PRE-CONCENTRATION STRATEGIES FOR MICROALGAE HARVESTING AS BIOREFINERY PROCESS CHAIN

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Dipòsit Legal: T. 1013-2013

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DOCTORAL THESIS

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Tarragona, 2013

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I STATE that the present study, entitled

"Pre-concentration strategies for microalgae harvesting as biorefinery process chain"

presented by Sema Şirin for the award of the degree of Doctor, has been developed under my supervision at the Department of Chemical Engineering of this university and that it fulfils all the requirements to be eligible for the degree of Doctor (Ph.D.) in Chemical, Environmental and Process Engineering.

Tarragona, April 2013

Doctoral Thesis Supervisor

Dr. Joan Salvadó Rovira

Dedicated to my family;

My dady, İbrahim,

My momy, Saime,

My sis Seda

Acknowledgements

I would like to thank those who helped me to make this thesis possible, without them which none of this would be possible.

First of all, I would like to express my sincere gratitude to my supervisor, to Dr. Joan Salvado, for all his support and optimistic point of view.

I would like to thank Universidad Rovira i Virgili, Fundacio de Universidad Rovira i Virgili for funding my research.

I would like to acknowledge the members of my jury to give me the honour to examine my research.

I would like to thank all members of Bioenergy and Biofuels Division of Institut de Recerca de l'Energia de Catalunya (IREC).

It was a chance to have some collaborators for this study. I would like to acknowledge the collaboration of Aquatic Ecosystems(AE) group of Institute for Food and Agricultural Research and Technology (IRTA) for their assistance of algal cultures within the scope of collaborative research project.

My acknowledgments go also to people from the Chemical Engineering Department of the Universidad Rovira i Virgili, however especially to Pepa Lazaro, for all her help especially at the beginning of my Spain adventure.

I would like to extend special thanks to my office mates for being my friend, resisting me in my stressful times, listening and encouraging me. All you made my doctoral study more tolerable.

My brother, Daniel, special thanks go to you for going into hot discussions with me about my soft spot – MatLAB- which is final part of my thesis. I am grateful to you for all your efforts. Your brother-ship, your help on MatLAB, your patience and encouragement are highly valued.

My dear Italian friends, I enjoyed every moment of the time we spent together, spoke about life. I am thankful to you to resist me in my crazy "how life is not fair" moments.

I would like to thank all Turkish community to be with me whenever I needed to discharge myself in my mother tongue.

Last, but certainly not least, my sincere thanks go to my mother and my sister for all their support and encouragement over the years, both emotional and financial. Without their support, I cannot have carried on that much strong, I would not be able to come so far. Without you none of my dreams would be possible. I love you.

Summary

Over the past few decades, microalgae become focus of the researches again as a possible raw material for the production of biodiesel due to the myth of the oil crisis and also Kyoto Protocol which entered into force in 2005. Although microalgae have proven to be very efficient at producing oil-rich lipids, the optimum conditions for algae cultivation and methods for harvesting and oil extraction have not been determined in detail yet.

In particular, the harvesting process is especially important to the effectiveness of the overall process because of the large volumes of water that must be processed. The requirements for downstream also makes complicated the chosen harvesting system.

Several solid-liquid separation technologies have shown some potential for achieving microalgae/water separation; however, application of these processes to biofuel production requires an evaluation of treatment effectiveness as a function of water quality, algae particle characteristics and process chemistry.

Pre-concentration (bulk harvesting) aims to separate the micro algae from culture medium. Flocculation, flotation or gravity sedimentation are the frequently used methods. Filtration, centrifugation and ultrasonic aggregation are mostly used in thickening. Of these technologies, from chemical and/or biological harvesting, by means of flocculants e.g. auto flocculation, chemical coagulation, inorganic coagulants, organic flocculants,

combined flocculation, electro-coagulation and ultrasonic aggregation. Flocculation is used in order to ease the following dewatering steps in this study

Flocculation is also a factor that affects the mechanical properties of the culture. Therefore, it is important to study the flow behaviour of flocculated suspensions by additives. The flocculation mechanism determines the properties of the flocs and therefore the rheological behaviour of the suspension. The rheological properties of algae slurries have a direct impact on the agitation and pumping power requirements as well as process design for producing algal biofuels.

Flocculation efficiency is commonly evaluated by measuring the settling rate of flocs, the percentage of solids settled, the sediment volume/weight, the moisture content and strength of flocs, and the suspension viscosity and turbidity in water treatment processes. Particle size analysis is usually applied in drinking water treatment for monitoring and controlling filtration process performance. Analysis of sizes of flocs formed in the coagulation and flocculation processes are not routinely conducted. However, particle size distribution (PSD) analysis can produce direct information about the flocs in the fluid. Through measuring and analysing the amount and size of particles, we can evaluate the efficiency of the process and assess the design of the treatment or harvesting systems.

A primary factor controlling the performance of sedimentation is the particle size distribution (PSD) of the incoming sediment. Particle size distribution information is needed to model the sedimentation process.

From biological harvesting methods, auto-flocculation is one of the promising methods for microalgae. The mechanism of auto-flocculation depends on the alkaline conditions. According to several studies two major reactions are effective: the precipitation of calcium carbonate ($CaCO_3$) and the precipitation of magnesium hydroxide ($Mg(OH)_2$), depending on the primary particles and the ions contained in the solution.

To sum up, this thesis aims;

(i) to determine a simple, rapid, efficient and cost effective pre-concentration method for *Phaeodactylum tricornutum* and *Nannochloropsis gaditana* through testing, (1) natural sedimentation (2) flocculation with commercial flocculants (aluminium sulphate, polyaluminium chloride) and chitosan and (3) pH induced flocculation. The drawbacks of the methods, state of the concentrated algae according to studied species after every studied treatment method, sedimentation rates of pH induced flocculation method were also shown;

(ii) to know more about pre-concentration processes of studied species, characteristic properties of downstream process after treating the pre-

concentration methods under author chosen conditions (viscosity, particle size and Ca and Mg ion determination analysis) were investigated after applying different methods of harvesting;

(iii) to try a mathematical model and a numerical simulation procedure for the flocculation process, using usual parameterization solutions to estimate the sedimentation rate and floc size distribution for pre-concentration process. The main objective is estimating sedimentation rate coupled with that of the particle size distribution (PSD), through a population balance equation (PBE).

From the thesis we could conclude; the selection of the proper harvesting method depends mainly on the process product targets. To decide which method is better for harvesting,it should be checked not only how efficient the agent as a flocculant and how appropriate to provide a cost effective harvesting, but also how feasible to use the agent in the case of using concentrated biomass in the further processes without any problem and managing the residual water after harvesting.

Depending on the chemical properties of the seawater and on the properties of the outer surface of the algae, pH induced flocculation seems promising for *P. tricornutum* and *N. gaditana* species. It is effective, economical and eco-friendly. Sedimentation rates of the author chosen method are also helpful to scale-up the process. The process is also very reproducible.

It is observed that after a certain pH, increasing the pH didn't change the flocculation efficiency, however changed the mass content of the concentrated sample (higher ash content).

The comparative study of pre-concentrated sample properties shows that; (I) Pre-concentration processes make both pumping and mixing easier because of the Newtonian behaviour of the samples than Non-Newtonian ones. (II) Particle size analysis of the pre-concentrated samples support settling properties sufficiently. (III) Ca and Mg ion concentrations of the pre-concentrated samples substantiate the fact that the Mg ion is the protagonist in the alkalinity-induced flocculation mechanism.

A population balance model (PBM) has been tried to build to predict the floc size distribution for *P. tricornutum* and *N. gaditana*. The model was ineffective to simulate alkalinity induced flocculation -a mimic of autoflocculation in lab scale. High shear rates were needed in the model to let the floc to appear. The mathematical model for flocculation is still a framework that should be developed. Additionally, the model needs to be further adjusted and integrated with the sedimentation model simultaneously with PBM.

List of Abbreviations

A: OD_{750} (optical density at 750nm) of sample

$A^{'}$ = the specific fragmentation rate for the largest particles present

AFDW: ash-free dry weight

AS: aluminium sulphate

B: OD_{750} of initial culture

C: algae culture

C: (in italics) OD_{750} of reference blanks

Ca: calcium

CF: concentration factor

d_i= the diameter of the particle of size *i* (m)

d_j =the diameter of the particle of size j (m)

D_f : mean diameter of floc

FC: filtered algae culture

FE: flocculation efficiency

G = the rate of dissipation (s^{-1})

GM: growth medium

HCl: hydrochloric acid

h_f: final height of concentrated algae solution

h_o: initial height of examined algae solution

(i+j=k)= the summation is over all pairs of particles generates a type-*k* particle

k: constant of viscosity

k_i = the number of particles in a floc of size *i*

k_{max}: the upper boundary of the classes

Mg: magnesium

n_i: the concentration of the particle number from the section i [in no.ml^{-1}]

n_j: the concentration of the particle number from the section j [in no.ml^{-1}]

$n_{k:}$ the concentration of the particle number from the section k [in no.ml^{-1}]

NaOH: sodium hydroxide

Nng: *Nannochloropsis gaditana*

OD: optical density

PAC: polyaluminium chloride

Pht: *Phaeodactylum tricornutum*

r_k: net rate of formation of aggregates of type k

S_k: the fragmentation rate

SR: sedimentation rate

SSVF: settleable solid volume fraction

u_1 = the volume of one single particle (m^3)

u_i = the volume of particle size *i* (m^3)

u_k = the volume of particle *k* (m^3)

u_{max} = the volume of the largest particle present

V(t)=the sedimentation velocity (m s^{-1})

μ = the viscosity of the fluid (Pas).

t =the time (s)

τ: shear stress

$\dot{\gamma}$: shear rate

γ_{jk} = the fragment distribution function of particle size *i* coming from size *k*.

$\propto_{ij}$= the collision efficiency between two colliding particles i and j

β_{ij}= collision frequency function

δ = constant

Δρ= the difference of densities between particle (ρ_p)and fluid ρ_f: (kg m^{-3})

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List of Publications and Contributions to Conferences

Publications

Şirin S, Clavero E, Salvado J. (2013).**Potential pre-concentration methods for *N. gaditana* and a comparative study of pre-concentrated sample properties –** Bioresource Technology 132, Jan,2013, doi: 10.1016/j.biortech.2013.01.037.

Şirin S, Trobajo R, Ibanez C and Salvado J. (2012). **Harvesting the microalgae *Phaeodactylum tricornutum* with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity induced flocculation.** J Appl Phycol 24, 1067-1080.

Articles in progress

Şirin S, Cortes D., Salvado J. **Flocculation kinetics and mathematical modelling of marine algae cells *Phaeodactylum tricornutum* and *Nannochloropsis gaditana***

Şirin S, Salvado J. **Pre-concentration strategies for marine alga *Chaetoceros calcitrans***

Posters and Presentations

Poster presentation: Nurra C , Rios SD, Şirin S, Salvadó J, Torras C. Microalgae concentration in the biorefining process. VII Barcelona Global Energy Challenges, Barcelona, Spain, 28-29 June 2012.

Poster presentation: Rios SD, Şirin S, Nurra C, Salvadó J and Torras C. Microalgae concentration: a step in the biorefining process. VI Barcelona Global Energy Challenges, Barcelona, Spain, 2-3 June 2011.

Poster presentation: Rios SD, Şirin S, Torras C, Clavero E, Salvadó J . Harvesting studies of Phaeodactylum tricornutum microalga, 1st International Conference on Algal Biomass, Biofuels and Bioproducts, Westin St Louis, St Louis, USA, 17-20 July 2011.

CHAPTER I

GENERAL INTRODUCTION

1.0 Introduction

Algal biofuel production has gained a great deal of interest in recent years not only due to the high photosynthetic efficiency of various algae strains and the ability of stressed algae populations to produce large quantities of lipids within their cells, but also due to their high content of other types of compound (vitamins, proteins, fatty acids, pigments, etc.) which are of considerable added value for the nutraceutical industry, pharmaceuticals, cosmetics, the human and animal food industry, etc.

The present thesis addresses the pre-concentration of algae cultures. In particular, the study focuses on the dewatering of two algal species in order to determine harvesting properties for use in biorefining practices.

The objective of this chapter is to present the motivations for the research from economic, social, and environmental perspectives. Moreover, the chapter describes the specific objectives of the research and concludes with the organization of the document.

1.1.State of the Art

Fossil fuels (oil, natural gas, and coal) contribute to 80% of total world energy supply (Chen et al., 2011). The U.S. Energy Information Administration (EIA) envisages that petroleum and non petroleum liquid fuel consumption will increase to 106.6 million barrels a day by 2030. This increase will largely take place in non-OPEC countries (emerging economies such as China and India) and the transportation sector will account for nearly 80% of the total liquid oil consumption.

The combustion of these petroleum derivatives leads to a considerable build up of CO_2 in the atmosphere. CO_2 is one of the main gases associated with the greenhouse effect (together with NO_x, SO_x and VPM), which most countries in the world are trying to revert. Biofuels have garnered worldwide interests for their potential to reduce GHG emissions, improve energy security, and enhance rural development. To mitigate the problem of environmental pollution and the exhaustion of petroleum supplies, it has been suggested that bio-fuels such as biodiesel and bio-ethanol can be used. In general, these fuels are carbon neutral since the raw materials on which they are based are

photosynthetic organisms such as the plants from which biodiesel oil is extracted (rapeseed, soya, sunflower, palm, etc.) or fermented bio-alcohols (corn, sugar cane, etc.).

The paths for producing biodiesel and bio-alcohols from different raw materials are well documented (Reijnders, 2006; Demirbas, 2007; Frondel and Peters, 2007; Narayanan et al., 2007; Palligarna and Brigg, 2008; Demirbas, 2009). Some authors discuss and compare the advantages of each fuel (Hill et al., 2006; Reijnders, 2006; Agarwal, 2007; Granada, 2007; Demirbas, 2008). Others integrate the two technologies (Gutierrez et al., 2009).

Bio-fuels have many advantages over fossil fuels: as well as being more environmentally friendly and a renewable source of resources, every region in the world has a raw material that can be used, to a greater or lesser extent, to produce some sort of bio-fuel, thus providing work for the population and improving the economies of the numerous countries that have so far been dependent on petroleum imports.

On the other hand, the sustainability of bio-fuels, many of first-generation, – which are produced primarily from food crops such as grains, sugar cane and vegetable oils – has been increasingly questioned over concerns. Several groups (Green Peace, Intermon Oxfam, etc) claim that the growing production of first generation bio-fuels is responsible for the increasing food and feed prices, and the destruction of forests –results as the

environmental and climate changes-. The ethical question was also brought along due to direct competition for land that is required for producing food.

The increasing criticism of the sustainability of many first-generation bio-fuels has raised attention to the potential of second-generation bio-fuels. Second-generation bio-fuels (those that do not threaten food supplies) were developed to overcome this obstacle. Depending on the feedstock choice and the cultivation technique, second-generation bio-fuel production has the potential to provide benefits such as consuming waste residues and making use of abandoned land. In this way, the new fuels could offer considerable potential to promote rural development and improve economic conditions in emerging and developing regions (OECD/IEA, 2010).

The particular interest of the possible feed stocks are the residual parts of agricultural and forest vegetation that contain cellulose for producing bio-alcohols, recycled oils, and other plants that contain oils and which do not threaten food supplies (*Jatropha curcas, Cynara cardunculus*, microalgae, etc).

Especially over the past few decades, microalgae become focus of the researches again as a possible raw material for the production of biodiesel due to the myth of the oil crisis (Kerr, 1998) and also Kyoto Protocol which entered into force in 2005. The use of algae as a bio-fuel seems to be a promising alternative to fossil fuels for various reasons.

(1) They have the ability to "harvest the sun" using inexpensive natural resources such CO_2 and H_2O (Carvalho et al., 2006).

(2) They are considered fast-growing photosynthetic organisms. They have shorter growth cycles as compared to other terrestrial plants (Sander and Murthy, 2010), so their productivities are higher. The best achieved productivity rates in outdoor facilities were about 20–22 gdry weight.$m^{-2}d^{-1}$ (averaged whole year) (Moheimani and Borowitzka, 2006).

(3) The total dry biomass of some microalgae contains up to 50% lipids. The lipid content of some genetically modified species or species submitted to some sort of stress during growth can exceed 80% (Packer 2009). However, in field studies the lipid content of dry biomass is much lower than high values reported in lab scales (i.e. 25-40%, Moheimani and Borowitzka, 2006).

(4) Their production does not compete with agriculture food crops for land or water use. Both are one of the major advantages often not adequately considered when the strategic implications of expanding biofuels production are taken into account (Searchinger et al., 2008).

Microalgae could not only be used as a raw material for biodiesel production, but also be used for purposes such as human and animal nutrition, animal feed, cosmetic product components in commercial applications(Mendola, 2003; Molina Grima, 2003; Wiffels, 2007; Lamers et al., 2008; Granado et al., 2009; Leary et al., 2009). Spolaore et al. (2006)

is summarized well the commercial applications of microalgae. Microalgae can also be used for treating waste waters (Vilchez et al.,2008).

Moreover, the history of microalgae has shown that research studies on microalgae have been numerous and varied but they have not always resulted in commercial applications. Commercial development of microalgal biotechnology is well documented in Olaizola, 2003.

Many authors suggested "bio-refinery concept" (Figure 1) is the possible solution to address the problem of costs associated with different stages of processing and production of different products from microalgae (Chisti, 2007; Powell et al., 2009; Rupprecht, 2009; Tran et al., 2010).

In this particular work, *Phaeodactylum tricornutum* and *Nannochloropsis gaditana* seawater species were chosen to focus on.

Phaeodactylum tricornutum species is an interesting microalgae candidate for biodiesel production because it grows well in culture and produces a significant amount of lipids (Alonso et al., 2000; Rodolfi, 2009). Moreover, *P. tricornutum* is one of the few microalgae species whose entire genome has been fully sequenced (Bowler et al., 2008), thus facilitating possible genetic modification for the purpose of increasing lipid productivity.

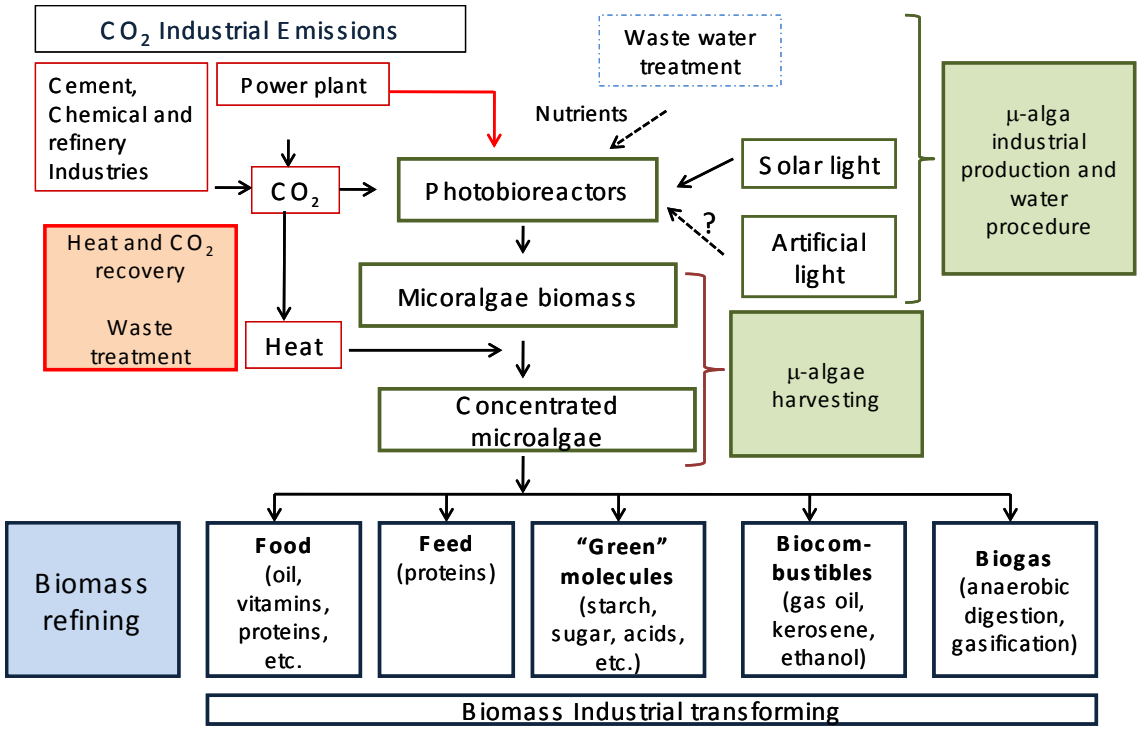

Figure 1.1 Possible integrated bio-refinery from microalgae as a raw material.

Furthermore, among the various diatom species, the ones belonging to the genus *Nannochloropsis* are particularly interesting because of their ability to accumulate large amounts of lipids, which can reach concentrations up to 65–70% of total dry weight (Boussiba et al., 1987; Hodgson et al., 1991; Rodolfi et al., 2009; Simionato et al., 2011). Details of microalgae species will be given in Chapter 2.

Producing microalgae is a well-known process (Molina Grima et al., 2003; Chisti, 2007; Schenk et al., 2008; Amin, 2009; Brennan and Owende, 2010; Mata et al. 2010; Scotts et al., 2010) but still expensive and challenging in large scales. It is estimated that at least

20-30% of the total cost of producing biomass must be attributed to the process of recovery (Gudin and Thepenier, 1986; Pushparaj et al., 1993; Lee et al., 1998; Oh et al., 2001; Kim et al., 2005; Christenson and Sims, 2011). The separation of the microalgae cells from the culture solution is particularly important for determining the cost and quality of the product.

Besides, microalgal harvesting is not a novel area for water treatment where the applied methods are similar as well for bio-fuel production with different concerns on harvested microorganisms. Regardless from the objective of harvesting process, small size of the algal cells, the low density difference between algae and growth medium, and dilute concentrations of algal cultures make harvesting process a key challenge especially in industrial scale. Consequently, the harvesting strategy has to be based on a low energy method in order to overcome the problems and make algal production economically feasible, which would in turn make the system commercially feasible (e.g. to produce biodiesel with algal technology). Combination of several separation strategies has been proposed where the selection of strategies depends on the species, desired final product etc (Brennan and Owende, 2010).

Microalgae harvesting can generally be divided into a two-step process:

1. Bulk harvesting: The purpose of this is to separate microalgal biomass from the bulk suspension. By this method, the total solid mater can reach 2–7% using flocculation,

flotation, or gravity sedimentation (Brennan and Owende, 2010). In this work, pre-concentration term will be used instead of bulk harvesting.

2. Thickening: The purpose of this harvesting is to concentrate the slurry, with filtration and centrifugation usually applied in the process. This step needs more energy than bulk harvesting (Brennan and Owende, 2010).

For instance; Ginzburg, 1993 mentioned two step harvesting processes for *Dunaliella* species; Rios et al., 2012 proved it for *P.tricornutum* and *N.gaditana* species.

Flocculation is one possible route to harvest microalgae and a such an important pre-treatment step. Because the microalgae cultures are stable for extremely long periods of time based on Stokes Law analysis in their natural way. Of the possible flocculation methods, auto-flocculation refers to the precipitation of algae and other particulate matters when the pH rises to a highly alkaline level (Lavoie and de la Noue, 1987). This phenomenon is related to the chemical make-up of the water, and in particular, the presence of calcium and magnesium carbonates. As the algae remove CO_2, the pH rises to a point at which precipitation of magnesium hydroxides and calcium carbonate along with algae occurs, causing removal of the particulate matters (Polprasert, 2007). Increases in pH can occur (I) naturally; such as during the diurnal variations in photosynthesis/respiration cycles(auto-flocculation) or (II) artificially by adding a base to the solution to increase the pH (alkalinity induced flocculation).

Alkalinity induced flocculation, pH modification with sodium hydroxide (NaOH) or lime ($CaCO_3$) to induce flocculation represents an inexpensive method of removal but the success varies according to the calcium, magnesium, and phosphate concentrations present in the water (Lavoie and de la Noue, 1987; Becker, 1994).

There are some studies on pH induced flocculation -mimic of auto-flocculation in lab scale- with several species (Spilling et al., 2011; Vandamme et al., 2012; Şirin et al., 2013). Although significant progress were observed especially in recent years, the understanding of auto-flocculation mechanisms is not clear yet. There are several factors affect the process of auto-flocculation as algae species, the medium characteristics, pH, time of the harvesting etc.

The role of magnesium hydroxide as a potential coagulant for algae removal has also been investigated recently (Vandamme et al., 2012; Şirin et al., 2013). Chemical coagulation with formation of magnesium hydroxide has been proved to be an effective alternative to conventional treatments for harvesting of algae. The harvesting mechanism may include: charge neutralization resulted from positively charged $Mg(OH)_2$ particles to destabilize algae cells and adsorptive coagulation by $Mg(OH)_2$ precipitates. It was also found that magnesium hydroxide could be recycled and reused in treating low concentration of heavy metals contaminated wastewater (Liu et al., 2011).

Flocculation efficiency is commonly evaluated by clearness of the residual solution/ turbidity (flocculation efficiency), measuring the settling rate of flocs (sedimentation rate), the percentage of solids settled (settleable solid volume fraction and/or concentration factor). However, these are only indirect indicators of the effectiveness of flocculation. Since flocculation is an aggregation process, clearly the most explicit and direct measure of flocculation efficiency is floc size distribution (Runkana et al., 2004). Previous investigators have studied the flocculation of algae and characteristics by conventional coagulants (Wu et al., 2011; Wang et al., 2012). However, although there are adequate amount of data on the characteristics of flocs using conventional coagulants, there have been limited studies focused on measuring and analyzing of flocs formed by auto-flocculation and their characteristics (Şirin et al., 2013).

Starting from the classical work of Smoluchowski (1917), population balance equation (PBE) has been used for modeling the kinetics of flocculation. The Smoluchowski number balance equation is universally used as the starting point for modeling flocculation in both liquid and gaseous phases (Smoluchowski, 1917; Letterman et al., 1998; Crittenden et al., 2005; Friedlander, 2000; Benjamin, 2011). It is the mathematical expression for collision frequency between particles in suspension for laminar flow. So, it should be also noted that the turbulent conditions exist in most cases of industrial flocculation (by mixers), and laminar flow conditions are limiting the design

considerations, therefore the Smoluchowski flow equation should be adapted to the case turbulent fluid motion.

1.2 Objectives and scope of the study

It is expected that there will be an optimum algae biomass pre-concentration that will minimize energy consumption biomass from the cultivation site to the biorefinery. In order to determine this optimum pre-concentration and accurately predict the process properties, accurate research on the algal species is necessary. This thesis reports:

- properties of microalgae species, namely (i) *Phaeodactylum tricornutum*, (ii) *Nannochloropsis gaditana* (Chapter 2);
- several harvesting methods for pre-concentration for maximizing algae recovery and reducing costs, drawbacks of the methods, state of the concentrated algae according to studied species after every studied treatment method. Sedimentation rates of pH induced flocculation method were shown which could help process scale up and design (Chapter 3);
- characteristic properties of downstream process after treating the pre-concentration methods under author chosen conditions. In order to know more about pre-concentration processes of studied species viscosity, particle size and Ca and Mg ion determination analysis were done after applying different methods of harvesting. With these analysis, we aimed to understand the flocculation

behaviours more, to give a step to mathematical modelling of pH induced flocculation by mean particle sizes, particle distributions etc. (Chapter 4);

- developing a mathematical model and a numerical simulation procedure for the flocculation process, using usual parameterization solutions to estimate the sedimentation rate and floc size distribution for pre-concentration process. Hence, a mathematical model was tried for particle coagulation for the author chosen harvesting techniques and applied to species *Nannochloropsis gaditana* and *Phaeodactylum tricornutum*. The main objective is estimating sedimentation rate coupled with that of the particle size distribution (PSD), through a population balance equation (PBE) (Chapter 5);
- conclusions of the all studies (Chapter 6).

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Introduction

CHAPTER II
MICROALGAE SPECIES

1.0 Introduction

Microalgae cells can be described as particles with distinct physical characteristics - morphology, surface charge, and density. The knowledge of such characteristics may provide advantage on the treatment requirements Table 2.1 shows the algae characteristics that affect the harvesting methods (Henderson et al., 2008).

Table 2.1 Algae characteristics affect treatment methods (Henderson et al., 2008).

Treatment Method	Algae Characteristics affect treatment method
Coagulation/Flocculation	Morphology, motility, extracellular organic matter (EOM), surface charge, cell surface area
Sedimentation	Morphology, motility, density
Filtration	Morphology, motility

This chapter provides background information about algae species that have been studied. Information on microalgae species; ***Phaeodactylum tricornutum*** and ***Nannochloropsis***

gaditana; characteristics, and how those properties may influence flocculation requirements.

2.0 Microalgae species

2.1 *Phaeodactylum tricornutum*

2.1.1 Taxonomy

Table 2.2 shows the taxonomy of *Phaeodactylum* species (Lewin et al., 1958; Cavalier-Smith and Chao, 2006).

Table 2.2 Taxonomy of *Phaeodactylum* species (Lewin et al., 1958; Cavalier-Smith and Chao, 2006).

Taxonomy	
Phylum	Bacillariophyta
Class	Bacillariophyceae
Order	Naviculales
Family	Phaeodactylaceae
Genus	*Phaeodactylum*
Species	***Phaeodactylum tricornutum*** is the only species in genus Phaeodactylum

2.1.2 Biology

Phaeodactylum belongs to the pennate diatoms (Bacillariophyceae). It grows fast and has emerged as a model system for physiological, biochemical, and molecular studies mainly because of its ease of culture and the ability to be routinely genetically transformed

(Scala et al., 2002, Montsant et al., 2005). *P. tricornutum* is one of the few microalgae species whose entire genome has been fully sequenced (Bowler et al., 2008), thus facilitating possible genetic modification for the purpose of increasing lipid productivity. *P. tricornutum* species is a seawater diatom.

Structural and morphological features

P. tricornutum is unicellular or forms small cell clusters. Cells are approximately 3 μm wide and 8 to 20 μm long (Lewin et al., 1958), and contain a single plastid (Round et al., 1990). Unlike other diatoms it can exist in different morphotypes (*i.e.* fusiform, triradiate or oval) (Borowitzka and Volcani, 1978). In the experiments **fusiform** morphotype of *P. tricornutum* was studied. Figure 2.1a shows microscope photos of the species.

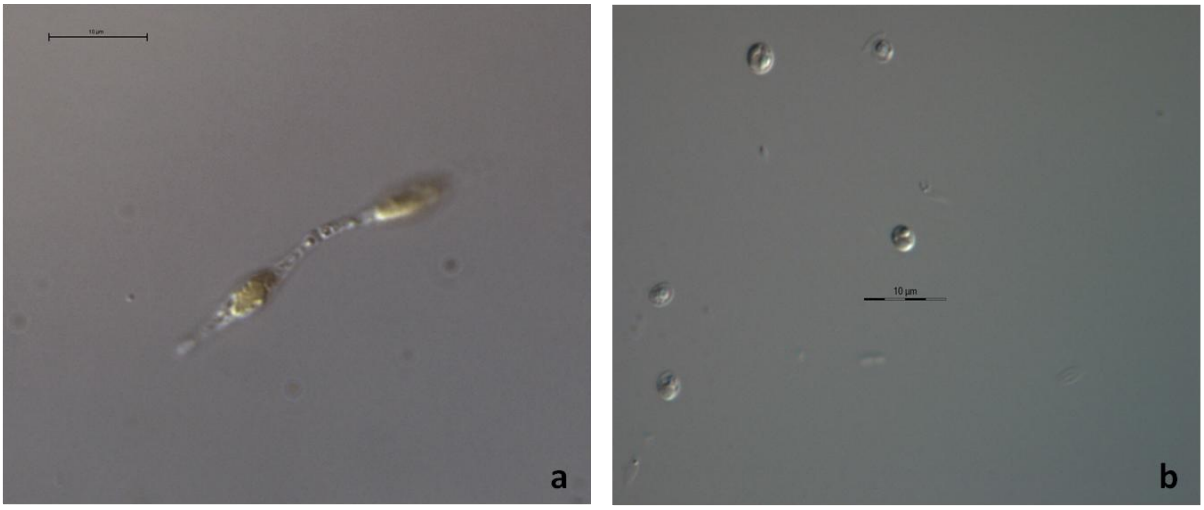

Figure 2.1 Microscopic photos of species a) *Phaeodactylum tricornutum* and b) *Nannochloropsis gaditana*.

Physiological characteristics

P. tricornutum has been found in several locations around the world, typically in coastal areas with wide fluctuations in salinity as well as in inland waters (Rushforth et al., 1988). They have ability of adaptation according to changing environmental conditions which could be related to the pleiomorphic character of the cells. The different morphotypes are also thought to be adapted for survival in different habitats. It is shown that the three morphotypes are physiologically different. Fusiform forms are common when grown in liquid media. Fusiform cells tend to transform into the ovoid morphotype when they are transferred on a solid medium (agar) while the reverse transformation occurs upon transfer in liquid medium. It has also been noted that fusiform and triradiate cells are more buoyant than oval cells (Lewin et al., 1958).

Biochemical composition

For *P. tricornutum,* different culture conditions result in significant variations in the biochemical composition of the cells of and, therefore, in their nutritious value.

The biomass of *P. tricornutum* contains on average; 36.4% crude protein; 26.1% available carbohydrates; 18.0% lipids, 15.9% ash. At low external irradiance, it was richer in protein and eicosapentaenoic acid (Rebolloso-Fuentes et al., 2001a).

The total lipid content and the fatty acid and lipid class (polar membrane lipids *vs* neutral storage lipids) composition are dependent on different chemical (nutrient starvation, salinity, growth-medium pH) and physical stimuli (temperature and light intensity). In addition growth phase and/or aging of the culture has an effect on oil content and composition. In *P. tricornutum* species, the major fatty acids could be obtained are; the very-long-chain (VLC) fatty acids (VLC-Polyunsaturated fatty acids (PUFA)), arachidonic acid (AA) (C20:4ω6), eicosapentaenoic acid (EPA) (C20:5x3) and docosahexaenoic acid (DHA) (C22:6ω3). They compose approximately 30% of the total fatty acid content (Hu et al., 2008).

P. tricornutum possess also important advantages as a potential commercial producer of EPA(3.9–5% of cell dry weight (CDW)) (Meiser et al., 2004). The TAG yield from *P. tricornutum* is about 14% of total dry weight (Yu et al., 2009). Unlike in some other diatoms, culture age had almost no influence on the total fatty acid content in *P. tricornutum* that remained around 11% of CDW. Conversely, culture age had a greater impact on lipid classes, producing changes in amounts of triacylglycerols (TAG) which ranged between 43% and 69%, and galactolipids (GLs) that oscillated between 20% and 40%. In general, the content of polar lipids of the biomass decreased with culture age (Alonso et al., 2000).

Carotenoids in *P. tricornutum* are low, ranging from 0.115 to 0.45 g/100g dry biomass. In *P. tricornutum*, chlorophylls are the main pigments (1.17 – 2.87g) (Rebolloso-Fuentes et al., 2001a).

Growth kinetics and productivity

Growth rate and biomass productivity are influenced by environmental conditions, available resources and choice of culture system.

In general, *P. tricornutum* shows good growth at temperatures between 15 and 25°C. For most isolates, growth ceases at temperatures above 30°C. Furthermore, aeration affects the growth of *P. tricornutum.* Higher growth rates were obtained under aerated conditions (Perez et al., 2008).

2.1.3 Summary of *P. tricornutum*

P. tricornutum is a popular species mostly used as a food source for the aquaculture industry because of its ease of cultivation and its rich oil content. It is widely used i as feed for penaid shrimp larvae, freshwater, prawn larvae, bivalve larvae and post larvae and marine zooplankton (Tredici et al., 2009).

Phaeodactylum has been proposed as a source of eicosapentaenoic acid (EPA, 20:5ω3) (Veloso et al., 1991; Molina Grima et al., 1994). *Phaeodactylum* extracts are also used in

cosmetics (Nizard et al., 2007) Fatty acids from *Phaeodactylum* have shown antibacterial activity (Desbois et al., 2009) and they were found in higher amounts in the fusiform than in the oval cell form (Desbois et al., 2010).

It is one of the popular species of interest for biodiesel production because of its ease of cultivation and its rich oil content. It is one of the few microalgae species whose entire genome has been fully sequenced that let also possible genetic modification for the purpose of increasing lipid productivity.

It is an example of a single celled, non-clumping, medium-sized diatom and is known that fusiform morhpothypes are more buoyant than others.

2.2 *Nannochloropsis gaditana*

2.2.1 Taxonomy

Table 2.3 shows the taxonomy of *Nannochloropsis* species (Karlson et al., 1996).

Table 2.3 Taxonomy of *Nannochloropsis* species (Karlson et al., 1996).

Taxonomy	
Phylum	Heterokontophyta
Class	Eustigmatophyceae
Order	Eustigmatales
Family	Monodopsidaceae
Genus	*Nannochloropsis*
Species	***N.gaditana***, *N.granulata, N.limnetica, N.oculata, N.salina*

2.2.2Biology

Nannochloropsis cells (Karlson et al., 1996; Fawley and Fawley, 2007) are non-motile, spherical to ovoid, 2–4 μm in diameter algae cells. This genus includes a variety of algal species which has taken the attention of aquaculture, food and biofuels industries as well as the nutraceutical market. *Nannochloropsis* has especially been proposed as feedstocks for biodiesel production because of its ability to accumulate up to 60% lipid under nitrogen starvation (Rodolfi et al., 2009). *Nannochloropsis* is considered a promising alga for industrial applications due to its ability to accumulate high levels of polyunsaturated fatty acids (eicosapentaenoic acid). *Nannochloropsis* is also used as an ingredient in cosmetic products (Tredici et al., 2009).

Structural and morphological features & Physiological characteristics

Nannochloropsis cells are non-motile, spherical to ovoid, 2–4 μm in diameter (Figure 2.1b). They poss a single chloroplast lacking a pyrenoid and containing chlorophyll *a*. Major carotenoids are violaxanthin, vaucheraxanthin and neoxanthin, besides β-carotene. Among others, algae of this genus can synthesize low amounts of canthaxanthin and astaxanthin. The composition of the cell wall is still unclear. Small refractile bodies can also be observed within the cytoplasm and can be both mobile (Brownian motion) or immobile.

Among the microalgae the most important group from the biotechnological point of view is represented by the eustigamotphytes.

Biochemical composition

Under artificial light in a photobioreactor, an average dry biomass composition of *Nannochloropsis* species was found as 29% protein, 36% carbohydrate, 18% lipid, 9% ash, and plamitic,palmitoleic, oleic and eicosapentaenoic acid (on average 2.2%) as major fatty acids (Rebelloso Fuentes et al. (2001b) where Brown et al. (1998) report a different composition for *Nannochloropsis* CS-246 tested for oyster rearing:17% protein, 23% carbohydrate, 26% lipid, 16% ash.

Nannochloropsis is widely cultivated for its high content of eicosapentaenoic acid (EPA; 20:5 ω3), present in amounts up to 4-5 % of the biomass, and because of its small cells (2-3 μm diameter) as feed for rotifers in the "green water" technique. Modulation of fatty acid composition by culture parameters such as light intensity, light-dark cycles, temperature, salinity and nutrients are also possible.

Patterns of variation in the lipid class and fatty acid composition during batch cultivation of *N. oculata* have also been investigated (Hodgson et al., 1991). Generally, the higher the biomass productivity, the higher is EPA productivity (Chini Zittelli et al. 1999). *Nannochloropsis* has been also proposed as source of lipid for biodiesel production

because it is able to reach over 60% lipids after nitrogen starvation (Rodolfi et al., 2009) mainly as TAGs containing saturated and monounsaturated fatty acids (Bondioli et al., 2010).

Growth kinetics and productivity

Nannochloropsis grows in seawater, with maximal growth rate in the salinity range from 25 to 30 g L^{-1}, but it can tolerate salinities between 10 and 35 g L^{-1} (Renaud and Parry, 1994).

Experiments with artificial or mixed (artificial and natural) light sources aiming to evaluate biomass productivity in different culture conditions have been carried out *Nannochloropsis* has also been proposed as source of oil for biodiesel production. Rodolfi et al. (2009) have suggested a "two phase strategy" (a first-stage cultivation of the microalga in the presence of nitrogen carried out in closed photobioreactors, followed by a second stage of nitrogen starvation, preferably carried out in open ponds), and have estimated a productivity of 20 ton of lipids per ha per year in the Mediterranean region. Lipid content increase after nitrogen starvation was not observed in *N. salina* (Boussiba et al., 1987), thus the selection of the right strain is of crucial importance for this application.

2.2.3 Summary of *N. gaditana*

The genus *Nannochloropsis* is particularly interesting because of their ability to accumulate large amounts of lipids, which can reach concentrations up to 65–70% of total dry weight (Boussiba et al., 1987; Hodgson et al., 1991; Rodolfi et al., 2009; Simionato et al., 2011). *Nannochloropsis gaditana* is a seawater microalga species that belongs to the class Eustigmatophycea which stands out as an important source of pigments of great commercial value besides its lipid content (Macias Sanchez et al., 2005). Therefore, this species is also considered as promising candidate for industrial applications of bio-fuel production where studies on its morphology, ultrastructure and growth physiology of its system have already been described (Lubian, 1982).

Nannochloropsis small cell size allows less energy consumption for mixing, but more energy is required for separating the cells from the culture medium. Thick cell wall, that allows mixing of the culture even with centrifugal pumps, but increases difficulty of cell breakage, which is necessary for oil extraction.

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Microalgae Species

CHAPTER III
PRE-CONCENTRATION

HARVESTING OF MICROALGAE SPECIES WITH PAC, ALUM, CHITOSAN AND ALKALINITY INDUCED FLOCCULATION

1.0 Introduction

The separation of the cells from the culture solution cost is estimated that at least 20-30% of the total cost of producing biomass must be attributed to the process of recovery (Gudin and Thepenier, 1986; Kim et al., 2005, Christenson and Sims, 2011).

The main engineering drawback is reducing the energetic consumptions of separating or isolating the micro algal cells from the culture medium which ultimately determines the production cost. This is mainly due to the size of the microalgae (0.5 –30 μm), the slight difference in their densities and the liquid in which they are contained, and the low concentrations in which they are found in the culture (0.5–5 kg m^3), which means that large amounts of water have to be processed.

The first difficulty is to concentrate dilute solutions of microalgae cultures (approximately 0.5–5 kg m^3 of dry weight) to concentrated solutions (between 20 and 100 percent more concentrated than the starting material). In a concentrated state, with 7–10% volume consist of cells, the rheology of the packed microalgae starts to become non-Newtonian and handling of the cells becomes problematic. At about 15–20% solids, the systems are no longer fluid and not amenable to pumping which makes handling even more difficult. It is generally preferable to maintain the system as liquid slurry showing Newtonian behaviour to facilitate efficient handling for further downstream processing using pumps (Greenwell et al., 2010).

As it is mentioned earlier, pre-concentration (bulk harvesting) aims to separate the micro algal biomass from the bulk suspension. Flocculation, flotation or gravity sedimentation is the frequently used methods where filtration, centrifugation and ultrasonic aggregation are used in thickening (Brennan and Owende, 2010).

The key harvesting and dewatering techniques currently used for microalgae are:

- **Settling and sedimentation – gravity sedimentation**: Gravity (natural) sedimentation can be the first step in concentration (pre-concentration). However depends on the species, sedimentation rate might be fast or slow (Shelef et al., 1984; Olaizola, 2003; Choi et al., 2006). Therefore more focused research is needed according to choice of species.

Some reports confirm that when CO_2 ceases to make a contribution to the culturing media, sedimentation occurs in a few hours because of the density difference of water and the microalgae species (Shelef et al., 1984; Heasman et al., 2001; Olaizola, 2003). This sedimentation might be due to the consumption of reserve sources such as lipids, which are necessary if biodiesel is to be produced, and a series of fatty acids with high value aggregates such as EPA, ß-carotene, etc (Chisti, 2007; Molina Grima et al., 2003). It is then necessary to determine the extent to which the intracellular content varies over a period of time without the contribution of the substrate being withdrawn. However, in some cases, depending on the species, sedimentation rate is very slow (0.1–2.6 cm h^{-1}) (Choi et al. 2006), and in high-temperature environments, much of the biomass produced will deteriorate during harvesting process. In consequence, sedimentation alone is largely dismissed as a viable harvesting method.

- **Chemical Methods** – chemical and/or biological harvesting, by means of flocculants e.g. auto flocculation, chemical coagulation, inorganic coagulants, organic flocculants, combined flocculation, electro-coagulation and ultrasonic aggregation. Flocculation is used in order to ease the following dewatering steps in this study.

Flocculation:

When natural sedimentation is not fast enough, flocculants can help to concentrate the particles more easily in suspension.

Knuckey et al. (2006) defined flocculation as the coalescence of finely divided suspended solids into larger loosely packed conglomerates, a process used widely in industry to remove suspended solids, e.g. for clarification of drinking water. In general, the first stage of flocculation is the aggregation of suspended solids into larger particles resulting from the interaction of the flocculants with the surface charge of the suspended solids. The second stage involves the coalescing of aggregates into large flocs that settle out of suspension.

The flocculation mechanism depends on cell and flocculant charges. Microalgae cells have negative charges on the surface of cell walls (at natural water pH) which prevent cells from aggregating in suspension, so they should be neutralized by using flocculants. The efficiency of flocculation is affected by the concentration, the ionic strength, the zeta potential (ξ) – which is also called the electro-kinetic potential (Samasundaran, 2006)–, the pH of the culture solution, the dosage of the flocculants and co-flocculants, the pH adjustment before or after the flocculants are added, and the mixing time and speed. The degree of salinity also influences the effectiveness of flocculants. If the ionic strength is high,

flocculants cannot make proper bridges between cells and this leads to low efficiency.

Metal salts (aluminium sulphate, ferric chloride, ferric sulphate, etc) are largely preferred in flocculation processes, because they lead to high harvesting efficiencies.

The application of polymers in flocculation is not a novel area. Ionic polymers are also called polyelectrolytes and characterised by their ionic properties. Compared with metal salts, cationic polyelectrolytes are less efficient at recovering microalgae (Pushparaj et al., 1993). Many studies have found that optimum flocculation occurs at polyelectrolyte dosages around the dosage that just neutralises the particle charge or gives a zeta potential close to zero (Kleimann et al., 2005; Bolto and Gregory, 2007).

Even though metal salts and polyelectrolytes are efficient at harvesting microalgae, they can also cause toxicity in the final product or the rest of the solution.

Nevertheless, natural polymers such as chitosan, cationic starches or bentonite, which are used in the agro food industry to clarify wines, have cationic properties. Chitosan (see Muzzarelli, 1977), a polymer that is obtained by deacetylating

chitin using the KOH process (Bough et al., 1978), is the most prominent of the natural cationic polymers. It is known to be non-toxic and non-hazardous to human health and also enables the medium to be recycled after the process has been completed.

It is clearly known that dosage of the flocculant strongly depends on microalgae species, concentration of the culture, medium and the process conditions. It is really hard to make a general mass balance calculation as how much flocculant would be needed per ton of culture from the literature.

One of the problems in large scale productions of microalgae is the development of efficient downstream processing to enable efficient separations of cells from culture broth as well as to maintain their viability and bioactivity (Harith et al., 2009).

If the chosen flocculant requires significantly lower dosages, as a result, it also improves the efficiency of downstream process due to improved supernatant clarities and also reduction of extra process costs such as product isolation and product purification in the stage of downstream process. Especially, if the flocculant that is added to the culture is toxic, after harvesting, it is essential to be purified, even if the rest of the solution has to recycle to process. It is a negative downstream effect that increases the capital cost of the process and also a problem

from the point of view of safety hazard. However, if the flocculant is biodegradable (such as microbial flocculants) their degradation products are harmless to the ecosystem and there is no need to purify (Salehizadeh and Shojaosadati, 2001).

In addition, if the chosen flocculant and/or flocculant dosage causes any damage on the cell wall, there might be two possibilities of negative downstream effect. If the content of the cell get mixed up with the solution, it might be hard to reduce the volume of slurry and concentrating the necessary part to get the final product. Another negative effect is the chemical change produced in the original lipids by the addition of flocculants.

Determining which flocculant is the most efficient for the whole process is an important parameter from the point of downstream process after the sedimentation and/or flocculation.

- **Filtration:** Filtration mainly separates solids from liquid by retaining those particles that are larger than the diameter of the pores of the permeable membrane (cut-off), which has different pressures on both sides of the membrane. The cost of the overall process is mainly due to the energy needed to apply the force to obtain an inter-membrane pressure difference—which can be achieved by means

of gravity, vacuum, pressure or centrifugal force—and the fact that the used membranes have to be replaced and cleaned periodically.

- **Centrifugation** – algae can also be harvested using centrifugation. Centrifugation is a method of separating algae from the medium by using a centrifuge to cause the algae to settle. The method is based on the principle that the gravitation field to which the cells are subjected is increased, then cell separation may be achieved faster. In most large-scale centrifuges, a centrifugal force equivalent to 5000–10000 g is possible, and this can achieve over 95 per cent removal under the correct operational conditions with large algal cells. However, at a large scale, the use of centrifuges become more problematic as the capital and operating costs increase with scale. This, together with the specialized materials of construction (high strength, corrosion-free alloys) and high maintenance costs required to operate in saline environments, means that these separations are expensive. Energy costs of about 1 kW h m^{-3} have been quoted for centrifuges (Molina Grima et al., 2003). Membrane filtration technology is an increasing trend to harvest microalgae because it is believed that capital, maintenance and management costs are lower (Wang et al., 2006).
- **Flotation** – froth flotation is a method whereby the water and algae are aerated into froth, with the algae then removed from the water; and

- **Electrolytic method** – such as the electrophoresis technique. Electrolytic flocculation is based on the movement of negatively charged microalgae toward the anode. Upon reaching the anode, the microalgal particles lose their charge and are able to form aggregates. Electrolysis of water produces bubbles that can aid the microalgal flocs to rise to the surface The main advantage of this process is that there is no need for flocculants. A disadvantage of this process is that the cathodes are prone to fouling (Vandamme et al., 2011a).

To overcome the difficulties of harvesting microalgae, at least two separation methods need to be combined. In-depth research is needed for every species if the best combination of separation methods would like to be determined. The objectives, then, are to increase the concentration of microalgae and reduce the volumes (or flows) to be handled in further steps of downstream.

Various general guidelines can be followed to determine which combination of methods is best for obtaining biomass that is ready to use and best suited to the desired final product.

1.1 Objectives and Scope

The goal of this study is to determine a simple, rapid and an efficient pre-concentration method -preferentially cost effective- for *Phaeodactylum tricornutum* and

Nannochloropsis gaditana through testing, (1) natural sedimentation; (2) flocculation with commercial flocculants (aluminium sulphate, polyaluminium chloride) and chitosan; and (3) pH induced flocculation.

Little research (Spilling et al., 2010; Vandamme et al., 2011b) has been published on pre-concentration by auto-flocculation of these species. Low-energy harvesting and optimal methods for maximizing algae recovery and reducing costs are worth investigating. This study also examined sedimentation rates with alkalinity induced flocculation (simulation of auto-flocculation) where satisfactory efficiencies were obtained with studied algae species. With the obtained results, we aimed to assist the designs to scale-up processes. To continue the further processes without any problem and to avoid possible problems on final product, the influence of the harvesting method on the cells were investigated by microscopic examinations as well.

2.0 Materials and Methods

2.1 Microalgal cultures

The marine phytoplankton species *Phaeodactylum tricornutum* and *Nannochloropsis gaditana* were obtained from the Institut de Recerca i Technologia Agromentaries (IRTA) in Sant Carles de la Rapita (Tarragona, Spain). They were selected because they

are easy to grow in large volumes, have high lipid contents, and examples of single celled, non-clumping, small medium-sized microalgae.

For cultivation of *P.tricornutum* species, f/2 medium of Guillard and Ryther (1962) was used in agar plates with 15 mL cultures. Cultures of 200 mL (conical flask) to 4 L (6 L volumetric flasks) were grown with Walne's medium (Walne et al., 1970) under the continuous illumination of daylight fluorescents TDL18W-840 with a 100-140 μmol photons m^{-2} s^{-1}. A stream of air was injected to provide atmospheric CO_2 and culture agitation.

For larger volume cultures (300 L), seawater was filtered through 25, 10, 5 and 1 μm pore sizes filters (polyKLEAN, MICRO-KLEAN, 3M/ Cuno). Then it was sterilized with bleach then neutralized with $Na_2S_2O_3$ respectively to eliminate biological contamination. Algae were grown in polyethylene bags with commercial fertilizer (0.3 mL L^{-1} Codafol 14.6.5, Sustainable Agro Solutions S.A., Lleida, Spain), B group vitamins (0.02 mL L^{-1} AquavitaB, Syva, Leon, Spain) and sodium silicate (107 μM, Sigma-Aldrich). The bags were illuminated at 100 μmol photon $m^{-2} \cdot s^{-1}$ with cool white fluorescents (24h light cycle). Aeration was also used to provide CO_2 and agitation. Temperature was regulated at 20±2 ^{o}C by means of light and aeration. The cultures were harvested on the first day of the stationary growth phase after six days of growth.

For *N.gaditana* species, only sodium silicate was not added. The cultures -slurry of 300 L- were harvested on the first day of the stationary phase after 10 days of growth. Cell density and $pH_{culture}$ were monitored offline by taking daily samples. The culture properties of species are detailed in Table 3.1.

Table 3.1 Characteristics of microalgal cultures

Species	Shape of cells	Mean size (µm)	Cell concentration (cells mL^{-1})	Dry weight (DW) (mg L^{-1})	Ash-free DW (mg L^{-1})	Salinity (‰)	pH	Mg &Ca ions composition (mM)
Pht†	Fusiform	Length:18 Width:3.5	$3.11\pm0.09x10^6$	104.62±2.5	63.43±1.92	~38	9-10	Mg:57.8±0.05 Ca:12.7±0.57
Nng‡	Spherical	3-4 (diameter)	$14.03\pm0.67x10^6$	132±3.32	89.92±1.61		8.5-10	Mg:57.8±0.0 Ca:9.8±0.51

*Cell concentration of the species at the time of harvesting.
†Pht-*Phaeodactylum tricornutum*
‡Nng- *Nannochloropsis gaditana*

Cell concentration

Cell growth was monitored by means of daily cell counts with a haemocytometer. Cell concentration for flocculation samples were monitored with a UV-visible spectrophotometer (Synergy HT Multi-Mode Microplate Reader, Biotek) using optical density (OD) measurements at 750 nm (wavelength for turbidity). Cell concentration was calculated by means of least square regression fitting by inter-plotting absorbance levels obtained by correlating cell counts taken with a Neubauer improved haemocytometer

(Brand) and a compound microscope (Olympus BH-2) after fixing the cells with formaldehyde (37%). The correlation of the measurements is described by the equation;

$y=65.135*10^{6}x+640519$ ($R^2= 0.99$) for *P.tricornutum* species, and

$y= 14.056*10^{7}x+928993$ ($R^2= 0.99$) for *N.gaditana* species

for the wavelength for turbidity, where y represents the cell density (cells mL^{-1}) and x is the absorbance value at the required wavelength. Formaldehyde solution (37%) was used as a fixative for turbidity samples (1 mL of sample was fixed with 10 µL of solution). Microscopic photos were taken using microscopes Leica DMI 3000B for *P. tricornutum* and Zeiss Axio Scope A1 for *N. gaditana.*

Ash-free dry weight (AFDW)

Aliquots of algal suspension were filtered through preweighed, precombusted (400^{o}C, 2 h) glass fibre filters (Whatman GF/F Glass Microfiber filter 4.7 cm, nominal pore size 0.7 µm). During filtration, vacuum pressure differentials were maintained at 35 to 55 mm Hg). After the cultures were filtered, the filters were washed with 20 mL 0.5 M ammonium bicarbonate to rinse out the seawater salts (Zhu and Lee, 1997). The filters were dried at 100^{o}C to constant weight, cooled down in a vacuum desiccator, and weighed. Dry weight (DW) was calculated by subtracting the oven dried filter weight from the preweighed, precombusted filter weight. These oven dried filters were then

ashed in a furnace at 400^{o}C for 4h, cooled down in a vacuum desiccator, and weighed to obtain the ash-free dry weight.

2.2 Natural sedimentation experiments

The parameters studied in the natural sedimentation experiments were daylight, darkness and temperature to see the effect of light and temperature on sedimentation rate. Experiments were planned to determine the degree of dependence of microalgal sedimentation on storage conditions. After harvesting, the microalgal culture was placed in two-litre transparent glass cylinders ([height/diameter] ratio≈6). They were allowed to settle under the conditions described in Table 3.2. All experiments were conducted at the original pH of the cultures (see Table 3.1).

Table 3.2 Experimental conditions for natural sedimentation

Experiment	Light conditions	Temperature (^{o}C)
1	Daylight	19±1(= T_{room})
2	Dark	19±1(= T_{room})
3	Dark	12±1
4	Dark	4±1

At predetermined time intervals, the samples were taken from the top third (1/3h) of the cylinders (Figure 3.1). At the end of the experiments, samples were also taken from the top two-thirds (2/3h) and the bottom of the cylinders. Cell concentrations of the samples were determined by absorbance with a spectrophotometer at 750 nm.

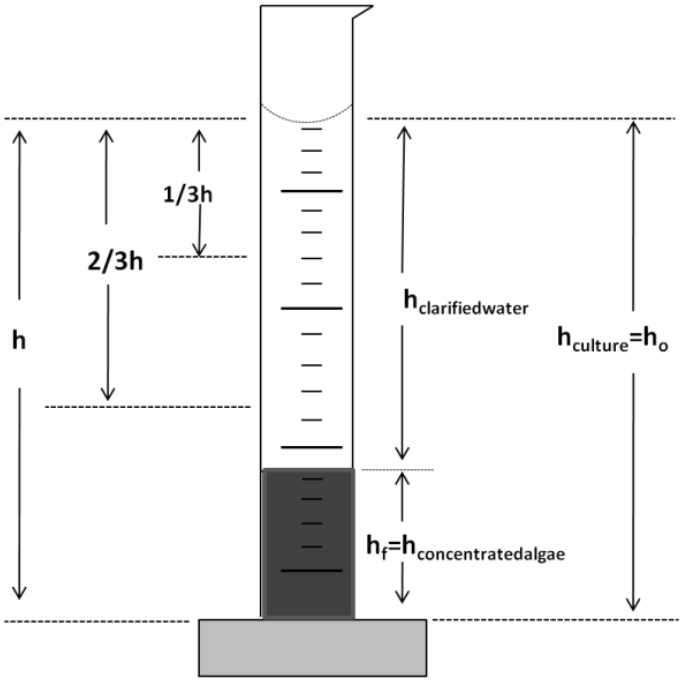

Fig. 3.1 Figure of sample heights for daylight-darkness, temperature difference and effect of pH experiments for flocculation

2.3 pH induced flocculation experiments

A wide range of pHs (pH=2 to pH=11) was tested to determine the effect of induced pH on flocculation. The experiments were done by adjusting the pH of the stock microalgae solutions to acidic and alkaline pHs with HCl or NaOH (0.1 and 1 N) solutions using the sedimentation experimental procedure in 250 mL cylinders. In order to obtain homogeneous pH, NaOH or HCl were added to cultures under agitation (300-350 rpm), followed by slower mixing and settling.

2.4 Flocculation experiments

50 mL of algal culture suspension was placed in a 100 mL beaker. When pH adjustment was needed, various amounts of HCl or NaOH solutions (0.1 and 1 N) were used. Different amounts of flocculants were added to 50 mL aliquots. The test beaker was stirred at 150 rpm for 2 min at room temperature, poured into polypropylene tubes with [height/diameter] ratio of ≈4, and left to settle. Samples were taken at 15 and 30 min. After the flocculation of the algal cells, an aliquot of the culture was drawn from 1/3h and 2/3h of tubes (see Figure 3.1). Finally, turbidity was measured using OD at 750 nm.

Flocculation efficiency (FE) was calculated using the following equation:

$$Flocculation\ efficiency(\%) = \left(1 - \frac{A-C}{B-C}\right) \times 100,$$

where A: OD_{750} (optical density at 750 nm) of sample, B: OD_{750} of initial culture and C: OD_{750} of reference blanks.

Reference blanks were used for every flocculating agent (seawater+used flocculant concentrations) in order to take into account the influence of dissolved flocculants on absorbance levels.

The concentration factor (CF), which is the ratio of the final product concentration to the initial concentration, was calculated as (Lee et al., 2009):

$$Concentration\ factor = \left(\frac{h_o}{h_f}\right) \times flocculation\ efficiency,$$

where h_o: initial height of examined algae solution and h_f: final height of concentrated algae solution (Figure 3.1).

Settleable solid volume fraction (SSVF) was calculated from the volume occupied by the settled algal precipitates as a fraction of the initial algal culture volume (Graves et al., 1979):

$$Settleable\ solid\ volume\ fraction = \left(\frac{h_f}{h_o}\right)$$

The SSVF is a fraction of the initial volume to be further processed, leading to a lower energy path of harvesting; the lower the SSVF, the better.

Flocculants

Polyaluminium chloride (PAC), aluminium sulphate (AS) and chitosan were used as flocculants.PAC and AS flocculants were purchased from Kemira Iberica S.A., Spain. The specifications for the products are shown in Table 3.3a. Chitosan was purchased from Sigma Aldrich Co., Spain. The stock solution of chitosan (5 g L^{-1}) was prepared by dissolving it in 1% acetic acid solution during continuous agitation until a clear solution

was obtained. The pH of 5 g L^{-1} stock solution of chitosan was approximately 4. The properties of chitosan are presented in Table 3.3b.

Table 3.3a Properties* of polyaluminium chloride and aluminium sulphate

	Active substance	**Percentage (%) of active substance**	**Density (25°C) (g cm $^{-3}$)**	**Viscosity (25°C) (mPa.s)**	**pH 1 (v/v)**
Aluminium sulphate(AS)	Al_2O_3[1]	8.2±0.1[1]	1.33±0.02	20±5	3.5±0.5
	Al_2O_3 (free)[2]	0.12±0.08[2]			
Polyaluminium chloride (PAC)	Al_2O_3[3]	17.5±1[3]	1.36±0.03	<60	4.0±0.5
	Chlorides[4]	21±2[4]			

*taken from Kemira Iberica datasheets

Table 3.3b Properties of chitosan

Characteristic molecular weight	**Degree of deacetylation (%)**	**Viscosity (cP)**	**Molecular weight (Da)**	**Density (g cm $^{-3}$)**
Low	75-85	(20-300)†1	(5-19x10^4) †2	0.15-0.3

†1-1% in 1% acetic acid, Brookfield †2- based on viscosity

3.0 Results

3.1 Natural sedimentation experiments

In all the natural sedimentation experiments we performed, no significant sedimentation of *P. tricornutum* nor *N.gaditana* was detected in either dark or light conditions or when different temperatures were tested (Figure 3.2).

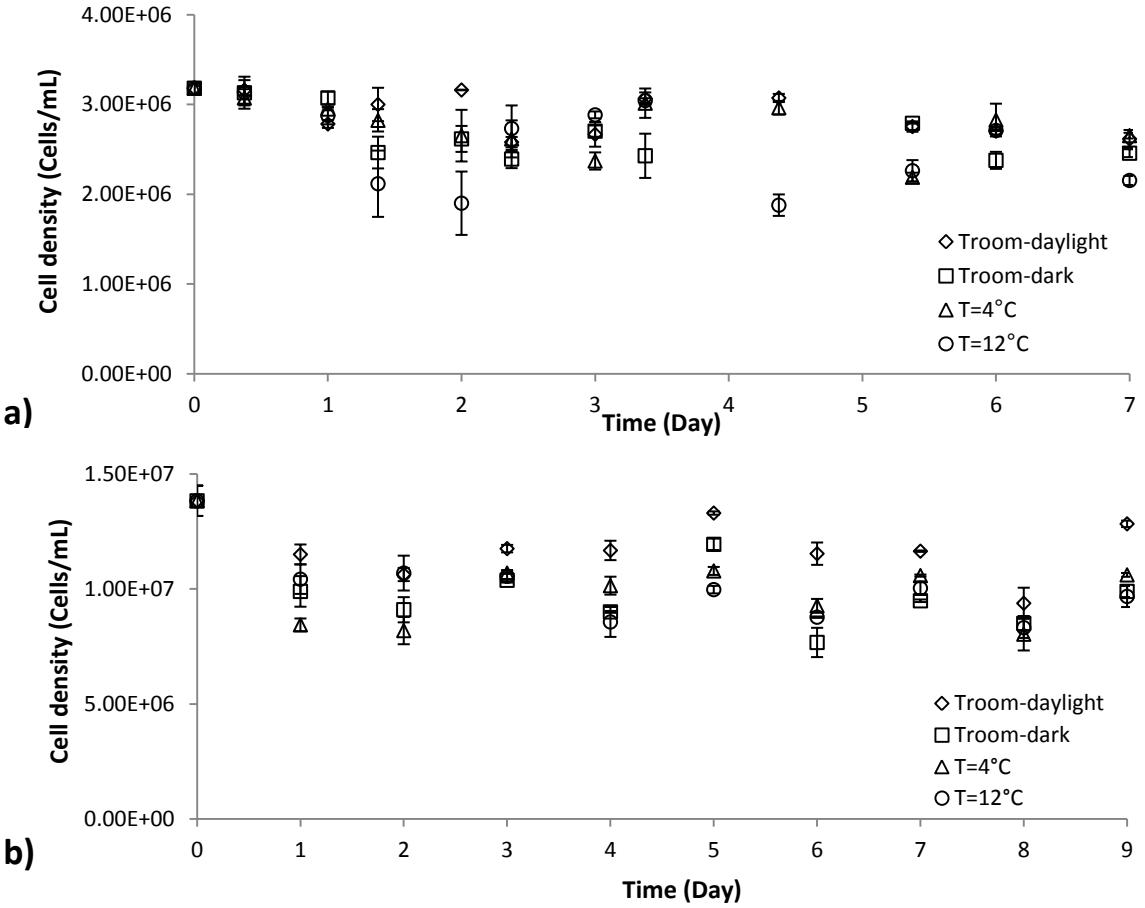

Fig. 3.2 Natural sedimentation of **a)** *P. tricornutum* **b)** *N. gaditana* cells under studied conditions in Table 3.2. Data are represented with ±standard error (SE) (n=3)

3.2 pH induced flocculation experiments

3.2.1 *P.tricornutum*

Figure 3.3a shows the flocculation efficiency (FE) and concentration factor (CF) of *P. tricornutum* cells versus pH induced to acidic-alkaline values at 1hour of settling time.

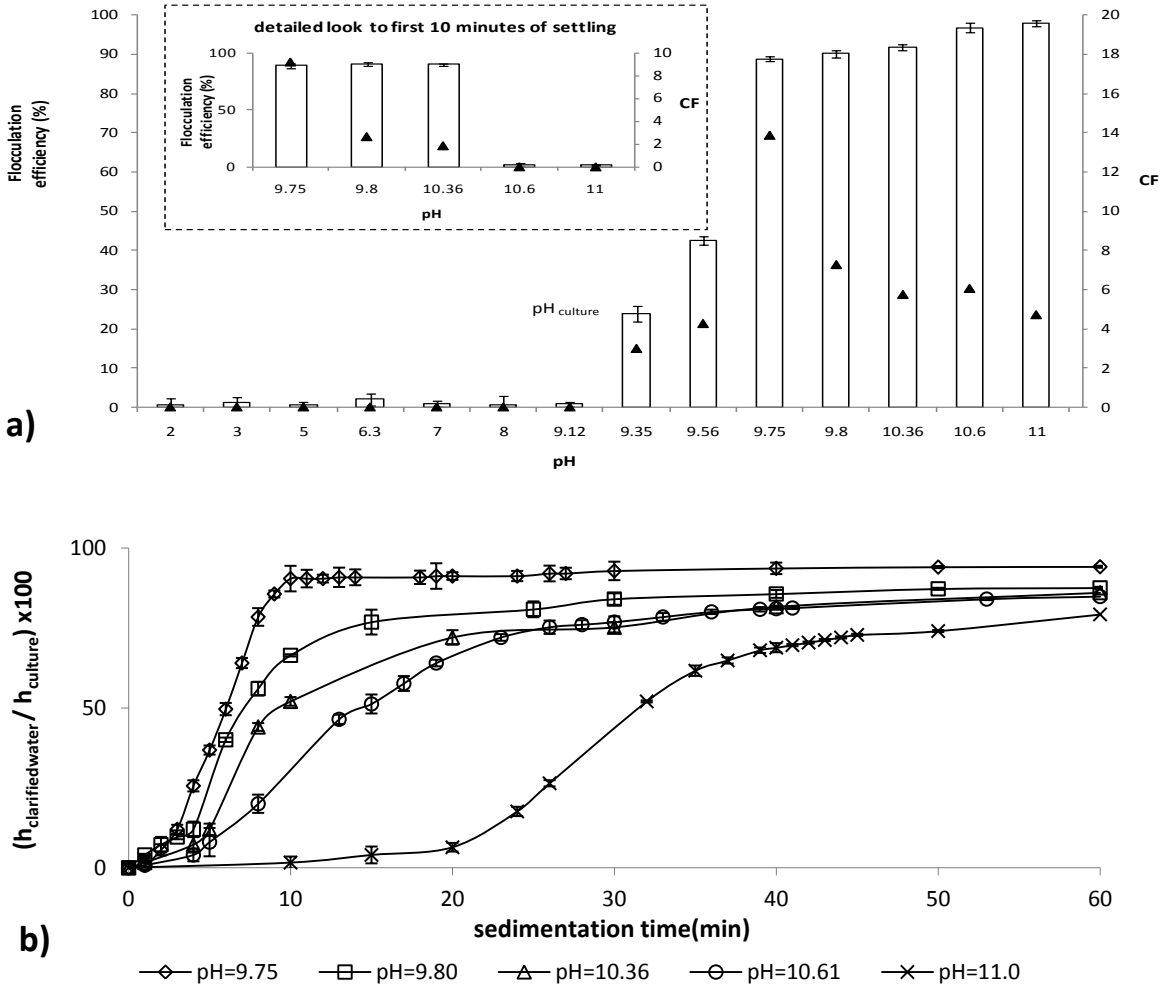

Fig. 3.3 a) Effect of pH change on flocculation efficiency and concentration factor (▲) of *P. tricornutum* culture at1hour settling, **b)** Graph of ($h_{clarified\ water}$/ $h_{culture}$) ratio versus sedimentation time to compare sedimentation rate for *P. tricornutum* culture. Data are represented with ±standard error (SE) (n=3)

Below $pH_{culture}$ (<9.12), satisfactory flocculation rates (CF≈0) were not observed with the addition of HCl. Even at pH=2 – the most acidic pH tested – no obvious visible change was observed in cell colour. However, a microscopic examination of *P. tricornutum* cells

under different pH treatments showed that chloroplasts deteriorated at low pHs (Figure 3.4).

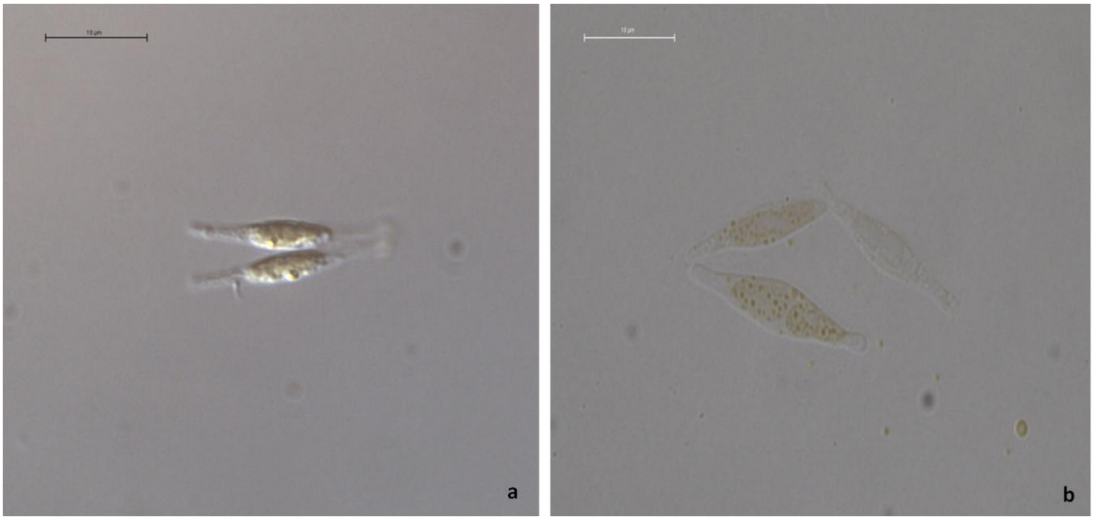

Fig. 3.4 Microscopic photographs of microalgal cells with acidic pH adjustment **(a)** culture without modified pH **(b)** culture with pH adjusted to 5. Scale bars = 10 μm.

Above $pH_{culture}$ (>9.12), after mixing NaOH (1 N) into the culture, a coarsening period was observed which was followed by sedimentation. During sedimentation, visibly larger flocs occurred and settled. An interface layer formed between the clarified solution and the concentrated algae cells. Between 9.12<pH<9.75, the displacement of concentrated cells occurred very quickly, but the FE was lower at the end of the settling period. With a slight increase in pH (to pH=9.75), harvesting efficiency was increased to 88% with a concentration factor (CF) of 9.3 and a settleable solid volume fraction (SSVF) of 0.064 in a short time interval (Table 3.4). A further increase in pH resulted in a slight increase in efficiency, but a decrease in CF (Figure 3.3a).

Table 3.4 Results for harvesting of *P. tricornutum* by inducing the pH to alkaline([height/diameter]ratio≈7)

pH	Max pH induced flocculation efficiency at 1hour (%)	Settleable Solid Volume Fraction (SSVF) at 10min (h_f/h_o)	Max SSVF at 1hour (h_f/h_o)	Concentration Factor (CF) at 10min	Cell condition after flocculation		Sedimentation rate ($cm\ h^{-1}$)
					Cell wall	Chloro-plast	
9.75	89	0.096	0.06	9.26	✓	✓	131
9.80	90	0.34	0.12	2.68	✓	✓	90
10.36	92	0.48	0.16	1.88	✓	✓	52
10.61	97	0.70	0.17	0.026	✓	✓	48
11.0	98	0.98	0.21	0.016	✓	✓	46

A threshold was reached at 0.5-0.7 degrees over the original pH of the culture (pH=9.75). At that threshold pH for *P. tricornutum* cells, the CF was at its highest value not only owing to an increase in FE, but also to a decrease in SSVF. After the threshold pH value was reached, increasing the alkalinity of the solution (9.75<pH) became more difficult. Up to pH=11, more efficient algae removal was obtained but with a much lower CF and SR.

In the experiments in which the algae started to settle efficiently, the height of the interface was recorded at regular time intervals. The sedimentation rate/settling velocity was taken as the slope of the straight-line portion of the plot of the interface height versus time (Graves, 1979). The sedimentation rates (SR) were compared within 9.75<pH<11.0 (Figure 3.3b). At the threshold pH (pH=9.75) the sedimentation rate (SR) was up to

131cm h^{-1}. When the pH increased to 11, the SR decreased to 46 cm h^{-1} (Table 3.4) with longer coarsening period at the beginning.

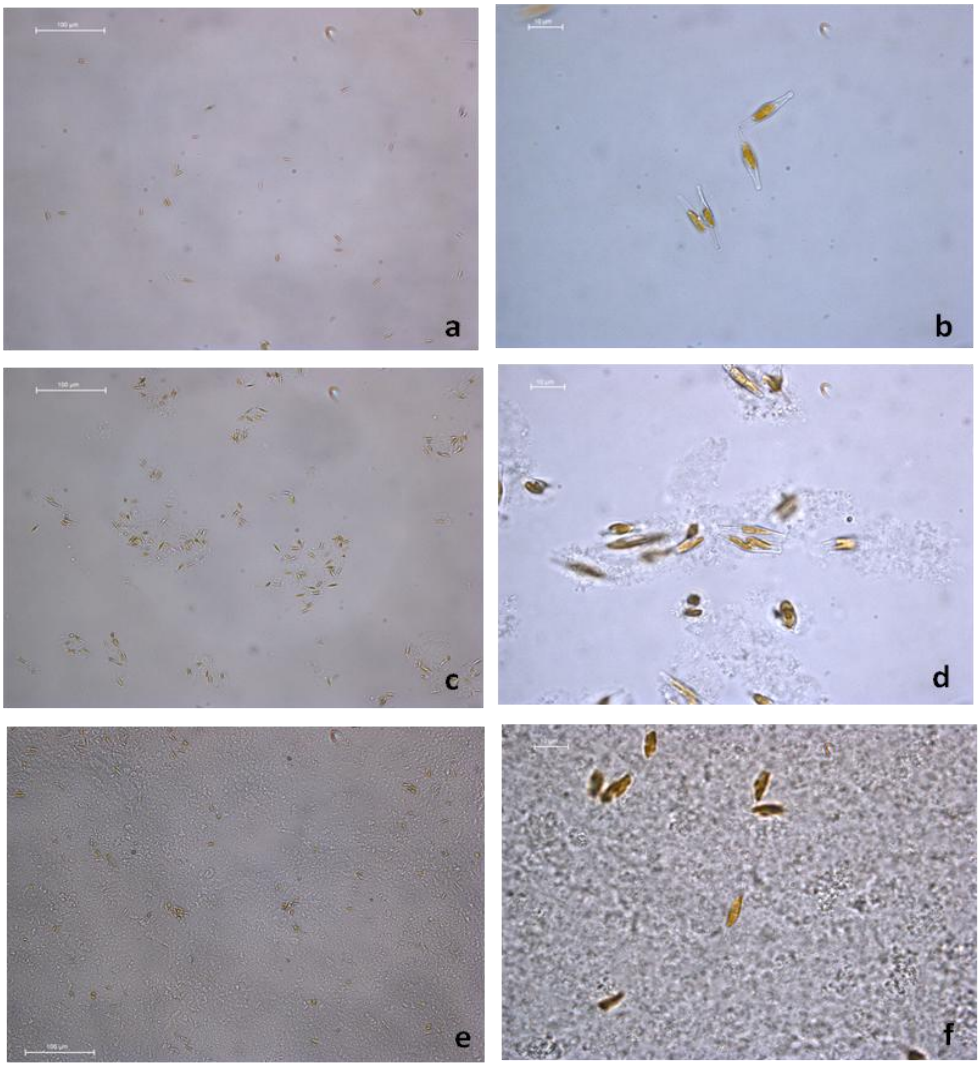

Fig. 3.5 Microscopic photographs of microalgal samples from the bottom of cylinders with alkaline pH adjustment **(a & b)** Cells of *P. tricornutum* in initial culture (pH=9.12), **(c & d)** flocculant mass containing algal cells of normal appearance after adjustment of pH to 9.75, **(e & f)** flocculated algal cells still of normal appearance, imbedded in a flocculant matrix after adjustment of pH to 11.0. Scale bars in figures a, c & e = 100 µm, in figures b, d & f = 10 µm.

3.2.2 *N.gaditana*

Figure 3.6a shows the flocculation efficiency (FE) and concentration factor (CF) of *N. gaditana* cells versus pH induced to acidic-alkaline values at one hour of settling time.

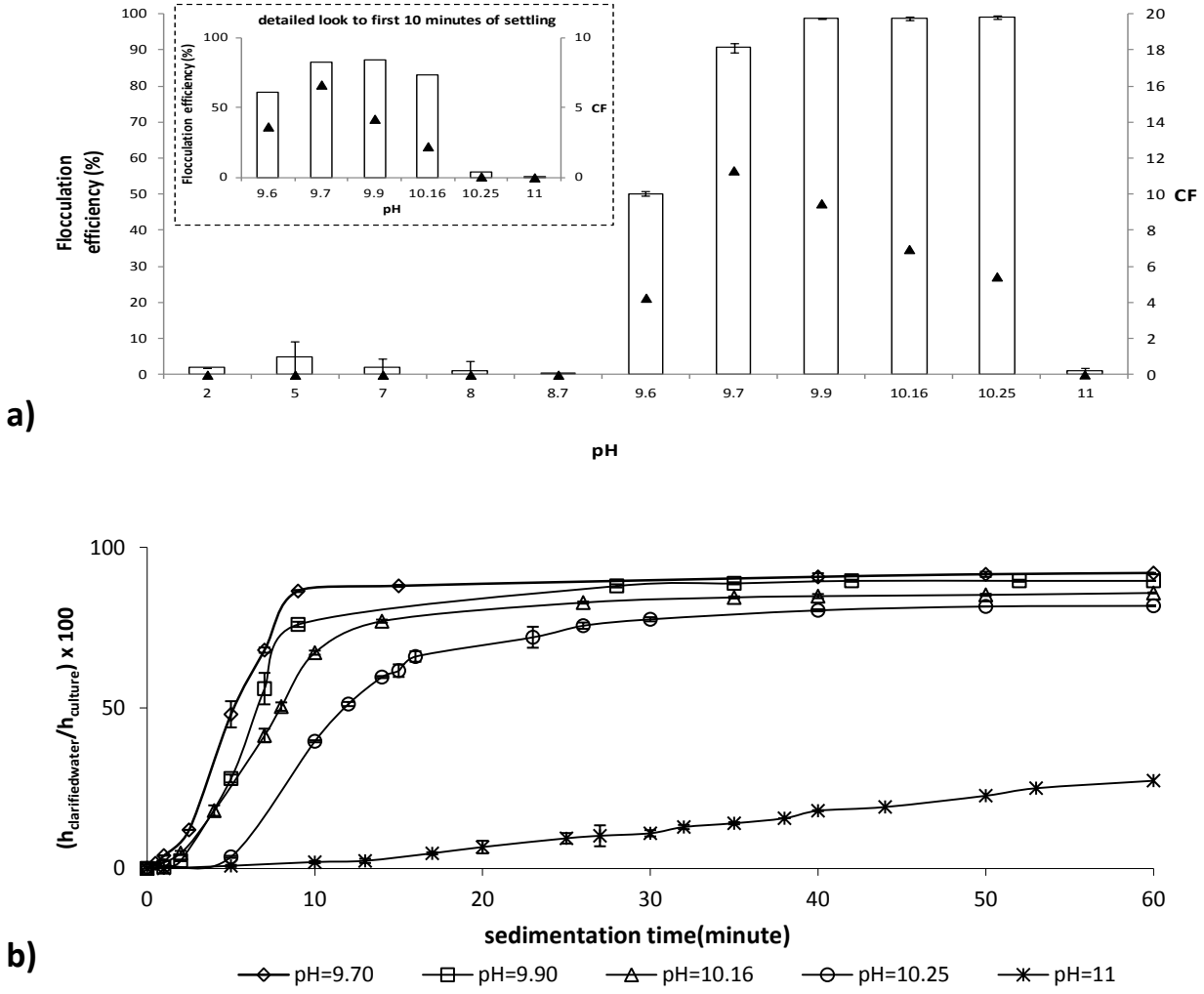

Fig. 3.6 a) Effect of pH change on flocculation efficiency and concentration factor (▲) of *N. gaditana* culture @1hour settling, **b)** Graph of ($h_{clarified\ water}$/ $h_{culture}$) ratio versus sedimentation time to compare sedimentation rate for *N. gaditana* culture. Data are represented with ±standard error (SE) (n=3).

No satisfactory flocculation efficiencies were observed at acidity-induced pHs (up to pH=8.7). As the acidity of the algae solution increased, the cell colour visibly changed and became lighter.

Microscopic examinations of acidic samples also show that under acidic conditions *N.gaditana* cells kept their shape and margin, however the chloroplast has shrunk (Figure 3.7; Figure 3.8b).

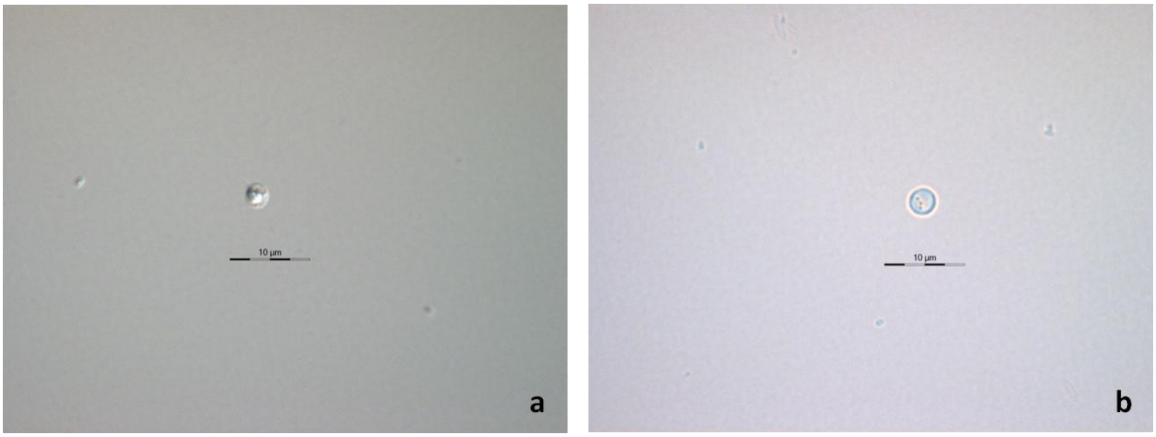

Fig. 3.7 Brightfield micrographs of microalgal samples of *N.gaditana* from cylinders with pH adjusted adjusted to 4.78.

When the pH of the culture was induced to alkaline pH values, the rapid increase in $pH_{culture}$ triggered floc formation. Near $pH_{threshold}$ values, $pH_{culture}<pH<pH_{threshold}$, rapid settling was observed, with low FE as in *P.tricornutum* cells. A threshold pH ($pH_{threshold}$=9.70) was reached at around 1 degree over the original pH of the culture ($pH_{culture}$=8.70). At that threshold pH for *N.gaditana* cells, harvesting efficiency (FE)

increased to 89% with a settleable solid volume fraction (SSVF) of 0.124 in 10 minutes (Table 3.5). The CF was at its highest value (6.65). Below the $pH_{threshold}$ value, FE and CF were both lower, whereas above the $pH_{threshold}$ FE was higher and CF lower. Higher flocculation efficiencies were observed up to pH=11 with slower sedimentation rates.

The height of the interface was recorded at regular time intervals as described previously. The sedimentation rates (SR) were compared between $pH_{threshold}$ and pH=11.0 (Figure 3.6b). At the threshold pH (pH=9.7) the sedimentation rate (SR) was up to 119 cm h^{-1}, whereas when pH was increased to 11, SR decreased to 6.3 cm h^{-1} (Table 3.5).

Table 3.5 Results for harvesting of *N.gaditana* by inducing the pH to alkaline ([height/diameter] ratio≈7)

pH	Max pH induced flocculation efficiency at 1hour (%)	Settleable Solid Volume Fraction (SSVF) at 10min (h_f/h_o)	Max SSVF at 1hour (h_f/h_o)	Concentration Factor (CF) at 10min	Cell condition after flocculation		Sedimentation rate (cm h^{-1})
					Cell wall	Chloro-plast	
9.70	90.61	0.124	0.08	6.65	✓	✓	119
9.90	98.78	0.200	0.104	4.20	✓	✓	96
10.16	98.88	0.328	0.142	2.23	✓	✓	78
10.25	99.07	0.604	0.182	0.07	✓	✓	54
11.00	1.20	0.979	0.723	0.00	✓	✓	6.3

The cells were in good condition after treatment with alkalinity induced flocculation. The *N.gaditana* cells maintained their colour. Microscopic examination showed that they kept their shape and the cells were also in good condition compared to those harvested at acidic pHs (Figure 3.8d-e).

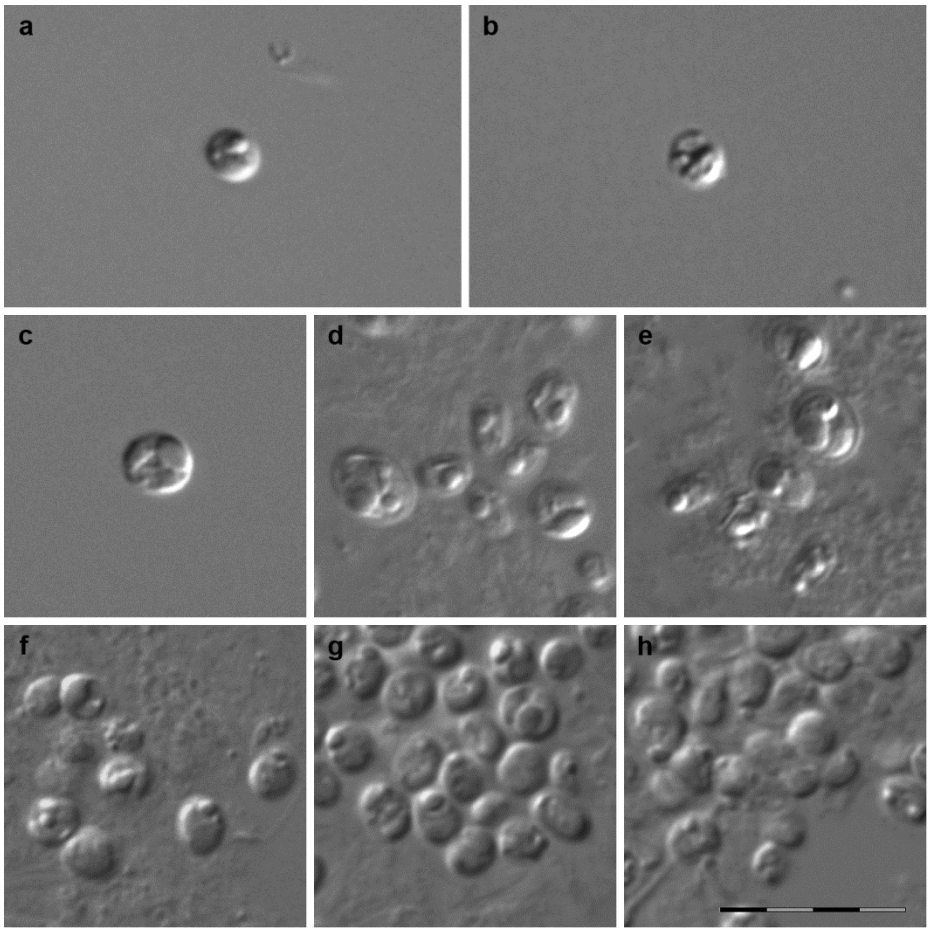

Fig. 3.8 Microscopic photographs of microalgal samples of *N.gaditana* from cylinders with pH adjustment **a)** culture without modified pH, **b)** culture with acidic pH adjusted to 4.78, **(c)** Cells of *N.gaditana* in initial culture (pH=8.7, for alkaline pH adjustments), **(d)** flocculant mass containing algal cells after adjustment of pH to 9.70, **(e)** flocculated algal cells still imbedded in a flocculant matrix after adjustment of pH to 11.0, (**f**) flocculated algal cells after addition of 20 ppm of AS, (**g**) flocculated algal cells after addition of 20 ppm of PAC, (**h**) flocculated algal cells after addition of 30 ppm of chitosan. Scale bars in figures = 10 μm. Microscopic photos of microalgal cells are from the bottom of tubes/cylinders (for d,e,f,g,h).

3.3 Sedimentation with flocculants

Ferriclar ($Fe_2(SO_4)_3$), ferric chloride ($FeCl_3$), aluminum sulphate ($Al_2(SO_4)_3$), polyaluminum chloride (PAC) ($Al_nCl_{(3n-m)}(OH)_m$), chitosan, Praestol-650, Magnafloc-

368, Magnafloc-1011, bentonite and polielectrolite novus were tried to harvest *P.triconutum* and *N.gaditana* species.

Praestol 650, Magnafloc 368, Magnafloc 1011, Bentonite and polielectrolite novus showed inefficient results with both species. Those flocculants are produced for waste water treatment and they have high moleculer weight to treat water with high waste initial concentrations. This might be the possible reason for low flocculation efficiency.

Ferriclar, ferric chloride, aluminum sulphate, polyaluminum chloride showed satisfactory efficiency for harvesting. We decided to focus on harvesting by aluminium sulphate ($Al_2(SO_4)_3$) and polyaluminum chloride (PAC) due to their costs and varied behaviour on concentration.

Primarily, the trials were conducted by adjusting the pH before and after the addition of flocculants depending on the pH-flocculant dosage effect. When pH was adjusted to lower values than $pH_{culture}$, chloroplast damage was observed at the time of the experiments or shortly thereafter for both species. Moreover, when the pH of the culture increased with NaOH solutions above $pH_{culture}$, alkaline-induced flocculation began or the turbidity of solutions increased. Based on the results of these pH adjustment experiments, we decided to perform alum/PAC experiments without adjusting pH.

3.3.1 *P.tricornutum*

3.3.1.1 Polyaluminium chloride (PAC) and aluminium sulphate (AS) flocculation

Figure 3.9a shows the changes in flocculation efficiency (FE) of *P. tricornutum* using PAC without adjusting the pH versus flocculant concentrations. The change in pH due to the addition of PAC is also shown. Samples were taken at 1/3h and 2/3h (see figure 3.1) after 15 and 30 min of settling.

For 30 min settling time, no significant differences were found in the FEs of samples that were taken at 1/3h and 2/3h. However, a marked difference was found in the FEs of samples taken at 1/3h after 15 min settling time, especially at PAC concentrations of between 20 and 50 mg L^{-1} (ppm). When settling time was doubled, FE considerably increased at both heights for each concentration. With PAC concentrations between 50 and 90 ppm, FEs increased by 15-20%; increases of nearly 100% or more were observed in all other ranges, especially for 2/3h samples. The most satisfactory results were achieved with the addition of between 30 and 70 ppm of PAC with final pH range of 6.5-7.6.

Figure 3.9b shows flocculation efficiency (FE) versus variety of AS concentrations. pH change due to AS addition is also reported. For 30 min settling time, the FEs of the AS

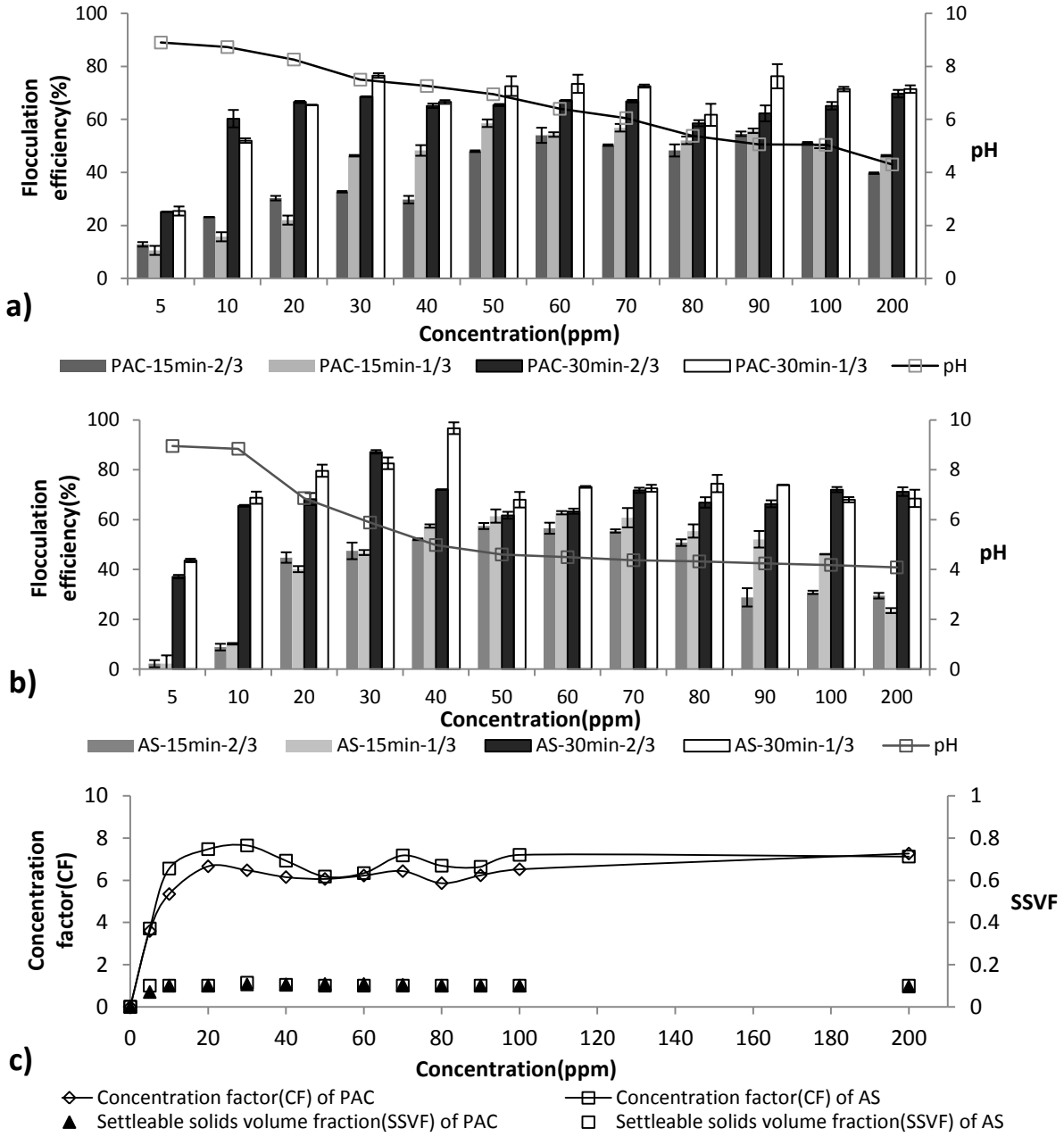

Fig. 3.9 Flocculation efficiency of *P. tricornutum* **a)** with PAC, **b)** with AS. Data are represented with ±SE (n=3). The abbreviations 1/3 and 2/3 represent one-third or two-thirds from the top of the tubes c) CF and SSVF according to PAC and AS concentration at 30 min settling time (FEs of samples taken from the top 2/3rds of the tubes were used to calculate CF).

samples did not show any remarkable differences between sampling heights. However, marked variances were seen at 15 min and 90 and 100 ppm concentrations.

When PAC and AS were used, the colour of the cells changed slightly from brownish to greenish at flocculant concentrations of over 90 ppm (naked eye examination). Microscopic examinations (Figure 3.10) shows that at higher concentrations of PAC and AS the condition of the chloroplasts deteriorated, cells changed colour and it appeared that the cytoplasm became more granular at higher concentrations.

With an initial concentration of ($3.11\pm0.09 \times 10^6$ cells mL^{-1}) of *P. tricornutum*, optimum results for PAC and AS flocculants were obtained at concentrations of over 20 ppm with algae recovery ranged between 60 and 80%. Our experiments using two commercial flocculants lead us to conclude that longer settling times (enough time for the flocs to stabilize and collapse) yield the same efficiencies between different sampling heights (Figure 3.9a-b). Removal efficiencies, CFs and SSVFs were the nearly the same after reaching proper concentration for commercial flocculants (Figure 3.9c).

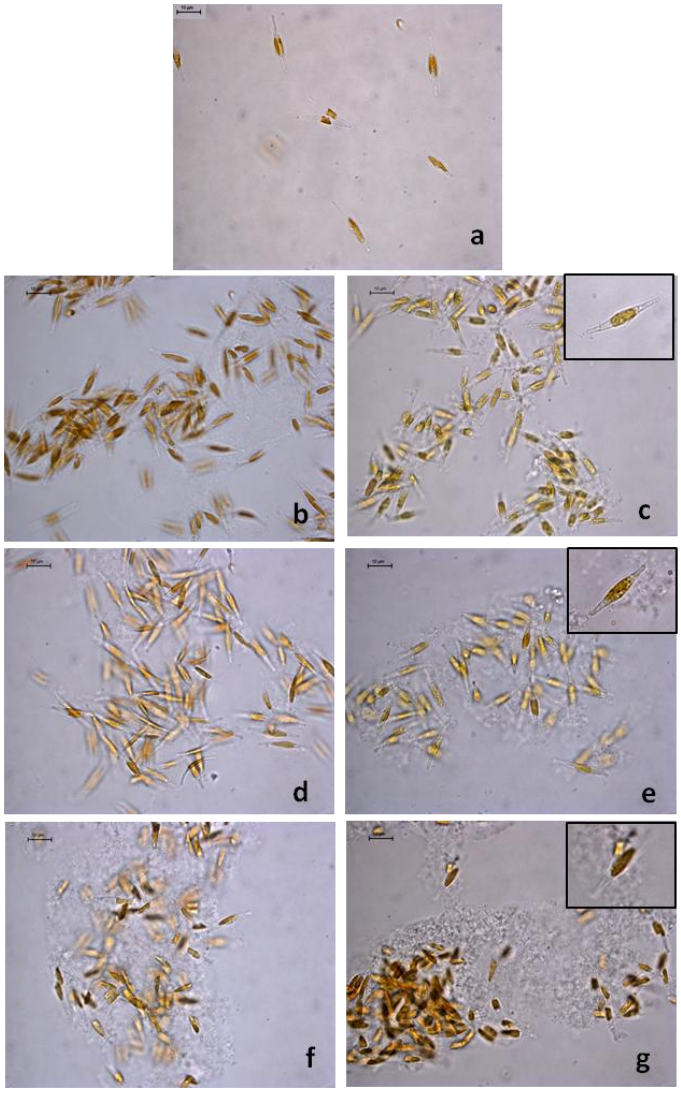

Fig. 3.10 Microscopic photographs of microalgal cells from the bottom of tubes **(a)** initial culture: scattered individual cells of *P. tricornutum*, **(b)** flocculated algal cells after addition of 40 ppm of AS, **(c)** flocculated algal cells after addition of 200 ppm of AS, **(d)** flocculated algal cells after addition of 40 ppm of PAC, **(e)** flocculated algal cells after addition of 200ppm of PAC, (**f)** flocculated algal cells after addition of 40ppm of chitosan **(g)** flocculated algal cells after addition of 200 ppm of chitosan. Scale bars= 10µm

3.3.1.2 Chitosan flocculation

Our results found no efficient flocculation using only chitosan in culture conditions (FE~15%). To improve flocculation efficiency, pH was adjusted within the range of 3 and 10.4. Flocculation efficiency and the concentration factor of *P. tricornutum* cells with pH adjustments to different values after the addition of 30 ppm chitosan are shown in Figure 3.11a. OD measurements were taken with 1/3h samples.

At acidic and slightly acidic pHs, flocculation efficiency was not high (<30%). However, a huge increase in efficiency was obtained by increasing the pH only to 9.9 in both sampling heights. We therefore decided to conduct further experiments to find optimum chitosan concentrations for the cultures at an adjusted pH level of 9.9.

In addition, even when pH was adjusted to 10.35, no change was seen in cell colour via naked eye examination. The cells maintained their shapes and chloroplasts seemed normal based on microscopic examinations.

Determination of optimum concentration for chitosan

The flocculation efficiencies of different concentrations of chitosan at adjusted pH (≈9.9) are shown in Figure 3.11b. Samples were taken at 1/3h and 2/3h (see figure 3.1) after 15 and 30 min of settling.

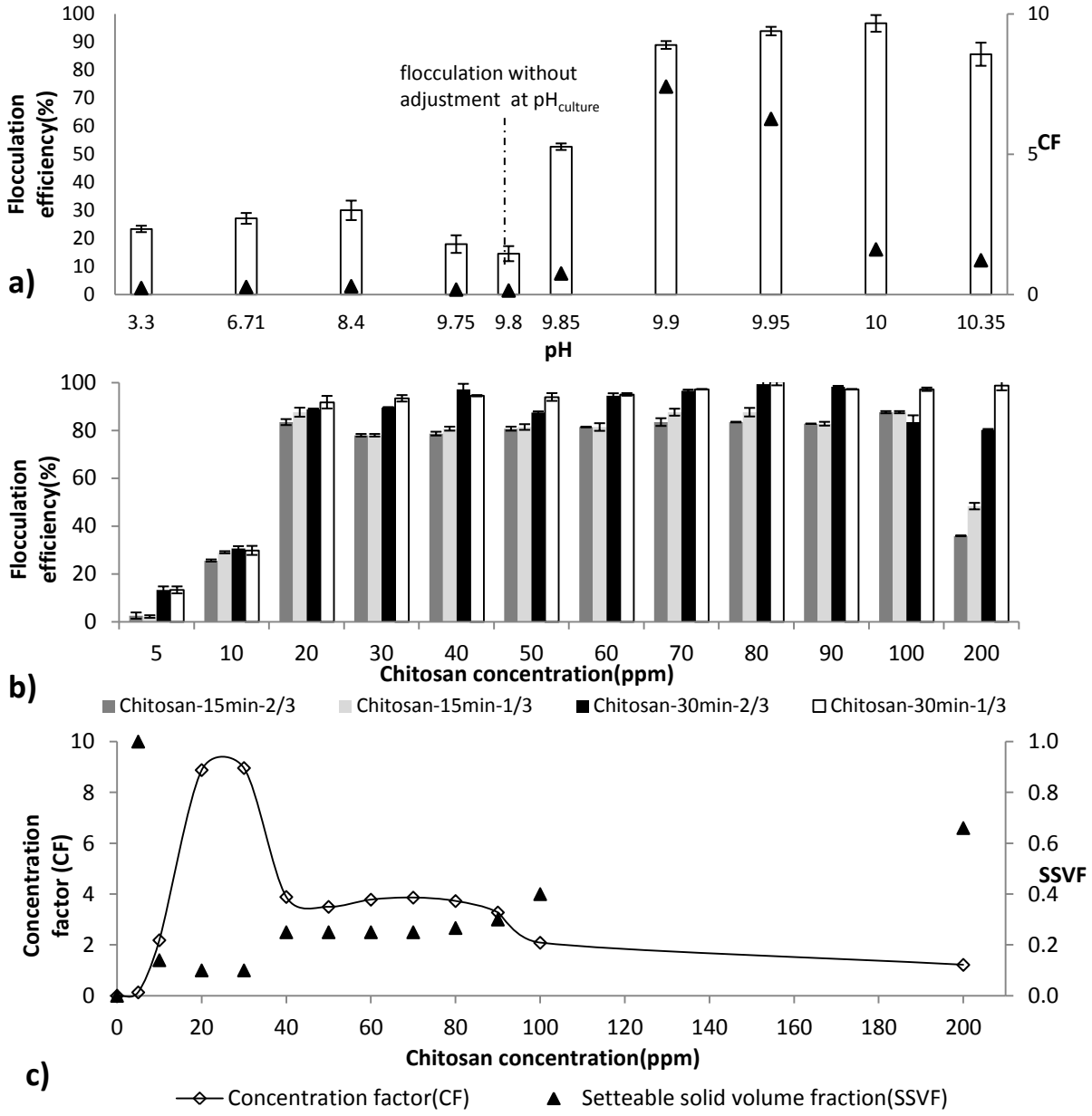

Fig. 3.11 a) Changes in the flocculation efficiency (FE) and concentration factor (CF) (▲) of *P. tricornutum* flocculated with 30ppm of chitosan according to pH at 15 min settling time. **b)** Flocculation efficiency of *P. tricornutum* versus chitosan concentration at pH=9.9. The abbreviations 1/3 and 2/3 represent one-third or two-thirds from the top of the tubes. Data are represented with ±SE (n=3) **c)** CF and SSVF according to chitosan concentration at adjusted pH=9.9 at 30 min settling time (FEs of samples taken from the top 2/3rds of the cylinders were used to calculate CF).

For both sampling heights, increasing the concentration of chitosan from 10 to 20 ppm resulted in an obvious increase in efficiency. On the other hand, flocculation efficiency did not show a marked increase at chitosan concentrations of 20 to 100 ppm. Furthermore, FE decreased considerably when the concentration was increased from 100 to 200 ppm.

At chitosan concentrations exceeding 20 ppm, removal efficiencies were the nearly the same, but CF decreased with increasing dosages of chitosan due to the increase in SSVF (Figure 3.11c). Meanwhile, the sedimentation rate decreased.

In opposed to the PAC and AS experiments, in the chitosan experiments, no conspicuous changes were observed in cell colour (via naked eye) or the chloroplast of the cells (by microscope) (Figure 3.10f-g).

3.3.2 *N.gaditana*

3.3.2.1 Polyaluminium chloride (PAC) and aluminium sulphate (AS) flocculation

Aluminium sulphate (alum) and poly-aluminium chloride (PAC) were tested for use in the harvesting of *N.gaditana* species.Figures 3.12a and 3.12b show the changes in the flocculation efficiency (FE) of *N.gaditana* cells using AS and PAC, respectively. The change in pH due to the addition of the flocculants is also shown in the figure. Samples

were taken after 15 minutes; 30 minutes at 1/3h and 2/3h and after 2 hours of settling at 2/3h.

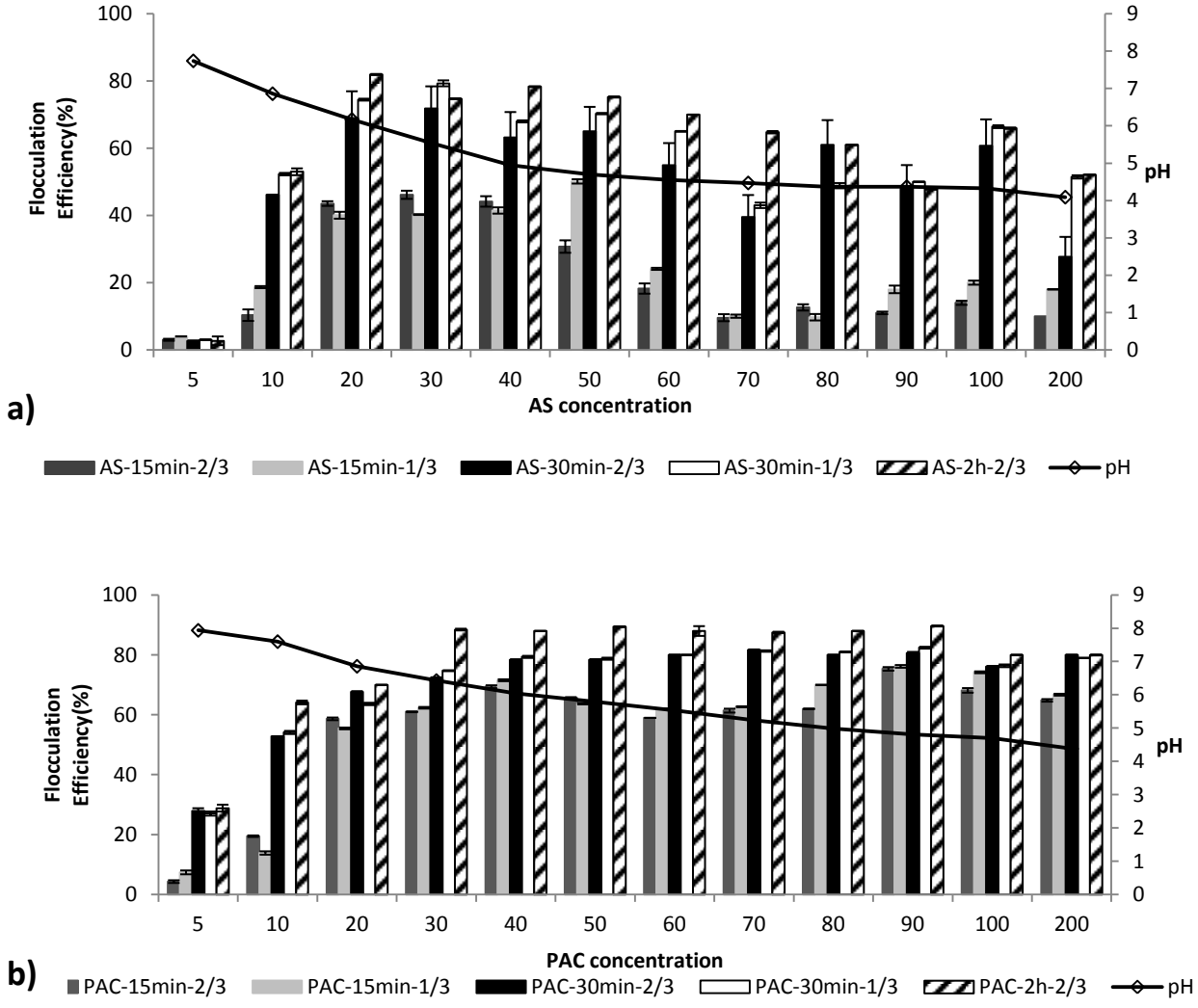

Fig. 3.12 Flocculation efficiency of *N. gaditana* **a)** with PAC, **b)** with AS,

After 15 min of settling time, no efficient flocculation was found for the AS samples, especially above 40 mg L^{-1} (ppm). The FEs of the AS samples did not show any great differences between sampling heights either. It seemed not enough time had passed for particles to appear. Very few flocs settled to the bottom of the tubes. When the settling time in the AS experiments was doubled, the increase in FEs was satisfactory. For AS concentrations above 50 ppm, 2 hours of prolonged settling time was also tested. No great differences in FEs were observed between settling times of 30 min and 2 hours at either sampling height. AS has an optimum pH range of 4.0 to 7.0, whereas the optimal coagulation with AS takes place at pH values between 5 and 7 (Wang et al., 2005). PAC is a flocculant with a wide pH adaption range of 5.0~9.0 and its flocculation efficiency is better (between 6 and 7.5) (Şirin et al., 2011). The optimum dosages found for the flocculants PAC and AS indicate that these final pH values are also suitable for *N.gaditana* cells.

The optimum concentration for AS flocculant with *N.gaditana* cells was found to be 20 ppm, regardless of the settling time. At 30 min settling time, the FE was around 70%. The FE for an AS concentration of 20 ppm at 15 min settling time was 30% lower than at a settling time of 30 min.

The FEs of samples taken at 1/3h after 15 min settling time were clearly different, especially above 20 ppm. When the settling time was doubled (30 min), and then

extended to 2 hours, no great differences were observed in the FEs at any of the PAC concentrations at any sampling height. Therefore, it seems that 30 min of settling time is sufficient to determine the optimum concentration of PAC for the flocculation efficiency of the *N.gaditana* species studied. With PAC concentrations between 10 and 20 ppm, FEs tripled for the 15 min samples. However, no great difference was found in the 30 min samples. Results were most satisfactory with the addition of 20 ppm of PAC and the final pH range was between 6.5 and 7.6 as it was in the *P.tricornutum* culture (Şirin et al., 2011).

In the *N.gaditana* cultures studied, the optimum results for PAC depended on the choice of settling time. At 10 ppm of PAC, 30 min settling time was found to be the optimum time with an algae recovery range of around 55%, whereas for 15 min settling time, 20 ppm was a better choice with an algae recovery range of around 60%.

Colour changes in the concentrated algae cells were observed at flocculant concentrations of over 90 ppm for both PAC and AS. This is most probably due to an effect of the low pH caused by flocculant additions as observed before in *P.tricornutum* samples. Rest of the concentrations, the cells maintained their colour. The chloroplasts seemed normal based on microscopic examinations (Figure 3.10f-g).

3.3.2.2 Chitosan flocculation

Chitosan, a cationic polyelectrolyte, has been tested for use in harvesting *N.gaditana* cultures. Figure 3.13a shows the FE and CF of *N.gaditana* cells versus adjustments to different values after the addition of 30 ppm of chitosan. OD measurements were taken for 1/3h samples. At acidic and slightly acidic pHs, no efficient flocculation was found. On the basis of flocculation efficiency, unsatisfactory results were obtained using chitosan alone. After the addition of chitosan, acidic and slightly acidic pH adjustments also failed to provide adequate FEs. However, when pH was adjusted to over $pH_{culture,}$ a noticeable increase in efficiency was obtained.

When the pH of the solution was induced to alkaline pH values over $pH_{culture}$ after the addition of 30 ppm of chitosan, the algae cells started to flocculate. An appreciable increment in flocculation efficiency was observed. The highest FEs and CFs were reached by increasing the pH to 9.9 in both sampling heights. We therefore decided to conduct further experiments to find the optimum chitosan concentrations at an adjusted pH level of 9.9.

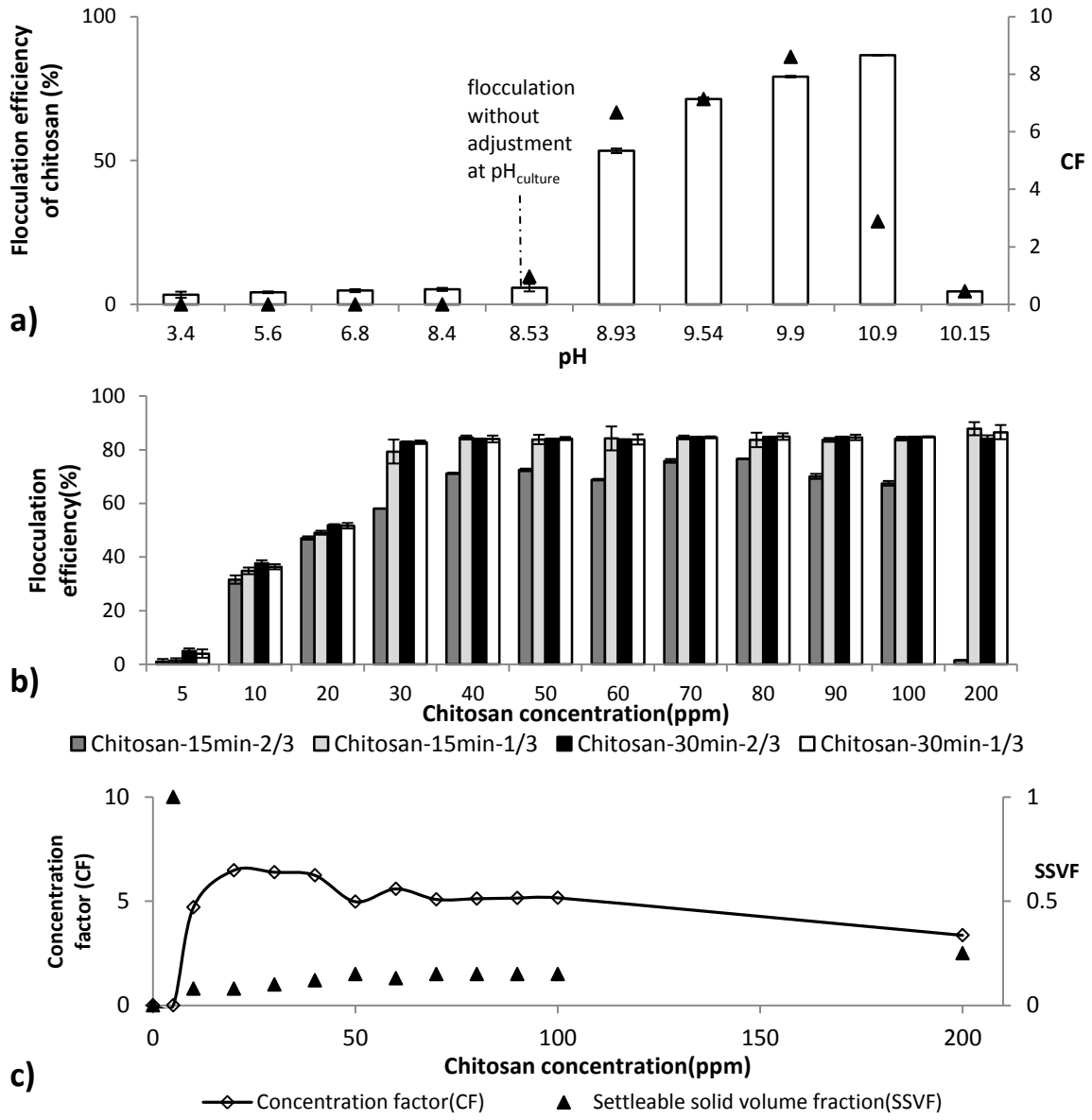

Fig. 3,13 a) Changes in the flocculation efficiency (FE) and concentration factor (CF) (▲) of *N.gaditana* flocculated with 30ppm of chitosan according to pH at 15 min settling time, **d)** Flocculation efficiency of *N.gaditana* versus chitosan concentration at pH=9.9 **e)** CF and SSVF according to chitosan concentration at adjusted pH=9.9 at 30 min settling time (FEs of samples taken from the top 2/3rds of the tubes were used to calculate CF). Data a,b,c,d are represented with ±SE(n=3). The abbreviations 1/3 and 2/3 represent one-third or two-thirds from the top of the tubes.

Determination of optimum concentrations of chitosan

The flocculation efficiencies of different concentrations of chitosan at adjusted pH (≈9.9) are shown in Figure 3.13b. Samples were taken at 1/3h and 2/3h after 15 and 30 min of settling.

The flocculation efficiency clearly increased after a chitosan concentration of 10 ppm, however the highest FEs and CFs were reached after 30 ppm. Additionally at chitosan concentrations exceeding 30 ppm, removal efficiencies and concentration factors did not significantly improve. Only at 200 ppm of chitosan was a settling time of 15 min insufficient to yield efficient CFs.

No conspicuous changes were observed in cell colour (via the naked eye) or the chloroplast of the cells (by microscope) (Figure 3.8h).

4.0 Discussion

Natural sedimentation can be the first step in concentration (pre-concentration). But, from the natural sedimentation experiments of *P. tricornutum* and *N. gaditana* species, it might be concluded that it was not efficient and fast enough method for these species.

The effective sedimentation of microalgae in natural conditions depends primarily on the species involved. The aquatic media in which microalgae are grown affects the charge of

the solution as well as the density of the culture, which are the most important parameters for natural sedimentation. Furthermore, light conditions are an important parameter in microalgae growth. Microalgae maintain photosynthetic activity in the presence of light and continue their metabolic activity. In dark conditions, they cannot perform photosynthesis and their metabolic activity decreases. Such lower metabolic activity can allow cells to agglomerate and settle faster in dark conditions (Danquah et al. 2009).

When acidic HCl solution was added to both cultures, no flocculation was observed. There was no connection between flocculation efficiency and acidic pH levels. Additionally, cultures cannot tolerate sudden and/or drastic changes in pH to acidic values.

There is a clear effect of pH on harvesting when $pH_{culture}$ was induced to alkaline pHs.

For *P.tricornutum* species with $3.11\pm0.09x10^{6}$ cells mL^{-1} cell density and $pH_{culture}$=9.12, $pH_{threshold}$ was found 0.5-0.7 degree over original pH of culture (pH=9.75) with max flocculation efficiency of 89%, max concentration factor of 14.83. The sedimentation rate was also the highest (131 cm h^{-1}) at threshold pH, because the algal cells started to form larger precipitates.

For *N.gaditana* species, when we increased pH even a little higher than culture pH, some chemical ions in the medium started to precipitate. The rapid increase in $pH_{culture}$ triggered

floc formation. Especially between $pH_{culture}$ and $pH_{threshold,}$ close to $pH_{threshold}$ values rapid settling observed with low FE. For *N. gaditana* species with $14.03\pm0.67x10^6$ cells mL^{-1} cell density and $pH_{culture}$=8.7, $pH_{threshold}$ was found 0.8-1.0 degree over original culture pH (pH=9.7) with flocculation efficiency of 89% and concentration factor of 4.5 in 10 minutes of settling time. Sedimentation rate was also the highest in the tested pH range. At $pH_{threshold}$, max FE was obtained with optimum sedimentation rate.

Under alkaline conditions, some chemical ions in the medium precipitate along with the algal biomass and help to harvesting process. Two major reactions are effective in liquid-solid separation when pH is increased: the precipitation of calcium carbonate $CaCO_3$ and the precipitation of magnesium hydroxide $Mg(OH)_2$ (Alexeyev, 1979). The role played by each reaction depends on the primary particles and the ions contained in the solution. $Mg(OH)_2$ precipitate has a positive superficial charge which attracts the colloid particles, inducing adsorption and then agglomeration which explains why significant efficiencies are reached when magnesium hydroxide is precipitated (Leentvaar and Rebhun, 1982). Calcium phosphate precipitates are also considered flocculating agents, which react with the negatively charged surface of the algae and promote aggregation and flocculation (Sukenik and Shelef, 1984). Precipitation of magnesium begins at approximately pH 9.5, becomes significant above pH 10.5, and is essentially complete at pH 11.0–11.5 (Merril and Jorden, 1975; Dziubek and Kowal, 1984; Semerjian and Ayoub, 2003).

The pH of the algae solution is depending on the species, algae population, growth medium which makes harder to predict the sedimentation behaviour and rate of the algae. Nevertheless, pH induced flocculation mechanism was depending not only on growth medium chemical properties (Mg ion content etc), but also to the algae species. Each alga species reacts individually/differently to addition of alkaline solutions to culture due to having different electrokinetic charges. In fact, all species have charges usually negative with different zeta potentials and charge densities (Sukenik and Shelef, 1984). The structure of the cell wall and materials in every microalgae species are different and it affects alga's sedimentation behaviour.

P. tricornutum and *N. gaditana* cultures reach high alkaline pHs during the exponential growth phase due to photosynthesis (CO_2 consumption). Therefore, the threshold pH can be used to achieve auto-flocculation by adjusting the air or CO_2 supply. There must be a certain specific threshold pH where auto-flocculation begins for each different species (Yahi et al., 1994; Blanchemain and Grizeau, 1999; Spilling et al., 2010). Additionally, it should only be noted that each growth medium has unique electrical properties. Therefore, even identical species being cultured in different algal culture mediums might also have a specific threshold pH that triggers auto-flocculation (Şirin et al., 2012). These findings are parallel to results of Spilling et al. (2010) for *P. tricornutum* and Vandamme et al. (2011) for *Chlorella vulgaris*. However, if artificial pH manipulation will be used to

reach alkaline pHs, the environmental impact of alkalinity in residual water must also be considered.

Flocculation caused by alkaline adjustment has not only been used effectively by Sukenik and Shelef (1984), Ayoub et al. (1986) and Elmaleh et al. (1991) but were also applied in last decade by Horiuchi et al. (2003) for *Dunalliella testolata* sp., by Csordas & Wang (2004) for *Chaetoceros sp.*, by Spilling et al. (2010) and Vandamme et al. (2011a) for *P. tricornutum*.

Harvesting *P. tricornutum* and *N. gaditana* with the addition of commercial flocculants was tested as a potential concentration step. Among the available coagulants, aluminium sulphate (AS) and polyaluminium chloride (PAC) are the most extensively used coagulants/flocculants for sludge conditioning and dewatering coagulation processes. In water treatment processes to find the optimum conditions for chemical flocculation, pH is adjusted -especially priorly of flocculant addition-. It is known that flocculants/coagulants are pH dependent for performance and non-optimal pH causes excessive dosages. Mostly, they work in a pH range of 4-10. Since the goal is treating water, conditions of the organisms are not the main objective in the process. However, in our study it is important the quality of algal biomass after flocculation. After harvesting, to keep concentrated algae in good conditions was one of our first priorities -to avoid any problem in the further processes and final product-.

Both two flocculants are widely used in water treatment processes. AS has an optimum pH range of 4.0 to 7.0 where the optimal coagulation with AS takes place at pH values near 5 and 7 (Wang et al. 2005). PAC is a flocculant with a wide pH adaption range of 5.0~9.0 and shows a better flocculation efficiency between 6-7.5. The found optimum dosages for the flocculants PAC and AS supports that those final pH values are also suitable for *N.gaditana* cells.

The use of polymerized forms of aluminium (e.g. PAC) has become more common because of their higher charge density compared to AS. Additionally, PAC and polyaluminium sulphate (PAS) often result in a decrease in coagulant doses and the associated production of solids (Zhao, 2003) compared to AS.

The optimum dosage for the flocculants PAC and AS was found to be 30ppm for *P. tricornutum* cultures with final pHs of 7.5 and 5.9 respectively. But for *P. tricornutum*, AS provided a moderately higher degree of efficiency with a higher CF. For *N.gaditana*, AS provided a moderately faster sedimentation according to PAC with better concentration factors and FEs as well. However, subsequent to this potential harvesting approach using commercial flocculants, a downstream flocculant removal process may be required. The need for this treatment would increase the process costs. Otherwise, the presence of flocculants in further downstream, extraction and/or fuel conversion

processes must also be understood and checked. Moreover, if residual water is intended for recycling, the effect on culture growth and lipid production must be clarified.

At flocculant concentrations of over 90 ppm for PAC and AS for *P.tricornutum* and *N.gaditana*, the colour change in the concentrated algae cells were observed which most possibly an effect of the low pH (down to 4) caused by flocculant additions. It appeared that the cytoplasm became more granular, possibly as result of lipid drop formation at higher concentrations of PAC and AS treatments (Figure 3.8c, 8e for *P.tricornutum*).

Chitosan was also tested for its harvesting ability on *P. tricornutum* and *N. gaditana* cells. No efficient flocculation was observed using only chitosan in culture conditions for both species. When we adjusted the pH to acidic and slightly acidic pHs afterwards adding chitosan, flocculation efficiency was not high as well.

Chitosan flocculation presumably takes place by charge neutralization and bridging between algal cells by chitosan chains, as in the case of other polyelectrolytes (Divakaran and Pillai, 2002). In acidic conditions, chitosan is a linear chain because $-NH_2$ groups carry positive charges and are therefore clustered tightly together. The positively charged $-NH_2$ and $-NH^{3+}$ groups repel one another and while this is occurring, chitosan remains dispersed (Gualteri et al., 1988). Therefore, in acidic conditions the degree of flocculation is weak (Harith et al., 2009). When pH was adjusted to alkaline pHs, noticeable increase in efficiency was obtained. It is possible that with an alkaline pH, the positive charge

gradually disappears (neutralization point at pH 7.9) and chitosan tends to coil and precipitate (Gualteri et al., 1988).

For *P.tricornutum* species, at higher concentrations of chitosan experiments, FE increased only slightly until reaching a plateau. FE decreased considerably when the concentration was increased from 100 to 200 ppm. This drastic decrease in performance was brought about when the chitosan overdose caused an overload of positive charges, which were retained on the surface of the cell wall. It causes repulsion between positively charged microalgae cells resulting in re-stabilization. Adding more than the optimal dosage of chitosan to the solution positively affects flocculation and settling rate up to a certain degree, possibly depending on the initial algae concentration.

When chitosan concentration was increased, more NaOH solution was needed to reach optimum pH. So, not only might algal biomass flocculate with chitosan, but some medium chemical ions might also precipitate in the presence of the optimum alkaline condition of pH=9.9. This might also explain the increase in SSVF and the decrease in sedimentation rate as occurs in alkalinity-induced flocculation.

For *N.gaditana*, above 30 ppm of chitosan concentrations, FE did not show a significant increase with increasing dosages. It arrived to a plateau as well. Kaseamchochoung et al. (2006) concluded that FE of chitosan depends on its characteristics, the pH and ionic strength of medium .Our results also supports that FE of *N. gaditana* cells with chitosan

is more affected by ionic strength than by flocculant concentration the same way as FE of *P.tricornutum* species. Nevertheless, distinctively from previous *P.tricornutum* results, FE of *N. gaditana* cells did not show a decrease when the concentration of chitosan was increased above 90 ppm. SSVFs did not show a distinct difference either after reaching optimum chitosan dosages. We might conclude that chitosan concentrations more than optimal dosage still keep the charges stabilized without overloading due to higher initial algae concentration of *N. Gaditana*. These results also supports that the type of the harvesting microorganism is also important on FE (Strand et al., 2003).

Chitosan is a favorable flocculant in waste water treatment because of giving high FEs, low-toxicity, biocompability and biodegradability (Lertsutthiwong et al., 2009). Despite of well defined uses of chitosan flocculation in wastewater sytems, there is a gap between research and industrial application of harvesting of algae with chitosan, most probably due to the price of the chitosan. However, it is not necessary to use a very high quality/pure chitosan for harvesting process. Additionally, flocculation with chitosan let us to use the concentrated biomass without any problem for the further processes, to have problem-free final product and also use the residual water directly via recycling. It is not the object of this study, but further research is needed to understand the effect of recycled water, after flocculation using chitosan, on cultivation.

Several authors (Lavoie and Note, 1983*;* Buelna et al., 1990; Divakaran and Pillai 2002) have published similar optimum results for chitosan concentrations between 20-40ppm chitosan for several species which refers the importance of pH in chitosan flocculation from our point of view.

5.0 Conclusion

The selection of the proper harvesting method depends mainly on the process product targets. However, to decide which method is better for the process to harvest, it should be checked not only how efficient the agent as a flocculant and how appropriate to provide a cost effective harvesting, but also how feasible to use the agent in the case of using concentrated biomass in the further processes without any problem and managing the residual water after harvesting.

The results obtained in this study suggest that harvesting by autoflocculation is a promising and sufficiently effective method. From the economical point of view, it seems also feasible because it is does not cause any additional cost in the industrial scale as flocculant agent and treatment processes. Besides, there is no problem to re-use residual water if the autoflocculation is induced naturally. Microscopic observations were also showed that there might be no problem with the harvested cells for further downstream processes and final product/s as well.

Additionally, the optimum flocculant dosages required for the synthetic coagulants PAC and AS are higher than that of chitosan pH adjusted, although they achieve lower removal efficiencies for *P. tricornutum* and *N.gaditana* .

6.0 References

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CHAPTER IV
COMPARATIVE STUDY OF PROPERTIES OF PRE-CONCENTRATED SAMPLES

1.0 Introduction

Sedimentation is applied in many chemical engineering operations and processes such as filtration, -fluidization, two- phase flow and environmental engineering. Industrial sedimentation operations may be carried out batch- wise or continuously and the rate data of sedimentation process is very important for designing equipment used in chemical practices.

The sedimentation of microalgae cells involves the mechanics, flow and transport properties of mixtures of medium, cells and/or exo-cellular substances. Fundamental aspects of sedimentation and related solid-liquid separation processes such as flocculation, filtration or centrifugation include properties of solutions such as rheology, viscosity, particle size and shape, particle-particle interaction, surface characteristics,

yield stress, concentration. These processes are of critical importance for solid-liquid separations in the chemical, pulp and paper, wastewater, food, pharmaceutical, ceramic and other industries. For instance, viscosity of the medium is the main physical property, which affects the sedimentation or density of particles is again an important factor that affects the settling velocity of the particles. When the density of the particles is bigger, then the settling rate is higher.

During harvesting of microalgae cultures, flocculants are added to flocculate the cells. Even though chemically induced flocculation is a common practice in the water treatment industry, induced flocculation is still considered a novel approach to enhancing (especially in sediment pond performance) as well in algae harvesting. This flocculation is also a factor that affects the mechanical properties of the culture. Therefore, it is of real interest to study flow behaviour of flocculated suspensions by additives. The flocculation mechanism determines the properties of the flocs and therefore the rheological behaviour of the suspension. The rheological properties of algae slurries have a direct impact on the agitation and pumping power requirements as well as process design for producing algal biofuels.

Below solid concentration of about 4% by weight most sludges exhibit Newtonian fluid behaviour, which is a linear relationship exists between shear stress and shear rate where the constant of proportionality, μ, is the viscosity of the fluid, i.e water. Algae slurries are

complex fluids composed of a liquid phase that contain water, polymeric substances and dissolved salts, algae cells, and other insoluble solids (Wileman et al., 2012). However, the presence of polymeric substances in this phase, such as extracellular polymeric substances (EPS), has shown to make this fluid non-Newtonian (Clementi and Moresih, 1998).

The mixed liquor is a flocculant suspension in which larger particles can be formed by the coalescening of particles which have collided. These larger particles generally enhance settling characteristics. The particle distribution is bimodal with primary particles (microflocs) in the 0.5 to 5 μm and flocs (macroflocs) in the 10 to 5000 μm range. The settling properties depends both on the distribution of primary and floc particles and on how easily the primary particles are entrapped into larger flocs (Holenda et al., 2006).

The particle separation system processes are difficult to describe by a theoretical analysis due to involved particles are not regular in shape, density, or size. Consideration of the theory of ideal systems is a helpful and a useful guide to interpreting observed behaviour in more complex cases.

Depending on the application, flocculation efficiency is commonly evaluated by measuring the settling rate of flocs, the percentage of solids settled, the sediment volume/weight, the moisture content and strength of flocs, and the suspension viscosity

and turbidity in water treatment processes. However, these indices are only indirect indicators of the effectiveness of flocculation.

Since flocculation is an aggregation process, clearly the most explicit and direct measure of flocculation efficiency is floc size distribution. Not surprisingly, then, quite a few studies have focused on measuring and analyzing the influence of various process variables on floc size distribution (Koh 1984; Amal et al., 1990a; Amal et al.,1990b, Zhang and Buffle, 1995; Kusters et al.,1997; Das, 1998; Ferretti et al.,1998; Flesh et al., 1999; Biggs et al., 2000; Rattanakawin and Hogg, 2001; Selomulya et al., 2002; Somasundaran and Runkana, 2003)

There are many important properties of colloidal systems that are determined directly or indirectly by the interaction forces between particles. These colloidal forces consist of the electrical double layer, van der Waals, Born, hydration, and steric forces. The measurement of particle size is also a defining property. However, there is some argument in the literature as to whether the maximum floc size remaining in the system should be measured or the average floc size (Leentvaar and Rebhun, 1983; Francois, 1987; Bache et al., 1999; Jarvis et al, 2006).

Particle size analysis is usually applied in drinking water treatment for monitoring and controlling filtration process performance. Analysis of sizes of flocs formed in the coagulation and flocculation processes are not routinely conducted. However, particle

size distribution (PSD) analysis can produce direct information about the flocs in the fluid. Through measuring and analysing the amount and size of particles, we can evaluate the efficiency of the process, assess design of the treatment or harvesting systems. Zeta potential and the particle size distribution measurements are reported and used to relationalise the observed optimal conditions for the algal flocculation studies (Phoochinda et al., 2004).

A primary factor controlling the performance of sedimentation is the particle size distribution (PSD) of the incoming sediment. Particle size distribution information is needed to model the sedimentation process. The accuracy of sedimentation models that consider particle-to-particle interaction is enhanced with a continuous representation of the PSD.

The basis of the current theories used in modelling changes in particle size distribution (PSD) are usually based on the work by Smoluchowski (1917) which employs the coalesced sphere assumption. More detailed study on Smoluchowskis' work will be given in chapter 5. Few cases can be found in the literature where sediment pond flocculation has been modeled (Tambo andWatanabe, 1979; Vialoulis and List, 1984; Krishnappan and Marsalek, 2002). However, no known successful flocculation model has been developed that can be used to design and predict the performance of even in a sediment pond employing induced flocculation.

1.1 Objectives and Scope

The aim of this chapter is, to know more about pre-concentration processes of *Nannochloropsis gaditana* and *Phaeodactylum tricornutum.* Pre-concentrated sample characteristics such as viscosity, particle size distribution (PSD) and magnesium and/or calcium concentrations that comparatively little research has been published about were investigated to provide insight into flocculation behaviours.

With these analyses, we aimed to better understand the flocculation behaviour, to give a step to flocculation modelling of pH induced flocculation by means of knowledge of particle sizes, particle distribution size.

2.0 Materials and Methods

In order to better understand the pre-concentration processes of *Nannochloropsis gaditana* and *Phaeodactylum tricornutum*, we used different techniques (Table 4.1) to harvest the following three types of sample under a variety of conditions;

(1) Growth medium (GM), which was obtained by means of the procedure described in the microalgal cultures section (for 300 L slurries of algae), but skipping the algal inoculation step so that the effect of the growth medium on the harvesting processes could be observed.

(2) Microalgal cultures (C), which were obtained as described in the microalgal cultures section (for 300 L slurries of algae) so that the effect of the algae species could be observed.

(3) Filtered culture (FC), which was obtained by filtering microalgal culture through glass fibre filters (Whatman GF/F Glass Microfiber filter 4.7 cm, nominal pore size 0.7 μm), cell-free culture, to observe the effect of other materials in the culture medium (e.g. extracellular organic matter).

All experiments in this section were conducted using the sedimentation experimental procedure in 250 mL cylinders as performed in pH-induced flocculation experiments (chapter 3).

Table 4.1 Experimental conditions to obtain concentrated samples of *Phaeodactylum tricornutum* and *Nannochloropsis gaditana* species.

Sample treated	**Treatment method**	**Condition**
(1) Growth medium (GM)	Alkalinity induced flocculation	$pH_{threshold}$ pH=11
(2) Microalgae culture (C)	Flocculation with commercial flocculant PAC	30ppm for *P.tricornutum* 20ppm for *N.gaditana*
(3) Filtered culture (FC)	Flocculation with commercial flocculant AS	30ppm for *P.tricornutum* 20ppm for *N.gaditana*
	Flocculation with chitosan	20ppm for *P.tricornutum* 30ppm for *N.gaditana*

2.1Viscosity analysis of samples after harvesting

The rheological properties of algae slurries play a role in agitation and pumping power requirements and must be considered as one of the design aspects for algae production processes. Research should be carried out into downstream processes and increasing cell concentrations during harvesting. The rheology of cell cultures is also influenced by cell morphology (e.g. size, shape and aggregation) as well as the biomass concentration and the process applied.

The samples of two marine species pre-concentrated with different treatment methods were tested for rheometric characteristics. Viscosities were measured over a range of shear rates (245-2700 s^{-1}) with a rotational viscometer (Thermo-Haake VT550) using an NV sensor at 30^{o}C. According to the shear rates, the range of flows could be assumed to be between 45 and 500 L min^{-1} (~3-30 m^3 h^{-1}). The culture densities of both species were also similar to productivity rates found in outdoor facilities (especially in raceway ponds). All the rheometric measurements studied were taken in triplicate and the viscosities were calculated using the averages of the measurements. Under the shear rates used, the following assumptions were made: (i) algal flocs did not change their shape; (ii) no cell settling occurred during measurements; (iii) the algae were not damaged.

The kinematic viscosities of the residual solutions after every harvesting technique were monitored by means of an Oswald-Cannon-Fenske viscometer (at 30^{o}C) to check for any differences in viscosity in relation to the culture medium.

There are no straightforward techniques for experimentally characterising floc strength without destroying the flocs to some extent, and there is little information in the literature on researching floc structure and floc strength under different coagulation mechanisms. So, we tried to understand floc strength by applying different agitation speeds (max 1500 rpm) and shear rates (2700 s^{-1}). The settling efficiencies of the samples were checked after the viscosity measurements had been taken.

2.2 Particle size analysis of samples after harvesting

Particle/floc size distribution was analysed in order to make an in-depth study of the diameters of the particles/flocs. Understanding the characteristics of the floc (e.g. size, shape, density) is essential if the settling characteristics are to be understood and design processes developed. The particle sizes of the concentrated samples were measured using a Coulter Multisizer 3 (Beckman Coulter Inc, USA) with a 1000 μm orifice tube capable of characterising flocs ranging in size between 20 and 600 μm, depending upon the density of the flocs being analysed. Gibbs (1982) mentioned the possibility of floc breakage when the particles pass through a small orifice. To prevent this from happening, an orifice of 1000 μm was used to characterise flocs. Stirring was applied so that the flocs

could not settle. The average/mean diameter of the flocs (D_f) was calculated using the overlays of three samples. Seawater was used as the conductive electrolyte and filtered with glass fibre filters (Whatman GF/F Glass Microfiber filter 4.7 cm, nominal pore size 0.7 µm) prior to use. Multisizer AccuComp 1.19 software provided by Coulter Limited was used for further data analysis. All data given in this study are the average of three measurements.

2.3 Ca and Mg ion analyses of samples after harvesting

The concentration of Ca and Mg ions in residual water were measured by means of atomic absorption spectroscopy (model 3110; Perkin–Elmer) after harvesting the algae culture in order to better understand the mechanism at work in alkalinity-induced flocculation. The Ca and Mg contents of the monitored samples were compared with the content of the culture medium to help establish the mechanism.

3.0 Results

3.1 Viscosity analysis

The rheological properties of the concentrated algae suspensions of two microalgal species, *P. tricornutum.*and *N.gaditana*, were studied after being harvested by different treatment methods (Table 4.1).

Table 4.2 Dynamic viscosities and Mg and Ca contents of samples

Species	Method of concentration	Sample	Viscosity* (µPas)	R^{2**}	Mg content†,‡ (%)	Ca Content†,‡ (%)
Phaedactylum tricornutum	$pH_{threshold}$	C	2.1	0.9867	84±0.91	97±2.86
		GM	2.1	0.9792	97±1.77	97±1.92
		FC		0.9898	94±1.85	97±1.29
	pH=11	C	2.2	0.9840	0±0.00	86±1.29
		GM		0. 9898	5±1.40	81±5.92
		FC	2.3	0.9681	0.32±0	82±4.34
	PAC	C	2.2	0.9939	98±1.60	98±1.84
		GM		0.9898	98±1.80	98±1.80
		FC		0.9867	98±1.85	98±1.80
	AS	C	2.3	0.9898	98±1.60	98±0.00
		GM	2.2	0.9968	98±1.60	98±0.80
		FC		0.9968	98±1.60	98±1.80
	Chitosan	C	2.2	0.9730	97±0.91	94±5.75
		GM		0.9898	98±0.40	95±1.60
		FC		0.9898	98±1.67	97±0.45
Nannochlorpsis gaditana	$pH_{threshold}$	C	2.1	0.9791	87±1.91	98±0.00
		GM	2.0	0.9970	92±2.50	99±0.31
		FC		0.9968	90±3.28	97±0.00
	pH=11	C	2.2	0.9774	0±0.00	77±1.59
		GM	2.1	0.9923	0.5±0.68	72±5.35
		FC	2.4	0.9748	0.7±0.91	77±1.81
	PAC	C	2.0	0.9912	97±0.70	98±0.88
		GM		0.9730	98±0.40	98±0.88
		FC		0.9939	97±0.40	98±0.88
	AS	C	2.1	0.9730	95±2.60	98±0.88
		GM		0.9856	97±1.60	98±0.00
		FC		0.9800	97±1.60	97±1.60
	Chitosan	C	2.2	0.9843	98±0.93	97±1.31
		GM	2.1	0.9886	97±1.60	97±0.47
		FC		0.9800	97±1.53	97±1.31

* Viscosity measurements were done at 30°C with concentrated algae samples after harvesting.
** R^2 is the linear regression determination coefficient of viscosity.
†Mg and Ca ion measurements were done with residual solution samples after harvesting.
‡ Mg and Ca ions percentages were calculated by comparison to culture medium ion contents.

For all the samples tested, the apparent viscosity was constant when measured at different rotational speeds corresponding to different shear rates (Figure 4.1a-b).

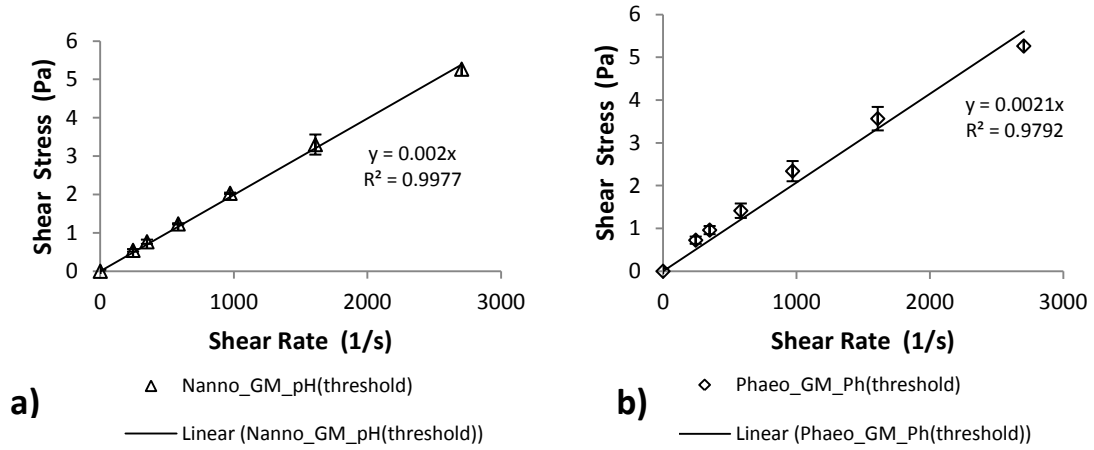

Figure 4.1 Examples of shear stress (τ) as a function of shear rate ($\dot{\gamma}$)for **a)** *P.tricornutum* and **b)***N.gaditana* cultures of growth medium (GM) samples harvested at $pH_{threshold}$ at 30°C. Data are represented with ±standard error (SE) (n=3).

Our experimental results establish that the samples showed the characteristics of a Newtonian fluid in the range of shear rates tested, where the constant of proportionality relating the shear rate and shear stress is viscosity.

It was calculated as:

$$\tau = k\dot{\gamma}$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}) and *k* is the constant of viscosity (Pa s).

The samples measured had a dynamic viscosity range of approximately 2-2.4x10^{-3} Pa.s at 30°C after the pre-concentration process, whereas the dynamic viscosity of seawater is

given as 0.87 $x10^{-3}$ Pa.s (temperature=30^{o}C; salinity:38 ppt). The linear regression determination coefficients for the viscosity (R^2) of the samples were higher than 0.96.

Samples C, GM or FC treated with the same harvesting methods displayed no major differences in viscosity (Table 4.2). Additionally, the kinematic viscosity data of residual solutions after the algae had been harvested were also monitored and compared with kinematic viscosity data of the culture medium and no differences were found in viscosities before or after treatment. Similarly, in alkalinity-induced flocculation experiments, no marked difference in viscosity was observed at $pH_{threshold}$ and pH=11.

To measure floc strength, we applied different agitation speeds (max 1500 rpm) and shear rates (2700 s^{-1}). The settling efficiencies of the samples were checked after the viscosity measurements had been taken. No precise differences were observed in terms of settling rates. The turbidity of the residual solutions was satisfactory, as it was before the procedure. Microscopic observations backed these settling results.

3.2 Particle size analysis

Table 4.3 shows the variations in the particle diameter of the concentrated samples after harvesting using the methods shown in Table 4.1. The particle size distribution graph for *P. tricornutum* and *N. gaditana* samples can be seen in Figures 4.2a and 4.2b, respectively.

Table 4.3 Particle diameter variations of concentrated samples after harvesting of cultures with different methods.

Species	Sample	Treatment	Mean dia-meter (µm)	Standart error (SE)	Coefficient variation (%)	d_{10}±SE (µm)	d_{50} ±SE (µm)	d_{90} ±SE (µm)
Phaeodactylum tricornutum	GM	pH=11	41.5	3.64	17.5	34.8±0.07	39±0.30	52.4±1.20
	C		43.1	5.40	25.0	35.3±0.07	40.3±0.30	54.2±0.46
	FC		43.1	6.41	25.7	35.0±0.14	39.0±1.10	54.7±1.53
	GM	$pH_{threshold}$	47.8	6.45	27.0	35.5±0.07	43.6±0.48	66.4±0.53
	C		47.7	6.60	27.6	35.4±0.20	43.5±0.52	67.0±0.47
	FC		47.3	7.43	31.4	35.3±0.16	41.9±1.16	66.80±1.5
	C	pH=9.30	45.8	8.34	25.7	34.8±0.03	42.1±0.27	62.6±0.84
		pH=9.54	46.6	9.33	27.0	35.9±0.20	45.1±0.61	63.8±0.38
		AS	40.3	3.07	14.9	34.8±0.07	38.2±1.13	48.8±4.12
		PAC	39.8	3.53	17.7	34.8±0.02	37.6±0.01	47.0±0.78
		Chitosan(1) (pH=9.85)	39.2	3.18	16.2	34.8±0.02	37.4±0.21	45.4±0.27
		Chitosan(2) (pH=9.9)	40.6	3.16	15.5	35.0±0.02	38.7±0.31	48.6±0.29
Nannochloropsis gaditana	GM	pH=11	38.5	2.87	25.6	34.8±0.02	36.7±0.13	42.3±0.27
	C		40.5	5.11	17.8	34.9±0.03	38.4±0.05	47.8±0.91
	FC		40.1	2.65	13.2	35.0±0.02	38.6±0.08	47.8±0.31
	GM	$pH_{threshold}$	40.2	5.06	17.8	34.9±0.07	38.0±0.16	47.7±0.17
	C		42.9	3.99	18.6	35.3±0.05	40.7±0.16	53.5±0.37
	FC		41.3	6.78	23.2	35.0±0.03	38.6±0.27	49.3±0.63
	C	AS	40.00	4.50	16	34.8±0.07	38.2±1.13	52.6±4.20
		PAC	44.32	8.18	23.0	35.4±0.02	40.3±0.01	56.3±0.80
		Chitosan(1) (pH=9.74)	43.00	4.90	19.0	34.9±0.10	38.8±0.14	55.1±1.32
		Chitosan(2) (pH=9.9)	44.10	6.50	20.9	34.9±0.12	40.5±0.66	57.7±2.37

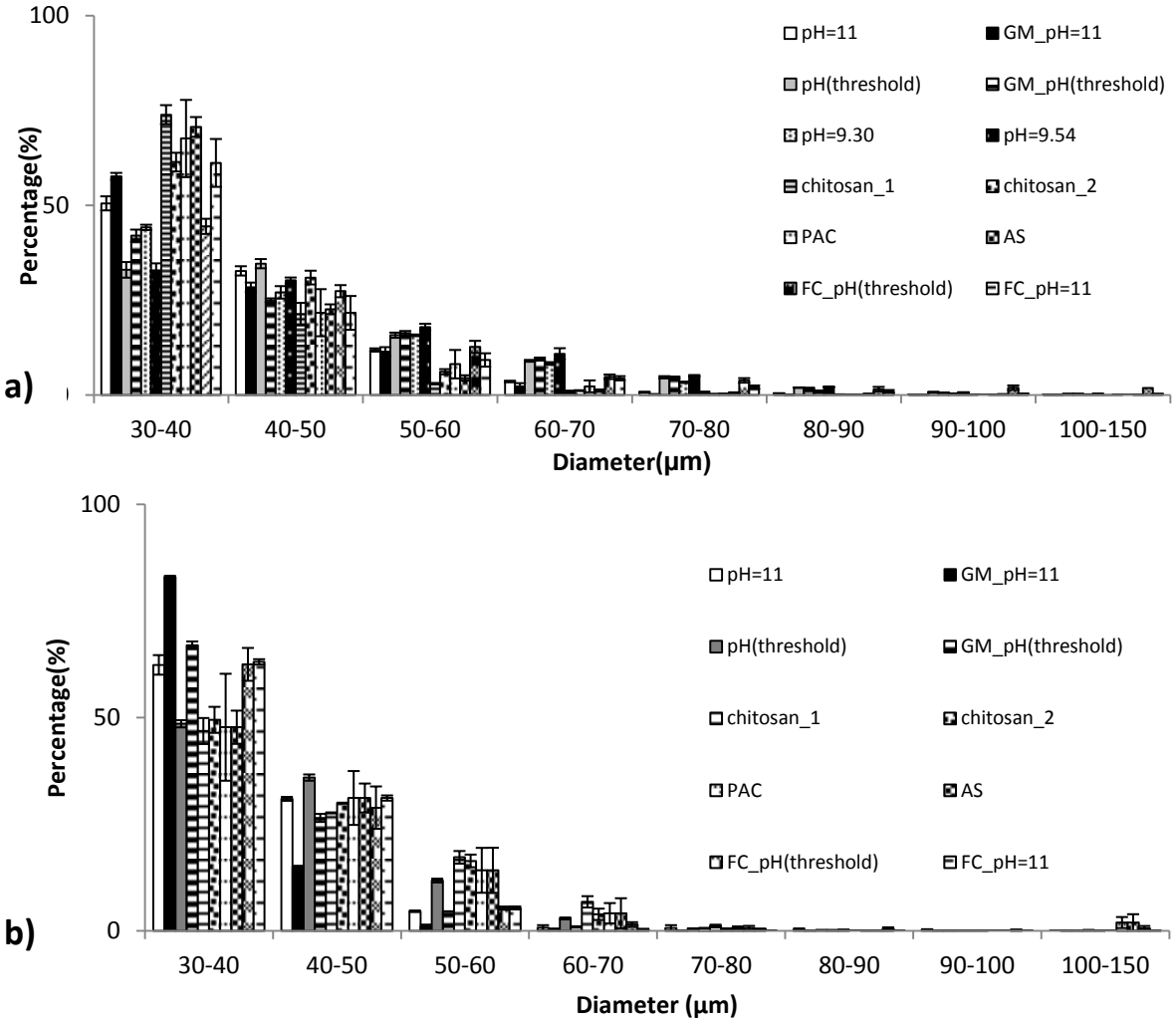

Fig 4.2 Percentage particle size distribution of cultures after harvesting methods **a)** *P.tricornutum* and **b)** *N.gaditana.* Data are represented with ±standard error (SE) (n=3).

On average, the diameter of the *P. tricornutum* flocs was greater than that of the *N.gaditana* flocs and, because of the larger size of this species, the standard deviation was higher.

The size distribution graph for the *P. tricornutum* samples showed that the $pH_{threshold}$ samples contained a higher percentage of particles with diameters in the range 50-80 μm whereas pH=11 samples contained a higher percentage of particles with shorter diameters (30-40 μm). For samples harvested at pH=11, the mean diameters (D_fs) were approximately 10% smaller.

The D_fs of *N. gaditana* were in the range 38-44 μm (i.e. smaller than that found for the *P. tricornutum* samples). The most noticeable difference in the particle size distribution of *N. gaditana* was few particles were over 70 μm (especially in $pH_{threshold}$).

The size distribution graph for this species showed that $pH_{threshold}$ samples contained a higher percentage of particles in the 50-70 μm diameter range, whereas pH=11 samples contained higher percentages of lower range particles (30-40μm). The settling properties of alkalinity induced flocculation for *P. tricornutum* and *N. gaditana* flocs support the size distribution graph (Figure 4.2). The $pH_{threshold}$ samples showed higher SRs than pH=11 samples where the FEs were almost the same with high CFs. pH=11 alkalinity-induced flocculation samples contained higher percentages of lower range particles. In addition, the D_fs of the flocs of *P. tricornutum* was found higher than *N. gaditana*.

No distinct differences were found in the D_fs of the C and FC samples. However, after a detailed examination, some diversity in the particle size distribution was observed. For instance, for *P. tricornutum* samples, at pH=11, the C sample contained a higher

percentage of particles between 40-50 μm while the FC sample contained higher percentages in the 30-40 μm range. When compared to the GM particles, they were found to have a lower percentage of 40-50 μm particles as well. The D_fs of the GM samples were a little smaller. The same nuances in particle ranges were also observed in the *N. gaditana* samples.

For AS, PAC and chitosan flocculant treatments of *P. tricornutum* at optimum concentrations, D_fs were measured as 40.3±3.07 μm, 39.8±3.53 μm and 40.6±3.16 μm, respectively. The D_fs of *N. gaditana* samples treated with AS, PAC and chitosan flocculants at optimum concentrations measured 40.0±4.50 μm, 44.32±8.18 μm and 44.10±6.50 μm, respectively with high SEs.

3.3 Ca and Mg ion analysis

The calcium and magnesium ion concentrations in the residual solutions after harvesting were monitored and compared with the ion content of the culture medium so that the effect of Mg/Ca ions on flocculation mechanisms with different treatment methods could be estimated.

At $pH_{threshold}$, magnesium ion concentrations were lower than in the culture medium, although no distinctive differences in the calcium ion concentration were found (Table 4.2) in comparison with the culture medium.

After both algae species at $pH_{threshold}$ had been harvested, the residual solutions contained lower concentrations of magnesium ions than the culture medium, although no distinctive decrease was found in the concentration of calcium ions. When pH was induced to pH=11 for harvesting, no magnesium ions were detected and the calcium ion concentrations in the residual samples decreased in both algae species compared to initial Ca concentrations (Table 4.2).

For instance, after harvesting *P.tricornutum* at $pH_{threshold}$, the residual water contained 84±0.9% of magnesium ions of the initial concentration where the calcium ion concentration did not show distinctive decrease. When the pH increased to pH=11, no magnesium ions were detected in the residual water. The calcium ion concentration decreased by 14±1.3% in comparison to initial Ca concentration as well.

No changes in Mg or Ca ion concentrations were detected in the residual solutions in the algae cultures harvested with AS, PAC or chitosan flocculants (at the concentrations given in Table 4.1). Under both conditions, neither the GM nor the FC samples demonstrated a distinct change in Ca or Mg ion concentration.

4.0 Discussion

The rheological properties of algae slurries play a role in agitation and pumping power requirements and must be considered as one of the design aspects for algae production processes.

The samples showed the characteristics of a Newtonian fluid in the range of shear rates tested. Newtonian fluids are very similar to water, making both pumping and mixing relatively easy. Additionally, the mixing times for Newtonian fluids are lower than those of non-Newtonian fluids.

Although the rheology of cell cultures is influenced by cell morphology (for example, size, shape and aggregation) as well as biomass concentration, after the pre-concentration process no significant differences in viscosities were observed in either *N. gaditana* or *P. tricornutum*, regardless of the method used. The reason for this might be that the cultures were grown with the same seawater, that the initial concentration of microalgae cultures was lower or that the viscosity of $Mg(OH)_2$ was low (even at high concentrations it flows like water).

The viscosities of the supernatant of the algal culture suggest that no viscous substances such as extracellular polysaccharide (EPS) were excreted by the species during the pre-concentration process due to stress. The microalgae species used in this study are all

unicellular species with negligible EPS production. Hence, the effect of EPS was not measurable in our viscosity analyses. Below a solid concentration of about 4% by weight, most sludges exhibit Newtonian fluid behaviour: that is, there is a linear relationship between shear stress and shear rate where the constant of proportionality, μ, is the viscosity of the fluid (i.e. water). Algae cultures are generally accepted to behave like Newtonian fluids (Clementi and Moresih, 1998). During batch culture, the rheological behaviour of the medium can change from Newtonian to non-Newtonian due to EPS production during growth; however, in long-term cultures, the rheology reverts to Newtonian as a result of the hydrolysis of the polymer (Lupi et al., 1991).

Coagulation/flocculation is a well-known process used in water treatment to remove suspended particles by combining smaller particles into larger aggregates. The mechanisms involved in this can include the compression of the diffuse (double) layer, charge neutralisation, enmeshment or 'sweep floc', and adsorption and interparticle bridging (Tchobanoglous and Schroeder, 1987). These mechanisms result in flocs of different sizes, strengths and structures. However, it is difficult to state and characterise the size and shape of flocs because the different generation mechanisms mean they are so irregular. Particle shape affects the behaviour of aggregated particles, particularly in terms of collision efficiency (Wiesner 1992) and settling rates (Li and Logan, 1997). Particles of different sizes settle at different rates. The larger particles will settle more rapidly than the smaller ones (assuming similar density and shape).

On average, the diameter of the *P. tricornutum* flocs was greater than that of the *N. gaditana* flocs and, because of the larger size of this species, the standard deviation was higher. Coulter methods use the equivalent resistance diameter, which is the diameter of a spherical particle that has the same resistance as the particle tested. Therefore, *N. gaditana* samples yielded results with standard errors that were lower than expected. The sphericity of *Nannochloropsis* cells is higher than that of *Phaedactylum* cells, so the standard errors were lower.

The mechanism of alkalinity induced flocculation is the same for both species which is enmeshment of algae cells into magnesium hydroxide particles. Alkalinity-induced flocculation can result from an increase in medium ionic strength, which causes double layer compression. By linking our viscosity- floc strength results, it is unlikely that this mechanism was involved in our experiments, as the change in ionic strength caused by pH increase is limited. As Vandamme et al. (2012), we also credit that flocculation in our experiments was caused by the mechanism of charge neutralisation.

The mean particle size of magnesium hydroxide seems to decrease as the pH value of the solution and the Mg^{2+} concentration increase under particular experimental conditions (Xu and Deng, 2006). Therefore, for pH=11 alkalinity-induced flocculation samples contained higher percentages of lower range particles. In addition, the D_fs of the flocs of

P. tricornutum was found higher than *N. gaditana* most probably only due to this species has bigger spherical particle diameter than *N. gaditana* species.

Generally, C and FC samples contained microalgae cells and other materials in the culture medium (e.g. extracellular organic matter) which might let the magnesium hydroxide neutralize the negative surface charge of them (Semerjian and Ayoub, 2003) resulting a little higher D_fs.

For AS, PAC and chitosan flocculant treatments of the species, the diameters were similar because all three flocculants had almost the same percentage of particles in the 30-40 μm range. However, chitosan had more particles in the 40-50 μm range, which resulted in faster sedimentation rates and higher CFs than for other flocculants which might indicate the mechanisms of flocculation are different. By aluminum ions, metal hydroxides bind to the negative surface of the microalgal cells and destabilize the microalgal suspension by charge neutralization resulting in enmeshment of microalgae and insoluble precipitates Insoluble metal hydroxides can destabilize the microalgal suspension through a mechanism known as sweeping flocculation (Duan and Gregory, 2003). In the case of chitosan, it can easily react with microalgal particles by interparticle bridging behaviour and may even remove smaller particles by an enmeshment mechnaism where the interparticle bridging capability should be the main mechanism (Chung et al., 2005).

Several studies have mentioned that under alkaline conditions two major reactions are effective: the precipitation of calcium carbonate ($CaCO_3$) and the precipitation of magnesium hydroxide ($Mg(OH)_2$), depending on the primary particles and the ions contained in the solution (Vandamme, 2011).

The analyses of Mg ions in residual solution of $pH_{threshold}$ showed a decrease in magnesium concentration which indicates $Mg(OH)_2$ formation. By rising the pH of the culture to pH=11, all magnesium ions converted into $Mg(OH)_2$ and formation of $CaCO_3$ started. This fact resulted as lower CF and SR values at higher alkaline pHs where Mg and Ca precipitates sediment with algae cells (chapter 3). This sufficiently explains the flocculant matrix differences and also the colour change in the precipitate to whitish at pH=11 in chapter 3 in Figure 3.5d and 5e.

The analysis of Ca and Mg ion concentrations with alkalinity-induced flocculation substantiate the fact that the Mg ion is the protagonist in the flocculation mechanism at alkaline pH. The flocs are assumed to be a mixture of Mg^{+2} ions generated by the reaction of the NaOH, which causes the precipitation of $Mg(OH)_2$.

$$Mg^{+2} + 2OH^- \rightarrow Mg(OH)_2 \downarrow$$

When the culture pH was induced to pH=11, no magnesium ions were detected, and the calcium ion concentration decreased. All magnesium ions converted into $Mg(OH)_2$ and $CaCO_3$ started to form. This resulted in a time lag for the initiation of settling and lower

CF and SR values at higher alkaline pH at which Mg and Ca precipitated sediment with algae cells. The production of $(OH)^-$ ions, especially at pH values higher than $pH_{threshold}$, changes the inorganic carbon equilibria in the seawater and facilitates the following buffering reaction (Turnbull and Ferriss, 1986);

$$OH^- + HCO_3^- \leftrightarrow H_2O + CO_3^{2-}$$

It is also an explation of why it was difficult to increase the alkalinity of the solution. As a result of the continuing increase in pH, $CaCO_3$ also precipitates at higher alkaline pH values.

$$Ca^{+2} + CO_3^{-2} \rightarrow CaCO_3 \downarrow$$

The pH is buffered by a set of reactions that take place between carbon dioxide and water. The formation of the alkaline scales $CaCO_3$ and $Mg(OH)_2$ strongly depends on temperature, pH, the release rate of CO_2 and the concentrations of HCO_3^- , CO_3^{2-},Ca^{2+}, and Mg^{2+} ions.

Because the concentrations of magnesium ions in the culture media were approximately the same, it is not surprising that similar results were found for both *P. tricornutum* and *N. gaditana* species. This supports the hypothesis that the Mg ion is the triggering ion in the flocculation mechanism.

5.0 Conclusion

Viscosity results show that pre-concentration processes make both pumping and mixing easier because of the Newtonian behaviour of the samples than Non-Newtonian ones. Particle size analysis supports settling properties sufficiently, however further research on density and mechanism of the flocs is recommended. The analysis of Ca and Mg ion concentrations substantiates the fact that the Mg ion is the protagonist in the alkalinity-induced flocculation mechanism.

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Comparative study of pre-concentrated samples

CHAPTER V
A MODELING APPROACH TO ALGAL AUTO-FLOCCULATION

1.0 Introduction

Flocculation is an important pre-treatment step because the microalgae cultures are stable for extremely long periods of time based on Stokes Law analysis.

The following subdivisions can be made for coagulation/flocculation mechanism:

- electrostatic coagulation: compression of the double layer.
- adsorptive coagulation: adsorption and interparticle bridging; adsorption and charge neutralization
- precipitation coagulation. enmeshment/sweep flocculation.

One or more of these mechanisms may dominate for a given algae harvesting process.

- **Charge neutralization** This happens when a charge flocculant is adsorbed to aparticle surface of the opposite charge and the charges neutralize. When the

flocculate is small relative to the particle and when the flocculant is evenly distributed on the surface of the particle a homogeneous reduction of the surface charge will take place. This lowers the repulsive electrostatic forces between the particles, and once sufficiently reduced the attractive van der Waals forces will dominate: this will result in a weak aggregation of the particles (Gregory, 2006).

- **Inter-particle bridging** This occurs when the flocculant exceeds the size of the particle. Adsorbed polymer chains may reach far into the bulk of the suspension and thus form bridges to other particles(Gregory, 2006).
- **Enmeshment in a precipitate, or sweep flocculation**: destabilizes particles when the dose of inorganic salt is sufficient to cause precipitation at the pH of the solution. Initially, dissolved cations are attracted to the negative particle surface, as occurs in adsorption and charge neutralization, and, as precipitation occurs, the original particles become trapped in the newly formed precipitate. Transition metal hydroxide precipitates are often amorphous in nature (non-crystalline) and entrap large amounts of water and other dissolved ions. Aluminum (III) and iron (III) salts are often employed, but in greater concentrations and at slightly higher pH values than used for charge neutralization.
- **Compression of double layer:** high ionic strength solutions cause compression of the double layer and reduction in net repulsion of the particles. DLVO theory (named for Derjaguin, Landau, Verwey, and Overbeek) (Russel et al., 1989)

adequately predicts that compression of the double layer is more effective when ions of higher charge are present. The reduced double layer yields a lower energy barrier as the balance between attractive and repulsive forces is shifted. At a high enough ionic strength there is a net attractive force throughout the double layer.

A diversity of water treatment processes have been employed for algae removal. Autoflocculation is one of those which refers to destabilization of algae particles by raising the pH to the point at which the solution is supersaturated with respect to divalent cations (e.g., Mg^{2+} and Ca^{2+}). Increases in pH can occur naturally, such as during the diurnal variations in photosynthesis/respiration cycles or by adding a base to the solution to increase the pH. Though this method of harvesting is typically very economical, its effectiveness is highly variable with water chemistry. (Lavoie and de la Noue, 1986; Becker, 1994).

Three primary flocculation treatments were investigated in this research: commercial flocculants (PAC&AS), chitosan, and autoflocculation. PAC and AS represent a well-described flocculant that is used in many areas of water treatment; however, their inorganic composition is not typically considered amenable to algae treatment because of limitations to potential by product uses of the algae biomass. Chitosan is a natural polymer that can be produced relatively inexpensively and does not interfere with byproduct uses of algae. Using chitosan as a flocculant has many benefits over other

flocculants such as alum, ferric chloride, and synthetic cationic polymers such as cetyltrimethylammonium bromide (CTAB). Because of its natural origin, it is non-toxic and results in algae concentrates can be used as food in marine aquaculture or other food processing industries. Further, it is naturally abundant, though commercial production is still limited. (Muzzarelli, 1977; Divakaran, R., and Sivasankara Pillai, V.N., 2001) . However, its commercial use and availability is still limited.

pH modification with sodium hydroxide (NaOH) or lime ($CaCO_3$) to induce autoflocculation represents an inexpensive method of removal but the success varies according to the calcium, magnesium, and phosphate concentrations present in the water.

Autoflocculation, as mentioned previously, is flocculation induced from the precipitation of divalent cations. The solubility of such ions as a function of pH has been well characterized in the literature. Raising the pH induces precipitation of calcium and magnesium carbonates, hydroxyapapties and/or hydroxide solids. These precipitates; (1) create nucleation sites for adsorption of algae particles, and (2) promote algae enmeshment within the precipitate.

As such, removing algae via a pH-induced process is merely a function of promoting precipitation of species naturally present in the water. Some of the important equilibrium reactions for calcium and magnesium solids that control autoflocculation are presented in previous chapter. This phenomenon is related to the chemical properties of the water, and

in particular, the presence of calcium and magnesium carbonates. As the algae remove CO_2, the pH rises to a point at which precipitation of magnesium hydroxides and calcium carbonate along with algae occurs, causing removal of the particulate matters (Polprasert, 2007). Increases in pH can occur (I) naturally; such as during the diurnal variations in photosynthesis/respiration cycles (auto-flocculation) or (II) artificially by adding a base to the solution to increase the pH (alkalinity induced flocculation).

There are some studies on pH induced flocculation -mimic of autoflocculation in lab scale- with several species (Vandamme et al., 2012; Şirin et al 2013). Although significant progress were observed especially in recent years, the understanding of autoflocculation mechanisms is not clear yet. There are several factors affect the process of autoflocculation as algae species, the medium characteristics, pH, time of the harvesting etc. Chemical coagulation with formation of magnesium hydroxide has been proved to be an effective alternative to conventional treatments for harvesting of algae. The harvesting mechanism may include: charge neutralization resulted from positively charged $Mg(OH)_2$ particles to destabilize algae cells and adsorptive coagulation by $Mg(OH)_2$ precipitates. It was also found that magnesium hydroxide could be recycled and reused in treating low concentration of heavy metals contaminated wastewater (Liu et al., 2011).

Flocculation efficiency is commonly evaluated by clearness of the residual solution/ turbidity (flocculation efficiency), measuring the settling rate of flocs (sedimentation rate), the percentage of solids settled (settleable solid volume fraction and/or concentration factor). However, these are only indirect indicators of the effectiveness of flocculation. Since flocculation is an aggregation process, clearly the most explicit and direct measure of flocculation efficiency is floc size distribution (Runkana et al., 2004). Previous investigators have studied the flocculation of algae and characteristics by conventional coagulants (Wu et al., 2011; Wang et al., 2012). Nevertheless, although there are adequate amount of data on the characteristics of flocs using conventional coagulants, there have been limited studies focused on measuring and analyzing of flocs formed by autoflocculation and their characteristics (Şirin et al., 2013).

Mathematical modelling of the settling process of small particles inside a vertical column is in interest of several diverse areas of science: separation of solids in waste water treatment plants, polymer science, thickening in mineralogy, sediment pond design in environmental engineering, etc. To represent the processes of flocculation more realistically, various models have been developed on (Letterman et al. 1998; Crittenden et al. 2005; Friedlander 2000; Benjamin, 2011) the basis of the Smoluchowski equation (1917) in order to simulate evolution of aggregate size distribution such as Population Balance Model (PBM).

Models based on Smoluchowski and models based on population balance considers microscopic phenomena such as particle collisions, particle concentration and flocculation rate. In the modelling experiments- in situ experiments-, mostly idealised artificial particle suspensions were used where no exterior complications are allowed. However, in real experiments/processes, alkalinity, phosphate ions, extracellular organic matter, etc complicated modelling and also hard to guess the process. Therefore, it could be challenging to determine the parameters. Additionally, Smoluchowski equation are limited by the constraints imposed by Smoluchowski´s principal assumptions (Thomas et al., 1999) and the population balance models have often been conditional on the floc size.

The flocculation is accompanied by sedimentation of the microalgae. Sedimentation is a process where solid particles, known as sediment, settle in a fluid due to gravitational forces (Julien, 1994). The particles travel downward through the fluid while interacting with natural movement of the fluid until the particles ultimately deposit on the bed of the water body. This model is based on Stoke's law, describing the rate at with which particles sediment due to gravity. It should be only kept in mind that the Stokes law becomes invalid in more concentrated suspensions found in commercial and research settling (such as the processing of minerals, the processing of colloidal ceramics, and the treatment of industrial and municipal waste streams). In these cases, interactions between particles modify the settling behaviour of a given particle, relative to that of an isolated particle (Abel et al., 1994).

1.1 Objectives and Scope

The aim of this study is to implement population balance model to check that is able to describe the flocculation process of algae cells induced by alkalinity, using usual parameterization solutions to estimate floc size distribution and the sedimentation rate at steady state for concentration process. There is no study in the literature on the floc formation of mimic of autoflocculation-alkalinity induced flocculation experiments- which made the study worth to investigate.

The model parameters were taken from literature, in order to obtain a model that can predict the aggregates' characteristics (size and structure) or the operating conditions which produce aggregates with the characteristics required. Hence, a mathematical model has been built for particle coagulation for the author chosen harvesting techniques and applied to species *Nannochloropsis gaditana* and *Phaeodactylum tricornutum*. The main objective is estimating PSD, through a population balance equation (PBE).

2.0 Theory

2.1. Population Balance Model

The rate of the particle number balance, the rate of change of concentration of particle size k is given by, r_k , (Barbu et al., 2010);

$$\frac{dn_k}{dt} = r_k = \frac{1}{2}\sum_{i+j=k} \propto_{ij} \beta_{ij} n_i\, n_j - n_k \sum_{i=1}^{max} \propto_{ik} \beta_{ik} n_i - S_k n_k + \sum_{j=k+1}^{max} \gamma_{jk}\, S_j n_j$$

(1) (2) (3) (4)

where r_k =net rate of formation of aggregates of type k; n_i, n_j, and n_k, are the concentration of the particle number from the section [in number of cells.ml^{-1}] i, j, and k; respectively; $\propto_{ij}$= the collision efficiency between two colliding particles i and j, β_{ij}= collision frequency function; and the expression *i+j=k* indicates that the summation is over all pairs of particles whose combination generates a type-*k* particle. S_k is the fragmentation rate and γ_{jk} is the fragment distribution function of particle size *i* coming from size *k*. The first and second terms of this equation simply states that r_k equals the rate of formation of such particles by collisions of smaller particles minus the rate of their disappearance by collisions with others to form larger particles (forming of particles type-*k*). The third and fourth terms are the rate of fragmentation of particles of size *k* and the rate of production of particles of size *i* due to fragmentation of larger particles of size *k* (destroying of particles type-*k*) (Smoluchowski, 1917; Han et al., 2003).

Typically, particle "types" are defined on the basis of their size, and volume is assumed to be conserved in collisions. Formation and disappearance of type-k particles by breakup of existing particles could be incorporated into the equation, but rarely are; i.e.,

aggregation is treated as an irreversible process. β_{ij} is related to the properties of the particles and the suspending medium, the mixing regime, and the transport mechanism that brings the colliding particles together (Benjamin, 2011). In the case of aggregation is reversible, fragmentation term comes out and r_k is defined as including the third and fourth terms.

The total number of particles present is not constant, but is a function of time for a given set of experimental conditions.

Maximal floc size: Defining the upper boundary of the classes (k_{max}) is one of the key issues. The initial division of flocs in the classes is known. It equals to size of the species size where the initial concentration is equal to cell density (k=1; t=0). It indicates that single cells are present already before the model starts to work but not flocs or aggregates.

The index max (equation 1) represents the largest particle size. k_{max} is defined as the maximal amount of the cell in the biggest floc size. k_{max} was calculated by the mass balance equation, dividing the volume of the biggest floc to a single cell *(* $u_i = k_i * u_1$ where *u* equals to volume of a sphere). It is assumed that the density of the floc particles is constant.

Collision efficiency: In this work, we consider only two body interactions, as many previous investigators (Battherham et al., 1981; Han et al., 2003). Collision efficiency of 1 ($\propto_{ij}= 1$) is assumed which indicates that if two particles were to collide, they will stick together (Spicer and Pratsinis, 1996).

Collision frequency: The collision frequency depends upon the dominant hydrodynamic conditions. Orthokinetic flocculation refers to contacts or collisions of colloidal particles resulting from bulk fluid motion, such as stirring. In systems of stirring, the velocity of the fluid varies both from point to point and temporally (from time to time). Flocculation under laminar conditions is useful in experimental studies since the flow field is well defined. However, mostly industrial applications, flocculation occurs under turbulent conditions, resulted as rapid floc growth. Saffman and Turner (1956) represented an equation for the collision frequency of small particles in turbulent flow which is;

$$\beta_{ij=}\frac{2.3}{8}G(d_i + d_j)^3$$

This equation is based on two major assumptions; (I) the particles are approximately equal in size (II) the particles are much smaller than the smallest turbulent eddies present. Even so, when the floc particles start to grow, it is unlikely that they will be much smaller than the smallest turbulent eddies present. Although the high probability of invalid assumptions, this equation were used by several authors (Spicer and Pratsinis, 1996;

Binbing et al., 2003). Consequently, collision frequency is mostly depending on the rate of dissipation and the dimensions of the particles which have the same impact in both equations. We preferred to use the Saffman and Turner equation, due to let us to make our study comparable with the literature results.

Fragmentation rate: For fragmentation of the flocs, the fragmentation rate is given by the equation (Pandya and Spielman, 1982);

$$S_k = A' G^{\delta'} {u_k}^{\delta''}$$

where A is the specific fragmentation rate for the largest particles present, δ is a constant, u_k is the volume of particle k and $u_{\max}$ is the volume of the largest particle present. $1/3$, 1.6 and 0.0047 were used for δ'' δ' and for A'respectively. (Han et al., 2003).

Fragment distribution function: Numerous fragment distribution functions have been described in the literature. From all, binary fragmentation is the simplest one where one floc fragment into two approximately equally sized particles. It is also probably that algae flocs can fragment into 2, 3 or 4 particles, however to make the equation easier binary fragmentation was preferred (Nopens et al. 2005; Han et al., 2003).

$$\gamma_{jk} = c \frac{u_j}{u_k}$$

where u_j is volume of the particle j. For binary fragment, c=1 j=k+1; for ternary fragment c=2 j=k and j=k+1; and for quaternary fragment c=2; j=k+2. In our calculations, binary fragmentation was tried due to being the simplest.

2.2. Sedimentation model

Sedimentation is the settling of a particle, or suspension of particles, in a fluid due to the effect of an external force such as gravity, centrifugal force or any other body force.The method that has been applied previously in the literature uses Stokes' law (Stokes, 1844) for the settling rate of a sphere where the gravitational force acting on the sphere balances the drag force of the fluid and limited with Stokes' assumptions. Concha and Christiansen (1986), first solve the settling of one particle in a fluid, then, they introduce corrections for the interaction between particles, through which the settling velocity of a suspension is drastically reduced. Finally, the settling of isometric and non-spherical particles was treated. a gravity field, it is written as (Concha and Christiansen,1986);

$$V(t) = \frac{1}{18}\frac{\Delta\rho d^2 g}{\mu}\left(1 - exp\left(-\frac{2}{3}\frac{\mu}{18\rho_p d^2}t\right)\right)$$

where *V(t)* is the sedimentation velocity (m s^{-1}), $\Delta\rho = \rho_p - \rho_f$; the difference of densities between particle and fluid (kg m^{-3}), μ is the viscosity of the fluid (Pas).

The term inside the exponential term multiplying the time t is called *Stokes number* and the term outside the parenthesis is the *terminal velocity*, as mentioned *Stokes Equation* and is valid for small Reynolds numbers. Although Stokes equation is widely used, it should be kept in mind that Stokes law becomes invalid in more concentrated suspensions found in commercial settling for waste water treatment processes.

2.3 Experimental data

Data; cell counts, viscosities, particle size distributions and mean diameters and also sedimentation rates, used for pre-concentration process of *P.tricornutum* and *N. gaditana* cells were obtained in previous studies (Şirin et al 2012; Şirin et al., 2013). The data can be seen for each experimental set in Table 5.1a-b.

For particles to occur, collisions are essential between particles. Thus, in the lab experiments for both species we conducted, after addition of NaOH, the suspension was stirred in order to ensure collisions through mixing (1500 rpm rapid mixing followed by 300 rpm slow mixing). Additionally, it should be keep in mind that during pH adjustment, in the time of adding NaOH to the solution, the culture was stirred gently to be ensure of reading pH of the solution correctly. The time that the solution was stirred during the addition of NaOH depends on the pH we would like to achieve and volume of the sample. The higher is the desired pH, the longer the time we need (buffering effect etc.). These steps made hard to calculate the energy given to the system by stirring. Eventually, we tested different stirring speeds and times in the model to let the flocs appear and to see the change in the particle size distribution graphs.

Table 5.1a. Data for the experimental sets for *P.tricornutum* and *N.gaditana*

Species	Shape of cells	Mean size (µm)	Spherical diameter	Treatment	Viscosity* (µPa.s)	Cell density† (Cellsml^{-1})
P.tricornutum	Fusiform	Length:18 Width: 3.5	18µm	$pH_{threshold}$	2.1	$3.2x10^6$
				pH=11	2.2	
N.gaditana	Sphere	3-4 (diameter)	4µm	$pH_{threshold}$	2.1	$15x10^6$
				pH=11	2.2	

*Data obtained after treatment †Data obtained before treatment

Table 5.1b Particle diameter variations of concentrated samples after harvesting of cultures with alkalinity induced methods.

Species	Treatment	Mean diameter (µm)	d_{10}±SE (µm)	d_{50} ±SE (µm)	d_{90} ±SE (µm)	Largest floc size (exp)(µm)
P.tricornutum	pH=11	43.1±5.40	35.3±0.07	40.3±0.30	54.2±0.46	115
					Modelling k_{max}	261
	$pH_{threshold}$	47.7	35.4±0.20	43.5±0.52	67.0±0.47	102
					Modelling k_{max}	182
N.gaditana	pH=11	40.5	34.9±0.03	38.4±0.05	47.8±0.91	83
					Modelling k_{max}	4574
	$pH_{threshold}$	42.9	35.3±0.05	40.7±0.16	53.5±0.37	77
					Modelling k_{max}	3652

2.4 Mathematical modelling for floc occurrence

To develop a mathematical model for autoflocculation of microalgal cultures is needed to understand the system, the microalgal suspension, and how flocs form during the flocculation process. A suspension of microalgal cells consists of single cells, and /or aggregations of microalgal cells. When the flocculants are added to the system, all cells can collide with each other to create flocs. This means that one cell has infinite possibilities to collide with other cells to form an infinite number of flocs, which lead to programme unsolvable in time. Under these circumstances, for a flocculation model, following set of assumptions were taken into consideration.

Assumptions:

1. There are several floc classes which are different in size depending on the range of diameters. The floc diameter can be calculated from the amount of cells that is in a floc. Every class has a lower and upper boundary, defining the class size.
2. The steady state floc size distribution is based on the floc diameter (d_k).
3. During the flocculation, there is no net growth or loss of culture.
4. The initial class size assumes that only single cells, not aggregates are present already before the model starts to work. So, the range of the lower class boundary is already known.
5. The collision efficiency, α, is taken as 1 which means when a cell or floc collides with another cell or floc, the cells or flocs will stick.

With all assumptions, the rate of particle number could be calculated by population balance model. MATLAB's most frequently used standard solver for ordinary differential equations (ODEs), ode45 was used to obtain the particle size distribution at each time step from a given initial condition. This function implements a Runge-Kutta method with a variable time step for efficient computation.

For the flocculation model, one of the important parameter is time that the program runs (t_{max}). The pH adjustments and mixing the solution to let the cells flocculate depends on the volume used and desired pH where during the agitation flocculation occurs. Therefore, different t_{max} values were tested.

The energy dissipation term- shear rate (G) is another important parameter of the model. The more energy is given into the system the more chance that the particle will have chance to collide due to faster movement. It is difficult to estimate the average shear rate for the alkalinity-induced experiments, since different energies is brought into the system during pH adjustment, rapid stirring and low stirring.

To see the effect of mixing on particle size distribution, , energy dissipation rates of; 25 s^{-1} (corresponds to 1500rpm), 30 s^{-1} (corresponds to 1800rpm), 100 s^{-1} (recommended G in lime flocculation) (Camp 1955), 3000 s^{-1} and 42000 s^{-1}(G in Rulyov, 2010 for ultra-dispersed systems) have been tested.

$k_{max,}$ which is defined as the maximal amount of the cell in the biggest floc size, is also tested by using the max diameter value obtained from experiments (Table 5.1.b). Additionally to see the effect of k_{max} on the model, different values were also tested (Table 5.2).

The code for the model was written in Mat-LAB programming language and is presented in Appendix A.

2.5 Sedimentation model after flocculation

To see the sedimentation profile of the flocs appeared with PBM, Stokes' equation was used. We assumed the particles didn't collide with other particles and form larger particles during sedimentation during the sedimentation process and didn't show hindered settling, so only the first part of the became valid. Actually, this assumption is only valid when the collision of these particles goes slowly compared to their sedimentation which in our case we supposed. At a certain moment the particles have reached the bottom and are thus out of suspension. The rate at which a particle sinks is linearly dependent on its diameter square. Larger and heavier the particles, the faster they sink. Density of the flocs was assumed as 1300 kg m^{-3} by considering our experimental results and densities in Henderson et al., 2008. The terminal velocities of the flocs obtained from the previous section were calculated. The cylinder that the flocs were let to settle (0.21m) divided into

21 slabs to see how to flocs change their position by the time and concentration factor at the end of the sedimentation.

3. 0Results

3.1 Population balance model for *P.tricornutum& N.gaditana*

Alkalinity induced flocculation -mimic of autoflocculation in lab scale were tried to model with the programme. Experimental data of $pH_{threshold}$ and pH=11 were taken as reference. The volume concentration in each section can be calculated from a set of initial conditions. After the treatments of harvesting, the particle size distribution of each experiment showed different dispersions which directly affected the sedimentation rate (Figure 4.2 chapter 4).

In the model, all data are thus dependent on the number initial cell number, all generation and disappearance terms which are also dependent on the collision efficiency, α and collision frequency, β. The modelling is also dependent on the energy dissipation (G) which is defining the amount of work that is put into the system in the form of stirring, shaking etc.

Table 5.2 shows the particle size distribution of *P.tricornutum* cells obtained by the model according to different parameters without fragmentation terms.

Table 5.2. Particle size distribution with different parameters studied in the model for *P.tricornutum*

G	Time	k_{max}	Ranges(%)								
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
s^{-1}	s		**30-40***	**40-50***	**50-60***	**60-70***	**70-80***	**80-90***	**90-100***	**100-150***	**150-600***
25	600										
	1200	182									
30	1800		0	0	0	0	0	0	0	0	0
	2700	261									
100	3600										
	5400										
30000	600	182	99.14	0.86	0	0	0	0	0	0	0
	1200		90.38	8.99	0.62	0	0	0	0	0	0
	1800	261	76.70	18.22	4.42	0.6	0	0	0	0	0
	2700		60.36	22.46	10.89	4.27	1.48	0.43	0.1	0	0
	3600	182	51.78	21.65	12.97	7.19	4.07	2.33	0	0	0
		261	51.59	21.52	12.82	7.02	3.88	2.09	1.08	0	0
	5400	182	42.65	18.29	12.07	8.12	6.25	5.6	7.01	0	0
		261	46.08	19.45	12.5	8.02	5.7	4.43	3.8	0	0
42000	600	182	96.8	3.15	0.05	0	0	0	0	0	0
	1200		79.41	16.78	3.42	0.36	0	0	0	0	0
	1800	261	62.99	22.3	9.98	3.45	1	0.23	0.04	0	0
	2700	182	49.92	21.04	12.85	7.35	4.36	2.65	1.82	0	0
		261	50.54	21.24	12.89	7.29	4.23	2.43	1.37	0	0
	3600	182	43.89	18.83	12.31	8.09	6	5.07	5.8	0	0
		261	46.63	19.75	12.64	7.99	5.53	4.12	3.33	0	0
	5400	182	36.82	15.71	10.76	7.9	7.05	7.89	13.85	0	0
		261	44.06	18.21	11.84	7.98	6.25	5.64	6	0	0
experimental		$pH_{threshold}$	33.00	34.63	15.77	4.67	9.01	1.94	0.75	0.24	0
		SE	2.07	1.22	0.68	0.30	0.25	0.06	0.06	0.02	0
		$GM_{threshld}$	42.00	25.00	16.33	9.47	4.60	1.76	0.61	0.24	0
		SE	1.61	0.49	0.53	0.40	0.26	0.16	0.04	0.07	0
		$FC_{threshold}$	44.48	27.38	12.68	4.80	3.84	1.54	1.92	1.83	0
		SE	2.00	1.60	1.60	0.60	0.60	0.60	0.60	0.60	0
		pH=11	50.55	32.73	11.87	3.56	0.74	0.39	0.08	0.10	0
		SE	1.86	1.22	0.43	0.21	0.10	0.09	0.03	0.02	0
		GM_{11}	57.66	28.41	11.45	2.23	0.19	0.04	0.02	0.01	0
		SE	0.91	1.24	1.17	0.89	0.05	0.01	0.01	0.00	0
		FC_{11}	61.20	21.65	9.24	4.41	2.06	0.94	0.28	0.23	0
		SE	6.31	4.46	1.76	0.56	0.45	0.37	0.06	0.10	0

*****Ranges are corresponds to floc diameters which are written in micrometers.
† Experimental values and the meaning of abbreviations can be find in chapter 4.

No flocs appeared in the ranges at G rates of 25, 30 and 100 s^{-1} for all t values and for all k_{max} values. To check what is the breakage point of G value to let the flocs to appear in

the model, really high energy dissipations (G rates of 30000 s^{-1} and 42000 s^{-1}) used in the literature to flocculate ultra-dispersed particles with flocculant of cationic polymer Zetag were tried with different stirring times. Over high energy dissipations (e.g., G= 20000 s^{-1}), the flocs started to appear in the model which is found as the breakage energy dissipation of this model for *P.tricornutum* particles.

For G values of 30000 s^{-1} and 42000s^{-1}, at t=600 s, regardless of the k_{max}, almost all flocs appeared in range 1 (30-40 μm). Neither the k_{max} increased nor the G, no distinct differences were observed. Stirring times of 30 min (1800 s), 45 min (2700s), 1 hour and 1.5 hour also tested. For G=30000 s^{-1} and G=42000 s^{-1}, by the time increased the percentage of range 1 (30-40μm) was decreased and bigger floc ranges appeared. For instance, at G= 30000 s^{-1} for t=1200 s, percentage of range 1 in the total flocs decreased ~10% and range 2 was started to appear; for t=1800 s, range 1 percentage decreased ~25%, then range 2 and 3 appeared. When the G rate was constant and t increased, the flocs have longer time to collide with high energies, which results as higher concentrations in bigger floc range. For stirring times of t=3600s and longer at G=30000 s^{-1}, the biggest range of floc didn't change, however the percentages of the ranges changed for the benefit of bigger flocs. The higher is the G and the longer is the t, the flocs were formed max to range 7 (90-100 μm). When the time that model run was increased, it is observed that k_{max} didn't affect much the particle size distribution

percentage over k_{max}=150. Although some small differences were observed between the ranges, those were not that much remarkable.

The model is dependent on the floc diameter, the flocculation frequency parameter. The model showed behaviors as expected with high shear rates. The smallest floc classes decrease and the larger ones increase in time (Appendix A Figrue A.1-2). At the end, after the model was run, the total cell numbers were the same as the initial cell density which means the mass is conserved. Although really high shear rates were tried in the model, the experimental and theoretical results didn't match for particle size ranges. In the experimental data, a lower percentage of range 1 and a higher percentage of range 2 were measured for $pH_{threshold}$ which is the reason that the fastest sedimentation rate obtained. By the way, the differences between the experimental and theoretical results were almost equal to percentage differences of range 1-2 and higher ranges. Therefore, we tried the PBM with fragmentation rate terms, to check the flocs of range between 4 and 9 would have fragmentised and let the range 2 flocs appear in higher percentage. The data for fragmentation rate and distribution function were taken from literature where mostly applied for polymer particles in silico experiments (Han et al., 2003; Binbing 2003). However, when the fragmentation term was included and calculated with the literature data, it started to appear as imaginary number where giving us negative values (third term of equation 1) which theoretically cannot be possible. This result most probably obtained due to really high G values, energy dissipation rates. Nevertheless, on

the other hand, without high G values, no flocs could be able to appear in the model which is a dilemma in the model.

The model was then run for *N.gaditana* samples with initial cell concentration of $1.5x10^7$ cell mL^{-1} with size of 4-5 μm cells (Table 5.3).

Table 5.3. Particle size distribution with different parameters studied in the model for *N.gaditana*.

G s^{-1}	**Time** s	k_{max}	**Ranges(%)** 1 30-40*	2 40-50*	3 50-60*	4 60-70*	5 70-80*	6 80-90*	7 90-100*	8 100-150*	9 150-600*
25	600	1000									
	1200										
30	1800	3652	0	0	0	0	0	0	0	0	0
	2700										
100	3600	4574									
	5400										
	600	1000									
30000	1200										
	1800	3652	100	0	0	0	0	0	0	0	0
42000	2700										
	3600	4574									
	5400										
experimental†		$pH_{thresho}$	48.56	35.9	11.79	2.91	0.52	0.15	0.04	0.07	0.00
		SE	0.84	0.74	0.44	0.22	0.07	0.07	0.03	0.03	0.00
		GM_{thres}	67.00	26.4	4.55	0.98	0.64	0.15	0.05	0.15	0.00
		SE	0.88	0.95	0.04	0.10	0.05	0.05	0.05	0.05	0.00
		$FC_{thresho}$	62.50	28.8	5.34	1.50	0.42	0.53	0.21	0.61	0.00
		SE	0.00	3.85	4.98	0.38	0.54	0.09	0.29	0.05	0.53
		pH=11	62.37	30.9	4.60	0.75	0.71	0.33	0.23	0	0
		SE	2.30	0.46	0.18	0.59	0.63	0.24	0.15	0.00	0.05
		GM_{11}	82.98	15.1	1.08	0.39	0.03	0.04	0.05	0.06	0
		SE	0.29	0.15	0.39	0.15	0.03	0.06	0.05	0.06	0
		FC_{11}	63.08	31.1	5.38	0.39	0	0	0	0	0
		SE	0.63	0.61	0.35	0.09	0	0	0	0	0

*****Ranges are corresponds to floc diameters which are written in micrometers.
† Experimental values and the meaning of abbreviations can be find in chapter 4.

The model was run for max 5400 seconds with typical lab scale G values, and then with high G values used in literature, however no satisfactory results were able to obtain. For

the G values between 25-100s^{-1}, the model didn't give any results of any aggregation in the expected ranges. For the G rates of 32000 and 42000 s^{-1}, all flocs were appeared in range 1 which means of flocs between 30-40μm (Table 5.3). Longer stirring rates or even really high energy dissipation rates didn't give any promising results for *N.gaditana* cells to achieve experimental data or even close.

3.2 Sedimentation model for *P.tricornutum& N.gaditana*

We considered the cases of settling of a homogeneous suspension with solid particles of different sizes obtained from PBM (section 3.1). It is customary to use the radius of the smallest particles as a length unit, while the time unit is the Stokes time t_{St}.

With k_{max}=182 and G=30000s^{-1}, $pH_{threshold}$ values were used to check the sedimentation time amd concentration factor at the end of the process (see in Appendix B).

According to floc sizes, the velocities were calculated in the range of $2.5x10^{-5}$ (for k=1) - $8.10x10^{-4}$ ms^{-1} (k_{max} =182).

The cylinder devided into 21 slabs to observe the sedimentation time according to settling. It was observed that the smallest ranges' sedimentation time was 36min (average) where the biggest ranges' was 4 min(average) to reach bottom of the cylinder. In 10 min of settling time, it was observed that all bigger flocs (over range 4) already sedimented.

4.0 Discussion

The model used, development of different floc classes in time using a collision frequency, is dependent on the floc diameter, the flocculation frequency parameter.

Complete flocculation (formation-fragmentation) depends on floc chacteristics, G (the higher is the G, the smaller is the floc) and Gt (Camp number which gives information about collisions). The general design criterion for a basic rectangular flocculation tank is known as Gt=10,000 to 100,000 for energy dissipation /input (e.g., for metal salts with typical t range between 15 to 30 min and typical G range between 20 to 75sec^{-1} and for lime with typical T range between 1 to 10 min and typical G range between $G \geq 100$/sec).

The G rates of 25 and 30s^{-1} were tested because of using 1500rpm-1800rpm stirring range in lab experiments. These values were also perfectly matched with the Camp number (Camp, 1955) that is used in flocculation tanks. However, no flocs formed in the desired ranges at G values of 25, 30 and 100s^{-1} for all t and k_{max} values of both algae species.

Really high energy dissipation/shear rate values were required most probably due to big floc sizes were aimed to obtain according to experimental data with the model. In the literature, that much high shear rates were applied for silica particles and ultra-dispersed particles (Raghavan et al., 2008, Rulyov, 2010).

The programme written in the Mat-Lab showed behaviour as expected with high shear rates (The smallest floc classes'/ ranges decrease and the larger ones increase by the time) where we could conclude that the programme is working. However, the PBM was ineffective to simulate mimic of auto-flocculation process -alkalinity induced flocculation- for both *P.tricornutum* and *N.gaditana* cells in the used G rates.

The reason of ineffectiveness of PBM to simulate auto-flocculation might be the mechanism itself. Autoflocculation consists two mechanism (*i*) embedding of algae cells into $Mg(OH)_2$ precipitates -sweep flocculation- and/or (*ii*) charge neutralization. It might be possible that larger flocs were formed in the experimental study as the Mg ions precipitated as $Mg(OH)_2$, then microalgae cells were embedded into that matrix. The particle size distribution results of growth medium (GM) and culture (C) (in chapter 4, Figure 4.2), the flocculation efficiency results of commercial flocculants (in chapter 3) also supports that probability.

Additionally, not only the sweep flocculation mechanism, but also the charge neutralization should be taken into account to built a new model for the further studies to simulate autoflocculation. Moreover, the dominant mechanism depending on the process parameters (alkalinity, algae species, stirring rate and time, etc) comes into question which makes the algorithm harder to explain in mathematic language.

Experimental results showed that auto-flocculation should be preferred because of providing low energy demand, chemical free harvest. However, in the model for the flocs to form, really high shear rates were required. That kind high G rates cause high energy consumptions for energy calculations. "rpm" - in our case stirring rate of agitator- can be converted to shear rate in reciprocal seconds by the use of appropriate equations or factors which gave us 25-30 s^{-1} for G values. But, in real, autoflocculation happens because of pH increase as a result of ceasing air or CO_2 injection. There is no stirring except air/CO_2 injection during algae growth, neither no addition of NAOH.

On the other hand, Tesson et al. (2008) concluded that the excretion of polysaccharides of acidic nature by *Phaeodactylum* which was related to pH increase led to cells aggregation and correlated well with $Mg(OH)_2$ brucite formation. This phenomenon would make the work more complicated than it is. Because the release of exopolysaccharides by microorganisms depends on species, physiological status and environmental conditions (Passow and Alldredge, 1995; Passow et al., 200; Passow, 2002).

Unfortunately, the interactions among the algae during autoflocculation process are complex and poorly known. For this reason, it could be difficult to determine the parameters and dependency of parameters on each other. In addition, flocculation models adopting the Smoluchowski equation are limited by the constraints imposed by Smoluchowski´s six principal assumptions and the population balance models have often

been conditional on the floc size. k_{max} is an important parameter in the model for the particle size distributions. In the method is also an important step to decide the largest size. However, G and t terms seem the key parameters in our trials.

The sedimentation model with Stoke's equation shows promising and sufficient results. Some differences between the experimental results were obtained due to diversity of models particle size distribution results.

5.0 Conclusions

The mathematical model for flocculation is still a framework that should be developed further. The model is based on models in the literature and it has been used to test for large colloids and small microorganisms (<5 μm). It is a widely applied model, for yeast in beer brewing processes up to meteorological models for droplet formation.

However, the model has been ineffective to simulate alkalinity induced flocculation of microalgae cells of *P.tricornutum* and *N.gaditana* . Due to auto-flocculation is a complex process, further kinetic studies are needed to built a new model. The future model also needs to be further adjusted and integrated with the sedimentation model simultaneously to complete the model of autoflocculation followed by sedimentation.

Moreover, it should be keep in mind that even the different methods are used for particle dynamics, numerically simulated PSD slopes do not always agree with those obtained by

experimental results. The assumptions underlying population balance model analysis are mainly responsible for the disagreement. In real experiments/processes, alkalinity, phosphate ions, extracellular organic matter, etc complicated modelling and also hard to guess the process.

6.0 References

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CHAPTER VI
CONCLUSIONS

For decades, algae biodiesel has been announced as one option to alter fossil fuels. However, it does not appear that a feasible process for large-scale production of algae oil to bio-diesel is available at present. Further research on the technology is needed.

The selection of the proper harvesting method depends mainly on the process product targets. To decide which method is better for harvesting, it should be checked not only how efficient the agent as a flocculant and how appropriate to provide a cost effective harvesting, but also how feasible to use the agent in the case of using concentrated biomass in the further processes without any problem and managing the residual water after harvesting.

Dependenting on the chemical properties of the seawater and on the properties of the outer surface of the algae, pH induced flocculation seems promising for *P.tricornutum* and *N.gaditana* species. It is effective, economical and eco-friendly. Sedimentation rates

of the author chosen method are also helpful to scale-up the process. The process is also very reproducible. It is observed that after a certain pH, increasing the pH didn't change the flocculation efficiency, however changed the mass content of the concentrated sample (higher ash content).

The comparative study of pre-concentrated sample properties shows that;

(I) Pre-concentration processes make both pumping and mixing easier because of the Newtonian behaviour of the samples than Non-Newtonian ones.

(II) Particle size analysis of the pre-concentrated samples support settling properties sufficiently.

(III) Ca and Mg ion concentrations of the pre-concentrated samples substantiate the fact that the Mg ion is the protagonist in the alkalinity-induced flocculation mechanism.

A population balance model (PBM) has been tried to built to predict the floc size distribution for *P.tricornutum* and *N.gaditana*. The model was ineffective to simulate alkalinity induced flocculation -a mimic of autoflocculation in lab scale. High shear rates were needed in the model to let the floc to appear. The mathematical model for flocculation is still a framework that should be developed. Additionally, the model needs to be further adjusted and integrated with the sedimentation model simultaneously with PBM.

APPENDIX

A. Codes for floc formation

```
function r = ratio(t,v)

global g;
global M;
global R;
global it;
global Mp;
global rhop;

%% inputs
%kmax=input('kmax: ');
kmax=182;
%G=input('G: ');
GG=[5,25,30,100,30000,42000, 60000];
G=GG(g);
%dp=input('dp :');
dp=18e-6;

%% constants
rhop=1300;
A=0.0047;
deltap=1.6;
deltapp=1/3;
c=1; %For binary fragment
alfa=1; %collision efficiency


%% Equations
Mp=rhop*(4/3)*pi*(dp/2)^3;
vp=(4/3)*pi*(dp/2)^3;
%vt=vp*M(1,1);

%% Matrix
%particle size distribution
```

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```
for i=1:kmax
    d(i)=dp*nthroot(i,3);
end

%Particle volume distribution
for i=1:kmax
    u(i)=(4/3)*pi*(d(i)/2)^3;
end

%Collision frequency
Beta=zeros(kmax,kmax);
for i=1:kmax
    for j=1:kmax
        Beta(i,j)=(2.3/8)*G*(d(i)+d(j))^3;
    end
end

%Fragmentation rate
S=zeros(kmax,1);
for k=1:kmax
    S(k)=A*G^deltap*((4/3)*pi*(d(k)/2)^3)^deltapp;
end

%Fragment distribution function
y=zeros(kmax,kmax);
for k=1:kmax
    for i=1:kmax
        y(k,i)=c*u(i)/u(k); %check!
    end
end

%% code
r=zeros(kmax,1);
ctr1=0;
ctr2=0;
ctr3=0;
ctr4=0;

for k=1:kmax
    for i=1:kmax
        %% ctr2 Dissapearance of small flocs due to colision
        if i+k>kmax
            dis=0; %for concervation of mass!!
        else
            dis=alfa*Beta(i,k)*v(i);
        end
        ctr2=ctr2+dis;
        %% ctr4 Appearance of flocs due to fragmentation
```

```
%          if k<kmax
%              jj=k+1
%              if v(jj)==0%v(k)==0
%                  form=0
%              else
%                  form=y(k,jj)*S(jj)*v(jj)
%              end
%              ctr4=ctr4+form; % (fragmentation)
%          end

    %% Ctr1 Formation of big flocs from the colision of smaller flocs
    for j=1:kmax
        if i+j==k
            gen=alfa*Beta(i,j)*v(i)*v(j);
            ctr1=ctr1+gen;
        end
    end
end
%% ctr3 Dissapearance of flocs due to fragmentation
% if k>1
%     ctr3=S(k)*v(k);
% end


%% r
%r(k)=0.5*ctr1-v(k)*ctr2-ctr3+ctr4;
r(k)=0.5*ctr1-v(k)*ctr2;

clear ctr1 ctr2 ctr4 ;
ctr1=0;
ctr2=0;
ctr4=0;
end
end


ODE45-----
%clear all;
%clc;
global v;
global g;
global M;
global R;
global it;
global Mp;
global rhop;
global initial
it=it;
```

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```
for g=5;

kmax=182;
M=zeros(1,kmax);
M(1,1)=3.2e6;

[t,v] = ode45(@Phaeo_6th_march,[0 5400],M);
plot(t,v)
% time=cputime;
diameter=[30 40 50 60 70 80 90 100 150 600]; %experiment diameter in
microns
rad=diameter/2000000;

clear ranges range1 range2 range3 range4 range5 range6 range7 range8
range9 ;
ss=size(v);
p=ss(1);
final=v(p,:);
int1=round((rhop*(4/3)*pi*rad(1)^3)/Mp);
int2=round((rhop*(4/3)*pi*rad(2)^3)/Mp);
int3=round((rhop*(4/3)*pi*rad(3)^3)/Mp);
int4=round((rhop*(4/3)*pi*rad(4)^3)/Mp);
int5=round((rhop*(4/3)*pi*rad(5)^3)/Mp);
int6=round((rhop*(4/3)*pi*rad(6)^3)/Mp);
int7=round((rhop*(4/3)*pi*rad(7)^3)/Mp);
int8=round((rhop*(4/3)*pi*rad(8)^3)/Mp);
int9=round((rhop*(4/3)*pi*rad(9)^3)/Mp);
int10=round((rhop*(4/3)*pi*rad(10)^3)/Mp);

range1=0;
range2=0;
range3=0;
range4=0;
range5=0;
range6=0;
range7=0;
range8=0;
range9=0;

if kmax>=int2
    for i=int1:int2
        r1=final(i);
        range1=range1+r1;
    end
end

if kmax>=int3
    for i=(int2+1):int3
```

```
        r2=final(i);
        range2=range2+r2;
    end
end

if kmax>=int4
    for i=(int3+1):int4
        r3=final(i);
        range3=range3+r3;
    end
end

if kmax>=int5
    for i=(int4+1):int5
        r4=final(i);
        range4=range4+r4;
    end
end

if kmax>=int6
    for i=(int5+1):int6
        r5=final(i);
        range5=range5+r5;
    end
end

if kmax>=int7
    for i=(int6+1):int7
        r6=final(i);
        range6=range6+r6;
    end
end

if kmax>=int8
    for i=(int7+1):int8
        r7=final(i);
        range7=range7+r7;
    end
end

if kmax>=int9
    for i=(int8+1):int9
        r8=final(i);
        range8=range8+r8;
    end
end
```

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```
if kmax>=int10
    for i=(int9+1):int10
        r9=final(i);
        range9=range9+r9;
    end
end

ranges=[range1,range2,range3,range4,range5,range6,range7,range8,range9]
;

ranges=ranges*100/sum(ranges);
 RANGES(g,:)=ranges;

%TIME(g,1)=time;
%bar(ranges)
end
initial=v(length(v),:);
```

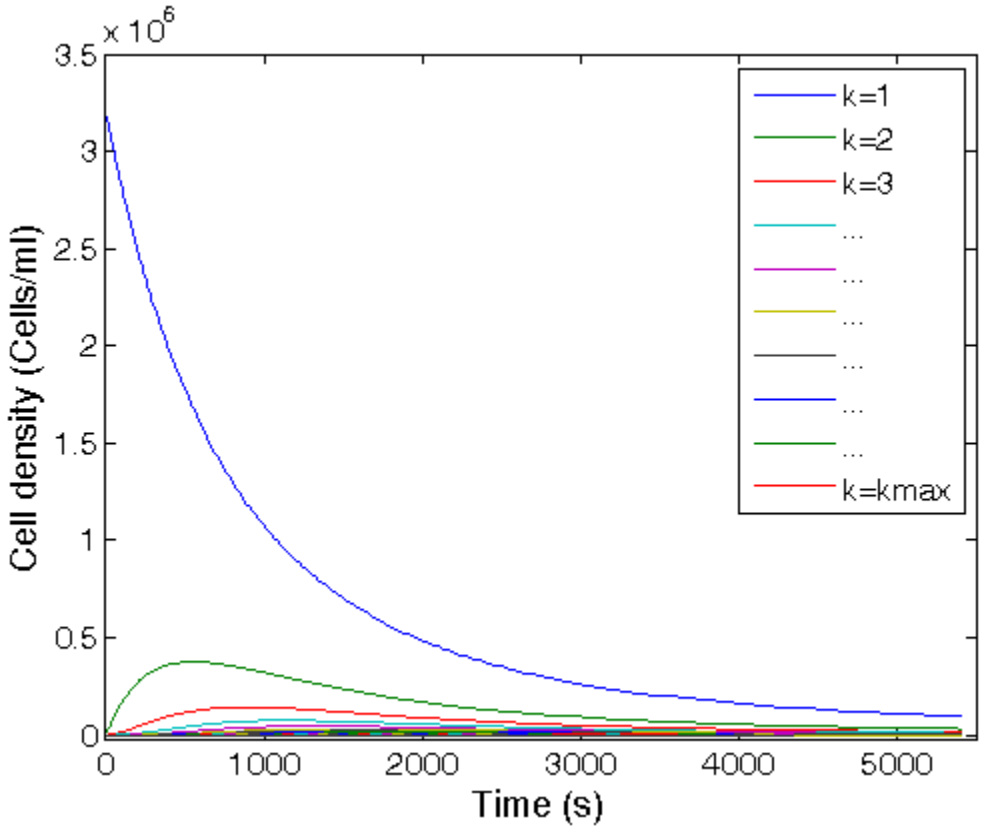

Figure A.1. Cell density graphs for the culture(k=1) and the flocs by the time model runs (Initial cell density= $3.2*10^6$ cells mL^{-1} ; G=$30000s^{-1}$; k_{max}=182; t=5400s) of *P.tricornutum*

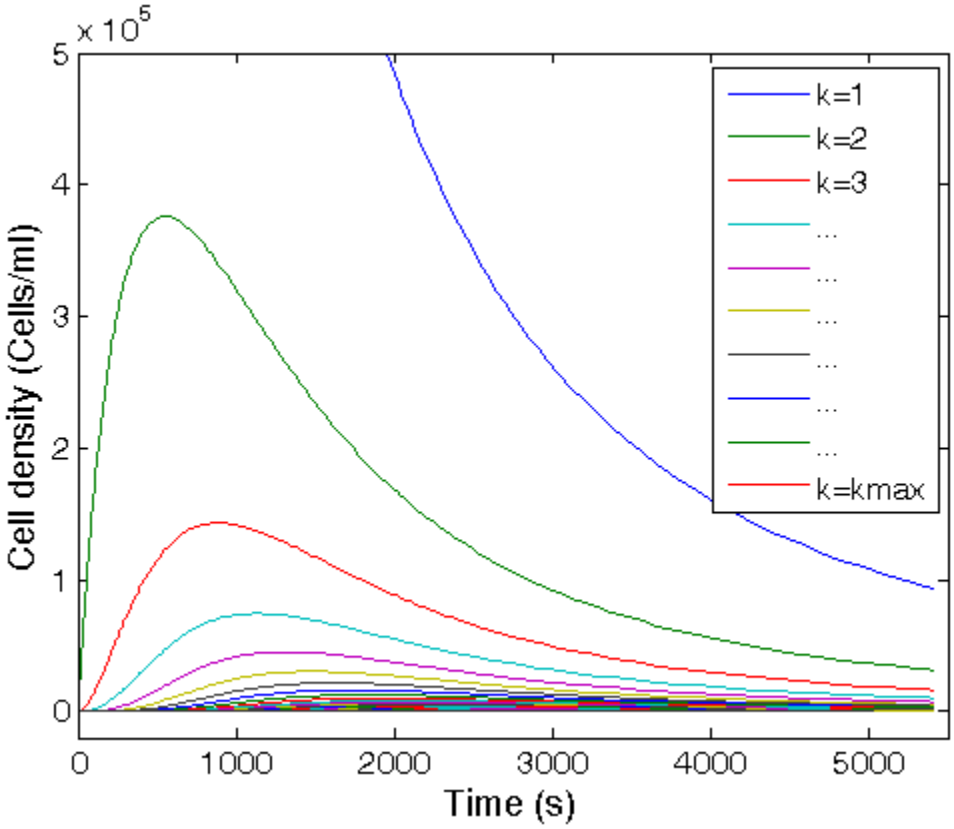

Figure A.2. A close look to cell density graphs for the culture(k=1) and the flocs by the time model runs (Initial cell density= $3.2*10^6$ cells mL^{-1} ; G=$30000s^{-1}$; k_{max}=182; t=5400s) of *P.tricornutum*

B. Sedimentation

Table B.1.Sedimentation profile example of range 1- flocs occurred with the programme ($G=30000s^{-1}$; $k_{max}=182$) for *P.tricornutum cells*

	Flocs not sedimenting				**Flocs belonging to Range 1 (30-40µm)**						
	Number of cells in flocs										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Velocity $x10^{-5}$ (ms^{-1})	2.52	4.00	5.24	6.35	7.37	8.32	9.22	10.08	10.903	11.697	12.464
Slabs	**Time needed for the flocs to travel slabs (m)**										
1	6.61	4.17	3.18	2.62	2.26	2.00	1.81	1.65	1.53	1.42	1.34
2	13.23	8.33	6.36	5.25	4.52	4.01	3.61	3.31	3.06	2.85	2.67
3	19.84	12.50	9.54	7.87	6.79	6.01	5.42	4.96	4.59	4.27	4.01
4	26.46	16.67	12.72	10.50	9.05	8.01	7.23	6.61	6.11	5.70	5.35
5	33.07	20.83	15.90	13.12	11.31	10.01	9.04	8.27	7.64	7.12	6.69
6	39.68	25.00	19.08	15.75	13.57	12.02	10.84	9.92	9.17	8.55	8.02
7	46.30	29.16	22.26	18.37	15.83	14.02	12.65	11.57	10.70	9.97	9.36
8	52.91	33.33	25.44	21.00	18.09	16.02	14.46	13.23	12.23	11.40	10.70
9	59.52	37.50	28.62	23.62	20.36	18.03	16.27	14.88	13.76	12.82	12.03
10	66.14	41.66	31.80	26.25	22.62	20.03	18.07	16.53	15.29	14.25	13.37
11	72.75	45.83	34.98	28.87	24.88	22.03	19.88	18.19	16.81	15.67	14.71
12	79.37	50.00	38.15	31.50	27.14	24.04	21.69	19.84	18.34	17.10	16.05
13	85.98	54.16	41.33	34.12	29.40	26.04	23.50	21.49	19.87	18.52	17.38
14	92.59	58.33	44.51	36.75	31.67	28.04	25.30	23.15	21.40	19.95	18.72

15	99.21	62.50	47.69	39.37	33.93	30.04	27.11	24.80	22.93	21.37	20.06
16	105.8	66.66	50.87	41.99	36.19	32.05	28.92	26.46	24.46	22.80	21.39
17	112.4	70.83	54.05	44.62	38.45	34.05	30.73	28.11	25.99	24.22	22.73
18	119.1	75.00	57.23	47.24	40.71	36.05	32.53	29.76	27.51	25.65	24.07
19	125.7	79.16	60.41	49.87	42.98	38.06	34.34	31.42	29.04	27.07	25.41
20	132.3	83.33	63.59	52.49	45.24	40.06	36.15	33.07	30.57	28.50	26.74
21	138.9	87.49	66.77	55.12	47.50	42.06	37.95	34.72	32.10	29.92	28.08

Table B.2.Sedimentation profile example of range 8- flocs occurred with the programme ($G=30000s^{-1}$; $k_{max}=182$) for *P.tricornutum cells*

	Flocs belonging to Range 8 (100-150µm)										
	Number of cells in flocs										
	172	**173**	**174**	**175**	**176**	**177**	**178**	**179**	**180**	**181**	**182**
Velocity $x10^{-5}$ (ms^{-1})	77.94	78.24	78.54	78.84	79.14	79.44	79.74	80.04	80.34	80.63	80.93
Slabs	**Time needed for the flocs to travel slabs (m)**										
1	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
2	0.43	0.43	0.42	0.42	0.42	0.42	0.42	0.42	0.41	0.41	0.41
3	0.64	0.64	0.64	0.63	0.63	0.63	0.63	0.62	0.62	0.62	0.62
4	0.86	0.85	0.85	0.85	0.84	0.84	0.84	0.83	0.83	0.83	0.82
5	1.07	1.07	1.06	1.06	1.05	1.05	1.05	1.04	1.04	1.03	1.03
6	1.28	1.28	1.27	1.27	1.26	1.26	1.25	1.25	1.24	1.24	1.24
7	1.50	1.49	1.49	1.48	1.47	1.47	1.46	1.46	1.45	1.45	1.44
8	1.71	1.70	1.70	1.69	1.68	1.68	1.67	1.67	1.66	1.65	1.65
9	1.92	1.92	1.91	1.90	1.90	1.89	1.88	1.87	1.87	1.86	1.85
10	2.14	2.13	2.12	2.11	2.11	2.10	2.09	2.08	2.07	2.07	2.06

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11	2.35	2.34	2.33	2.33	2.32	2.31	2.30	2.29	2.28	2.27	2.27
12	2.57	2.56	2.55	2.54	2.53	2.52	2.51	2.50	2.49	2.48	2.47
13	2.78	2.77	2.76	2.75	2.74	2.73	2.72	2.71	2.70	2.69	2.68
14	2.99	2.98	2.97	2.96	2.95	2.94	2.93	2.92	2.90	2.89	2.88
15	3.21	3.20	3.18	3.17	3.16	3.15	3.14	3.12	3.11	3.10	3.09
16	3.42	3.41	3.40	3.38	3.37	3.36	3.34	3.33	3.32	3.31	3.29
17	3.64	3.62	3.61	3.59	3.58	3.57	3.55	3.54	3.53	3.51	3.50
18	3.85	3.83	3.82	3.81	3.79	3.78	3.76	3.75	3.73	3.72	3.71
19	4.06	4.05	4.03	4.02	4.00	3.99	3.97	3.96	3.94	3.93	3.91
20	4.28	4.26	4.24	4.23	4.21	4.20	4.18	4.16	4.15	4.13	4.12
21	4.49	4.47	4.46	4.44	4.42	4.41	4.39	4.37	4.36	4.34	4.32