively charged microalgal cells. Under these conditions, coagulation flocculation of microalgal cells is probably mostly due to sweeping coagulation flocculation by insoluble aluminum hydroxide $Al(OH)_3$. In their study on the use of ECF for removal of microalgae from eutrophic surface waters, Gao et al. (2010) also noted that a low pH had a positive effect on the recovery efficiency of microalgae during ECF [64]. Because of this positive effect of a low initial pH, an initial pH value of 4 was used in all subsequent experiments.

Figure 3.4 illustrates the influence of stirring during the ECF process on η_a. For an increase in stirring speed from 0 to 60 and 150 rpm, the time required to achieve destabilization of the microalgal suspension decreased by almost a factor two. At the maximum stirring speed of 200 rpm, however, the time

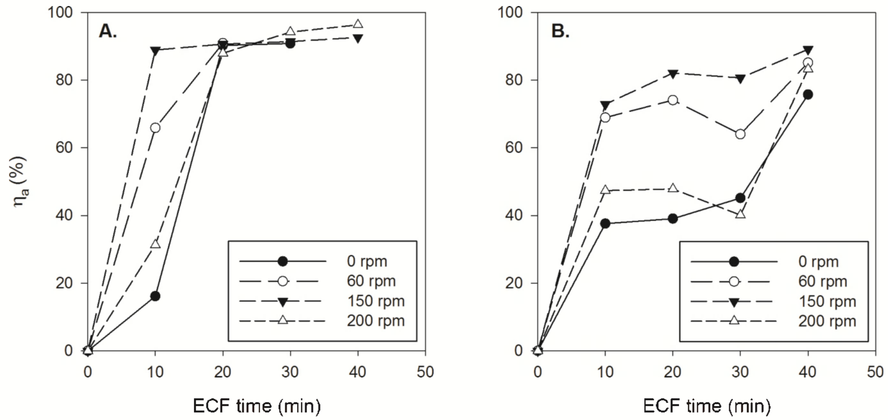


Figure 3.4: Microalgae recovery efficiency η_a as function of ECF time at different stirring speeds. Conditions: (A) *Chlorella vulgaris*, (B) *Phaeodactylum tricornutum*, 3 mA cm^{-2}, pH = 4, sedimentation time = 30 min.

required to achieve destabilization increased again. Previous studies on ECF for other applications have also demonstrated that stirring can improve the coagulation flocculation efficiency [33]. Stirring improves the recovery efficiency by enhancing contact rates between the coagulants and the microalgal cells [122]. The highest stirring rate, however, probably caused break-up of microalgal flocs due to the high shear forces applied, resulting in a longer time needed to achieve a similar recovery efficiency. Because the time needed to achieve a maximal η_a was shortest for a stirring speed of 150 rpm, this stirring speed was used in subsequent experiments.

The reproducibility of the ECF process was evaluated in a new set of experiments in triplet, working under the following (optimal) experimental conditions: Aluminum anode, pH 4, sedimentation time of 30 min, and stirring speed of 150 rpm. For both types of microalgae, the two lowest current densities from the range tested above were used (1.5 and 3 mA cm^{-2} for *Chlorella vulgaris* and 0.6 and 1.5 mA cm^{-2} for *Phaeodactylum tricornutum*). Figure 3.5 illustrates that, for both species, the time required to initiate flocculation as well as the final recovery efficiencies are reproducible.

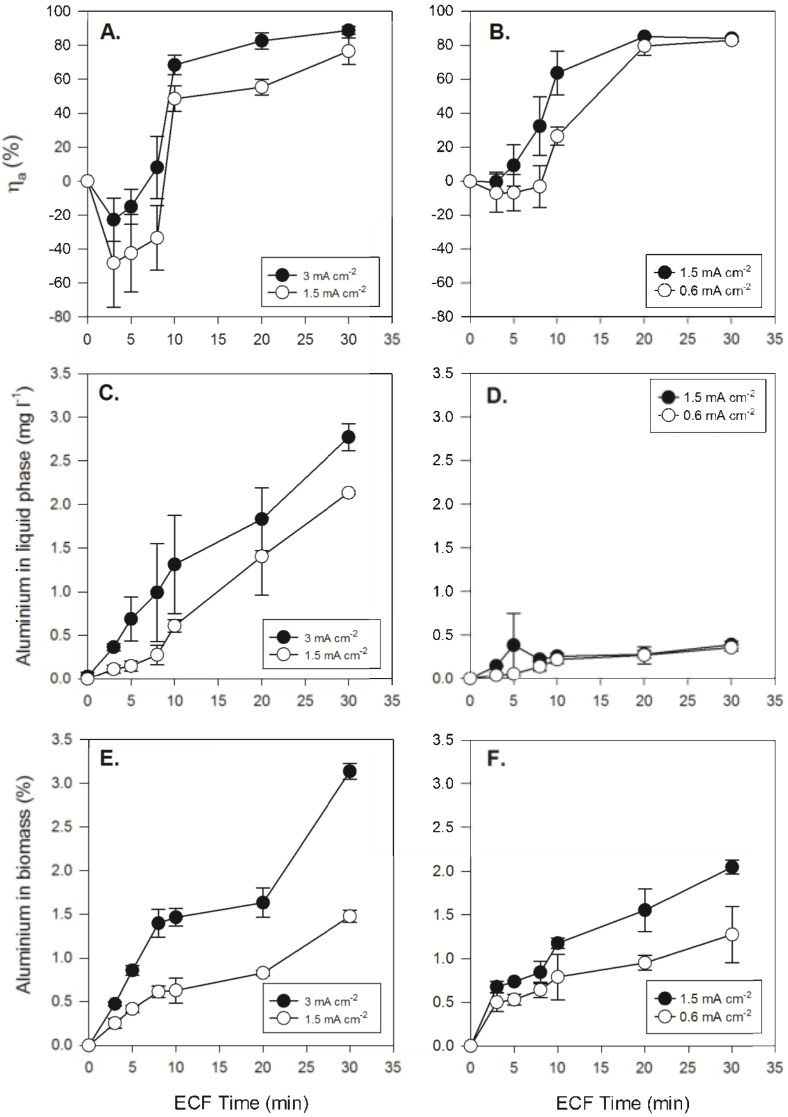


Figure 3.5: (A and B) Microalgae recovery efficiency η_a, Al in (C and D) liquid phase, and (E and F) in residual biomass measured using two different current densities. Conditions: (A, C, E) *Chlorella vulgaris*, (B, D, F) *Phaeodactylum tricornutum*, pH = 4, stirring speed = 150 rpm, sedimentation time = 30 min (n = 3).

3.3.2 Accumulation of aluminum during ECF

During the above-mentioned experiment, we also investigated the accumulation of aluminum in both the recovered microalgal biomass and in the liquid phase during the course of the ECF process (Fig 3.5). As predicted by Faraday's law, the concentration of aluminum in both the biomass and the liquid phase increased with time and with current density. Aluminum content in the recovered microalgal biomass was about twice as high at the higher current density than at the lower current density. For both species, the aluminum content in the microalgal biomass continued to increase after the maximal η_a was reached, which can be ascribed to continued precipitation of aluminum hydroxides. In the experiment with *Chlorella vulgaris*, aluminum concentration in the liquid phase was relatively high and continued to increase after the maximal η_a was reached. In *Phaeodactylum tricornutum*, on the contrary, the Al concentration in the liquid phase was much lower and appeared to stabilize when the maximal η_a was reached.

The difference in aluminum concentration in the water between the marine and freshwater species are most likely due to differences in the chemical composition of the freshwater and the seawater medium. The seawater medium contains high concentrations of sulphate anions. These sulphate anions are known to facilitate precipitation of aluminum hydroxides [74, 54]. This probably explains the low residual aluminum concentrations in the process water in the experiments with *Phaeodactylum tricornutum*. The seawater medium also contains high concentrations of magnesium and calcium cations (Table 3.1). Electrolytic release of hydroxyl anions at the cathode may lead to high pH levels near the cathode. This is known to cause precipitation of carbonates and hydroxides of calcium and magnesium [116, 193]. We monitored calcium and magnesium concentrations in the experiments with *Phaeodactylum tricornutum* at a current density of 1.5 mA cm^{-2}. Calcium concentrations did not decrease appreciably in the medium during the course of the experiment but magnesium concentrations decreased by about 15%, suggesting that precipitation of magnesium carbonates or hydroxides did indeed occur. Magnesium concentrations in the biomass did not increase during the experiment, most likely because magnesium was precipitated on the cathode. In long-term operation, this may lead to an increased current consumption during the ECF process.

Both in the marine and the freshwater medium, it is clear that the aluminum content in both the water and the microalgal biomass can be kept low by using a lower current density. To avoid accumulation of excess aluminum in either the liquid phase, the biomass, or both, ECF should not be continued beyond the point where η_a reaches the saturation phase. Taking this into account, the aluminum content in the microalgal biomass could be kept below 1% in the harvested biomass. In the process water it could be kept below 2 mg L^{-1} for *Chlorella vulgaris* or 0.5 mg L^{-1} for *Phaeodactylum tricornutum* in the process water.

In the experiments described in this research, microalgae were coagulated-flocculated by aluminum hydroxides. This mechanism of coagulation–flocculation is comparable to coagulation–flocculation of microalgae using aluminum salts like alum. According to the literature [167], 80-250 mg alum L^{-1} corresponding to 7.2-23 mg Al L^{-1} is needed to coagulate/flocculate a microalgal suspension. For harvesting *Chlorella minutissima*, Papazi et al. (2009) used 750 mg L^{-1} alum, which corresponds to 120 mg L^{-1} of aluminum [137]. If we assume that only aluminum oxidation occurred at the anode, we estimated that in the experiments in which the lowest current density was used, only 3.5 mg Al L^{-1} was released in the experiment with *Chlorella vulgaris* and 1.7 mg Al L^{-1} in the experiment with *Phaeodactylum tricornutum*. This suggests that ECF is more efficient in terms of aluminum consumption than coagulation–flocculation using alum. These findings coincide with the results of Cañizares et al. (2009) on the use of ECF for treatment of textile waters [34].

3.3.3 Power Consumption

The experimental results indicated that similar microalgal recovery efficiencies could be obtained by applying a high current density during a short time as by applying a low current density during a longer time. From an energy consumption point of view, it is unclear which strategy is best. Therefore, for the data presented in Figure 3.2, the global power consumption, expressed as kWh kg^{-1} dry weight microalgal biomass recovered during the ECF process was calculated using Equation 3.10 for each sampling time (Tables 3.4 and 3.5). For each ECF run, a point in time could be identified at which the power consumption per unit of microalgal biomass recovered was minimal. This point in time generally corresponded to the time at which η_a reached the saturation phase. For *Chlorella vulgaris*, for instance, this corresponded to an ECF time of 40 min at a current density of 1.5 mA cm^{-2} and 20 min at 6 mA cm^{-2}. For *Phaeodactylum tricornutum*, this point in time was situated at 20 min at a current density of 0.6 mA cm^{-2} and 3-5 min at 3 mA cm^{-2}.

These analyses clearly indicated that the minimal power consumption per unit of microalgal biomass recovered is much lower if lower current densities are used than when higher current densities are used. For *Chlorella vulgaris*, 1.3 kWh kg^{-1} recovered microalgae was consumed at a current density of 1.5 mA cm^{-2} while 9.5 kWh kg^{-1} recovered microalgae was consumed at 6 mA cm^{-2}. For *Phaeodactylum tricornutum*, the difference was smaller,

Table 3.4: Power consumption (kWh kg^{-1} dry weight recovered microalgae) using different current densities (CD) for *Chorella vulgaris* based on previous experiment (Fig 3.2).

	ECF time (min)							
CD (mA cm^{-2})	10	20	30	40	50	60	70	80
1.5	5.3	8.4	2.3	1.3	1.5	1.9	2.1	2.4
3	11.3	3.6	4.1	5.7	7.0	8.3	-	-
6	13.4	9.5	14.1	-	-	-	-	-
12	25.4	34.3	-	-	-	-	-	-

- = no data available: ECF process completed

Table 3.5: Power consumption (kWh kg^{-1} dry weight recovered microalgae) using different current densities CD for *Phaeodactylum tricornutum* based on previous experiment (Fig 3.2).

	ECF time (min)					
CD (mA cm^{-2})	3	5	8	10	20	30
0.6	-	-	-	0.4	0.2	0.3
1.5	-	-	1.1	0.4	0.5	0.8
3	0.4	0.4	0.5	0.5	0.8	1.7

- = insufficient microalgae recovery achieved to calculate realistic values

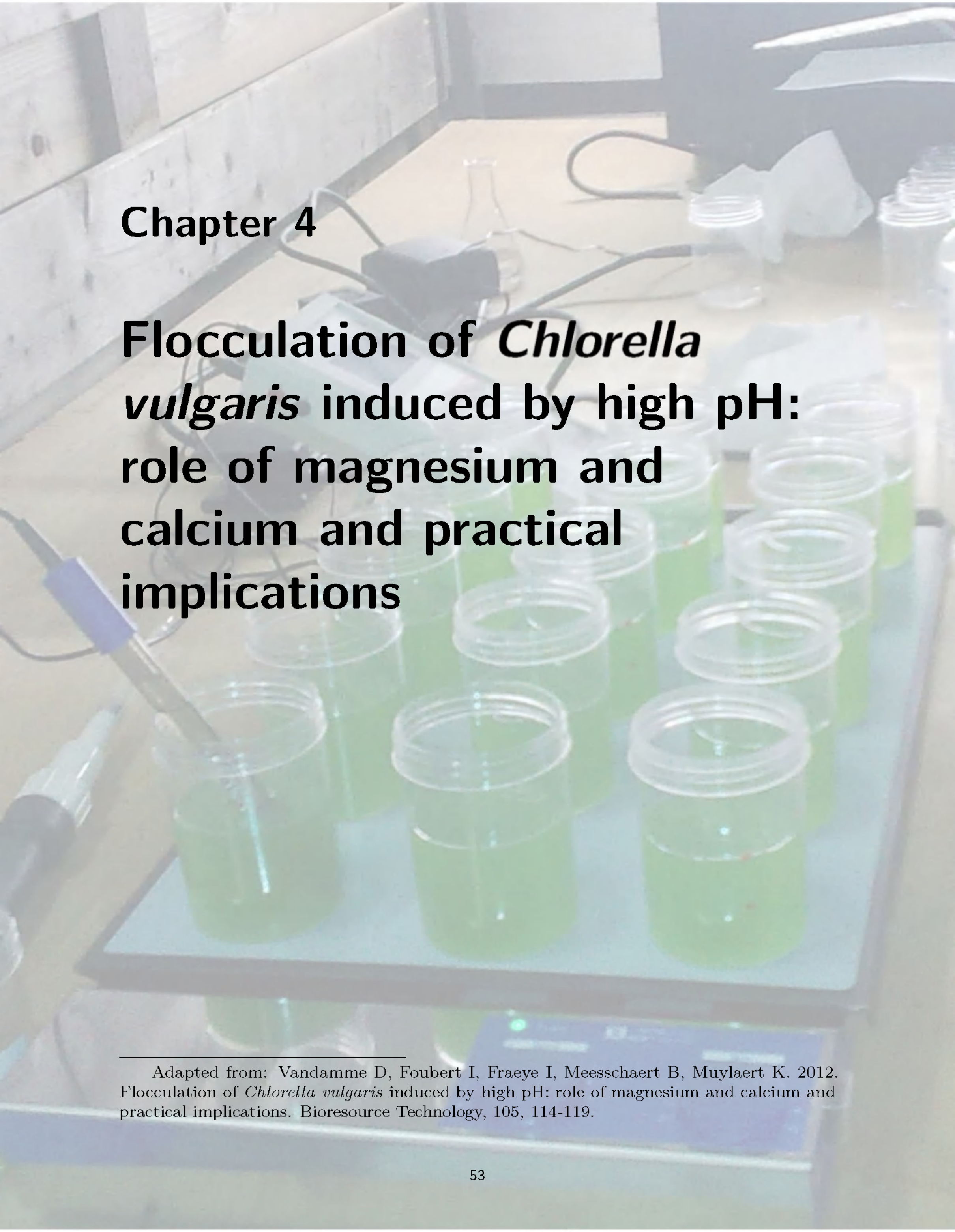
with 0.2 kWh kg^{-1} recovered microalgae consumed at 0.6 mA cm^{-2}, and 0.4 kWh kg^{-1} recovered microalgae were consumed at 3 mA cm^{-2}. Previous studies, in which ECF was used to remove microalgae from surface waters, have also indicated that the energy consumption to achieve coagulation–flocculation is lower when a lower current density is used [63]. Although a higher current density thus leads to a more rapid coagulation–flocculation of the microalgae, the use of a low current density is more efficient, from an energy consumption point of view. It should be noted, however, that the use of a low current density requires relatively long retention times of the water in the reactor. It is not unusual, however, to use long retention times in other applications of ECF [50, 208]. Nevertheless, the retention time should be taken into account when the process is applied at an industrial scale. A long retention time will require a larger reactor to process the same volume of water. A long retention time may also influence the quality of the algal biomass that is harvested.

For the experiments depicted in Figure 3.5, the minimum value of the power consumption was 2.1 kWh kg^{-1} of biomass harvested for *Chlorella vulgaris* and 0.2 kWh kg^{-1} of biomass harvested for *Phaeodactylum tricornutum*, at a current density of 1.5 and 0.6 mA cm^{-2}, respectively. These data confirm the low power requirements of ECF, especially for the marine species. The lower power consumption needed for the marine species is mainly due to the higher conductivity of the marine medium when compared to the freshwater medium, which results in a higher efficiency of the electrolytic release of aluminum from the anode [96], but other phenomena could also play a role here. Mouedhen et al. (2008) reported that chloride ions present in seawater attack the aluminum oxide layer formed on the surface of the anode, thereby enhancing the release of aluminum from the anode [126].

In existing microalgal production systems for high value applications, centrifugation is currently the most commonly used technology for harvesting microalgae. For low value applications, however, the use of conventional centrifuges is not economically feasible [121]. Power consumption of conventional centrifugation has been estimated at 8 kWh m^{-3} of microalgal suspension [48]. Assuming a microalgal biomass concentration of 0.5 kg m^{-3}, which is typical for microalgal production systems and comparable to the microalgal biomass concentration used in our experiments, this would correspond to a power consumption of 16 kWh kg^{-1} microalgal biomass recovered. The experiments in this study indicate that, for the freshwater microalgae *Chlorella vulgaris*, power consumption of ECF is an order of magnitude lower than for centrifugation. For *Phaeodactylum tricornutum*, the difference is nearly two orders of magnitude. Because ECF is a complex process involving electrolysis, coagulation–flocculation and sedimentation/flotation, there is no straightforward approach for estimating the challenges and costs associated with scaling-up of the technology [88]. Pilot-scale tests are therefore required to confirm whether rates of power consumption can be extrapolated to industrial scale ECF reactors, and to estimate additional costs of a full-scale setup. An important parameter that will influence power consumption in large-scale systems which was not investigated in this study is the distance between the electrodes, which has an important influence on power consumption [88, 96]. Nevertheless, our results indicate that ECF may be a promising technology for harvesting microalgae, in particular for species cultivated in seawater.

3.4 Conclusions

Although both aluminum and iron anodes achieved destabilization of the microalgal suspensions, aluminum anodes proved to be more efficient. During ECF, Al^{3+} and Fe^{2+} are released from the sacrificial anode and form metal hydroxides in the solution. Destabilization of the microalgal suspension was probably achieved through a combination of charge neutralization by positively charged metal hydroxides and sweeping coagulation–flocculation by insoluble metal hydroxides. The efficiency of the ECF process using aluminum as an anode could be significantly improved by reducing the initial pH and by increasing the turbulence. It is also recommended to include a sedimentation period between ECF and the removal of the microalgal flocs as destabilization of the microalgal suspension continues after removal of the microalgal suspension from the ECF reactor. Although higher current densities resulted in a more rapid destabilization of the microalgal suspension, this also resulted in a higher power consumption and release of aluminum from the sacrificial anode. Release of aluminum in the process water is lower, probably due to enhanced precipitation of aluminum hydroxides related to the presence of sulphates in seawater. When ECF is compared to chemical coagulation–flocculation using alum, consumption of aluminum appears to be lower when ECF is used. Power consumption of ECF was an order of magnitude lower than centrifugation when applied to the freshwater microalgae *Chlorella vulgaris* and nearly two orders of magnitude lower when applied to the marine microalgae *Phaeodactylum tricornutum*. ECF is therefore an attractive technology for harvesting microalgae, particularly for harvesting marine microalgae.



Chapter 4

Flocculation of *Chlorella vulgaris* induced by high pH: role of magnesium and calcium and practical implications

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Adapted from: Vandamme D, Foubert I, Fraeye I, Meesschaert B, Muylaert K. 2012. Flocculation of *Chlorella vulgaris* induced by high pH: role of magnesium and calcium and practical implications. Bioresource Technology, 105, 114-119.

4.1 Introduction

Several studies have demonstrated that flocculation of microalgae can also be induced by increasing the medium pH, a phenomenon that is often referred to as 'autoflocculation'. Golueke and Oswald (1970) observed that microalgae in waste stabilisation ponds flocculated on warm and sunny days, when CO_2 was depleted and the pH increased [68]. Microalgal suspensions are generally stabilised by a negative surface charge of the cells which is generated by carboxyl and/or sulphate groups [81]. The fact that flocculation of microalgae occurs at a high pH is therefore surprising, since the surface charge of microalgal cells is expected to become more negative at a high pH and flocculation is thus inhibited [103]. It has been suggested that flocculation at high pH is caused by chemical precipitation of calcium and/or magnesium salts at a high pH [167]. Indeed, Nurdogan and Oswald (1995) noted that autoflocculation did not occur in waters poor in calcium and magnesium [132]. They demonstrated that flocculation in such waters could be induced by addition of lime. Sukenik and Shelef (1984), on the other hand, suggested that flocculation at high pH was caused by precipitation of calcium phosphate [176]. High phosphate concentrations (0.1-0.2 mM) are required for this process to be effective. Lavoie and De la Noüe (1987) could not induce flocculation of *Scenedesmus* in a medium that was low in phosphate, which supports the role of calcium phosphate [103].

Although it appears from these previous studies that calcium and/or magnesium play a role in flocculation of microalgae at high pH, there is still uncertainty about the general underlying mechanism. Moreover, the practical implications and the potential for reducing the cost of harvesting microalgae have not been fully explored. The goal of this study is therefore, (1) to investigate the role of calcium and magnesium in the flocculation process, and (2) to evaluate the practical implications for flocculation induced by high pH for harvesting microalgae.

4.2 Materials and methods

4.2.1 Culturing of microalgae

We used *Chlorella vulgaris* (211-11b SAG, Germany) as a model species for investigating the mechanism of and practical implications for the use of pH induced flocculation in freshwater medium. *C. vulgaris* is a promising species for the production of microalgal biomass for food, feed or fuel, and is currently intensively studied [60]. *C. vulgaris* as cultured in dechlorinated

Table 4.1: Concentrations of the main ions in the medium used to culture *Chlorella*.

	Freshwater (mM)
Cl	1.7
Na	1.9
Mg	1.2
Ca	2.0
K	0.3
P	0.06
SO_4	1.3
Conductivity (mS cm^{-1})	0.8

tap water enriched with inorganic nutrients according to the concentrations of the Wright's cryptophytes medium [79]. Table 4.1 shows the concentrations of the main ions in this medium. The microalgae were cultured in 30 L bubble column photobioreactors that 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. Flocculation experiments were conducted at a microalgal density of approximately 0.5 g dry weight per litre.

4.2.2 General setup of flocculation experiments

Flocculation of the microalgal suspensions induced by high pH was investigated using jar test experiments (n = 2). These experiments were carried out in 100 mL beakers that were stirred using a magnetic stirrer. pH was adjusted by addition of 0.5 M sodium hydroxide. The microalgal suspension was mixed intensively (1000 rpm) for 10 min during and just after pH adjustment. Then, the suspensions were mixed gently (250 rpm) for another 20 min, after which they were allowed to settle for 30 min. The flocculation efficiency η_a was estimated by comparing absorbance at 550 nm between the pH-adjusted treatment and a control treatment. Samples (3.5 mL) were collected in the middle of the clarified zone. The flocculation efficiency η_a was calculated as:

$$\eta_a = \frac{OD_i - OD_f}{OD_i} \tag{4.1}$$

where OD_i is the optical density of the suspension after 30 min sedimentation without pH adjustment, and OD_f is the optical density of the suspension after the complete treatment.

4.2.3 The role of magnesium and calcium

Flocculation induced by high pH was tested at different pH levels between 9 and 12. To unequivocally demonstrate the role of bivalent cations in the flocculation process, we tested if flocculation induced by high pH could be inhibited by addition of EDTA (10 mM), a chelating agent that can sequester polyvalent cations. This was done at the three pH levels where flocculation occurred (pH 11, 11.5 and 12).

To investigate the fate of calcium and magnesium during pH increase, we monitored concentrations of these cations in the dissolved and particulate phase before and after pH induced flocculation at pH 11. Concentrations of cations in the dissolved phase were measured after removal of algal cells by centrifugation followed by filtration over Whatman GF/C glass microfibre filters. Concentrations in the particulate phase were measured in the pellet obtained during centrifugation. The pellet was first dried at 100 °C for 24 h, then incinerated at 450 °C for 24 h and the ashes were dissolved quantitatively in HNO_3:HCl 1:1. Calcium and magnesium concentrations were measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Jobin–Yvon Ultima, Horiba Scientific) and a mass balance was constructed. In addition to these measurements, we calculated the saturation index (SI) of $Ca_3(PO_4)_2$, $CaCO_3$, $CaMg(CO_3)_2$, $CaSO_4$ and $Mg(OH)_2$ at different pH levels between 10 and 11.5 using PHREEQC version 2 (USGS, USA) to estimate the influence of pH on precipitation of different calcium and magnesium salts.

To further investigate the relative importance of calcium and magnesium in the flocculation process, we tested whether flocculation at high pH could be induced in a medium lacking calcium or magnesium. To do so, *Chlorella* was separated from the medium using centrifugation and resuspended in fresh medium lacking calcium and magnesium. Preliminary experiments demonstrated that concentration of *Chlorella* using centrifugation and subsequent resuspension in the original medium had no influence on flocculation induced by high pH. In a first series, *Chlorella* cells were resuspended in a medium lacking magnesium and containing different calcium concentrations ranging between 0 and 2.5 mM. In a second series, calcium was omitted and magnesium was added in concentrations ranging between 0 and 1.5 mM. The range of calcium and magnesium concentrations used encompassed the range of calcium and magnesium observed in typical surface waters [30].

4.2.4 Practical implications of using flocculation induced by high pH for harvesting microalgae

The efficiency of several widely available bases (sodium hydroxide, potassium hydroxide, calcium hydroxide, magnesium hydroxide and sodium carbonate) was compared for flocculation induced by high pH. We gradually added a 0.5 M base solution to 100 mL beakers filled with a *Chlorella* suspension and monitored the increase in pH until coagulation could be visually observed. We estimated the ratio of base over microalgal biomass required for flocculation based on the quantity of base added and the biomass concentration of *Chlorella* in the medium.

We also investigated the influence of the pH increase on the viability of the cells. First, cell numbers were compared before and after flocculation. Flocculation tests were done by increasing the pH to 12, resulting in a flocculation efficiency of 99%. The medium was removed, the particulate phase was resuspended in distilled water, neutralised (pH 6.5) and stirred at 550 rpm for 60 min, resulting in a complete dissolution of the flocs and resuspension of the algal cells. Cell numbers were determined by counting intact cells using a Bürker count chamber. A minimum of 200 algal cells were counted in duplicate giving a counting error of maximum 5%. Average cell numbers before and after flocculation and resuspension were compared using a t-test with equal variances (as checked with an F-test; Sigmaplot 11, Systat Software, Inc.). Secondly, the quantum yield of photosystem II of the microalgal cells was compared before and after flocculation by pH increase (pH 10.5, 11, and 12). The quantum yield was measured after 20 min of dark adaptation of the cells using a PSI AquaPEN PAM fluorometer (sample size = 3.5 mL; n = 3). The quantum yield is a sensitive indicator for stress in microalgae [43].

4.3 Results and discussion

4.3.1 The role of magnesium and calcium

The influence of pH on flocculation efficiency was tested in a pH range from 9 to 12 (Fig 4.1 – pH treatment). No flocculation occurred up to pH 10.5. At pH 11, a flocculation efficiency of 75% was observed. At pH 11.5 and 12, the flocculation efficiency exceeded 95%. This indicates that *Chlorella* can be flocculated efficiently by increasing the pH of the culture to 11. This observation is in agreement with previous studies [21, 201].

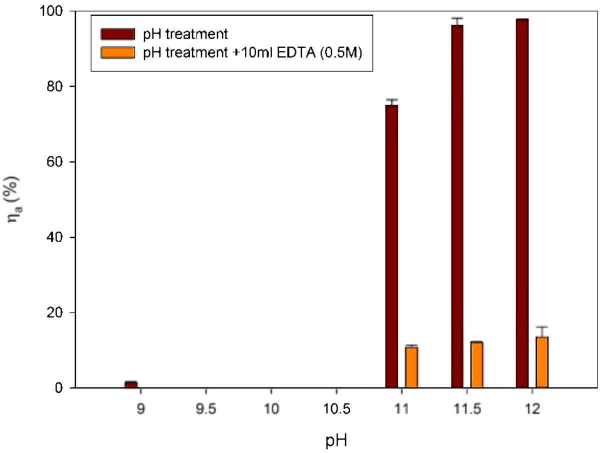


Figure 4.1: Flocculation efficiency η_a of *Chlorella vulgaris* as a function of pH in treatments with and without EDTA (0.5 M).

Several studies have suggested that bivalent cations such as calcium and magnesium play a role in the flocculation process at high pH [132, 167]. We wanted to unequivocally confirm the role of calcium and/or magnesium in pH-induced flocculation by addition of EDTA to remove bivalent cations from solution at pH levels 10.5, 11 and 12 (Fig 4.1 – pH treatment + EDTA). Addition of EDTA resulted in a strong and significant decrease in the flocculation efficiency η_a at the three pH levels tested, confirming that bivalent cations such as calcium and/or magnesium are indeed involved in flocculation at high pH.

To evaluate whether flocculation at high pH was related to precipitation of calcium and/or magnesium, we monitored both cations in the dissolved (medium) and particulate (biomass) phase before and after flocculation at pH 11 (Fig 4.2). Before flocculation, 97% of magnesium and 41% of calcium was in the dissolved phase. After flocculation, only 41% of the magnesium and 6% of the calcium was in the dissolved phase. This indicates that during flocculation, precipitation of both calcium and magnesium occurred. The fact that a substantial fraction of the calcium was already found in the particulate phase before the increase of pH to 11 suggest that some precipitation may already have occurred, probably due to increases in pH as a result of photosynthetic depletion of carbon dioxide. This observed precipitation of calcium and magnesium is in agreement with predictions of the PHREEQC model, which indicates that at pH 11 both calcium and magnesium are expected to precipitate as calcium carbonate, calcium magnesium carbonate, calcium phosphate and magnesium hydroxide.

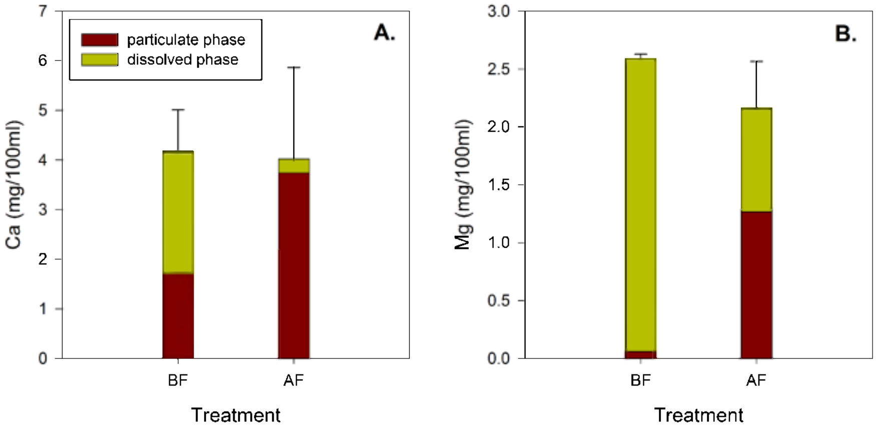


Figure 4.2: Mass balance of calcium (A) and magnesium (B), before treatment (BF) and after flocculation treatment (AF) at pH 11 for the dissolved phase and the particulate phase.

We then evaluated whether precipitation of calcium and magnesium salts separately were capable of inducing flocculation of the *Chlorella* suspension. We therefore isolated *Chlorella* cells from the growth medium and resuspended them in fresh medium lacking either calcium or magnesium or both and quantified the flocculation efficiency η_a at pH 10.5, 11 and 12. When *Chlorella* was resuspended in medium lacking both calcium and magnesium η_a was low at all pH levels (<20%) (Fig 4.3, control). This confirms the results of the EDTA addition experiment that calcium and/or magnesium are essential for the occurrence of flocculation at high pH. When calcium was added to the medium at concentrations ranging between 0.025 and 2.5 mM (Fig 4.3A), η_a remained low (<20%) at all pH levels. This indicates that, although calcium precipitated, it did not induce flocculation. In a similar experiment, magnesium was added to the medium at concentrations between 0.015 and 1.5 mM. At a magnesium concentration of 0.015 mM, the flocculation efficiency was below 20% at the three pH levels. At a concentration of 0.075 mM, the flocculation efficiency increased with pH, being about 25% at pH 10.5 and close to 85% at pH 12. At magnesium levels of 0.15 mM or higher, the flocculation efficiency was between 90% and 100% at the three pH levels tested. This indicates that in contrast to calcium, magnesium precipitation was capable of inducing flocculation of *Chlorella*.

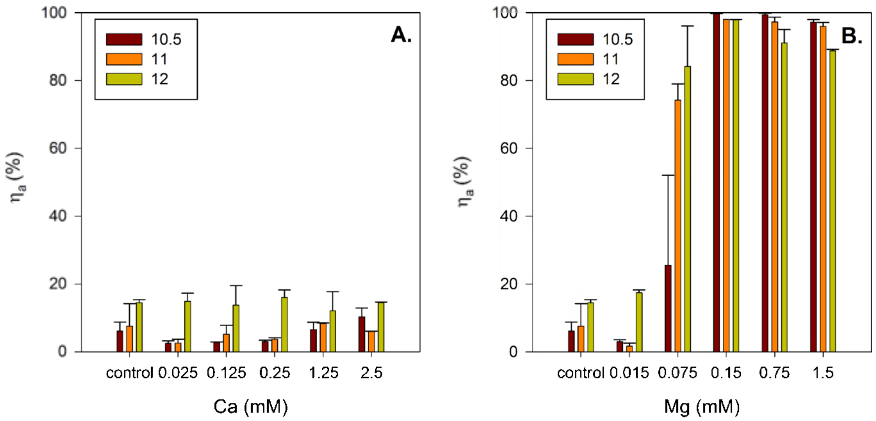


Figure 4.3: Flocculation efficiency η_a of *Chlorella vulgaris* at three pH levels as a function of the calcium concentration (A) and the magnesium concentration (B) in the medium. Control treatment is without addition of calcium or magnesium.

The DLVO theory states that suspensions are stabilised by surface charges of the particles [26]. In the case of microalgal cells, the surface charges are negative. The balance between the electrostatic repulsion and the Van der Waals attraction can be shifted towards attraction, causing coagulation and flocculation through several mechanisms. First of all, flocculation can result from an increase in medium ionic strength, which causes double layer compression. It is unlikely that this mechanism was involved in our experiments, as the change in ionic strength caused by pH increase is limited. Furthermore, this mechanism cannot explain the observed differences in flocculation behaviour upon addition of calcium versus magnesium. Another possible mechanism can be a reduction in surface charge of the microalgae. However, as microalgae generally carry a negative surface charge, an increase in pH will cause an increase in surface charge rather than a decrease, excluding this mechanism as a possible cause for flocculation induced by high pH.

In contrast, we propose that flocculation in our experiments was caused by a third mechanism, being charge neutralisation. During pH increase, magnesium hydroxide or brucite is formed. In brucite, some of the bivalent magnesium cations in the crystal structure are replaced by trivalent cations such as iron or aluminium, resulting in a layered double hydroxide crystal that carries positive charges [2]. These positive charges probably neutralise the negative surface charge of the microalgal cells, reducing the energy barrier between cells and thus causing destabilisation and subsequent flocculation of the microalgal suspension.

In wastewater treatment, precipitation of magnesium hydroxides at high pH is indeed sometimes used as an alternative for metal coagulants or synthetic polymeric flocculants to remove pollutants from wastewater [108, 164].

Although calcium also precipitated when pH was increased, this precipitation did not appear to induce flocculation of *Chlorella*. Calcium may have precipitated as calcium carbonate or calcium phosphate. In contrast to magnesium hydroxide, calcium carbonate crystals are not charged and are thus unlikely to induce coagulation by charge neutralisation. On the other hand, Sukenik and Shelef (1984) demonstrated that flocculation of microalgae at high pH was related to precipitation of calcium phosphate [176]. They found that the calcium phosphate precipitate had a positive surface charge, inducing flocculation through charge neutralisation, similar to the mechanism proposed here for magnesium hydroxide. A concentration above 0.1 mM phosphate was required to induce flocculation at high pH. It should be noted that in our experiments, the phosphate concentration before the flocculation treatment was only 0.006 mM. Therefore, calcium phosphate precipitation was probably insufficient to induce flocculation. Possibly, at higher phosphate concentrations, calcium phosphate precipitation plays a more important role in flocculation induced by high pH.

4.3.2 Practical implications of using flocculation induced by high pH for harvesting microalgae

Because flocculation of microalgae induced by high pH is correlated to precipitation of magnesium, the mechanism is dependent on the presence of magnesium in the growth medium. As magnesium is removed from the medium during flocculation, this may also have implications for the recycling of process water, which is required for sustainable cultivation of microalgae and their products. When *Chlorella* was flocculated induced by high pH, the magnesium concentration in the medium was reduced from an initial concentration of 1 mM to a final concentration of 0.35 mM. As we showed that a minimum concentration of about 0.15 mM of magnesium is required to induce flocculation at high pH, this means that repeated re-use of the water may lead to gradual depletion of magnesium, resulting in a failure of pH induced flocculation. Consequently, magnesium addition will be required from time to time.

Based on the mass balance in Fig 4.2, per 100 mL of microalgal culture, 1.2 mg of magnesium is transferred to the particulate phase after flocculation, which results in an increase of 30 mg Mg g^{-1} biomass dry weight. However, if the magnesium concentration in the medium is higher, the concentration of magnesium hydroxide that precipitates is also likely to be higher. Further research is required to evaluate whether this may interfere with specific

Table 4.2: Comparison of the use of four bases to induce coagulation at high pH.

	NaOH	KOH	$Ca(OH)_2$	$Mg(OH)_2$
$pH_{coagulation}$	10.8	10.8	10.8	9.7
Final base concentration (mM)	5.75	8.00	4.00	11.5
Required amount of base (mg g^{-1} biomass)	9	12	18	27

applications of the microalgal biomass (e.g. as animal feed) or with further processing of the biomass.

Harvesting of microalgae induced by high pH requires the addition of a base to raise the pH. Although we used sodium hydroxide to increase pH in our experiments, other bases may be cheaper or safer to use in an industrial environment. We thus tested whether other bases could be used to induce flocculation (Table 4.2). Sodium carbonate failed to induce flocculation, even when pH was increased to well above 11 (data not shown). Potassium hydroxide and calcium hydroxide induced flocculation at the same pH as sodium hydroxide (pH 10.8). The lowest quantity of base was required for sodium hydroxide (9 mg g^{-1} biomass), followed by potassium hydroxide (12 mg g^{-1} biomass) and calcium hydroxide (18 mg g^{-1} biomass). Magnesium hydroxide induced flocculation at a lower pH than the other bases (pH 9.7) but a relatively large quantity of base was required (27 mg g^{-1} biomass). In industrial applications, both the low cost and the low risk would favour calcium hydroxide or slaked lime over the other bases tested. Assuming a cost of 150$ ton^{-1} slaked lime, flocculating microalgae induced by high pH would cost approximately 18$ ton^{-1} biomass. Obviously, the sludge obtained after flocculation should be dewatered further using centrifugation. In our experiments with *Chlorella*, flocculation induced by high pH could be used to concentrate microalgal suspensions by a factor of about 50, which implies a large reduction in the electricity consumption during centrifugation.

An alternative to the use of lime may be to induce an increase in pH by intense microalgal photosynthesis. In cultures of *Phaeodactylum*, Spilling et al. (2011) observed that flocculation occurred without addition of base when CO_2 supply was interrupted and pH increased to 9.5 as a result of photosynthesis [172]. Nurdogan and Oswald (1995) also noted that flocculation of microalgae in high rate algal ponds occurred during warm and sunny days, when low solubility of CO_2 and high photosynthetic uptake of CO_2 resulted in a high pH. More research is required to investigate what conditions favour a natural increase in pH in microalgal cultures up to a point that precipitation of magnesium

Table 4.3: Cell numbers for untreated microalgae (control) and microalgae after flocculation at pH 12 (AF pH 12).

	Control	AF (pH 12)	p ($\alpha = 0.05$)
Cell number (#cells mL^{-1})	$6.10\times10^7 \pm 2.12\times10^6$	$5.15\times10^7 \pm 1.14\times10^6$	0.034

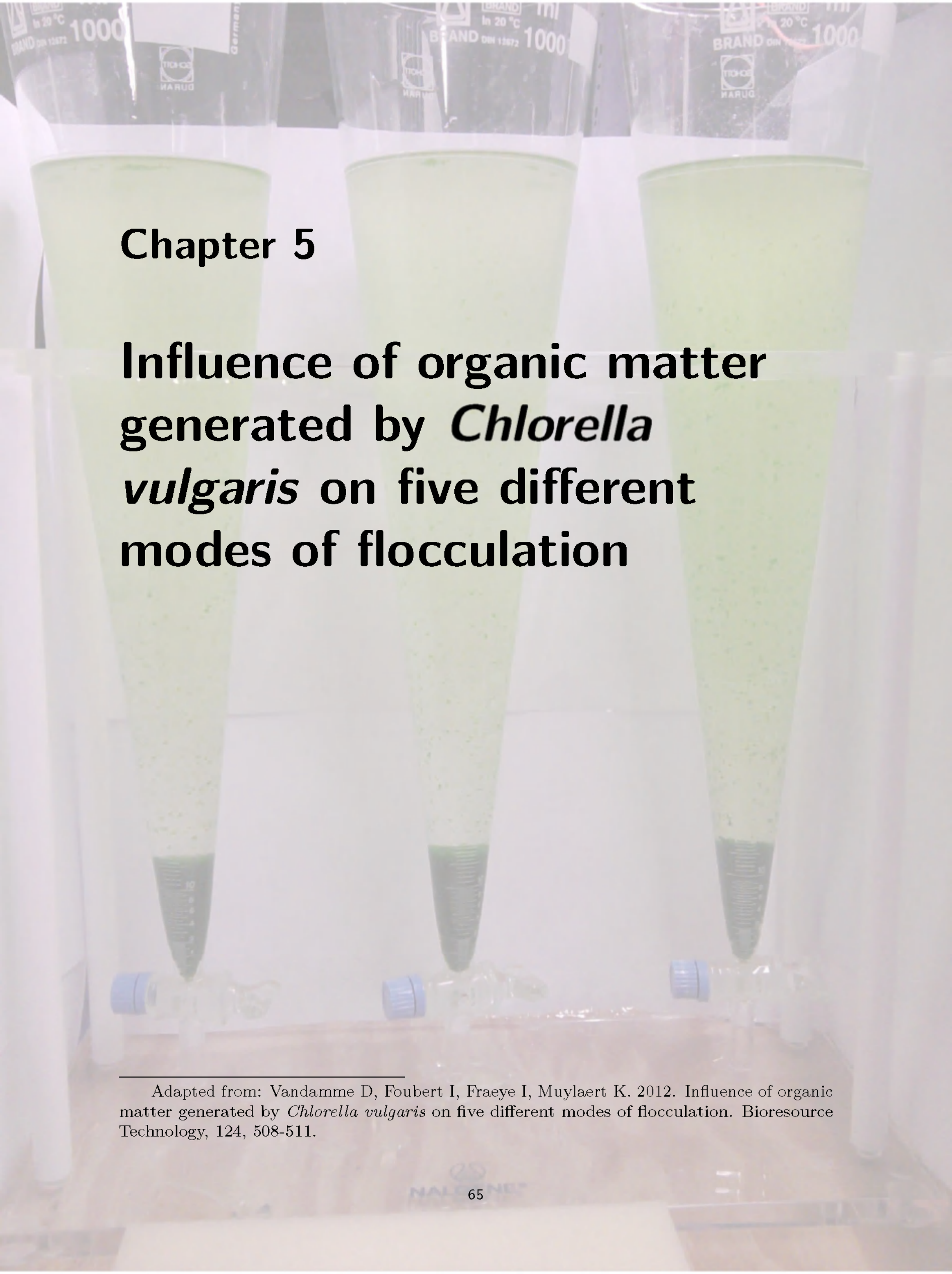
hydroxides occurs.

The use of flocculation induced by high pH for harvesting microalgae may have as advantage that the high pH effectively sterilises the microalgal biomass as well as the process water. This may be advantageous when microalgae are used in wastewater treatment, as the high pH may kill pathogenic microorganisms [164].

Care should however be taken that the high pH does not destroy the microalgal cells, as this may result in loss of useful bioproducts from the biomass. To evaluate whether the high pH causes cell lysis of *Chlorella*, the cell numbers were compared before and after flocculation by pH increase to pH 12 (Table 4.3). Although a significant decrease in cell numbers was detected upon flocculation ($p = 0.034$; $\alpha = 0.05$), it is clear that the major part of the microalgal cells was still intact (>85%). Secondly, we measured the maximum quantum yield of photosystem II in *Chlorella* biomass after flocculation induced by high pH using lime. We did not notice a significant decrease in the maximum quantum yield as long as pH remained below 12. The results of both methods suggest that the loss of useful bioproducts will most likely be minimal. Using light microscopy, Knuckey et al. (2006) also did not observe any apparent deterioration of microalgal biomass harvested after pH increase. But an earlier study by Blanchemain & Grizeau (1999) noted that cell lysis occurred after 1 h at pH 10.2 for *Skeletonema costatum* based on colour change of the algal culture from brown to green [99, 20]. Taken together, these findings suggest that operation time will be an important process parameter to take into account in the development of a pH induced harvesting process for microalgae. However more exact and standardized methods to measure cell lysis are needed for an in-depth and quantitative analysis of its influence on the cell viability.

4.4 Conclusions

Our experiments demonstrate that the terminology ‘autoflocculation’ is misleading because flocculation induced by high pH is not related to changes in properties of the microalgal cells, but is a form of chemical flocculation in which precipitation of magnesium is involved. Flocculation induced by high pH is a potentially useful method to preconcentrate microalgal biomass. However, the method depends on sufficiently high magnesium (>0.1 mM) concentrations. From a cost as well as safety perspective, pH is best increased using calcium hydroxide. Further research regarding the impact of pH induced flocculation on cell viability and recycling of process water is needed.



Chapter 5

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

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Adapted from: Vandamme D, Foubert I, Fraeye I, Muylaert K. 2012. Influence of organic matter generated by *Chlorella vulgaris* on five different modes of flocculation. Bioresource Technology, 124, 508-511.

5.1 Introduction

Primary concentration of microalgae can be achieved by flocculation, a phenomenon which can be induced by the addition of polyvalent metals such as aluminum sulfate (alum) or ferric chloride, or by electrochemical release of metal ions from a sacrificial anode in electro-coagulation flocculation (ECF) (Chapter 3). Alternatively, cationic biopolymers, such as chitosan or the cheaper alternative cationic starch (Chapter 2), 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 (Chapter 4). 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 neutralization and/or bridging.

During cultivation, microalgae release significant amounts of algal 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 [90]. 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 [127]. It is well-known that AOM interferes with the 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}; [15, 87]). 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* [39] and for the green alga *Chlorella zofingiensis* [204] 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 chapter, the influence of AOM on flocculation of *Chlorella vulgaris* using five different flocculation technologies was evaluated.

5.2 Materials and methods

5.2.1 Cultivation of *Chlorella vulgaris*

C. 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 medium [79]. 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 [75]. 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 the early stationary growth phase at a biomass concentration of 0.25 g L^{-1}.

5.2.2 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 (4,000g) and resuspended in fresh Wright's cryptophyte 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 [127]. Carbohydrates were measured using the phenol–sulfuric acid method [55]. 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 in Section 3.2.2 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:

$$\eta_a = \frac{OD_i - OD_f}{OD_i} \tag{5.1}$$

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 using 0.5 N HCl to 5.5 prior to addition of the coagulant. A solution of 0.015 M $Al_2(SO_4)_3 \cdot 18\,H_2O$ (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 (Section 2.3). 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 Equation 5.1.

5.3 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 5.1. 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 mg 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

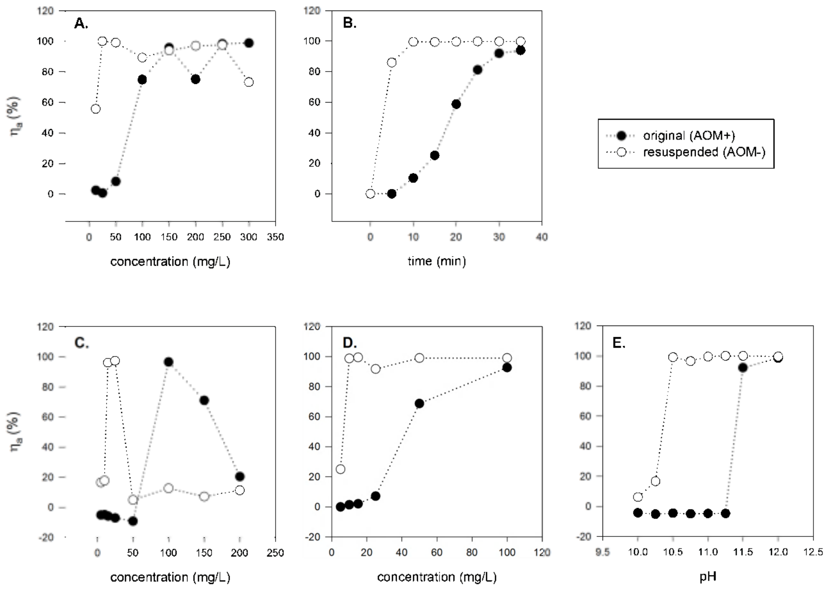


Figure 5.1: Dose response curves for different flocculation methods: for *Chlorella vulgaris* in original (AOM+) medium and resuspended (AOM-) in fresh medium: (A) alum, (B) ECF, (C) cationic starch, (D) chitosan, (E) pH induced flocculation.

Chlorella cells in their original medium were in the range of the doses mentioned in previous studies [184, 186, 188], 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 5-fold increase in energy consumption needed to achieve flocculation in the presence of AOM using ECF was in accordance to the 6-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 [14, 87]. 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 [15]. Both the number and position of these carboxyl groups and the size of the polysaccharide polymers influence the interaction with metal coagulants [15, 87]. 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 [143, 177]. 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 concentration of *Chlorella* cultivated in closed photobioreactors is at least an order of magnitude higher [90]. 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 threedimensional structure of both cationic biopolymers [27]. Overdosing cationic starch leads to a decrease in the flocculation efficiency. This phenomenon of dispersion restabilization is commonly observed with polyelectrolyte flocculants, including cationic starch and is probably the result of steric hindrance and/or electrostatic repulsion [186].

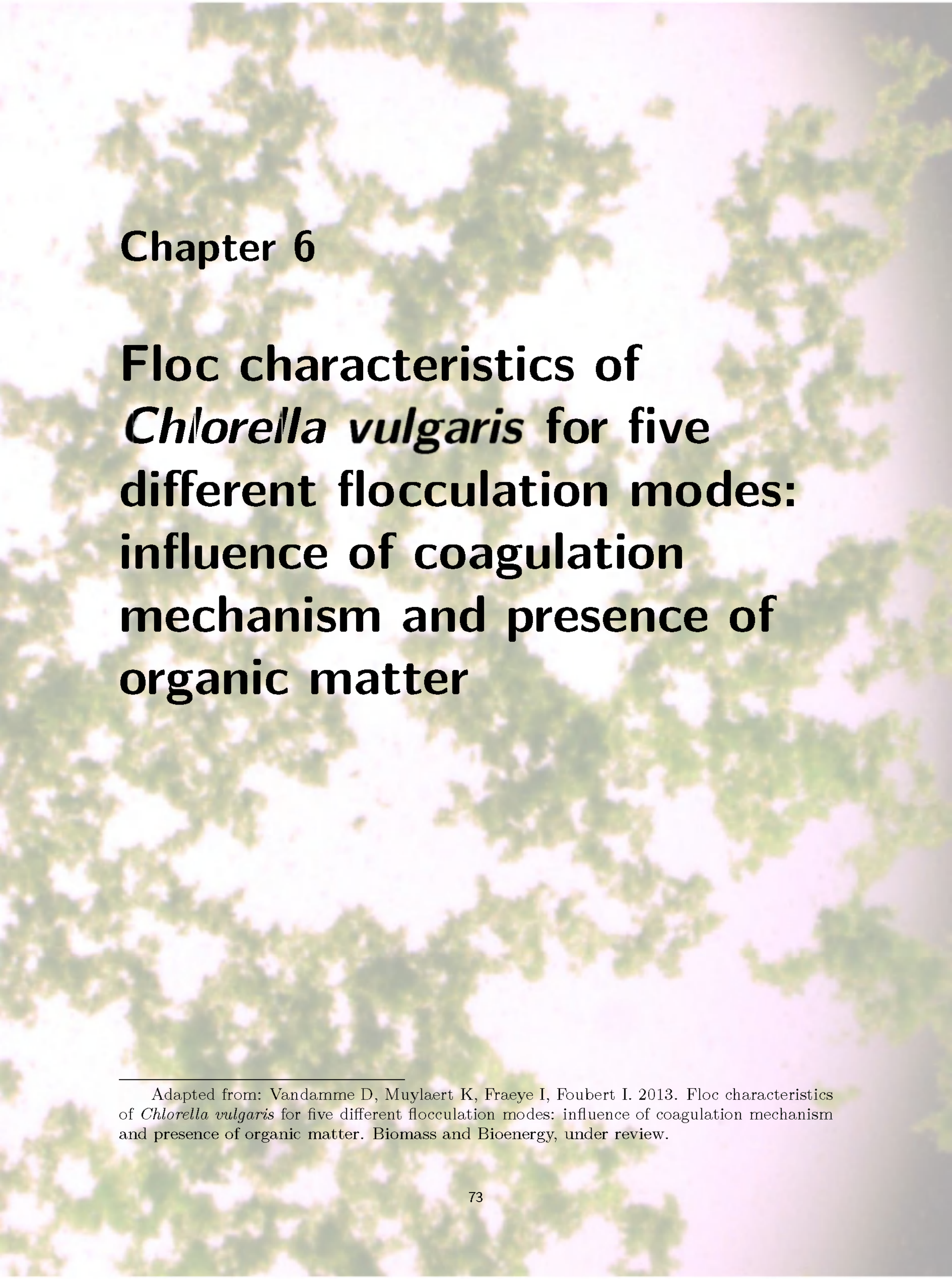
The present results demonstrated that AOM also interferes with flocculation by magnesium hydroxide precipitates formed at a high pH. Lee et al. (1998) also showed that flocculation induced by high pH in cultures of *Botryococcus braunii* was affected by growth stage [106], which is also known to have an influence on the amount of AOM [89]. Compared to the other flocculation technologies, the dose of sodium hydroxide required to induce flocculation was only about 2-fold higher in the presence of AOM. The reason for this is not clear. Possibly, it is due to the fact that sodium hydroxide is not the primary flocculant, but induces precipitation of magnesium hydroxides which act as the flocculant [184, 197]. As precipitation of magnesium hydroxides increases non-linearly with pH [108], a limited increase in the amount of base added may result in a strong increase in magnesium hydroxide precipitates.

Our results clearly showed that most of the flocculant dose required to flocculate *Chlorella* in an exponentially growing culture is lost on the AOM rather than being used for actual flocculation of the cells. The degree of inhibition of flocculation by AOM is most likely related to the quantity and the composition of the AOM present in the medium, and these variables will therefore have a major influence on the cost of harvesting microalgal biomass using flocculation. Although all microalgae produce AOM, the quantity and quality of the AOM differ between species and is influenced by culture conditions. For instance, cyanobacteria tend to produce AOM that is relatively rich in proteins compared to eukaryotic microalgae [177, 143]. The number and position of carboxyl groups on polysaccharides and the size of the polysaccharides differ between species of microalgae and also change with culture age [89, 143]. For *C. vulgaris* in stationary growth phase, the composition of AOM was analyzed in detail by Henderson et al. (2010) [87]. *Chlorella* produced about 0.0029 ng AOM per cell. The charge density of AOM was 3.2 meq g^{-1} and AOM contributed 84% of the total charge of the culture. The AOM had a protein:carbohydrate ratio of 0.4% and 60% of the AOM consisted of >30 kDa molecules. The current results suggested that some flocculation techniques are more sensitive to inhibition by AOM than others, although the degree of inhibition of the different techniques may be different with other species of microalgae and/or other culture conditions.

The present findings have important implications for the development of microalgal biofuels. To maximize lipid production in microalgae, cultures are often subjected to nutrient stress [194]. As nutrient-limited cells generally produce more AOM than exponentially growing cells [127], maximizing lipid production will most likely also result in a higher flocculant demand. Furthermore, medium recycling is essential to minimize the water demand and thus the ecological impact and production costs of microalgal biofuels [29]. During medium recycling, AOM is likely to accumulate in the medium, probably resulting in a gradual increase in flocculant demand. In wastewater treatment, several technologies, such as chlorine treatment or ozonation, have been proposed to reduce the load of AOM [86]. Given the high concentrations of AOM in dense microalgal cultures, it is questionable whether such technologies will be effective in sufficiently reducing AOM in intensive microalgal production systems. Oxidation of AOM may also result in lysis of algal cells, which might further increase flocculant demand [86].

5.4 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.



Chapter 6

Floc characteristics of *Chlorella vulgaris* for five different flocculation modes: influence of coagulation mechanism and presence of organic matter

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Adapted from: Vandamme D, Muylaert K, Fraeye I, Foubert I. 2013. Floc characteristics of *Chlorella vulgaris* for five different flocculation modes: influence of coagulation mechanism and presence of organic matter. Biomass and Bioenergy, under review.

6.1 Introduction

Flocs that are formed by various coagulation mechanisms can exhibit different floc characteristics such as floc size, structure and density and this will affect important parameters such as settling velocity and concentration factor [109]. The settling velocity is a key parameter in the design of large scale sedimentation units while the concentration factor is important to evaluate the water content of the particulate phase. The final water content of the particulate phase in addition to the flocculation efficiency will determine the overall efficiency of a flocculation mode. Most studies however only focus on the evaluation of flocculation based on flocculation efficiency. Recently, a few studies investigated these additional parameters for pH induced and chitosan flocculation [91, 171]. However, those results were not related to coagulation mechanism, floc size and structure. Therefore, in this study, the influence of coagulation mechanism on the floc characteristics of *Chlorella vulgaris* using five different flocculation modes was evaluated.

Microalgae are known to release significant amounts of organic matter (AOM). In microalgae cultivation systems, AOM can amount to 60-80 mg C L^{-1} [90]. The major fraction of this AOM consists of neutral or charged polysaccharides, but other compounds such as proteins, nucleic acids, lipids and other small molecules can be present as well [87, 127]. In Chapter 5, the effect of AOM on flocculation efficiency of *Chlorella vulgaris*, induced by five different flocculation modes, was evaluated. To the best of our knowledge, however, no publications have focused on the effect of the presence of AOM on the floc characteristics such as settling velocity, concentration factor and floc size. Therefore, in this chapter, also the influence of the presence of AOM on these floc characteristics of *Chlorella vulgaris* using five different flocculation modes was evaluated.

6.2 Materials and methods

6.2.1 Cultivation of *Chlorella vulgaris*

Chlorella vulgaris (211-11b SAG, Germany) was cultivated in dechlorinated tap water enriched with inorganic nutrients according to the concentration of the Wrights cryptophyte medium [79]. Bubble column photobioreactors (30 L) were used to cultivate the microalgae. The system was mixed by sparging with 0.2 μm filtered air (5 L $\min^{-1}$) and pH was controlled at 8.5 through 2-3% CO_2 addition using a pH-stat system. Growth of the microalgae was monitored by measuring the absorbance at 550 nm. Microalgal dry weight was determined gravimetrically by filtration using Whatman glass fibre filters (Sigma-Aldrich) and drying until constant weight at 105°C. Flocculation experiments were performed in the early stationary phase at a biomass concentration of 0.3 g L^{-1}.

6.2.2 Flocculation protocol

Five different flocculation modes were assessed: addition of alum (AL), electro-coagulation- flocculation using aluminum anodes (ECF), addition of chitosan (CH), addition of cationic starch (CS) and flocculation induced by high pH (pH). In addition, the influence of AOM on the flocculation process was studied for each flocculation mode. To do so, flocculation of *Chlorella* was compared in medium with and without AOM. To remove AOM, *Chlorella* was separated from the medium using centrifugation (4,000g) and resuspended in fresh medium. This approach has already been used successfully in previous studies and it has also been demonstrated that centrifugation and resuspension of *Chlorella*, as a treatment as such, has no influence on the flocculation efficiency [185].

In a preliminary study, the flocculation parameters (pH and dosage) resulting in a flocculation efficiency higher than 85% were determined in jar tests on 100 ml scale. For each flocculation mode, this was based on a protocol used in our previous study (Section 5.2.2). For alum ($Al_2(SO_4)_3 \cdot 18\,H_2O$; Sigma Aldrich), the pH was adjusted to 5.5 prior to and immediately after addition of the coagulant. For ECF, the setup described in a previous study was used (Section 3.2.2). 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}. For flocculation using chitosan (from crab shells, practical grade, Sigma Aldrich), the pH of the suspension was adjusted to 7.5 prior to and immediately after addition. 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 was not pH-dependent (2.3). For pH-induced flocculation, 0.5 N NaOH was used to increase the pH.

6.2.3 Sedimentation analysis

After flocculation, using each of the flocculation modes, sedimentation was followed in order to calculate the settling velocity. This analysis could not be performed for ECF because flotation simultaneously occurred with flocculation. For each flocculation treatment, coagulation was induced in 1 L cylindrical vessels according to the dosage obtained in the preliminary flocculation experiment.

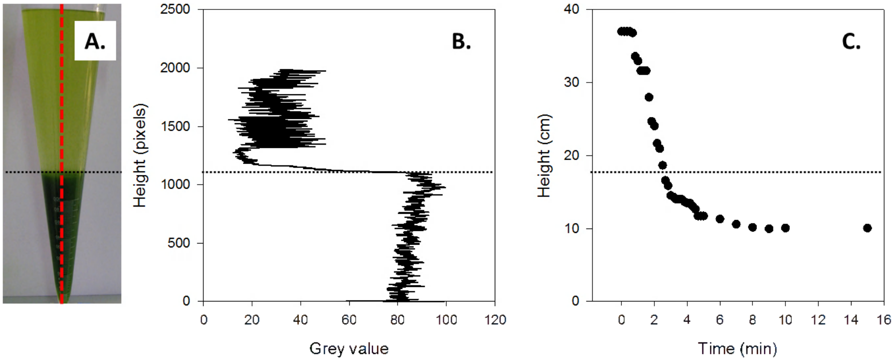


Figure 6.1: Example of sedimentation analysis: grey values were analysed in function of distance (A; red line). The interface of suspended and particulate phase could be detected by an increase in grey value (B). The corresponding height was plotted as function of time (C).

The suspension was stirred for 15 min at 300 rpm using an overhead stirrer. Then the suspension was transferred to 1 L imhoff cones, allowing sedimentation for 15 min. Images (Fig 6.1 A) were automatically taken at fixed time intervals using a webcam. Grey values were analysed as function of height using ImageJ (NIH, USA) allowing to determine the height of the interface between the suspended and particular phase at each time step (Fig 6.1 A; red line + 6.1 B). The corresponding height was then plotted as function of sedimentation time (Fig 6.1 C). The settling velocity is defined as the velocity in cm s^{-1} to achieve complete biomass settling without further observed increase of settled floc volume. To calculate this, the distance at which the moving front remained constant was divided by the corresponding settling time.

After 15 min of sedimentation, the suspension was allowed to settle an additional 15 min to determine the concentration factor (CF) and the aggregated volume index (AVI). Both parameters are related to each other and provide information about the residual water content of the particulate phase. The CF was determined by dividing the total volume of 1000 ml by the volume of the particulate phase after 30 min of sedimentation. The AVI was calculated according to the method of Javaheri and Dick [92]. It is defined as the volume in milliliters occupied by 1 g of algal suspension in the particulate phase after 30 min of settling and is calculated as:

$$AVI(mL\ g^{-1}) = \frac{volume\ of\ settled\ biomass\ (mL\ L^{-1})}{microalgal\ biomass\ dry\ weight\ (mg\ L^{-1})} \times 1000\ (mg\ g^{-1}) \tag{6.1}$$

6.2.4 Particle size analysis

After sedimentation, a subsample of the particulate phase was taken and diluted 10 times. The flocs were then analysed using a stereo zoom microscope (Olympus SZX10) and images were taken using a camera (Lumenera Infinity 2). Particle size analysis was conducted using ImageJ (NIH, USA). The original images were transformed to 8 bit, the background was substracted and particles smaller than 100 px^2 were thresholded (Fig 6.2). After transformation of the image, the average Feret's diameter was calculated.

6.3 Results

The optimal flocculation parameters, pH and dosage, in order to achieve a minimal flocculation efficiency of 85% were assessed in a preliminary study and this for all flocculation modes with and without the presence of AOM. Table 6.1 shows the pH and dosage selected for the remainder of the study to induce coagulation on 1 L scale for floc characterization based on sedimentation and particle size analysis. For all flocculation modes, the dosage to obtain a flocculation efficiency of 85% increased between 1.5 and 5-fold when AOM was present. This is in correspondence with previous results (Section 5.3).

Fig 6.3 presents floc front height as function of time for four different flocculation modes containing AOM (indicated as AOM+; Fig 6.3 A) and resuspended in fresh medium without AOM (indicated as AOM-; Fig 6.3 B). This analysis could not be performed for ECF because flotation occurred simultaneously with flocculation. For all flocculation modes with and without the presence of AOM, the biomass settled within 15 min of sedimentation. Alum and chitosan flocculation resulted in a faster sedimentation than pH induced flocculation. In both treatments, cationic starch flocculation resulted in the slowest sedimentation. The settling velocities were calculated for each flocculation mode and ranged between 0.06 and 0.6 cm s^{-1} with a maximal standard deviation of 12%. They confirmed the first observations made on the basis of the graphs. When AOM was present, the settling velocity was the highest for chitosan flocculation (0.4 cm s^{-1}), followed by alum flocculation (0.2 cm s^{-1}) and the lowest for pH induced flocculation (0.09 cm s^{-1}) and

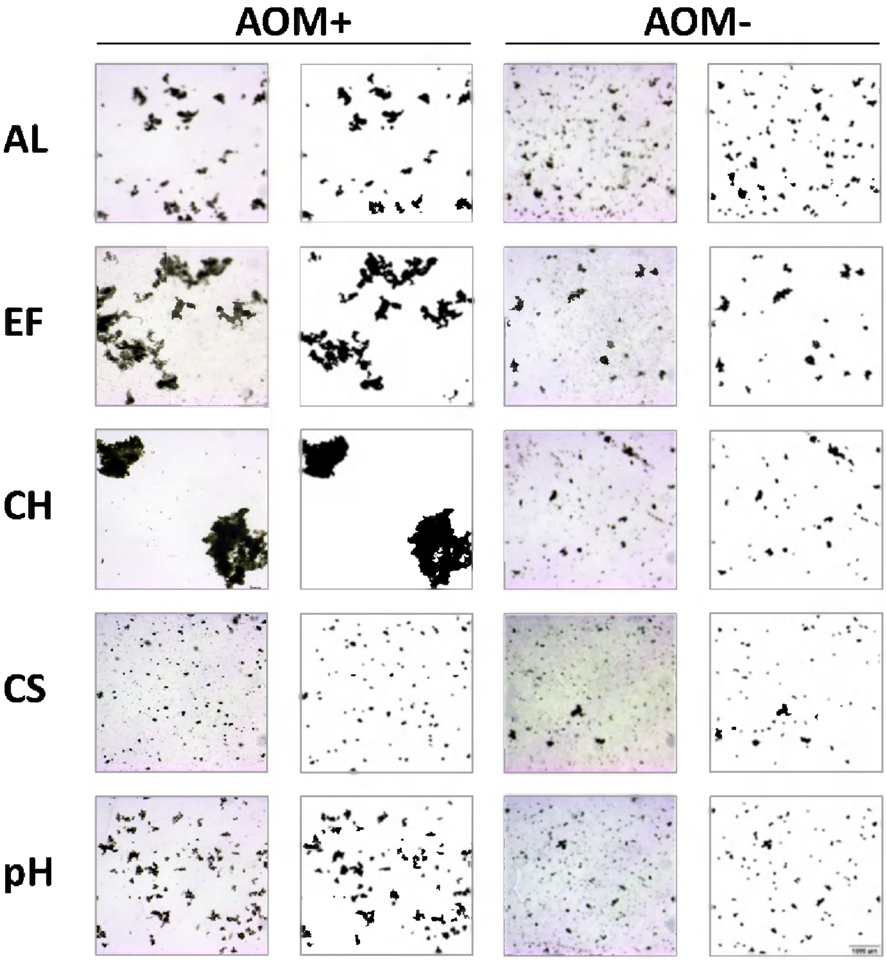


Figure 6.2: Original and transformed images used for particle size analysis for alum flocculation (AL), electro-coagulation-flocculation (ECF), chitosan flocculation (CH), cationic starch flocculation (CS) and pH induced flocculation (pH) for *Chlorella vulgaris* with and without the presence of AOM.

Table 6.1: Flocculation parameters (pH and dosage) of alum flocculation (AL), electro-coagulation-flocculation (ECF), chitosan flocculation (CH), cationic starch flocculation (CS) and pH induced flocculation (pH) for *Chlorella vulgaris* with and without the presence of AOM.

Flocculation mode	AOM+		AOM-	
	pH	Dosage ($mg\ L^{-1}$)	pH	Dosage ($mg\ L^{-1}$)
AL	5.5	100	5.5	20
ECF	8.5	1.2^1	8.5	0.4^1
CH	7.5	80	7.5	15
CS	8.5	150	8.5	100
pH	12	87^2	11.5	50^2

^{1}: Dosage for ECF expressed as power consumption (kWh kg^{-1} microalgae)
^{2}: Dosage for pH expressed as concentration added NaOH

cationic starch flocculation (0.06 cm s^{-1}). Without AOM, settling velocities mostly increased, but the order as function of flocculation mode in general remained the same. In the fast settling cases of chitosan and alum flocculation, the settling velocity increased to 0.6 cm s^{-1}. For pH induced flocculation, it increased to 0.2 cm s^{-1}. For cationic starch however, the settling velocity decreased to 0.04 cm s^{-1}.

Additional information about the residual water content of the particulate phase is provided by calculating the concentration factor (CF; Fig 6.4 A) and the aggregated volume index (AVI; Fig 6.4 B). A high concentration factor corresponds to a low AVI. Using cationic starch, the algal biomass could be concentrated more than 100 times, in the presence of AOM. After removal of AOM, this even increased to 180 times. This corresponds with an AVI lower than 25 mg g^{-1}. When AOM was present, the other flocculation modes resulted in a clearly lower CF of between 15 and 35, corresponding to a distinctly higher AVI of between 70 and 140 mg g^{-1}. In the absence of AOM, the CF increased and the AVI decreased for all flocculation modes. The Feret's diameter of the settled flocs for the five different flocculation modes was also determined (Fig 6.4 C). It varied between 130 and 2300 μm. When AOM was present, the flocs were larger, except for flocculation by cationic starch. For chitosan flocculation in the presence of AOM, flocs with a diameter higher than 2 mm were observed.

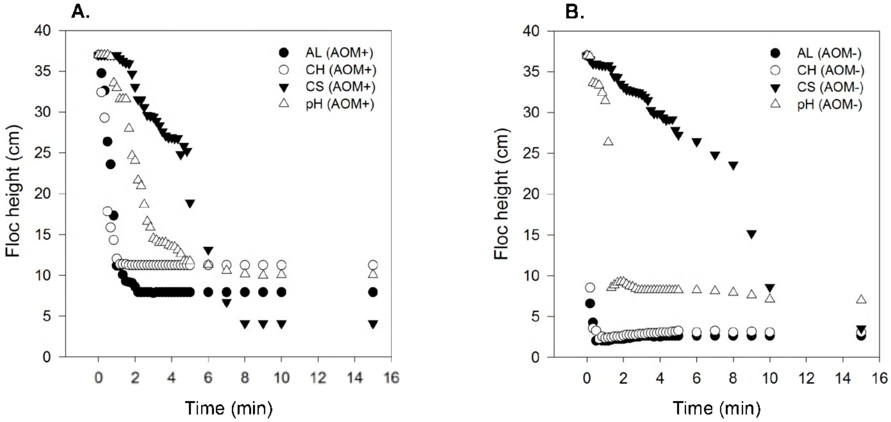


Figure 6.3: Sedimentation analysis for four flocculation modes: alum flocculation (AL), chitosan flocculation (CH), cationic starch flocculation (CS) and pH induced flocculation (pH) for *Chlorella vulgaris* (A) with (AOM+) and (B) without the presence of AOM (AOM-).

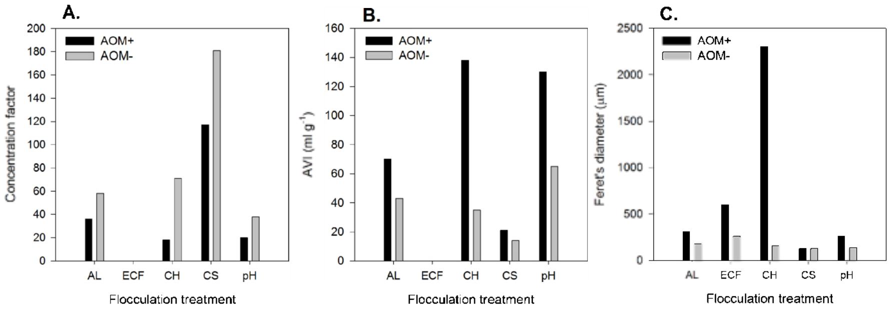


Figure 6.4: Concentration factor (A), AVI (B) and Feret's diameter (C) for five flocculation modes: alum flocculation (AL), electro-coagulation-flocculation (ECF), chitosan flocculation (CH), cationic starch flocculation (CS) and pH induced flocculation (pH) for *Chlorella vulgaris* with (AOM+) and without the presence of AOM (AOM-).

6.4 Discussion

The present results show that the type of flocculation mode affects the microalgal floc characteristics such as settling velocity, concentration factor and floc size. Flocculation of microalgae should not only be effective in terms of flocculation efficiency, but also in terms of settling rate and concentration of the biomass. Those parameters are important in the design of a harvesting process including a secondary harvesting step using for example centrifugation. As a consequence of this, the delivery of a fast settled and high concentrated biomass is desirable before centrifugation in order to improve overall energy consumption. Three mechanisms, i.e. charge neutralization, sweeping by precipitation enmeshment and bridging have been demonstrated in coagulation processes and differences in these mechanisms may explain the influence of flocculation mode on floc characteristics [22].

For flocculation modes using inorganic metal salts, the coagulation mechanism is absorption-charge neutralization or sweeping flocculation caused by precipitate enmeshment or a combination of both [7]. The usage of for example alum as coagulant introduces water-binding amorphous precipitates, which are present in large amounts especially when coagulation occurs by sweeping flocculation. During absorption-charge neutralization those precipitates are present in limited amounts and this has consequently less impact on the floc size [98, 6]. Absorption-charge neutralization may thus result in smaller flocs compared to flocculation based on sweeping. For alum flocculation, operating conditions such as biomass density, coagulation pH and coagulant dosage determine the coagulation mechanism [54]. In this study, pH was adjusted and controlled at 5 for alum flocculation, which is known to facilitate coagulation dominated by absorption-charge neutralization [98, 65]. In contrast, pH was not controlled during EC flocculation using aluminum anodes. The initial pH was 8.5 (Table 6.1) and is known to rise as function of operation time because of the hydroxide ions released at the cathode [188]. In those conditions, sweeping flocculation is the dominant flocculation mechanism [54]. This could explain the overall larger flocs for the EC flocculation mode when compared to alum flocculation (Fig 6.4 C).

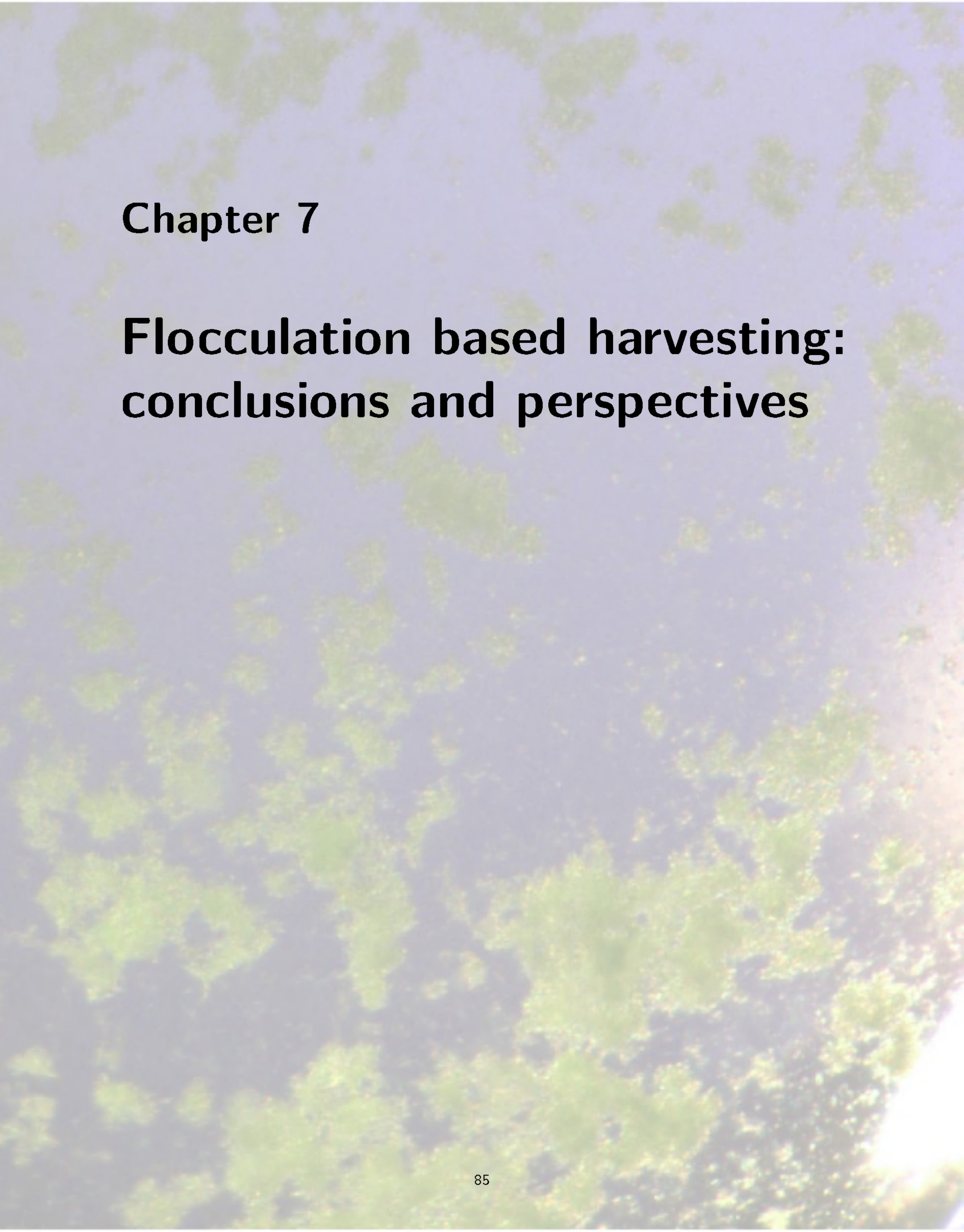
For flocculation induced by high pH, similar floc sizes as for alum flocculation were observed, but the floc compaction was inferior compared to alum flocculation (Fig 6.4 A/B). Positively charged magnesium hydroxides are involved in initiating coagulation by adsorption-charge neutralization and partially also by sweeping flocculation, depending on pH and magnesium concentration [184]. With an increasing pH, large quantities of amorphous hydroxides are allowed to precipitate. These are known to have a high affinity for water arising from mechanical trapping and hydrogen bonding and thus affect the water content of the particulate phase [6].

Cationic biopolymers have been used in coagulation/flocculation processes as flocculation aid for water purification and are known to lower flocculation dosage requirements, increase settlement and decrease sludge volume [22]. Generally, flocculation using biopolymers is induced by a bridging mechanism. This type of flocculation occurs when a polymer serves as a bridge after formation of a lot of particle-polymer-particle aggregates [109]. In general, the usage of polymers results in a higher effective density and thereby improved settling and a higher floc compaction [6]. In this study, chitosan and cationic starch flocculation indeed resulted in a higher compaction of the settled biomass, in absence of AOM (Fig 6.4 A/B). Furthermore chitosan flocculation indeed had the highest settling velocity, although cationic starch flocculation resulted in the lowest settling velocity (Fig 6.3). It must be noted that in this study both biopolymers were used as primary coagulant, while settling improvement is mostly achieved when biopolymers acts as flocculant aid in combination with a primary coagulant such as alum [151].

From previous studies [15, 185, 204], it is known that the flocculant dosage needed to achieve efficient flocculation is increased by the presence of AOM. In this study, similar results were obtained (Table 6.1). The present results however show that the presence of AOM also affects the microalgal floc characteristics such as settling velocity, concentration factor and floc size. It was found that in the presence of AOM the settled biomass has a greater water content, bigger floc size and that the settling velocity is lower (Fig 6.3 and 6.4). Henderson et al. (2010) characterized in detail the AOM produced by *Chlorella vulgaris* [87]. In the stationary growth phase, the AOM had a protein:carbohydrate ratio of 0.4 and a hydrophilicity of 71%. These hydrophilic attributes may contribute to the increase in water content of the settled biomass, but it is more likely that the increase in water content is caused by the increase of flocculation dosage due to AOM interference [6, 98]. As for the influence on needed flocculant dosage, also the importance of the influence of AOM on the floc characteristics was clearly depending on the floc mode. While cationic starch flocculation seemed to be hardly influenced by the presence of AOM, the characteristics of the flocs obtained by addition of chitosan were very much influenced by AOM. For chitosan, the presence of AOM resulted in the highest increase of flocculant dosage needed, while for cationic starch, this increase was the lowest (Table 6.1). Chitosan is known to have a high affinity for dissolved organic matter and this affected the required flocculant dosage in presence of AOM [22, 111, 129]. Abundant chitosan was as such available and flocs with a more open structure were thus allowed to form. This explains both the fact that chitosan based flocculation in the presence of AOM resulted in flocs with the lowest compaction and the largest floc size.

6.5 Conclusions

Floc characteristics such as settling velocity, concentration factor, aggregated volume index and floc size were studied for five different flocculation modes for *Chlorella vulgaris*. This study showed that coagulant dose and type determine coagulation mechanism and by this affect the floc characteristics of the settled microalgal biomass. The presence of AOM resulted in a lower concentration of the settled biomass. Our study thus showed that in addition to flocculation efficiency, the impact on the characteristics of the formed flocs needs to be taken into account as well in the overall assessment of flocculation based harvesting methods for microalgae.



Chapter 7

Flocculation based harvesting: conclusions and perspectives

7.1 Development of low-cost flocculation technologies

To produce microalgal biomass for bulk food, feed or biofuels, the scale of production has to be increased and the cost of production decreased by at least an order of magnitude. In particular, cost-effective harvesting is considered to be one of the biggest challenges to realize large-scale and low-cost production of microalgae biofuels. The energy needed for harvesting microalgae from typical open pond systems (0.03%) using centrifugation was calculated to be 14 MJ $kgDW^{-1}$ of microalgae [131]. When energy applications are the only focus, this would imply that more than 50% of the total combustion energy (estimated at 25 MJ $kgDW^{-1}$) is to be invested in a one step harvesting process using centrifugation. Moreover, if biodiesel (7-10 MJ $kgDW^{-1}$) is the only product, this would result in unsustainable production of biofuels [120]. This simple calculation demonstrates the urgent need for low-cost and energy-efficient harvesting methods. Flocculation is seen as a promising approach for reducing the cost and energy input of microalgae harvesting.

Flocculation is a widely used technology in different fields of industry. Increasing the particle size and concentration by flocculation increases the rate of settling and decreases the slurry volume that needs further dewatering. Flocculation processes are already frequently employed in water purification systems to remove microalgae or in wastewater systems to concentrate sludge. Similar to those systems, flocculation efficiency, settling rate and concentration factor are important in the design of an efficient flocculation method for harvesting microalgae. But in contrast to applications in other industries, the harvested biomass is the desired end-product during production of microalgae instead of the purified water. Consequently, the cost is related to the ratio of flocculant dose over the harvested biomass and not by the ratio of the flocculant dose over the volume of purified water. Secondly, because chemical flocculants added during the process end up in the biomass, contamination of the biomass is a critical evaluation criterion when developing flocculation technologies for harvesting microalgae. Contamination of the biomass with flocculation aids may limit the use of the biomass or fractions of the biomass for food and feed applications, or they may interfere with downstream processing of the biomass (e.g. lipid extraction). Moreover, the addition of chemicals should not limit the reuse of the cultivation medium after harvesting. The given arguments indicate that knowledge transfer from existing industries can certainly be interesting and useful, but needs to be applied within the boundaries of the production process of microalgae biomass.

Polymers are widely used flocculants in water and wastewater treatment. Synthetic polymers such as polyacrylamide are often used. However, because they may contain toxic acrylamide residues, their use for harvesting microalgae would result in contamination of the biomass as well as the culture medium. Natural biopolymers are therefore preferred. Chitosan is a widely used biopolymer made from shellfish waste and has been shown to be effective for harvesting microalgae [124, 51, 91]. The cost of chitosan, however, is too high to be used for harvesting microalgae. As an alternative biopolymer, cationic starch was evaluated in this study. Flocculation using cationic starch showed to be especially efficient for harvesting freshwater species and independent of pH, which is not common for biopolymers. The optimal dosage of cationic starch compared to chitosan is high but flocculant price is substantially lower, resulting in a much lower total flocculant cost. Additionally, this flocculation mode was capable of increasing the biomass concentration by the highest concentration factor. Cationic starches with a low degree of quaternary ammonium group substitutions are routinely used in wastewater treatment and do not pose environmental toxicity issues. Cationic starch is cheaper than reference flocculants such as alum and chitosan, but is probably still too expensive to use for biofuel production. Since our study, several others have continued to work with cationic starch. Recently, it was proposed to harvest freshwater microalgae using cationic starch in a biorefinery concept for the treatment of wastewater systems, production of biofuels and high added value products [133]. In a study addressing flocculant effects on the production of biodiesel, biosolvents and bioplastics, a novel designed and patent-protected cationic starch was claimed to be a more reliable and cheaper flocculant in comparison with alum [R.J. Anthony, PhD. Utah State University USA, personal correspondence]. Besides cationic starch, there is still a scope for research on the potential of other low-cost and non-toxic polymers based on proteins and peptides, tannins or *Moringa oleifera* seed extracts [139, 10, 179].

Secondly, as alternative for alum, electro-coagulation flocculation (ECF) was evaluated. During electrolysis, aluminum is dissolved in the medium at the anode and the released Al formes hydroxides that cause flocculation. An advantage of ECF over the use of metal salts is that no anions are released in the medium during this process. Our experiments indicated that Al anodes were more efficient than Fe anodes. ECF was capable of flocculating microalgae in both freshwater and marine media. Due to the low electrical resistance of seawater, the energy consumption was much lower in seawater than in freshwater. During our experiments, we encountered both flocculation and flotation, affecting the final concentration of the microalgae. Even in optimal conditions, residual aluminum was detected at relatively high concentrations (1%) in the biomass which can have limitations for food and feed related applications. On the other hand, a new study concluded that the biomass quality of *Nannochloropsis* after electro-coagulation was not significantly changed in terms of total lipids, fatty acid and pigment profile [118]. Two recent studies also showed that the concept of electro-coagulation flocculation can still be improved by using polarity exchange in a continuous harvesting system [94, 95].

It is a well-known phenomenon in open pond cultivation systems that microalgae sometimes flocculate spontaneously at high pH. Studies in the 1980's indicated that this phenomenon of 'autoflocculation' is related to precipitation of calcium phosphates at high pH. Flocculation by calcium phosphates is efficient, but it is not economically feasible nor sustainable due to the high phosphate requirements. But flocculation also occurred in our experiments in absence of calcium and phosphates in the medium. This was investigated further for the freshwater species *Chlorella vulgaris*. In our study, it has been shown to be associated with the formation of magnesium hydroxide precipitates. As pH is already quite high in microalgae cultivation systems due to CO_2 uptake by the microalgae during photosynthesis, the need for a base to increase pH is minimized. If calcium hydroxide or slaked lime is used to increase the pH, the cost of this flocculation method is very low. Although magnesium and calcium will precipitate and lime will be needed to increase pH, this method has significant potential in terms of cost effectiveness. The biomass will contain high concentrations of minerals with a low toxicity risk. For feed or food related applications, it will however be preferable to remove them from the biomass, which can imply an additional cost. Several other recent studies have highlighted the potential of this flocculation method including that the flocculated medium can be reused after neutralization [36, 162, 171, 197].

Table 7.1 summarizes the potential of the flocculation modes using cationic starch, electro-coagulation flocculation and pH induced flocculation compared with the two reference modes alum and chitosan flocculation as discussed above. The operating expenditures (OPEX) are estimated only based on the required dosage and the flocculant cost per ton and thus exclude operator costs and cost of mixing energy. Rapid mixing or flash mixing can be done in a flow-through stirred tank and average shear rates are usually in the region of 20-70 s^{-1}. For a water volume of 400 m^3 and using a motor with a power of 1 kW and an efficiency of 60%, the effective shear rate turns out to be about 40 s^{-1} [56]. By this, mixing energy for microalgae flocculation is calculated to be 1.5 kWh $tonDW^{-1}$. As this cost is a fraction of the flocculant cost it has been neglected in this comparison.

From the above it can be concluded that a single best flocculation mode is yet to be identified. There are however strong arguments presented in this work that no single mode will be suited for all microalgae species or applications. The choice will be largely dependent on the application and the cultivation conditions. As a consequence, it may be necessary to use a less efficient method to limit

Table 7.1: Potential of flocculation using cationic starch (CS), electro-coagulation flocculation (ECF), and pH induced flocculation (pH) compared with aluminum sulphate (AL) and chitosan (CH).

	CS	ECF	pH	AL	CH
Medium type^1	F	M	F	F	F
Dosage (ton tonDW^{-1})	0.1	0.02	0.02-0.05	0.15	0.03
Final concentration^2 (%)	3.8	0.4	0.6	1.2	0.6
Biomass contamination risk	low	medium	low	high	low
OPEX ^{3} (\$ tonDW^{-1})	150	80	<50	300	500

^{1}: freshwater (F) or marine (M)
^{2}: initial concentration is 0.04 %
^{3}: estimated by flocculant cost

restrictions on the further downstream processes or upstream medium recycling. Chemical flocculation has the disadvantage that it results in contamination of the biomass, although the use of natural polymers such as cationic starch may minimize this problem. Electro-coagulation flocculation has a low electricity demand when the method is used in seawater and may be a promising low-cost method for harvesting marine microalgae cultivated for biofuel production. pH induced flocculation holds promise as low-cost flocculation method but also results in contamination of the biomass, albeit with mineral precipitates with only low toxicity.

Other novel flocculation approaches are currently under development. The use of magnetic nanoparticles could facilitate magnetic separation of microalgae that are bound to those magnetic particles. Those nanoparticles are today prohibitively expensive but their cost may go down in the future if new methods for producing them become available. In addition, recycling these particles is needed to improve the feasibility of this method. Bioflocculation by addition of flocculating microalgae [158, 159], fungi [207] or bacteria [183] has also potential. This method requires cultivation of these flocculating microorganisms, but the cost for doing so is substantially lower than the cost of centrifugation. This cost can be avoided altogether if non-flocculating microalgae are co-cultivated with flocculating microalgae or bacteria. The latter is possible if the medium contains a carbon source for the bacteria, as is often the case in wastewater. This approach holds great potential and deserves further research. Additionally, controlled flocculation of microalgae through infochemicals or genetic modification is a promising technology but requires further basic research before it can be applied [67, 178]. The use of both infochemicals and genetic modification is likely to be highly species specific, which implies that research and development investments will have to be made for each species of microalgae that is used for large-scale production. So far, virtually no information is available on the identity of infochemicals that induce flocculation. For most species of microalgae, a toolbox for genetic modification is not yet available.

7.2 Influence of organic matter on flocculation

During our experiments, we observed large differences in flocculation efficiency of microalgae that were resuspended in fresh medium compared to microalgae that were left in their original cultivation medium. We suspected that this was due to organic matter produced by the microalgae. This was studied in detail by comparing five flocculation methods: the three modes developed in this thesis (CS, ECF, pH) and the two reference methods (AL and CH). Our results showed that algal organic matter (AOM) is important to take into account. AOM present in *Chlorella* cultures interfered with all tested flocculation modes which may have substantial consequences for the cost of flocculation. We showed that organic matter has an effect, but nothing is yet known about the differences in the effect of organic matter between species or about the influence of culture conditions on quantity and composition of organic matter. Although in our experiments, only inhibition of flocculation was observed, other studies have reported promotion of flocculation in the presence of AOM in specific conditions for *Scenedesmus obliquus* [15]. This demonstrates the need for further fundamental research to understand the interaction between AOM and flocculation as function of microalgae species and cultivation conditions.

7.3 Sludge and floc properties as a parameter for integration of flocculation as a two-step harvesting process

When flocculation is used for harvesting microalgae, it is part of a two-step harvesting process in which flocculation is used to preconcentrate the biomass to a slurry with a 1-4% dry matter content before final dewatering to a paste with a 25% dry matter content using a physical method (Fig 7.1). The more water can be removed during the first flocculation step, the lower the cost for the second mechanical dewatering step will be [93]. To minimize the cost for mechanical dewatering, it is also important that flocculation results in a small algal sludge volume [47]. Furthermore, the rate at which water can be removed during flocculation and sedimentation is important as rapidly settling flocs will require a smaller harvesting unit and thus incur lower investment costs for subsequent sedimentation by gravity thickeners or lamella separators.

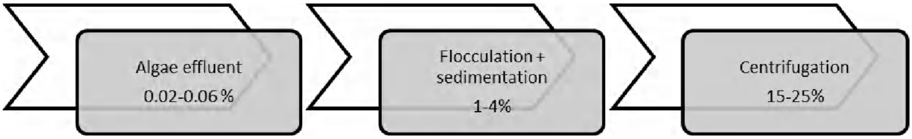


Figure 7.1: Flow diagram for the harvesting of microalgae biomass: integration of flocculation as part of a two-step harvesting process.

So far, few studies on flocculation of microalgae have taken parameters such as the sludge volume or the sedimentation rate into account [71]. In this study, we therefore evaluated slurry volume by assessing the concentration factor, floc size and settling rate for the three modes developed in this study (CS, ECF, pH) compared with two reference modes (Al, CH). Our studies showed that the introduction of a flocculation based concentration method is able to increase solid concentration from an initial concentration of 0.04% to maximal 3.8% (Table 7.1). Although there was a strong variation between the different flocculation modes, we minimally noted a 15-fold increase in solid concentration for every tested method. This significant reduction in microalgal biomass slurry that needs further dewatering (for example by centrifugation) underscores the advantage of using flocculation in a two-step harvesting process. Large differences in settling rates and floc size were observed. This has important implications for the choice of flocculation method. It is therefore recommended that the assessment of concentration factor, floc size and settling rate is included in future studies.

7.4 Short and medium term perspectives

This study has demonstrated the potential of flocculation based harvesting methods for microalgae biomass production. The proof of concept given in this study for flocculation using cationic starch, electro-coagulation flocculation and pH induced flocculation already have and will continue to trigger new initiatives towards the integration of flocculation in existing harvesting processes. Cationic starches can be designed tailored to a specific microalgae species, which also enables dose optimization. New types of electrodes or process regimes (e.g. polarity exchange) can further lead to improved harvesting by electro-coagulation flocculation. Preliminary results of pH induced flocculation for marine species offer favourable prospects. The high magnesium concentrations in marine media are beneficial in lowering the sensitivity of the harvesting system to mineral depletion. Moreover, the use of base or acids to apply pH induced flocculation or to recover magnesium will have less impact on the salinity in marine systems. Finally, the development of novel approaches such as recyclable magnetic nanoparticles as coagulants or bioflocculation using bacteria, other algae, infochemicals or flocculation induced by genetic modification could greatly reduce the costs and lower contamination risks in the medium term.

Algal organic matter (AOM) was shown to interfere with all tested flocculation modes, which may have a substantial impact on the cost of flocculation. At present, the underlying mechanisms of the production of AOM and the interference with flocculation remains a matter of conjecture. Further research is needed to understand the relationship between the dynamics of AOM released into the medium or attached to the cell wall and its interference with flocculation.

Our study showed that settling rate, floc size and floc compaction are important parameters next to flocculation efficiency. But after flocculation, additional dewatering is needed by for example centrifugation or filtration. Further insights in the flocculation mechanisms could improve the dewaterability of microalgal biomass during sedimentation. Therefore it would be interesting to assess floc density as well. This can result in the development of efficient flocculation aids. An integrated approach is therefore recommended in future work to study the impact of flocculation on the efficiency of subsequent dewatering methods. Finally, the biomass needs to undergo further handling which will imply shear stress during pumping. The stress resistance of the obtained flocs should be incorporated as well in future studies concerning the evaluation of floc characteristics of microalgal biomass.

7.5 Long term perspectives

Improved living standards in developing and transitional economies trigger an increasing demand for natural resources for food, feed and fuel. The worldwide concerns about CO_2 emissions and the need for resource security have boosted decision makers to increase research funding for projects concerning renewable sources of energy. Currently, microalgae are considered to be one of the most promising new sources for biofuels production. The responsibility of researches lies in the objective assessment of the potential of these new approaches including fuel from microalgae.

Several key publications in high impact scientific journals have projected scenarios to bring the promise of fuel from microalgae into reality. To replace 17% of petroleum that has been imported into the USA in 2008, an area the size of South Carolina ($= 8.2 \times 10^6$ ha) is needed for microalgae cultivation. Moreover, a quarter of the total amount of water the country currently uses for irrigated agriculture is needed when freshwater microalgae are used [192]. For Europe, if all transportation fuels need to be replaced by biodiesel from microalgae, the land the size of Portugal ($= 9.2 \times 10^6$ ha) is needed [194]. These numbers show that a change from fossil to renewable fuels from microalgae will still have an impact on society and environment.

These types of projections are probably important for decision makers, but from a scientific point of view, this kind of analyses contain a lot of uncertainty. Industrial experience on this large scale is not present and therefore the pre-assumptions made during those analyses are more important than the actual outcome. New initiatives taken to install demo plants worldwide will hopefully deliver additional data and remove uncertainty about productivities. It is reasonable to think that fuel from microalgae has a future as a by-product in an integrated process where value is created by extracted compounds. Nutrient and water recuperation will be crucial in order to meet sustainability criteria. Similar to other biorefinery concepts, the maturity of the industry together with a suited legislative governance will determine the success of microalgae as a commodity for the upcoming biobased economy.

Bibliography

[1] A.L. Ahmad, S. Sumathi, and B.H. Hameed. Coagulation of residue oil and suspended solid in palm oil mill effluent by chitosan, alum and PAC. *Chemical Engineering Journal*, 118(1-2):99–105, 2006.

[2] V. Alexeyev. *Quantitative Analysis*. University Press of the Pacific, 2000.

[3] C. G. Alfafara, K. Nakano, N. Nomura, T. Igarashi, and M. Matsumura. Operating and scale-up factors for the electrolytic removal of algae from eutrophied lakewater. *Journal of Chemical Technology & Biotechnology*, 77(8):871–876, 2002.

[4] Z. Amini Khoeyi, J. Seyfabadi, and Z. Ramezanpour. Effect of light intensity and photoperiod on biomass and fatty acid composition of the microalgae, Chlorella vulgaris. *Aquaculture International*, 20(1):41–49, 2011.

[5] G. H. Azarian, A. R. Mesdaghinia, F. Vaezi, R. Nabizadeh, and D. Nematollahi. Algae removal by electro-coagulation process, application for treatment of the effluent from an industrial wastewater treatment plant. *Iranian Journal of Public Health*, 36(4):57–64, 2007.

[6] D. H. Bache and R. Gregory. *Flocs in Water Treatment*. IWA Publishing, 2007.

[7] D. H. Bache, E. Rasool, D. Moffat, and F. McGilligan. On the strength and character of alumino-humic flocs. *Water Science and Technology*, 40(9):81–88, 1999.

[8] E. W. Becker. *Microalgae: Biotechnology and Microbiology*. Cambridge University Press, 1993.

[9] M. W. Beijerinck. Kulturversuche mit Zoochlorellen, Leichengonidien und anderen niederen Algen. *Botanisch Zeitschrift*, 48:725–739, 1890.

[10] J. Beltrán-Heredia and J. Sánchez-Martín. Municipal wastewater treatment by modified tannin flocculant agent. *Desalination*, 249(1):353–358, 2009.

[11] A. Ben-Amotz and M. Avron. The biotechnology of cultivating the halotolerant alga Dunaliella. *Trends in Biotechnology*, 8:121–126, 1990.

[12] M. Ben Sasson, W. Calmano, and A. Adin. Iron-oxidation processes in an electroflocculation (electrocoagulation) cell. *Journal of Hazardous Materials*, 171(1-3):704–9, 2009.

[13] H. Bernhardt and J. Clasen. Flocculation of micro-organisms. *Journal of Water Supply: Research and Technology - Aqua*, 40(2):76–87, 1991.

[14] H. Bernhardt, O. Hoyer, H. Schell, and B. Lusse. Reaction mechanisms involved in the influence of algogenic organic matter on flocculation. *Zeitschrift fur Wasser und Abwasser Forschung*, 18(1), 1985.

[15] H. Bernhardt, H. Schell, O. Hoyer, and B. Lüsse. Influence of algogenic organic substances on flocculation and filtration. *WISA*, 1:41–57, 1989.

[16] M. R. Bilad, D. Vandamme, I. Foubert, K. Muylaert, and I. F. J. Vankelecom. Harvesting microalgal biomass using submerged microfiltration membranes. *Bioresource Technology*, 111:343–52, 2012.

[17] D. Bilanovic, G. Shelef, and A. Sukenik. Flocculation of microalgae with cationic polymers - Effects of medium salinity. *Biomass*, 17(1):65–76, 1988.

[18] M. Björklund and L. Waberg. Flocculation of cellulosic fibres following addition of cationic starch. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 105(2-3):199–209, 1995.

[19] A. Black and M. Vilaret. Effect of particle size on turbidity removal. *Journal American Water Works Association*, 61:209–214, 1969.

[20] A. Blanchemain and D. Grizeau. Increased production of eicosapentaenoic acid by Skeletonema costatum cells after decantation at low temperature. *Biotechnology Techniques*, 13:497–501, 1999.

[21] A. Blanchemain, D. Grizeau, and J. Guary. Effect of different organic buffers on the growth of Skeletonema costatum cultures; further evidence for an autoinhibitory effect. *Journal of Plankton Research*, 16(10):1433–1440, 1994.

[22] B. Bolto and J. Gregory. Organic polyelectrolytes in water treatment. *Water Research*, 41(11):2301–24, 2007.

[23] M.A. Borowitzka and N.R. Moheimani. *Algae for Biofuels and Energy.* Developments in applied phycology. Springer, 2012.

[24] R. Bosma, W.A. Van Spronsen, J. Tramper, and R. H. Wijffels. Ultrasound, a new separation technique to harvest microalgae. *Journal of Applied Phycology*, 15:143–153, 2003.

[25] A. Bozarth, U. G. Maier, and S. Zauner. Diatoms in biotechnology: modern tools and applications. *Applied Microbiology and Biotechnology*, 82(2):195–201, 2009.

[26] J. Bratby. *Coagulation and flocculation in water and wastewater treatment.* IWA Publishing, London, UK, 2006.

[27] S. Bratskaya, S. Schwarz, J. Laube, T. Liebert, T. Heinze, O. Krentz, C. Lohmann, and W. M. Kulicke. Effect of Polyelectrolyte Structural Features on Flocculation Behavior: Cationic Polysaccharides vs. Synthetic Polycations. *Macromolecular Materials and Engineering*, 290(8):778–785, 2005.

[28] L. Brennan and P. Owende. Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews*, 14(2):557–577, 2010.

[29] L. B. Brentner, M. J. Eckelman, and J. B. Zimmerman. Combinatorial life cycle assessment to inform process design of industrial production of algal biodiesel. *Environmental Science & Technology*, 45(16):7060–7, 2011.

[30] P. Brezonik and W. Arnold. *Water Chemistry: An Introduction to the Chemistry of Natural and Engineered Aquatic Systems.* Oxford University Press, USA, 2011.

[31] M. R. Brown, M. A. McCausland, and K. Kowalski. The nutritional value of four Australian microalgal strains fed to Pacific oyster Crassostrea gigas spat. *Aquaculture*, 165(3):281–293, 1998.

[32] J.S. Burlew. *Algal culture from laboratory to pilot plant.* 1953.

[33] P. Cañizares, F. Martínez, C. Jiménez, J. Lobato, and M.A. Rodrigo. Coagulation and electrocoagulation of wastes polluted with dyes. *Environmental Science & Technology*, 40(20):6418–6424, 2006.

[34] P. Cañizares, F. Martínez, C. Jiménez, C. Sáez, and M. A. Rodrigo. Technical and economic comparison of conventional and electrochemical

coagulation processes. *Journal of Chemical Technology & Biotechnology*, 84(5):702–710, 2009.

[35] T. Cai, S. Y. Park, and Y. Li. Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews*, 19:360–369, 2013.

[36] M. Castrillo, L. M. Lucas-Salas, C. Rodríguez-Gil, and D. Martínez. High pH-induced flocculation-sedimentation and effect of supernatant reuse on growth rate and lipid productivity of Scenedesmus obliquus and Chlorella vulgaris. *Bioresource Technology*, 128:324–9, 2013.

[37] M. Cerff, M. Morweiser, R. Dillschneider, A. Michel, K. Menzel, and C. Posten. Harvesting fresh water and marine algae by magnetic separation: screening of separation parameters and high gradient magnetic filtration. *Bioresource Technology*, 118:289–95, 2012.

[38] C. Chen, K. Yeh, R. Aisyah, D. Lee, and J. Chang. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresource Technology*, 102(1):71–81, 2010.

[39] L. Chen, P. Li, Z. Liu, and Q. Jiao. The released polysaccharide of the cyanobacterium Aphanothece halophytica inhibits flocculation of the alga with ferric chloride. *Journal of Applied Phycology*, 21(3):327–331, 2008.

[40] Y. Chisti. Biodiesel from microalgae. *Biotechnology Advances*, 25(3):294–306, 2007.

[41] Y. Chisti. Biodiesel from microalgae beats bioethanol. *Trends in Biotechnology*, 26(3):126–31, 2008.

[42] L. Christenson and R. Sims. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances*, 29(6):686–702, 2011.

[43] A. Cid, C. Herrero, E. Torres, and J. Abalde. Copper toxicity on the marine microalga Phaeodactylum tricornutum: effects on photosynthesis and related parameters. *Aquatic Toxicology*, 31(2):165–174, 1995.

[44] J. Cohen. Improved jar test procedure. *Journal American Water Works Association*, pages 1425–1431, 1957.

[45] T. Coward, J. G. M. Lee, and G. S. Caldwell. Development of a foam flotation system for harvesting microalgae biomass. *Algal Research*, pages 1–10, 2013.

[46] R. Craggs, D. Sutherland, and H. Campbell. Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. *Journal of Applied Phycology*, 24(3):329–337, 2012.

[47] M. K. Danquah, L. Ang, N. Uduman, N. Moheimani, and G. M. Forde. Dewatering of microalgal culture for biodiesel production: exploring polymer flocculation and tangential flow filtration. *Journal of Chemical Technology & Biotechnology*, 84(7):1078–1083, 2009.

[48] M. K. Danquah, B. Gladman, N. Moheimani, and G. M. Forde. Microalgal growth characteristics and subsequent influence on dewatering efficiency. *Chemical Engineering Journal*, 151(1-3):73–78, 2009.

[49] J. A. Del Campo, M. García-González, and M. G. Guerrero. Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. *Applied Microbiology and Biotechnology*, 74(6):1163–74, 2007.

[50] W. Den and C. Huang. Electrocoagulation of silica nanoparticles in wafer polishing wastewater by a multichannel flow reactor: A kinetic study. *Journal of Environmental Engineering*, 132(12):1651–1658, 2006.

[51] R. Divakaran and V. S. Pillai. Flocculation of algae using chitosan. *Journal of Applied Phycology*, 14:419–422, 2002.

[52] R. B. Draaisma, R. H. Wijffels, E. Slegers, L. B. Brentner, A. Roy, and M. J. Barbosa. Food commodities from microalgae. *Current Opinion in Biotechnology*, pages 1–9, 2012.

[53] J. Duan and J. Gregory. Influence of soluble silica on coagulation by aluminium sulphate. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 107:309–319, 1996.

[54] J. Duan and J. Gregory. Coagulation by hydrolysing metal salts. *Advances in Colloid and Interface Science*, 100-102:475–502, 2003.

[55] M. Dubois, K.A. Gilles, J.K. Hamilton, and P.A. Rebers. Colorimetric method for determination of sugars and related substances. *Analytical Chemical Society*, 25(3):350–356, 1956.

[56] S. Elmaleh and A. Jabbouri. Flocculation energy requirement. *Water Research*, 25(8):939–943, 1991.

[57] M. M. Emamjomeh and M. Sivakumar. Fluoride removal by a continuous flow electrocoagulation reactor. *Journal of Environmental Management*, 90(2):1204–12, 2009.

[58] W. Farooq, Y. Lee, J. Han, C. H. Darpito, M. Choi, and J. Yang. Efficient microalgae harvesting by organo-building blocks of nanoclays. *Green Chemistry*, 15(3):749, 2013.

[59] C. M. Fellows and W. O. S. Doherty. Insights into bridging flocculation. In *Macromolecular Symposia*, volume 231, pages 1–10. John Wiley & Sons, 2006.

[60] Y. Feng, C. Li, and D. Zhang. Lipid production of Chlorella vulgaris cultured in artificial wastewater medium. *Bioresource Technology*, 102(1):101–5, 2011.

[61] J. A. Foley, N. Ramankutty, K. A. Brauman, E. S. Cassidy, J. S. Gerber, M. Johnston, N. Mueller, C. O'Connell, D. K. Ray, P. C. West, C. Balzer, E. M. Bennett, S. R. Carpenter, J. Hill, C. Monfreda, S. Polasky, J. Rockström, J. Sheehan, S. Siebert, D. Tilman, and D. P. M. Zaks. Solutions for a cultivated planet. *Nature*, 478(7369):337–42, 2011.

[62] I. Fraeye, C. Bruneel, C. Lemahieu, J. Buyse, K. Muylaert, and I. Foubert. Dietary enrichment of eggs with omega-3 fatty acids: A review. *Food Research International*, 48(2):961–969, 2012.

[63] S. Gao, M. Du, J. Tian, J. Yang, F. Ma, and J. Nan. Effects of chloride ions on electro-coagulation-flotation process with aluminum electrodes for algae removal. *Journal of Hazardous Materials*, 182(1-3):827–34, 2010.

[64] S. Gao, J. Yang, J. Tian, F. Ma, G. Tu, and M. Du. Electro-coagulation-flotation process for algae removal. *Journal of Hazardous Materials*, 177(1-3):336–43, 2010.

[65] A. J. Garzon-Sanabria, R. T. Davis, and Z. L. Nikolov. Harvesting Nannochloris oculata by inorganic electrolyte flocculation: effect of initial cell density, ionic strength, coagulant dosage, and media pH. *Bioresource Technology*, 118:418–24, 2012.

[66] R. Geider and J. Roche. Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *European Journal of Phycology*, 37(1):1–17, 2002.

[67] D. R. Georgianna and S. P. Mayfield. Exploiting diversity and synthetic biology for the production of algal biofuels. *Nature*, 488(7411):329–35, 2012.

[68] C. G. Golueke and W. J. Oswald. Surface properties and ion exchange in algae removal. *Journal Water Pollution Control Federation*, 42(8):304–314, 1970.

[69] L. Gouveia. *Microalgae as a Feedstock for Biofuels.* Springer, 2011.

[70] P. Govender, J. L. Domingo, M. C. Bester, I. S. Pretorius, and F. F. Bauer. Controlled expression of the dominant flocculation genes FLO1, FLO5, and FLO11 in Saccharomyces cerevisiae. *Applied and Environmental Microbiology*, 74(19):6041–52, 2008.

[71] M. R. Granados, F. G. Acién, C. Gómez, J. M. Fernández-Sevilla, and E. Molina Grima. Evaluation of flocculants for the recovery of freshwater microalgae. *Bioresource Technology*, 118:102–10, 2012.

[72] H. C. Greenwell, L. M. L. Laurens, R. J. Shields, R. W. Lovitt, and K. J. Flynn. Placing microalgae on the biofuels priority list: a review of the technological challenges. *Journal of the Royal Society, Interface*, 7(46):703–26, 2010.

[73] J. Gregory. *Particles in Water. Properties and Processes.* Number 20. International Water Association, May 2006.

[74] J. Gregory and J. Duan. Hydrolyzing metal salts as coagulants. *Pure and Applied Chemistry*, 73(12):2017–2026, 2001.

[75] M. J. Griffiths, C. Garcin, R. P. van Hille, and S. T. L. Harrison. Interference by pigment in the estimation of microalgal biomass concentration by optical density. *Journal of Mcrobiological Methods*, 85(2):119–23, 2011.

[76] M. J. Griffiths, R. P. Hille, and S. T. L. Harrison. Lipid productivity, settling potential and fatty acid profile of 11 microalgal species grown under nitrogen replete and limited conditions. *Journal of Applied Phycology*, 24(5):989–1001, 2011.

[77] J. U. Grobbelaar, L. Nedbal, and V. Tichý. Influence of high frequency light/dark fluctuations on photosynthetic characteristics of microalgae photoacclimated to different light intensities and implications for mass algal cultivation. *Journal of Applied Phycology*, 8(4-5):335–343, 1996.

[78] C. Guedes, H. M. Amaro, and F. X. Malcata. Microalgae as sources of high added-value compounds–a brief review of recent work. *Biotechnology Progress*, 27(3):597–613, 2011.

[79] R. R. L. Guillard and C. L. Lorenzen. Yellow-green algae with chlorophyllide c. *Journal of Phycology*, 8:1–14, 1972.

[80] G. Gutzeit, D. Lorch, A. Weber, M. Engels, and U. Neis. Bioflocculent algal-bacterial biomass improves low-cost wastewater treatment. *Water Science and Technology*, 52(12):9–18, 2005.

[81] S. Hadjoudja, V. Deluchat, and M. Baudu. Cell surface characterisation of Microcystis aeruginosa and Chlorella vulgaris. *Journal of Colloid and Interface Science*, 342(2):293–9, 2010.

[82] B. P. Han, M. Virtanen, J. Koponen, and M. Straškraba. Effect of photoinhibition on algal photosynthesis: a dynamic model. *Journal of Plankton Research*, 22(5):865–885, 2000.

[83] J. Hanotu, H. C. Bandulasena, and W. B. Zimmerman. Microflotation performance for algal separation. *Biotechnology and Bioengineering*, 109(7):1663–73, 2012.

[84] J. Haveren, E. L. Scott, and J. Sanders. Bulk chemicals from biomass. *Biofuels, Bioproducts and Biorefining*, 2:41–57, 2008.

[85] R. K. Henderson, S. A. Parsons, and B. Jefferson. Successful Removal of Algae through the Control of Zeta Potential. *Separation Science and Technology*, 43(7):1653–1666, 2008.

[86] R. K. Henderson, S. A. Parsons, and B. Jefferson. The impact of algal properties and pre-oxidation on solid-liquid separation of algae. *Water Research*, 42(8-9):1827–45, 2008.

[87] R. K. Henderson, S. A. Parsons, and B. Jefferson. The impact of differing cell and algogenic organic matter (AOM) characteristics on the coagulation and flotation of algae. *Water Research*, 44(12):3617–24, 2010.

[88] P. K. Holt, G. W. Barton, and C.A. Mitchell. The future for electrocoagulation as a localised water treatment technology. *Chemosphere*, 59(3):355–67, 2005.

[89] O. Hoyer, B. Lüsse, and H. Bernhardt. Isolation and characterization of extracellular organic matter (EOM) from algae. *Zeitschrift für Wasser und Abwasser*, 18(2):319–90, 1985.

[90] C. J. Hulatt and D. N. Thomas. Dissolved organic matter (DOM) in microalgal photobioreactors: a potential loss in solar energy conversion? *Bioresource Technology*, 101(22):8690–7, 2010.

[91] Sema Şirin, Rosa Trobajo, Carles Ibanez, and Joan Salvadó. Harvesting the microalgae Phaeodactylum tricornutum with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced flocculation. *Journal of Applied Phycology*, 24(5):1067–1080, November 2011.

[92] A. Javaheri and R. Dick. Aggregate size variations during thickening of activated sludge. *Journal of Water Pollution Control Federation*, pages 197–214, 1969.

[93] O. Jorquera, A. Kiperstok, E. A. Sales, M. Embiruçu, and M. L. Ghirardi. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresource Technology*, 101(4):1406–13, 2010.

[94] J. Kim, B. Ryu, B. Kim, J. Han, and J. Yang. Continuous microalgae recovery using electrolysis with polarity exchange. *Bioresource Technology*, 111:268–75, 2012.

[95] J. Kim, B. Ryu, K. Kim, B. Kim, J. Han, and J. Yang. Continuous microalgae recovery using electrolysis: effect of different electrode pairs and timing of polarity exchange. *Bioresource Technology*, 123:164–70, 2012.

[96] T. Kim, C. Park, E. Shin, and S. Kim. Decolorization of disperse and reactive dyes by continuous electrocoagulation process. *Desalination*, 150(2):165–175, 2002.

[97] A. M. J. Kliphuis, L. de Winter, C. Vejrazka, D. E. Martens, M. Janssen, and R. H. Wijffels. Photosynthetic efficiency of Chlorella sorokiniana in a turbulently mixed short light-path photobioreactor. *Biotechnology Progress*, 26(3):687–96, 2010.

[98] W. R. Knocke, J. R. Hamon, and B. E. Dulin. Effects of coagulation on sludge thickening and dewatering. *Journal of the American Water Works Association*, 79(6):89–98, 1987.

[99] R. Knuckey, M. Brown, R. Robert, and D. Frampton. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. *Aquacultural Engineering*, 35(3):300–313, 2006.

[100] D. O. Krentz, C. Lohmann, S. Schwarz, S. Bratskaya, T. Liebert, J. Laube, T. Heinze, and W. M. Kulicke. Properties and Flocculation Efficiency of Highly Cationized Starch Derivatives. *Starch/Stärke*, 58(3-4):161–169, 2006.

[101] M. Lagi, K. Bertrand, and Y. Bar-yam. The Food Crises and Political Instability in North Africa and the Middle East. *NECSI Food Crisis Research*, pages 1–15, 2011.

[102] A. W. D. Larkum, I. L. Ross, O. Kruse, and B. Hankamer. Selection, breeding and engineering of microalgae for bioenergy and biofuel production. *Trends in Biotechnology*, 30(4):198–205, 2012.

[103] A. Lavoie and J. Noüe. Harvesting of scenedesmus obliquus in wastewaters: Auto- or bioflocculation? *Biotechnology and Bioengineering*, 30:852–859, 1987.

[104] A. K. Lee, D. M. Lewis, and P. J. Ashman. Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel. *Journal of Applied Phycology*, 21(5):559–567, 2008.

[105] R. E. Lee. *Phycology*. Cambridge University Press, 2nd edition, 2008.

[106] S. J. Lee, S. Kim, J. Kim, G. Kwon, B. Yoon, and H. Oh. Effects of harvesting method and growth stage on the flocculation of the green alga Botryococcus braunii. *Letters in Applied Microbiology*, 27(1):14–18, 1998.

[107] Y. Lee, B. Kim, W. Farooq, J. Chung, J. Han, H. Shin, S. Jeong, J. Park, J. Lee, and Y. Oh. Harvesting of oleaginous Chlorella sp. by organoclays. *Bioresource Technology*, 132:440–5, 2013.

[108] J. Leentvaar and M. Rebhun. Effect of magnesium and calcium precipitation on coagulation-flocculation with lime. *Water Research*, 16:655–662, 1982.

[109] T. Li, Z. Zhu, D. Wang, C. Yao, and H. Tang. Characterization of floc size, strength and structure under various coagulation mechanisms. *Powder Technology*, 168(2):104–110, 2006.

[110] J. Lim, D. C. J. Chieh, S. A. Jalak, P. Yi. Toh, N. Hi. M. Yasin, B. W. Ng, and A. L. Ahmad. Rapid magnetophoretic separation of microalgae. *Small*, 8(11):1683–92, 2012.

[111] J. Lin, C. Huang, C. Chin, and J. R. Pan. Coagulation dynamics of fractal flocs induced by enmeshment and electrostatic patch mechanisms. *Water Research*, 42(17):4457–66, 2008.

[112] D. Liu, F. Li, and B. Zhang. Removal of algal blooms in freshwater using magnetic polymer. *Water Science and Technology*, 59(6):1085–91, 2009.

[113] L. M. Lubián. Concentrating cultured marine microalgae with chitosan. *Aquacultural Engineering*, 8:257–265, 1989.

[114] T. J. Lundquist, I. C. Woertz, N. W. T. Quinn, and J. R. Benemann. A realistic technology and engineering assessment of algae biofuel production. Technical Report October, Energy Biosciences Institute, 2010.

[115] F. X. Malcata. Microalgae and biofuels: a promising partnership? *Trends in Biotechnology*, 29(11):542–9, 2011.

[116] N. Mameri, A. R. Yeddou, H. Lounici, D. Belhocine, H. Grib, and B. Bariou. Defluoridation of septentrional Sahara water of north Africa by electrocoagulation process using bipolar aluminium electrodes. *Water Research*, 32(5):1604–1612, 1998.

[117] T. M. Mata, A. A. Martins, and N. S. Caetano. Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, 14(1):217–232, 2010.

[118] C.T. Matos, M. Santos, B.P. Nobre, and L. Gouveia. Nannochloropsis sp. biomass recovery by Electro-Coagulation for Biodiesel and Pigment production. *Bioresource Technology*, 2013.

[119] M. Mendez, C. Behnke, Y. Poon, and P. Lee. Induction of flocculation in photosynthetic organisms, 2009.

[120] J. J. Milledge and S. Heaven. A review of the harvesting of micro-algae for biofuel production. *Reviews in Environmental Science and Biotechnology*, 2012.

[121] E. Molina Grima, E. H. Belarbi, F. G. Acién Fernández, A. Robles Medina, and Y. Chisti. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology Advances*, 20(7-8):491–515, 2003.

[122] M. Y. Mollah, P. Morkovsky, J. A. G. Gomes, M. Kesmez, J. Parga, and D. L. Cocke. Fundamentals, present and future perspectives of electrocoagulation. *Journal of Hzardous Materials*, 114(1-3):199–210, 2004.

[123] M. Y. Mollah, R. Schennach, J . R. Parga, and D. L. Cocke. Electrocoagulation (EC)–science and applications. *Journal of Hazardous Materials*, 84(1):29–41, 2001.

[124] J. Morales, J. De La Noüe, and G. Picard. Harvesting marine microalgae species by chitosan flocculation. *Aquacultural Engineering*, 4:257–270, 1985.

[125] M. Morweiser, O. Kruse, B. Hankamer, and C. Posten. Developments and perspectives of photobioreactors for biofuel production. *Applied Microbiology and Biotechnology*, 87(4):1291–301, 2010.

[126] G. Mouedhen, M. Feki, M. De Petris Wery, and H. F. Ayedi. Behavior of aluminum electrodes in electrocoagulation process. *Journal of Hazardous Materials*, 150(1):124–35, 2008.

[127] S. M. Myklestad. Release of extracellular products by phytoplankton with special emphasis on polysaccharides. *Science of the Total Environment*, 165:155–164, 1995.

[128] A. Neori. “Green water” microalgae: the leading sector in world aquaculture. *Journal of Applied Phycology*, 23(1):143–149, 2011.

[129] W. Ngah and A Musa. Adsorption of Humic Acid onto Chitin and Chitosan. *Polymer*, pages 2305–2310, 1997.

[130] S. Nonhebel and T. Kastner. Changing demand for food, livestock feed and biofuels in the past and in the near future. *Livestock Science*, 139(1-2):3–10, 2011.

[131] N. Norsker, M. J. Barbosa, M. H. Vermuë, and R. H. Wijffels. Microalgal production–a close look at the economics. *Biotechnology Advances*, 29(1):24–7, 2011.

[132] Y. Nurdogan and W. J. Oswald. Enhanced nutrient removal in high-rate ponds. *Water Science and Technology*, 31(12):33–43, 1995.

[133] E. J. Olguín. Dual purpose microalgae-bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a biorefinery. *Biotechnology Advances*, 30(5):1031–46, 2012.

[134] W. J. Oswald and J. R. Benemann. A critical analysis of bioconversion with microalgae. In *Biological Solar Energy Conversion*, pages 379–396. Academic, New York, 1977.

[135] W. J. Oswald and C. G. Golueke. Biological transformation of solar energy. *Advances in Applied Microbiology*, 2:223–262, 1960.

[136] S. Pal, D. Mal, and R. Singh. Cationic starch: an effective flocculating agent. *Carbohydrate Polymers*, 59(4):417–423, 2005.

[137] A. Papazi, P. Makridis, and P. Divanach. Harvesting Chlorella minutissima using cell coagulants. *Journal of Applied Phycology*, 22(3):349–355, 2009.

[138] L. Peperzak. Phytoplankton sinking rates in the Rhine region of freshwater influence. *Journal of Plankton Research*, 25(4):365–383, 2003.

[139] G. J. Piazza and R. A. Garcia. Proteins and peptides as renewable flocculants. *Bioresource Technology*, 101(15):5759–66, 2010.

[140] P. Přibyl, V. Cepák, and V. Zachleder. Production of lipids in 10 strains of Chlorella and Parachlorella, and enhanced lipid productivity in Chlorella vulgaris. *Applied Microbiology and Biotechnology*, 94(2):549–61, 2012.

[141] P. T. Pienkos and A. Darzins. The promise and challenges of microalgal-derived biofuels. *Biofuels, Bioproducts and Biorefining*, 3(4):431–440, 2009.

[142] J. K. Pittman, A. P. Dean, and O. Osundeko. The potential of sustainable algal biofuel production using wastewater resources. *Bioresource Technology*, 102(1):17–25, 2011.

[143] M. Pivokonsky, O. Kloucek, and L. Pivokonska. Evaluation of the production, composition and aluminum and iron complexation of algogenic organic matter. *Water Research*, 40(16):3045–52, 2006.

[144] E. Poelman, N. Depauw, and B. Jeurissen. Potential of electrolytic flocculation for recovery of micro-algae. *Resources, Conservation and Recycling*, 19(1):1–10, 1997.

[145] C. Posten and G. Schaub. Microalgae and terrestrial biomass as source for fuels–a process view. *Journal of Biotechnology*, 142(1):64–9, 2009.

[146] A. Prakash, S. Solanski, and S. Zhu. Treatment of textile effluent by cationic starches: reclamation of wastewater. *Pollution Research*, 26:19–25, 2007.

[147] G. Prochazkova, I. Safarik, and T. Branyik. Harvesting microalgae with microwave synthesized magnetic microparticles. *Bioresource Technology*, 130C:472–477, 2012.

[148] R. Raja, S. Hemaiswarya, N. A. Kumar, S. Sridhar, and R. Rengasamy. A perspective on the biotechnological potential of microalgae. *Critical Reviews in Microbiology*, 34(2):77–88, 2008.

[149] M. N. V. Ravi Kumar. A review of chitin and chitosan applications. *Reactive and Functional Polymers*, 46(1):1–27, 2000.

[150] M. M. Rebolloso-Fuentes, A. Navarro-Pérez, J. J. Ramos-Miras, and J. L. Guil-Guerrero. Biomass nutrient profiles of the microalga Phaeodactylum tricornutum. *Journal of Food Biochemistry*, 25(1):57–76, 2001.

[151] F. Renault, B. Sancey, P. M. Badot, and G. Crini. Chitosan for coagulation/flocculation processes – An eco-friendly approach. *European Polymer Journal*, 45(5):1337–1348, 2009.

[152] A. Richmond. *Handbook of microalgal culture: biotechnology and applied phycology*. Wiley-Blackwell, 2008.

[153] R. J. Ritchie, K. Guy, and J. C. Philp. Policy to support marine biotechnology-based solutions to global challenges. *Trends in biotechnology*, 31(3):128–31, 2013.

[154] L. Roef, M. Jacqmain, and M. Michiels. Microalgae production in the selfsupported ProviAPT vertical flat-panel photobioreactor system. In *Microalgal Biotechnology: Potential and Production*, pages 243–246. 2012.

[155] N. Rossignol. Membrane technology for the continuous separation microalgae/culture medium: compared performances of cross-flow microfiltration and ultrafiltration. *Aquacultural Engineering*, 20(3):191–208, 1999.

[156] V. M. Rwehumbiza, R. Harrison, and L. Thomsen. Alum-induced flocculation of preconcentrated Nannochloropsis salina: Residual aluminium in the biomass, FAMEs and its effects on microalgae growth upon media recycling. *Chemical Engineering Journal*, 200-202:168–175, 2012.

[157] E. Ryckebosch, K. Muylaert, M. Eeckhout, T. Ruyssen, and I. Foubert. Influence of Drying and Storage on Lipid and Carotenoid Stability of the Microalga Phaeodactylum tricornutum. *Journal of Agricultural and Food Chemistry*, 59(20):11063–9, 2011.

[158] S. Salim, R. Bosma, M. H. Vermuë, and R. H. Wijffels. Harvesting of microalgae by bio-flocculation. *Journal of Applied Phycology*, 23(5):849–855, 2011.

[159] S. Salim, M. H. Vermuë, and R. H. Wijffels. Ratio between autoflocculating and target microalgae affects the energy-efficient harvesting by bio-flocculation. *Bioresource Technology*, 118:49–55, 2012.

[160] A. Sathish and R. C. Sims. Biodiesel from mixed culture algae via a wet lipid extraction procedure. *Bioresource Technology*, 118:643–7, 2012.

[161] N. Savage. Algae: The scum solution. *Nature*, 474:5–6, 2011.

[162] A. Schlesinger, D. Eisenstadt, A. Bar-Gil, H. Carmely, S. Einbinder, and J. Gressel. Inexpensive non-toxic flocculation of microalgae contradicts theories; overcoming a major hurdle to bulk algal production. *Biotechnology Advances*, 30(5):1023–30, 2012.

[163] M. Scholz, T. Hoshino, D. Johnson, Mark R. Riley, and J. Cuello. Flocculation of wall-deficient cells of Chlamydomonas reinhardtii mutant cw15 by calcium and methanol. *Biomass and Bioenergy*, 35(12):4835–4840, 2011.

[164] L. Semerjian. High-pH–magnesium coagulation–flocculation in wastewater treatment. *Advances in Environmental Research*, 7(2):389–403, 2003.

[165] B. R. Sharma, N. C. Dhuldhoya, and U. C. Merchant. Flocculants—an Ecofriendly Approach. *Journal of Polymers and the Environment*, 14(2):195–202, 2006.

[166] J. Sheehan, T. Dunahay, J. R. Benemann, and P. Roessler. *A look back at the US Department of Energy's Aquatic Species Program: Biodiesel from algae*, volume 328. National Renewable Energy Laboratory Denver, CO, 1998.

[167] G. Shelef, A. Sukenik, and M. Green. Microalgae harvesting and processing: a literature review. Technical Report 0, SERI/STR-231-2396, Technion Research and Development Foundation Ltd., Haifa (Israel), 1984.

[168] M. Shirzad-Semsar, S. Scholz, and W. M. Kulicke. Cationic starches as substitute for synthetic cationic flocculants in solid-liquid separation of harbor sludge. *The Journal of Physical Chemistry B*, 111(29):8641–8, 2007.

[169] R. P. Singh, T. Tripathy, G. P. Karmakar, S. K. Rath, N. C. Karmakar, S. R. Pandey, K. Kannan, S. K. Jain, and N. T. Lan. Novel biodegradable flocculants based on polysaccharides. *Current Science*, 78(7):798–803, 2000.

[170] K. Skjanes, C. Rebours, and P. Lindblad. Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. *Critical Reviews in Biotechnology*, pages 1–44, 2012.

[171] B. T. Smith and R. H. Davis. Sedimentation of algae flocculated using naturally-available, magnesium-based flocculants. *Algal Research*, 1(1):32–39, 2012.

[172] K. Spilling, J. Seppälä, and T. Tamminen. Inducing autoflocculation in the diatom Phaeodactylum tricornutum through CO2 regulation. *Journal of Applied Phycology*, 23(6):959–966, 2011.

[173] P. Spolaore, C. Joannis-Cassan, E. Duran, and A. Isambert. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, 101(2):87–96, 2006.

[174] P. Sridhar, C. Namasivayam, and G. Prabharan. Algae flocculation in reservoir water. *Biotechnology and Bioengineering*, 32:345–347, 1988.

[175] Y. Su, A. Mennerich, and B. Urban. Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture. *Water Research*, 45(11):3351–8, 2011.

[176] A. Sukenik and G. Shelef. Algal autoflocculation-verification and proposed mechanism. *Biotechnology and Bioengineering*, 26:142–147, 1984.

[177] T. Takaara, D. Sano, and H. Konno. Affinity isolation of algal organic matters able to form complex with aluminium coagulant. *Water Science and Technology*, 4:95–102, 2004.

[178] R. L. Taylor, J. D. Rand, and G. S. Caldwell. Treatment with algae extracts promotes flocculation, and enhances growth and neutral lipid content in Nannochloropsis oculata–a candidate for biofuel production. *Marine Biotechnology*, 14(6):774–81, 2012.

[179] C. M. Teixeira, F. V. Kirsten, and P. C. N. Teixeira. Evaluation of Moringa oleifera seed flour as a flocculating agent for potential biodiesel producer microalgae. *Journal of Applied Phycology*, 24(3):557–563, 2012.

[180] O. Tokuşoglu and M.K. Uunal. Biomass Nutrient Profiles of Three Microalgae: Spirulina platensis, Chlorella vulgaris, and Isochrisis galbana. *Journal of Food Science*, 68(4):1144–1148, May 2003.

[181] N. Uduman, Y. Qi, M. K. Danquah, G. M. Forde, and A. Hoadley. Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. *Journal of Renewable and Sustainable Energy*, 2(1):012701, 2010.

[182] J. B. van Beilen. Why microalgal biofuels won't save the internal combustion machine. *Biofuels, Bioproducts and Biorefining*, 4:41–52, 2010.

[183] S. Van Den Hende, H. Vervaeren, H. Saveyn, G. Maes, and N. Boon. Microalgal bacterial floc properties are improved by a balanced inorganic/organic carbon ratio. *Biotechnology and Bioengineering*, 108(3):549–58, 2011.

[184] D. Vandamme, I. Foubert, I. Fraeye, B. Meesschaert, and K. Muylaert. Flocculation of Chlorella vulgaris induced by high pH: role of magnesium and calcium and practical implications. *Bioresource Technology*, 105:114–119, 2012.

[185] D. Vandamme, I. Foubert, I. Fraeye, and K. Muylaert. Influence of organic matter generated by Chlorella vulgaris on five different modes of flocculation. *Bioresource Technology*, 124:508–511, 2012.

[186] D. Vandamme, I. Foubert, B. Meesschaert, and K. Muylaert. Flocculation of microalgae using cationic starch. *Journal of Applied Phycology*, 22(4):525–530, 2010.

[187] D. Vandamme, I. Foubert, and K. Muylaert. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. *Trends in Biotechnology*, 31(4):233–239, 2013.

[188] D. Vandamme, S. C. V. Pontes, K. Goiris, I. Foubert, L. J. J. Pinoy, and K. Muylaert. Evaluation of electro-coagulation-flocculation for harvesting marine and freshwater microalgae. *Biotechnology and Bioengineering*, 108(10):2320–2329, 2011.

[189] M. Vanthoor-Koopmans, R. H. Wijffels, M. J. Barbosa, and M. H. M. Eppink. Biorefinery of microalgae for food and fuel. *Bioresource Technology*, 2012.

[190] T. Viswanathan, S. Mani, K. C Das, S. Chinnasamy, A. Bhatnagar, R. K. Singh, and M. Singh. Effect of cell rupturing methods on the drying characteristics and lipid compositions of microalgae. *Bioresource Technology*, 126C:131–136, 2012.

[191] D. A. Walker. Biofuels, facts, fantasy, and feasibility. *Journal of Applied Phycology*, 21(5):509–517, 2009.

[192] M. S. Wigmosta, A. M. Coleman, R. J. Skaggs, M. H. Huesemann, and L. J. Lane. National microalgae biofuel production potential and resource demand. *Water Resources Research*, 47(3):W00H04, 2011.

[193] R. G. Wijesekara, N. Nomura, and M. Matsumura. Electrochemical removal of ammonia, chemical oxygen demand and energy consumption from aquaculture waters containing different marine algal species. *Journal of Chemical Technology & Biotechnology*, 80(12):1408–1415, 2005.

[194] R. H Wijffels and M. J. Barbosa. An Outlook on Microalgal Biofuels. *Science*, 329:796–799, 2010.

[195] R. H. Wijffels, M. J. Barbosa, and M. H. M. Eppink. Microalgae for the production of bulk chemicals and biofuels. *Biofuels, Bioproducts and Biorefining*, 4(3):287–295, 2010.

[196] P. E. Wiley, K. J. Brenneman, and A. E. Jacobson. Improved Algal Harvesting Using Suspended Air Flotation. *Water Environment Research*, 81(7):702–708, 2009.

[197] Z. Wu, Y. Zhu, W. Huang, C. Zhang, T. Li, Y. Zhang, and A. Li. Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. *Bioresource Technology*, 110:496–502, 2012.

[198] N. B. Wyatt, L. M. Gloe, P. V. Brady, J. C. Hewson, A. M. Grillet, M. G. Hankins, and P. I. Pohl. Critical conditions for ferric chloride-induced flocculation of freshwater algae. *Biotechnology and Bioengineering*, 109(2):493–501, 2012.

[199] L. Xu, C. Guo, F. Wang, S. Zheng, and C. Liu. A simple and rapid harvesting method for microalgae by in situ magnetic separation. *Bioresource Technology*, 102(21):10047–51, 2011.

[200] Y. Xu, S. Purton, and F. Baganz. Chitosan flocculation to aid the harvesting of the microalga Chlorella sorokiniana. *Bioresource Technology*, 129C:296–301, 2012.

[201] H. Yahi, S. Elmaleh, and J. Coma. Algal flocculation-sedimentation by pH increase in a continuous reactor. *Water Science & Technology*, 30:259–267, 1994.

[202] W. Yongmanitchai and O. P. Ward. Growth of and omega-3 fatty acid production by Phaeodactylum tricornutum under different culture conditions. *Applied and Environmental Microbiology*, 57(2):419–25, 1991.

[203] J. Zhang and B. Hu. A novel method to harvest microalgae via co-culture of filamentous fungi to form cell pellets. *Bioresource Technology*, 114:529–35, 2012.

[204] X. Zhang, P. Amendola, J. C. Hewson, M. Sommerfeld, and Q. Hu. Influence of growth phase on harvesting of Chlorella zofingiensis by dissolved air flotation. *Bioresource Technology*, 116:477–84, 2012.

[205] H. Zheng, Z. Gao, J. Yin, X. Tang, X. Ji, and H. Huang. Harvesting of microalgae by flocculation with poly (γ-glutamic acid). *Bioresource Technology*, 112:212–20, 2012.

[206] W. Zhou, Y. Cheng, Y. Li, Y. Wan, Y. Liu, X. Lin, and R. Ruan. Novel fungal pelletization-assisted technology for algae harvesting and wastewater treatment. *Applied Biochemistry and Biotechnology*, 167(2):214–28, 2012.

[207] W. Zhou, M. Min, B. Hu, Xi. Ma, Y. Liu, Q. Wang, J. Shi, P. Chen, and R. Ruan. Filamentous fungi assisted bio-flocculation: A novel alternative technique for harvesting heterotrophic and autotrophic microalgal cells. *Separation and Purification Technology*, 107:158–165, 2013.

[208] S. Zodi, O. Potier, F. Lapicque, and J. Leclerc. Treatment of the industrial wastewaters by electrocoagulation: Optimization of coupled electrochemical and sedimentation processes. *Desalination*, 261(1-2):186–190, 2010.

[209] I. Zongo, A. H. Maiga, J. Wéthé, G. Valentin, J. Leclerc, G. Paternotte, and F. Lapicque. Electrocoagulation for the treatment of textile wastewaters with Al or Fe electrodes: compared variations of COD levels,

turbidity and absorbance. *Journal of Hazardous Materials*, 169(1-3):70–6, 2009.

Curriculum vitae

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Profile

Personality determined - result-oriented - hands-on

Interests biotechnology - renewable energy - ICT - international politics

Sports cycling - mountainbiking - running

Education

Current education

PhD student in Bioscience Engineering
Microbial and Molecular Systems, Lab Aquatic Biology, *KU Leuven Kulak*, Kortrijk, Belgium

PhD Topic: Developing flocculation based harvesting methods for microalgae
Promotors: Prof. dr. ir. Imogen Foubert; Prof. dr. Koenraad Muylaert

Qualifications

2009	Master in Biosciences engineering: Environmental sciences and technology *KU Leuven*, Belgium Thesis project: Optimisation and feasiblity of biofuels from microalgae, *KU Leuven Kulak* - Promoter: Prof. I. Foubert
2007	Bachelor in Biosciences engineering: Environmental sciences and technology *KU Leuven*, Belgium
2002	High School degree (Latin-Mathematics) *Instituut Klein Seminarie*, Roeselare, Belgium

Additional courses and workshops

2013	BePCIS Particle Characterization Courses: Particle Size Analysis & Surface Charge Characterization. UGent, Belgium
2012	EPROBIO 2012 Erasmus intensive program: Biomass and Biofuels University of Foggia, Italy
2012	Exploitation of research - technology & knowledge transfer, KU Leuven, Belgium
2010	Initiation in entrepreneurship KU Leuven, Belgium
2010	Initiation in intellectual property management, Flemish innovation centre, Kortrijk

Work experience

Research experience

2009 - present Lab Aquatic Biology, KU Leuven Kulak, Belgium

For my PhD research, I am investigating the possibilities of flocculation based harvesting methods for microalgae. Working on this project, I have gained experience in the cultivation of microalgae, water processing and coagulation and flocculation methods. In addition to the development of an analytical and scientifically critical attitude.

Educational experience

2012 - 2013	Monitor of student projects, BSc Bioscience Engineering, KU Leuven Kulak
2009 - 2013	Practical courses Inorganic Chemical Analysis, KU Leuven Kulak
2009 - 2013	Practical courses zoology, KU Leuven Kulak
2010 - 2011	MSc thesis supervisor: Ruiming Zhang (MSc in Biology) KU Leuven Kulak
2009 - 2010	MSc thesis supervisor: Sandra Pontes (MSc in Chemical Engineering) Kaho Sint-Lieven Gent & KU Leuven Kulak
2009 - 2010	MSc thesis supervisor: Cynthia Aryshandy (MSc in Ecological Marine Management) VUB Brussels & KU Leuven Kulak

Skills

PC	Windows, Linux Ubuntu, Android MS Office, Open Office, LaTeX Statistica, SAS, Sigmaplot, R, Mathlab
Languages	Dutch: native speaker English: very good French: good German: basic

List of publications

Articles in internationally reviewed academic journals

Vandamme, D., Foubert, I., Meesschaert, B., Muylaert, K. (2010). Flocculation of microalgae using cationic starch. Journal of Applied Phycology, 22 (4), 525-530. IF(2010)=1.700.

Vandamme, D., Pontes, S., Goiris, K., Foubert, I., Pinoy, L., Muylaert, K. (2011). Evaluation of electro-coagulation-flocculation for harvesting marine and freshwater microalgae. Biotechnology and Bioengineering, 108 (10), 2320-2329. IF(2011)=3.946(Q1).

Bilad, M., Vandamme, D., Foubert, I., Muylaert, K., Vankelecom, I. (2012). Harvesting microalgal biomass using submerged microfiltration membranes. Bioresource Technology, 111, 343-352. IF(2011)=4.980(Q1).

Vandamme, D., Foubert, I., Fraeye, I., Meesschaert, B., Muylaert, K. (2012). Flocculation of *Chlorella vulgaris* induced by high pH: role of magnesium and calcium and practical implications. Bioresource Technology, 105, 114-119. IF(2011)=4.980(Q1).

Vandamme, D., Foubert, I., Fraeye, I., Muylaert, K. (2012). Influence of organic matter generated by *Chlorella vulgaris* on five different modes of flocculation. Bioresource Technology, 124, 508-511. IF(2011)=4.980(Q1).

Beuckels, A., Depraetere, O., Vandamme, D., Foubert, I., Smolders, E., Muylaert, K. (2013). Influence of organic matter on flocculation of *Chlorella vulgaris* by calcium phosphate precipitation. Biomass & Bioenergy, 54, 107-114. IF(2011)=3.646(Q1).

Bilad, M., Discart, V., Vandamme, D., Foubert, I., Muylaert K., Vankelecom, I. (2013). Harvesting microalgal biomass using a magnetically induced membrane vibration (MMV) system: filtration performance and energy consumption. Bioresource Technology, 138, 329-338. IF(2011)=4.980(Q1).

Discart, V., Bilad, M., Vandamme, D., Foubert, I., Muylaert, K., Vankelecom, I. (2013). Role of transparent exopolymeric particles in membrane fouling: *Chlorella vulgaris* broth filtration. Bioresource Technology, 129, 18-25. IF(2011)=4.980(Q1).

Vandamme, D., Foubert, I., Muylaert, K. (2013). Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. Trends in Biotechnology, 31 (4), 233-239. IF(2011)=9.148(Q1).

Bilad, M., Discart, V., Vandamme, D., Foubert, I., Muylaert K., Vankelecom, I. (2013). Coupled cultivation and pre-harvesting of microalgae in a membrane photobioreactor (MPBR). Bioresource Technology, under review. IF(2011)=4.980(Q1).

García-Pérez, J.S., Beuckels, A., Vandamme, D., Depraetere, O., Foubert, I., Parra, R., Muylaert, K. Influence of magnesium concentration and pH on flocculation of *Chlorella vulgaris* by magnesium hydroxide precipitation. Water Research, under review. IF(2011)=4.865(Q1).

Vandamme, D., Muylaert, K., Fraeye, I., Foubert, I. (2013). Floc characteristics of *Chlorella vulgaris* for five different flocculation modes: influence of coagulation mechanism and presence of organic matter. Biomass & Bioenergy, under review. IF(2011)=3.646(Q1).

Papers at international scientific conferences and symposia, published in full in proceedings

Vandamme, D., Foubert, I., Muylaert, K. (2009). Microalgal biomass production using wastewater: optimization and feasibility. In Verberk, J. (Ed.), Ross, P. (Ed.), Research projects book of IWA Benelux young water professionals. International Water Association Young Water Professionals Regional Benelux Conference. Eindhoven, The Netherlands. 30 September - 2 October 2009 (pp. 138-139) IWA Publishing. ISBN 978-90-8957-009-3.

Vandamme, D., Fraeye, I., Muylaert, K., Foubert, I. (2012). The potential of flocculation to harvest microalgae for biofuel production. Proceedings 17th Symposium on biological applied biological sciences: Vol. 77 (1). Symposium on applied biological sciences. Leuven, Belgium, 10 February 2012 (pp. 67-71). ISSN 1379-1176.

Meeting abstracts, presented at international scientific conferences and symposia, published or not published in proceedings or journals

Muylaert, K., Vandamme, D., Meesschaert, B., Foubert, I. (2009). Flocculation of microalgae using cationic starch. Phycologia: vol. 48 (4). International Phycological Congress. Tokyo, Japan, 2-8 August 2009, 63-63. (poster presentation).

Vandamme, D., Goiris, K., Pinoy, L., Meesschaert, B., Foubert, I., Muylaert, K. (2010). Harvesting microalgal biomass for biofuel production using cationic starch. International Conference on Innovation for Sustainable Production. Bruges, Belgium, 18-21 April 2010. (poster presentation).

Vandamme, D., Goiris, K., Pinoy, L., Meesschaert, B., Foubert, I., Muylaert, K. (2010). Harvesting microalgal biomass for biofuel production by flocculation. FEBS Workshop Microbial Lipids. Vienna, Austria, 13-15 May 2010. (poster presentation).

Vandamme, D., Goiris, K., Pinoy, L., Meesschaert, B., Foubert, I., Muylaert, K. (2010). Harvesting of microalgae for low value applications: the possibilities of flocculation. AOCS annual meeting & expo. Phoenix, Arizona, USA, 16-19 May 2010. (poster presentation).

Vandamme, D., Goiris, K., Pinoy, L., Meesschaert, B., Foubert, I., Muylaert, K. (2010). Developing flocculation-based harvesting techniques for microalgal biomass. European Workshop Biotechnology of microalgae. Nuthetal, Germany, 7-10 June 2010. (poster presentation).

Vandamme, D., Pontes, S., Pinoy, L., Muylaert, K., Foubert, I. (2010). Processing of microalgae for biofuel production: developing cost-efficient dewatering technologies based on electro-coagulation-flocculation. Euro Fed Lipid Congress. Munich, Germany, 21-24 November 2010. (poster presentation).

Vandamme, D., Muylaert, K., Foubert, I. (2011). Low cost harvesting of microalgae using flocculation for biofuel production. BIT's 1st Annual World Congress of Marine Biotechnology 2011. Dalian, China, 25-30 April 2011. (oral presentation).

Vandamme, D., Foubert, I., Muylaert, K. (2011). Advantages en disadvantages of using pH-induced auto-flocculation for harvesting microalgae. Congres of the international society for applied phycology. Halifax, Canada, 19-24 June 2011. (poster presentation).

Vandamme, D., Pontes, S., Goiris, K., Foubert, I., Pinoy, L., Muylaert, K. (2011). Evaluation of electro-coagulation-flocculation for harvesting marine and freshwater microalgae. Congres of the international society for applied phycology. Halifax, Canada, 19-24 June 2011. (poster presentation).

Vandamme, D., Foubert, I., Muylaert, K. (2011). Advantages and disadvantages of using pH-induced auto-flocculation for harvesting microalgae. Euro Fed Lipid Congress: Oils, Fats and Lipids for a Healthy and Sustainable World. Rotterdam, 18-21 September 2011. (poster presentation).

Bilad, M., Vandamme, D., Foubert, I., Muylaert, K., Vankelecom, I. (2011). Evaluation of submerged microfiltration process to harvest freshwater and marine microalgae. . BeNeLux Young Water Professionals Conference. Leuven, Belgium, 21-23 September 2011. (oral presentation).

Vandamme, D., Fraeye, I., Muylaert, K., Foubert, I. (2012). Flocculation induced by high pH to harvest microalgae for low value products. AOCS Annual Meeting & Expo. Long Beach, USA, april 29 - may 2 2012. (poster presentation).

Vandamme, D., Fraeye, I., Foubert, I., Muylaert, K. (2012). Magnesium flocculation at high pH: a cost-effective method for harvesting microalgae for biofuel production. European workshop biotechnology of microalgae. Nuthetal, Germany, 4-5 June 2012. (poster presentation).

Vandamme, D., Foubert, I., Fraeye, I., Muylaert, K. (2012). Harvesting microalgal biomass: preconcentration using flocculation. Young Algaeneers Symposium. Wageningen, The Netherlands, 14-16 June 2012. (oral presentation).

Vandamme, D., Foubert, I., Fraeye, I., Muylaert, K. (2012). pH induced flocculation: a cost-effective method for harvesting microalgae. International Diatom Symposium. Ghent, Belgium, 26-31 August 2012. (poster presentation).

Discart, V., Bilad, M., Vandamme, D., Foubert, I., Muylaert, K., Vankelecom, I. (2012). Direct role of transparent exopolymeric particles (TEP) on membrane fouling of micro- and Ultrafiltration. Euromembrane 2012, London, UK, 23-27 September 2012. (oral presentation).

Bilad, M., Discart, V., Vandamme, D., Foubert, I., Muylaert, K., Vankelecom, I. (2012). Simultaneous cultivation and pre-harvesting of microalgae in a lab-scale membrane photobioreactor (MPBR). Euromembrane 2012, London, UK, 23-27 September 2012. (poster presentation).

Vandamme, D., Pohl, P., Fraeye, I., Foubert, I., Brady, P., Hewson, J., Muylaert, K. (2012). Harvesting microalgal biomass: the potential of flocculation based preconcentration. Algal Biomass, Biofuels and Bioproducts. Westlin San Diego, USA, 10-13 June 2012. (oral keynote presentation).

Vandamme, D., Foubert, I., Muylaert, K. (2013). Design and evaluation of flocculation based harvesting processes for microalgae biomass production. Symposium Biorefinery for Food, Fuel and Materials. Wageningen, The Netherlands, 7-10 April 2013. (oral presentation).

Meeting abstracts, presented at international professionally oriented conferences and symposia, published or not published in proceedings or journals

Vandamme D., Foubert I., Muylaert K.(2011). Developing flocculation based harvesting methods. Flemish Algae Platform: separation of microalgae. Gent, Belgium, 31 maart 2011. (oral presentation).

Foubert, I., Vandamme, D., Muylaert, K. (2011). Flocculation based harvesting methods. Algae Technology Platform - Practical Short Course: Algae Harvesting and Processing for Value Added Applications. Brussels, Belgium, 26-27 May 2011. (oral presentation).

Vandamme, D., Foubert, I., Muylaert, K. (2012). Harvesting microalgal biomass: flocculation based harvesting. Algae Technology Platform. Ghent, Belgium, 5-7 December 2012. (oral presentation).

Meeting abstracts, presented at other scientific conferences and symposia, published or not published in proceedings or journals

Vandamme, D., Pontes, S., Pinoy, L., Foubert, I., Muylaert, K. (2010). Electro-coagulation-flocculation based dewatering technologies for microalgae. CINBIOS, Forum for Industrial Biotechnology. Mechelen, Belgium, 9 November 2010. (poster presentation).

Vandamme, D., Depraetere, O., Muylaert, K. (2011). Valorisatie afvalstromen uit landbouw via micro-algen. VCM-conferentie: een nieuwe markt voor mest. Gent, Belgium, 16 March 2011. (poster presentation).

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