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An assessment of inexpensive methods for recovery of microalgal biomass and oils

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ABSTRACT

Inexpensive processes for harvesting the microalgal biomass from the culture media and recovering oils from the harvested biomass are necessary for economically viable production of low-value products such as fuels. This study focused on harvesting of microalgae biomass from the culture broth by flocculation–sedimentation and recovery of oils from the harvested biomass using solvent-based extraction. Flocculation–sedimentation was explored for several marine and freshwater microalgae including *Choricystis minor* (freshwater), *Neochloris* sp. (freshwater), *Chlorella vulgaris* grown in freshwater; *C. vulgaris* grown in seawater; *Nannochloropsis salina* (seawater) and *Cylindrotheca fusiformis* (seawater), as a means for substantially concentrating the biomass prior to further dewatering by other methods. Aluminum sulfate and ferric chloride were investigated as cheap, highly effective, readily available in large quantities and innocuous flocculants. Flocculation–sedimentation behavior of the microalgae was evaluated with several flocculation conditions. The optimal microalgal biomass harvesting conditions identified in batch flocculation studies were applied to design and characterize a continuous flocculation–sedimentation system. The effect of the flocculant used and the water in the biomass paste on the extraction of oils were assessed in comparison with controls. The optimal solvent composition for extraction of the biomass paste was established. Using this solvent composition, the optimal extraction conditions (i.e. the volume of the solvent mixture relative to biomass, the extraction temperature and time) were identified using a 2^3 factorial experimental design.

Removal of more than 95% of the biomass from the broth by flocculation–sedimentation was shown to be possible for all the microalgae, but the required dosage of the flocculant depended on the following factors: the microalgal species; the ionic strength of the

suspending fluid; the initial concentration of the biomass in the suspension; and the nature of the flocculant. Irrespective of the algal species, the flocculant dosage was found to increase linearly with increasing concentration of the biomass in the culture broth. The flocculant dosage for a given level of biomass recovery under standardized processing conditions increased with an increase in the cell specific surface area in the range of 26–450 μm^2 $cell^{-1}$. Al^{3+} was a better flocculant than Fe^{3+} for some algae, but the situation was reversed for some others. The continuous flow biomass recovery was performed with *N. salina*, as this alga had the highest oil productivity among the species studied. With an aluminum sulfate dosage of 229 mg L^{-1} and a total flow rate of 22.6 mL min^{-1}, almost 86% of the *N. salina* biomass could be recovered from the broth within 148 min in the sedimentation tank. A prior flocculation–sedimentation treatment could greatly reduce the energy demand of subsequent dewatering by other methods. The flocculants adsorbed to the biomass were not removed by washing, but this did not hinder oil recovery from the biomass paste by solvent extraction. A modification of well-known Bligh and Dyer method could be used to recover more than 96% of the oils from *N. salina* biomass paste. The single-step modified extraction procedure was much superior to the Bligh and Dyer original. The optimal extraction conditions for *N. salina* biomass paste included a solvent mixture (chloroform, methanol and water in the volume ratio of 5.7:3:1) volume of 33 mL per g (dry basis) of the algae biomass; an extraction temperature of 25°C; and an extraction time of 2 h.

This work represents the first detailed study of the continuous flocculation–sedimentation process for harvesting *N. salina* biomass from the culture broth and the specific suitable solvent combination of chloroform, methanol and water for extracting algal crude oils from the *N. salina* biomass paste without a prior drying step.

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CHAPTER 1

INTRODUCTION

Microalgae have been identified as a potentially highly productive source of fuel oils (Chisti, 2007; Schenk et al., 2008; Brennan & Owende, 2010; Chisti, 2012; Bellou et al., 2014). Renewable oil production using microalgae has numerous advantages compared to production from oil crops (Chisti, 2007, 2008, 2010; Mata et al., 2010; Packer et al., 2011; Chisti, 2012, 2013; Kiran et al., 2014). Microalgae grow rapidly and many are rich in lipids. Growing microalgae for oil does not require fertile cropland. Algae can be grown in freshwater, brackish water, seawater, and municipal wastewater (Posten, 2012; Sastre, 2012). In addition to oil, microalgae can provide various types of value-added co-products (Metting, 1996; Hu et al., 2008; Huang et al., 2010; Borowitzka, 2013b). Economic production of oils from microalgae requires inexpensive methods of recovering the algal biomass from the culture broth and low-cost methods for extracting the oil from the wet biomass.

The low concentration of biomass (0.5 g L^{-1} dry biomass in some commercial production systems) and the small size of microalgae cells (typically 3–30 μm in diameter) make large scale recovery of the biomass expensive (Shelef et al., 1984; Gudin & Therpenier, 1986; Grima et al., 2003b; Uduman et al., 2010a; Pragya et al., 2013a). The biomass recovery from the broth contributes about 20–30% to the total cost of biomass production (Grima et al., 2003b; Danquah et al., 2009b; Mata et al., 2010; Salim et al., 2011). Furthermore, the recovered microalgae biomass has a high moisture content and drying it is expensive (Gudin & Therpenier, 1986; Li et al., 2008b; Cheng et al., 2013; Dejoye Tanzi et al., 2013). A major

challenge for microalgae biomass recovery processes for producing fuel oils is the concentration of the biomass from a very low level in the broth to a point where oil extraction becomes possible at the lowest possible cost (Pienkos & Darzins, 2009; Halim et al., 2011). Moreover, the oil in the microalgal biomass needs to be extracted from a moist biomass paste without a prior drying step, to minimize costs (Chisti, 2008; Lardon et al., 2009; Taher et al., 2014a; Yang et al., 2014). However, suitable extraction methods have been barely investigated.

This work is focused on flocculation–sedimentation as an inexpensive method for the recovery of microalgal biomass and extraction of oils. Some freshwater and marine microalgae which were identified as good oil producers (Chisti, 2007; Gouveia & Oliveira, 2009; Rodolfi et al., 2009; Huerlimann et al., 2010; Mata et al., 2010; Gouveia, 2011), are used for the study. Flocculation followed by gravity sedimentation is considered to be an inexpensive and highly effective technique for harvesting microalgal biomass from the culture media (Uduman et al., 2010a; Chen et al., 2011; Milledge & Heaven, 2013; Pahl et al., 2013a; Vandamme et al., 2013; Barros et al., 2015). Batch and continuous flocculation–sedimentation are studied using commercial low-cost and safe flocculants (aluminum sulfate and ferric chloride). Based on the data obtained from batch studies, a continuous flocculation–sedimentation process is designed and characterized. Although batch flocculation of algae using various methods has received some attention in the literature (Vandamme et al., 2012; Wu et al., 2012; Pahl et al., 2013a; Udom et al., 2013; Vandamme et al., 2013; Weschler et al., 2014; Misra et al., 2015; Shen et al., 2015), continuous flocculation–sedimentation has not been discussed for microalgae. As flocculants may interfere with subsequent oil recovery, impact of the adsorbed flocculant on subsequent oil extraction was examined. The extraction conditions (e.g. solvent composition, solvent volume, extraction time, extraction temperature) for oil recovery from biomass paste were

optimized based on a modified extraction method of Bligh & Dyer originally established for lipid extraction from fish tissue. The study is organized into five chapters. Chapter 2 reviews the relevant literature. Chapter 3 describes the materials and methods in detail. Chapter 4 reports the results and discusses the important issues. Chapter 5 summarizes the key findings, the conclusion of the study and their implications.

CHAPTER 2

LITERATURE REVIEW

2.1 Microalgae and their applications

Microalgae or microphytic algae are microscopic photosynthesis eukaryotic microorganisms (Sastre, 2012) that are found in ecosystems including freshwaters, marine environments, and brackish waters (Becker, 1995; Gouveia, 2011; Varfolomeev & Wasserman, 2011). Microalgae can be grown photoautotrophically, i.e. using carbon dioxide as the sole source of carbon and light as the sole source of energy (Becker, 1995). Some microalgae can be grown heterotrophically using organic compounds to provide energy and carbon (Grobbelaar, 2007; Brennan & Owende, 2010). Microalgae can grow rapidly, complete their life cycle in period ranging from 24 h to several days and adapt quickly to unfavourable environmental conditions (Varfolomeev & Wasserman, 2011).

Microalgae produce a great diversity of biochemical compounds including pigments, starches, sugars, proteins, lipids, hydrocarbons and bioactive compounds (Metting, 1996; Spolaore et al., 2006; Stephens et al., 2010). Some of the commercial products obtained from microalgae include β-carotene, astaxanthin, docosahexaenoic acid, eicosapentaenoic acid and phycobilin pigments (Eriksen, 2008; Borowitzka, 2013a). Table 2.1 shows some of the major products of microalgae and their applications. Microalgae products have been extensively discussed in the literature (Borowitzka, 1992; Pulz & Gross, 2004; Spolaore et al., 2006; Varfolomeev & Wasserman, 2011; Sastre, 2012; Borowitzka, 2013a; Markou & Nerantzis, 2013; Skjanes et al., 2013).

Table 2.1 Microalgal products and applications (Sastre, 2012)

Microalga	Cultivation system and producer countries	Product	Application areas	Market price (US$/kg)
Chlorella vulgaris	Open pond: China, Japan, Taiwan; tubular photobioreactor: Germany	Biomass	Food supplements, cosmetics	5-60
		β-Glucan	Cosmetics	2000
Dunaliella salina	Open pond: Israel, Hawaii, India, China; shallow lagoons: Australia	Biomass	Food supplements, animal feed, aquaculture	300-3000
		β-Carotene	Food colorant, cosmetics	
Haematococcus pluvialis	Open pond: Hawii, India, China, Japan, Taiwan; tubular photobioreactor: Israel, India	Astaxanthin	Feed colorant (salmon), food supplement, cosmetics, pharmaceuticals	3000-10000
Isochrysis galbana	-	Fatty acids	Animal nutrition	-
Odontella aurita	Open pond	Fatty acids	Pharmaceuticals, cosmetics, baby food	-
Phaeodactylum tricornutum	Open pond, basin	Lipids, fatty acids	Nutrition, biofuels	-
Porphyridium sp.	Tubular photobioreactor	Polysaccharides	Pharmaceuticals. Cosmetics, nutrition	
		Phycoerythrin	Food colorant	$3\text{-}25\times10^{6}$

Source: Adapted from Sastre (2012)

In the last few years microalgae have attracted much attention as a potential source of oils for making liquid biofuels (Chisti, 2007, 2010; Borowitzka, 2013a; Chisti, 2013). This is because algae grow more rapidly than many oil crops, can have a high oil content in their biomass (Table 2.2) and algal oils can be converted to liquid transport fuels such as biodiesel. Potential fuels such as bioethanol, biohydrogen and biogas can also be made from algae (Chisti, 2007; Brennan & Owende, 2010; Chisti, 2010; Stephens et al., 2010). Unlike land plants, algae do not require fertile cropland. In a suitable climate, algae may be grown in freshwater, brackish water, seawater and municipal wastewater (Metting, 1996; Chisti, 2007; Hu et al., 2008; Brennan & Owende, 2010; Huang et al., 2010). As fuels are extremely low-value products, algae for producing fuels must be grown and harvested cheaply. This is possible only if freely available sunlight is used to grow algae photoautotrophically (Chisti, 2007, 2010, 2013).

Table 2.2 Oil content (% of dry biomass) of some microalgae

Species	Oil content (% of dry biomass)
Botryococcus braunii	25 – 75
Chlorella sp.	28 – 32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16 – 37
Dunaliella primolecta	23
Isochrysis sp.	25 – 33
Monallanthus salina	>20
Nannochloris sp.	20 – 30
Nannochloropsis sp.	31 – 68
Neochloris oleoabundans	35 – 54
Nitzschia sp.	45 – 47
Phaeodactylum tricornutum	20 – 30
Schizochytrium sp.	50 – 70
Tetraselmis sueica	15 – 23

Source: Chisti (2007)

2.2 Microalgal biomass production

2.2.1 Photoautotrophic cultivation

Photoautotrophic cultivation of microalgae needs light, carbon dioxide and water. In addition, essential inorganic nutrients (e.g. N and P) must be provided (Becker, 1995). Most commercial scale production of microalgae relies on this method. Open ponds and closed photobioreactors are the main technologies used in photoautotrophic production (Pienkos & Darzins, 2009; Brennan & Owende, 2010).

2.2.1.1 Open ponds

Almost all commercial-scale production is presently in open outdoor ponds. Ponds may be natural (e.g. lakes, lagoons) or purpose built artificial ponds such as shallow circular ponds and raceways (Borowitzka, 2005; Brennan & Owende, 2010). Figure 2.1 shows the different types of large scale open pond systems used in commercial production. Raceways are the most commonly used open culture system. Raceways are cheaper to build and operate than photobioreactors, but have important limitations such as a relatively low productivity and susceptibility to contamination (Borowitzka, 2005). The maximum attainable microalgae biomass concentration in an open pond system is typically 0.5-1.0 g L^{-1} (Chisti, 2007). Open ponds culture systems have been extensively reviewed in the literature (de la Noue & de Pauw, 1988; Becker, 1995; Richmond, 2004; Andersen, 2005; Borowitzka, 2005; Chisti, 2007; Eriksen, 2008; Li et al., 2008; Gouveia & Oliveira, 2009; Mata et al., 2010; Oh et al., 2010; Chisti, 2012).

Figure 2.1. Different types of large-scale open pond systems used in commercial production: a) paddle-wheel driven raceway pond (http://algaeforbiofuels.com/category/column-of-dr-john-kyndt/); b) shallow lagoons of the *Dunaliella salina* (http://en.wikipedia.org/wiki/Salt-_evaporation_pond); c) plastic lined raceway-type pond with water-jet circulation (http://-biofuelsdigest.com/bdigest/wp-content/uploads/2011/05/AuroraAlgae.jpg); d) circular ponds with rotating agitator for *Chlorella pyrenoidosa* biomass production, Japan (http://www.-sunchlorella.com/corporate-activity.html).

2.2.1.2 Closed photobioreactors

Closed photobioreactors (PBR) overcome some of the major limitation of open pond systems (Brennan & Owende, 2010), but are expensive to build and operate. Photobioreactor may use natural or artificial light (Behrens, 2005). Various kinds of photobioreactors are available (Figure 2.2) and have been reviewed in the literature (de la Noue & de Pauw, 1988; Becker, 1995; Tredici, 2003; Lee & Shen, 2004; Andersen, 2005; Behrens, 2005; Chisti, 2007; Tredici, 2007; Eriksen, 2008; Rodolfi et al., 2009; Brennan & Owende, 2010; Huang et al., 2010; Mata et al., 2010; Chen et al., 2011; Acién Fernández et al., 2012; Yen et al., 2014). The maximum microalgae biomass concentration in a typical closed photobioreactor is about 5.0 g L^{-1} (Chisti, 2007), but may be higher if the light path is short, e.g. 1 cm.

2.2.2 Heterotrophic cultivation

Heterotrophic cultivation requires an organic carbon feedstock (e.g. sugars) as a source of carbon and energy. The organic carbon compounds used in heterotrophic growth are ultimately obtained through photosynthesis. Heterotrophic growth uses bioreactors similar to the ones used in fermentation technology (Eriksen, 2008). Heterotrophic cultivation offers certain advantages (Eriksen, 2008; Huang et al., 2010), but in view of the need for a carbon source it is not expected to be competitive with photoautotrophic growth for really large-scale production of low value products such as oil for biofuels. Heterotrophic growth does achieve a high biomass concentration and productivity. For example, according to Xiong et al. (2008) with fed-batch cultivation of *Chlorella protothecoides*, the cell density achieved was 51.2 g L^{-1} in 167 hours in a 5 L bioreactor. The alga was grown in a basal salts medium with glucose. Heterotrophic growth does require a suitably formulated nutrient medium and

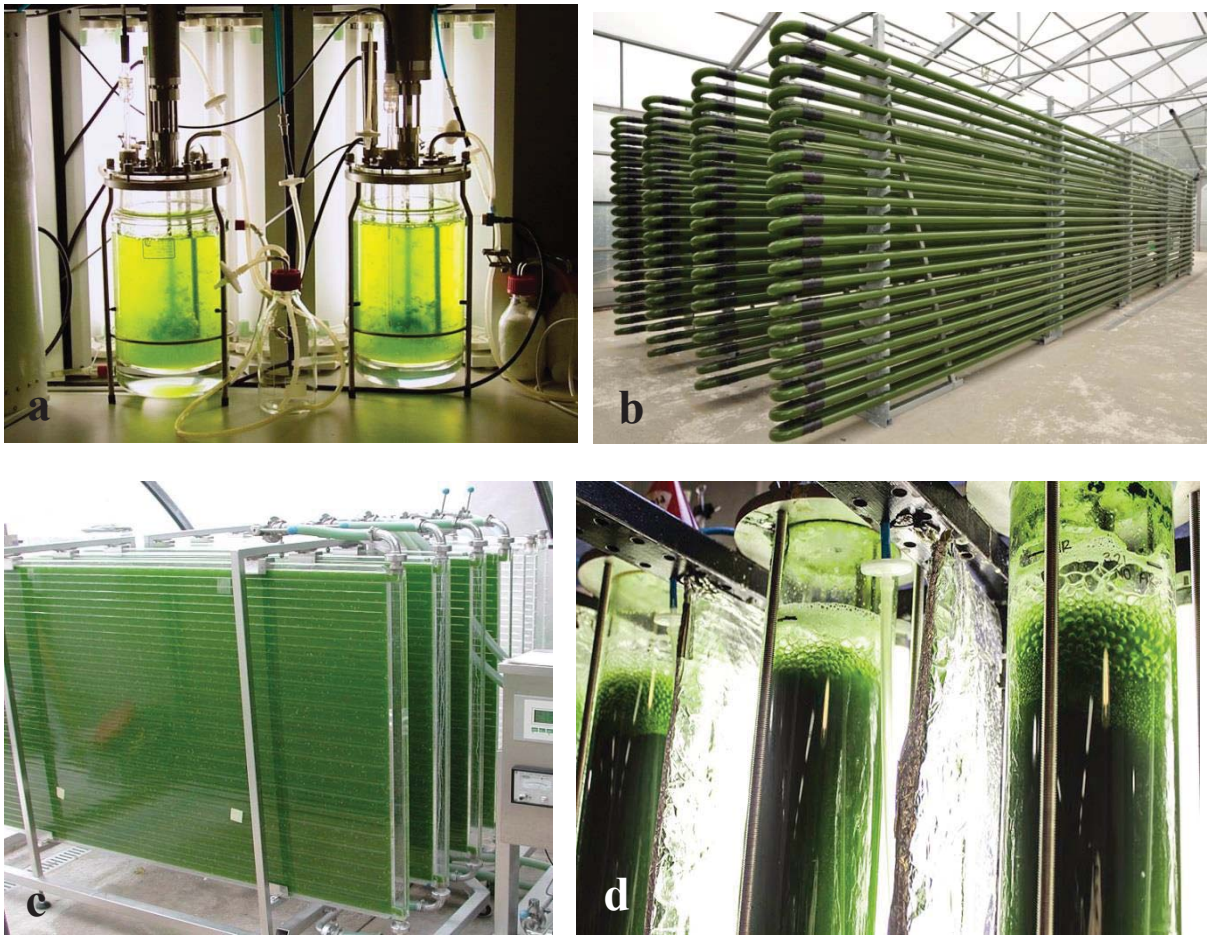


Figure 2.2. Photobioreactor systems used for microalgal biomass production: a) a stirred tank photobioreactor (en.wikipedia.org/wiki/photobioreactor); b) tubular photobioreactors with tubes made of glass or plastic (en.wikipedia.org/wiki/photobioreactor); c) flat-plate photobioreactors (en.wikipedia.or/photobioreactor); d) bubble-column photobioreactors at the University of Cape Town, South Africa (http://www.uct.ac.za).

control of the environmental factors (Huang et al., 2010). In addition, heterotrophic cultures inevitably require rigorous aseptic operation to prevent growth of the other unwanted microorganisms.

2.3 Microalgal growth

In a photoautotrophic batch cultivation system, algal growth post inoculation follows a typical pattern of a lag phase follow by an exponential growth phase, an stationary phase and finally a death phase (Becker, 1995). During cultivation, algae grow by increasing cell size through biosynthesis and increase the cell number by cell division (Becker, 1995; Lee & Shen, 2007). The cell size of algae in the stationary phase is often larger than the cell size in the exponential phase (Danquah et al., 2009b). For example, the average cell size of *Chlorella zofingiensis* grown in modified BG-11 medium was 3.0 μm in the exponential phase (Zhang et al., 2012), but increased to 3.2 μm in the stationary phase (Zhang et al., 2012). The microalgal growth rate and the cell composition depend on the compositions of growth medium and the cultivation conditions (Chen et al., 2010; Yeh & Chang, 2012). Growth may be measured in terms of biomass concentration (g L^{-1}), cell number (cells mL^{-1}), and optical density, for example (Becker, 1995). Algal growth is often expressed as biomass productivity, that is the biomass produced per unit culture volume per unit time (g L^{-1} d^{-1}) (Griffiths & Harrison, 2009).

2.4 Microalgal lipids production

Microalgae grown photosynthetically use solar energy to convert carbon dioxide and water to oxygen and carbohydrates (Becker, 1995; Grobbelaar, 2007; Brennan & Owende, 2010). The latter then provide the energy and carbon for the synthesis of the other biochemicals such as

lipids and proteins (Spolaore et al., 2006; Chisti, 2010; Stephens et al., 2010). Lipids are the essential structural components of a microalgal cell and are used to build the cell membrane, membrane of the chloroplast, mitochondria and the other structures (Thompson Jr, 1996; Guschina & Harwood, 2013). Chlorophylls and some of other light harvesting accessory pigments are also lipids (Masojídek et al., 2013). Many microalgae accumulate lipids to levels above those necessary for normal growth (Chisti, 2007; Mata et al., 2010). Certain lipids are accumulated to store energy. Others are made to enhance the cell's ability to capture light. Yet other lipids protect the cell against oxidative damage and photooxidation. Some lipids may serve as flotation aid (Thompson Jr, 1996; Hu, 2007).

Lipids in microalgae cells occur in various forms. The cell contains nonpolar lipids such as acylglycerols, sterols, free (nonesterified) fatty acids, waxes, steryl esters, and hydrocarbons (Sharma et al., 2012). Also present are the polar lipids such as phosphoglycerides (phospholipids) and glycosylglycerides (glycolipids) (Becker, 1995). Triacylglycerols (TAG or triglycerides) are accumulated as storage products and these are the main type of lipid used in making biodiesel (Huang et al., 2010). The accumulation of lipids is influenced by a number of chemical and physical factors which can act independently or in combination (Hu, 2007; Hu et al., 2008). The chemical factors include nutrient starvation, salinity and pH (Guschina & Harwood, 2013). The physical factors are temperature and light intensity (Hu et al., 2008). Light intensity affects lipid metabolism and lipid composition (Guschina & Harwood, 2009). Extended exposure to low light decreases the level of the total phospholipids, whereas a high light intensity tends to increase the level of TAGs (Hu et al., 2008; Guschina & Harwood, 2009). In addition, the phase of growth also affects lipid accumulation (Hu et al., 2008). In many algae, the TAG level in a biomass is higher in the stationary phase (Huerlimann et al., 2010).

Cultivation temperature affects the biochemical composition of algae. In general, a decrease in growth temperature to below the normal level increases the content of unsaturated lipid in the membrane systems. This helps in retaining the membrane fluidity as unsaturated lipids remain in a liquid state at relatively low temperatures. Microalgae from cold water habitat tend to be especially rich in lipids containing polyunsaturated fatty acids. Moreover, in many algae, as temperature increases, the saturated fatty acid content also increases (Hu, 2007; Hu et al., 2008).

The limitation of nutrients such as nitrogen, phosphorus and iron significantly affects lipid accumulation in microalgae (Grobbelaar, 2007; Hu, 2007; Hu et al., 2008). Nutrient limitation reduces growth rate and the need to make new membrane structures. The lipids that would otherwise go into making the membrane structures are then accumulated (Hu et al., 2008; Hu, 2013). Nitrogen limitation is known to increase the lipid content of biomass of many algae by several fold compared to the biomass grown in non-limited conditions (Becker, 1995). Deprivation of phosphate and sulfate can also enhance lipid accumulation (Hu et al., 2008; Rodolfi et al., 2009).

Microalgae triglycerides are potentially attractive for biodiesel production (Chisti, 2007). An alga of choice for lipids production must have a high lipid productivity (Griffiths & Harrison, 2009). The lipid productivity is a measure that combines both the biomass productivity and the lipid content of the biomass (Rodolfi et al., 2009). Generally microalgae rich in lipids, have a low biomass productivity (Griffiths & Harrison, 2009; Rodolfi et al., 2009; Huerlimann et al., 2010; Doan et al., 2011) because synthesis of lipids diverts energy from biomass growth. Based on lipid productivity, *Chlorella vulgaris* and *Nannochloropsis salina* are known to be some of the most attractive algae so far studied (Rodolfi et al., 2009; Doan et al., 2011; Yeh & Chang, 2012). Lastly, any alga suitable for a large-scale production process, must have a high productivity of the target product under the available

environmental conditions. Ease of recovery of the target product is also an important criterion for selecting an alga for large scale operations (Borowitzka, 2013b).

2.5 Harvesting of microalgal biomass from culture broth

Large scale harvesting of microalgal biomass can be expensive because the concentration of the biomass in photoautotrophic culture tends to be low (0.5 g L^{-1} typically in an outdoor pond). Also the microalgal cells are small and their density is similar to water (Shelef et al., 1984; Gudin & Therpenier, 1986; Grima et al., 2003b; Granados et al., 2012), therefore they do not settle easily under gravity. The recovery of biomass from the broth contributes about 20-30% to the total cost of biomass production (Grima et al., 2003b; Mata et al., 2010). As the broth is dilute, a large volume of broth must be processed to recover a small quantity of biomass. Harvesting of the biomass for a low value product such as fuel oil must be done especially cheaply and use a process that is capable of handling large volumes of broth (Pienkos & Darzins, 2009). Harvesting methods for recovering microalgal biomass include flocculation, gravity sedimentation, flotation, centrifugation and filtration (Grima et al., 2003b).

Figure 2.3 shows the possible stages in harvesting of the biomass from a dilute broth with approximately 0.02-0.06% (w/v, g/100 mL) total suspended solid (TSS) to the wet paste stage with a biomass concentration of 15-25% TSS, or more. The desired biomass concentration can be achieved in a single step involving centrifugation or filtration, or in a multiple step harvesting process (stage 1, 2 and 3) (Shelef et al., 1984). Combinations of these methods can greatly enhance harvesting efficiency. For example a flocculation step followed by sedimentation, or flotation, or centrifugation steps (Grima et al., 2003b; Mata et al., 2010).

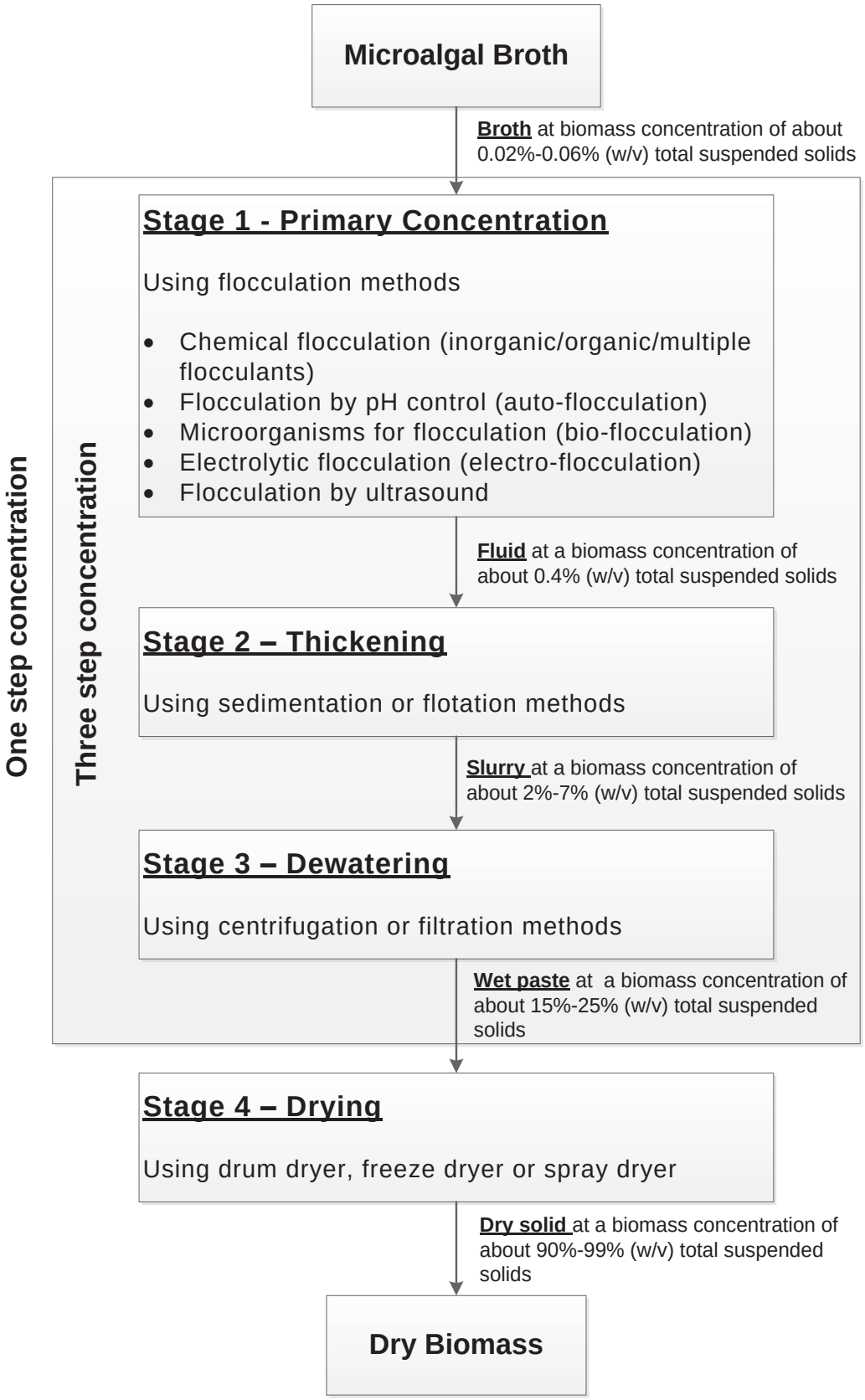


Figure 2.3. An overview of microalgal biomass recovery technologies. Source: adapted from Shelef et al. (1984), Uduman et al. (2010a), and Pahl et al. (2013a).

There is no single method, or combination of methods that is suited to all microalgae (Brennan & Owende, 2010; Milledge & Heaven, 2013). The concentration of the biomass at the end of the harvesting process is important as it influences the subsequent operations such as drying and extraction (Uduman et al., 2010a). The choice of a suitable harvesting method also depends on the microalgal species (i.e. the cell size, density and morphology) (Becker, 1995), the acceptable moisture content in the final product (Lee & Shen, 2004; Mata et al., 2010) and the production system being used (Shelef et al., 1984). Notwithstanding this, based on efficiency and cost, flocculation followed by sedimentation is the most suitable primary concentration method for recovering microalgal biomass from the culture media (Grima et al., 2003a; Grima et al., 2003b; Uduman et al., 2010a; Chen et al., 2011; Milledge & Heaven, 2013; Pahl et al., 2013a; Vandamme et al., 2013; Barros et al., 2015).

Harvesting of the microalgal biomass by flocculation followed by gravity sedimentation is the focus of this work. Flocculation must overcome the stability of a microalga suspension so that sedimentation by gravity becomes feasible. Stability of algal suspension is discussed in the next section.

2.5.1 The stability of microalgal suspension

Microalgal suspensions may be viewed as hydrophilic bio-colloids. The dispersed particles (i.e. the discontinuous phase, or dispersed phase) are distributed uniformly through an aqueous medium (i.e. the continuous phase) (Tenney et al., 1969; Pahl et al., 2013a). These suspensions are generally quite stable. That is, the cells typically do not settle out, or coagulate, if the suspension is left standing. Two factors affect the stability of the suspension. The first is the electric charge on the surface of the cells. Usually, the cells carry a net negative electric charge on the surface due to ionization or dissociation of the surface

functional groups such as –COOH and $–NH_2$. The second factor responsible for stabilizing the suspension is the small size of the cells and a cell density that is close to the density of the suspending medium (Shelef et al., 1984; Henderson et al., 2008c). The negatively charged cells mutually repel due electrostatic repulsion (Figure 2.4). This keeps the cells apart and prevents them from coalescing into larger aggregates. The combination of the dispersed small cells and a small density difference between them and the surrounding fluid prevents settling under the normal gravitational field. There is also a mutual attraction between particles arising from the van der Waals forces (Tenney et al., 1969), but this is insufficient to overcome electrostatic repulsion.

In summary, microalgal cells are typically surrounded by an electrical double layer (EDL) (Figure 2.5) (Bratby, 2006; Gregory, 2006; Henderson et al., 2008c, 2008b). The negative charge on the surface of the cells pulls positive ions (cations) from the surrounding medium and repels the negative ions (anions) from the vicinity. This results in the formation of a tight fitting layer of cations around each cell. This is the rigid Stern layer (static layer) that is attached to the cell (Tenney et al., 1969; Shelef et al., 1984; Kaisha, 1999). The Stern layer is surrounded by a "diffusion" layer, that is relatively rich in the positive ions in the immediate vicinity of the Stern layer, but has an almost zero charge at the interface between the diffusion layer and the medium (Pahl et al., 2013a). Within the diffusion layer the concentration of the cations declines from the interface with the Stern layer outwards and the concentration of anions increase until at the outer surface the concentration of the charges are nearly equal (Kaisha, 1999). When a microalgal cell moves in the culture medium, a part of culture medium moves with the cell. The outer surface of this medium moving with the algal cell is known as the electro-kinetic face or the plane of shear (the interface between the Stern layer and the diffusion layer). The electrical potential at the plane of shear is known as the zeta potential (Speers et al., 1992; Kaisha, 1999).

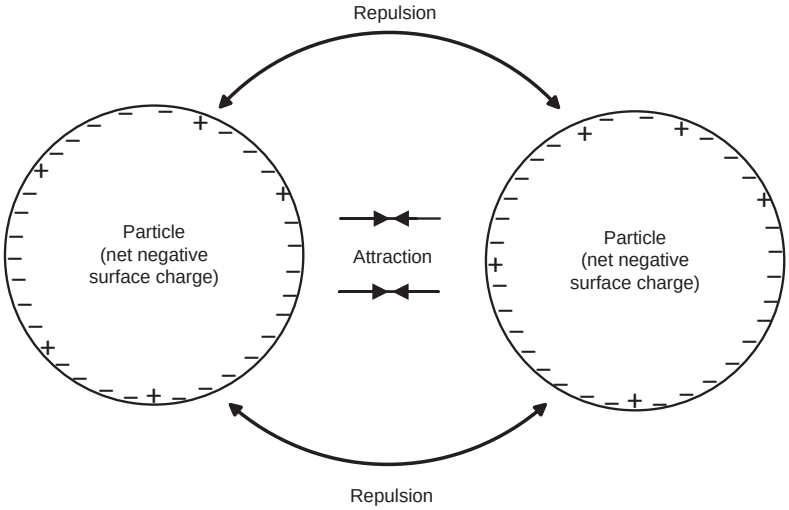


Figure 2.4. Forces between two adjacent like-charged spherical particles. Source: Adapted from Hughes (2001).

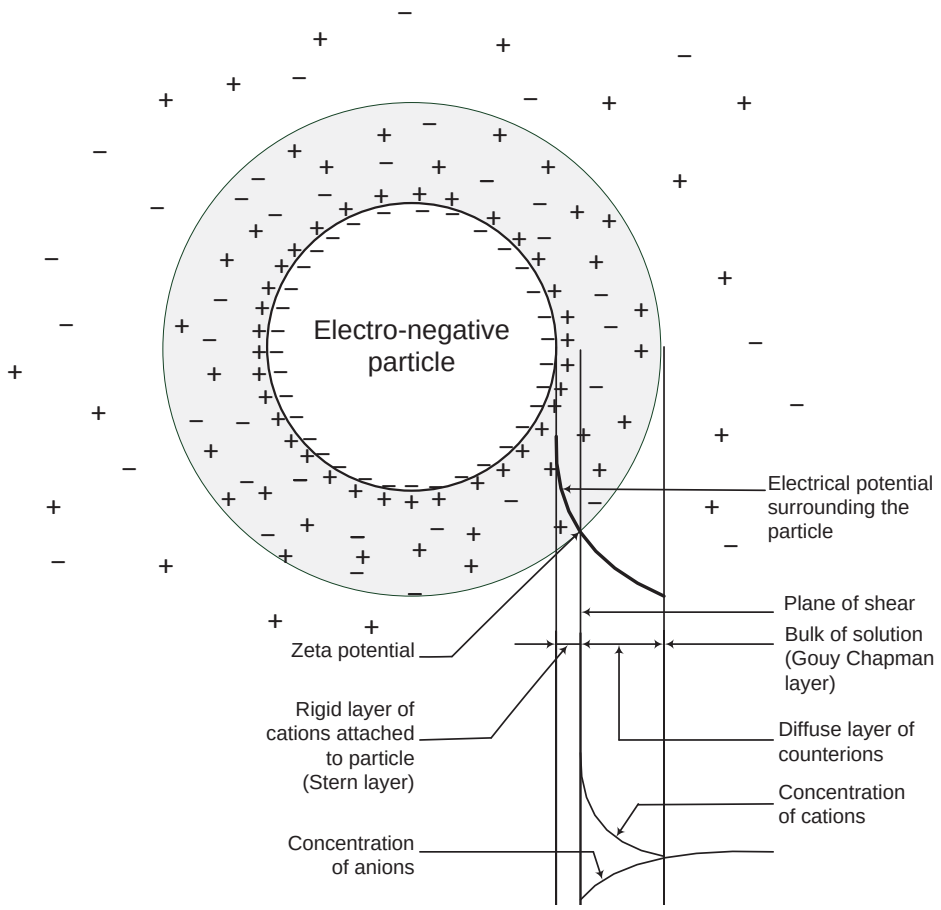


Figure 2.5. A conceptual representation of the electrical double layer. Source: Adapted from Bratby (2006).

The zeta potential is an index for characterising the electrical charge of the particle surrounded by its double layer. A reduction in the magnitude of the negative zeta potential signifies a reduction in the repulsive electrostatic forces between particles. A critical zeta potential can be reached where the attractive van der Waals forces overcome the electrostatic repulsive forces and therefore the particles agglomerate (Henderson et al., 2008c). The zeta potential of microalgae is usually in the range of −10 to −35 mV (Henderson et al., 2008b). The intensity of the surface electric charge, or the areal density of the charge, is a function of the microalgal species, the phase of growth, the ionic strength of the medium, the pH, and other environmental conditions (Shelef et al., 1984; Danquah et al., 2009b; Zhang et al., 2012). For example, Danquah et al. (2009b) reported that the zeta potential value of *Tetraselmis suecica*/*Chlorococum* sp. decreased from −43.2 mV in the exponential phase to −34.5 mV in the stationary phase. Similarly results of Zhang et al. (2012) showed a reduction in the value of the zeta potential of *Chlorella zofinginesis* in the stationary phase. This was associated with changes in the areal density of the surface functional groups in differnce phases of growth.

Changes in zeta potential during growth are at least partly linked to changes in the surface functional groups and the cell size during growth. The functional groups known to occur on cell walls of microalgae include the carboxyl (–COOH), phosphate ($–PO_4$), amine ($–NH_3$) and hydroxyl groups (–OH) (Hadjoudja et al., 2010). In one study, the areal concentration of these groups was found to decrease slightly in going from the exponential phase to the declining phase (Zhang et al., 2012). The microalgal average cell size may increase with time during batch cultivation (Danquah et al., 2009b; Zhang et al., 2012) through accumulation of lipids and carbohydrates for example.

The ionic strength of the culture medium also affects the stability of the microalgal cell suspension (Figure 2.6). In a low ionic strength medium (i.e. low salt concentration), the diffusion layer around the particle is thicker and prevents the cells from coming into contact. In a high salt concentration medium, the diffusion layer thins and the particles can come closer (Gregory, 1993, 2006). This may allow the van der Waals attraction force to overcome the double layer repulsion. In a medium of high ionic strength, the zeta potential of a cell is typically lower than the zeta potential of the same cell in a low ionic strength medium (Figure 2.7).

2.5.2 Microalgal flocculation

Flocculation enhances the average size of the suspended particles by agglomerating them to improve the speed of settling (Bratby, 2006; Chen et al., 2011). Flocculation is achieved by adding chemicals, or flocculants, to a microalgal suspension. Flocculants reduce or neutralize the negative surface charge that prevents the cells from coming together (Becker, 1995; Grima et al., 2003a). Flocculation is essentially a pretreatment step to improve cell harvesting by methods such as gravity sedimentation, filtration, centrifugation, and flotation (Grima et al., 2003b; Brennan & Owende, 2010). Flocculation is applicable to a wide range of microalgal species and is readily scalable (Uduman et al., 2011). The flocculation methods that have been used for microalgae include chemical flocculation (Sukenik et al., 1988; Gao et al., 2010; Şirin et al., 2012; Zheng et al., 2012), microbial flocculation (bio-flocculation) (Lee et al., 2009a; Salim et al., 2011), electrolytic flocculation (electro-coagulation-flocculation) (Gao et al., 2010; Uduman et al., 2011; Vandamme et al., 2011), ultrasonic flocculation (Bosma et al., 2003), and autoflocculation (Uduman et al., 2010a).

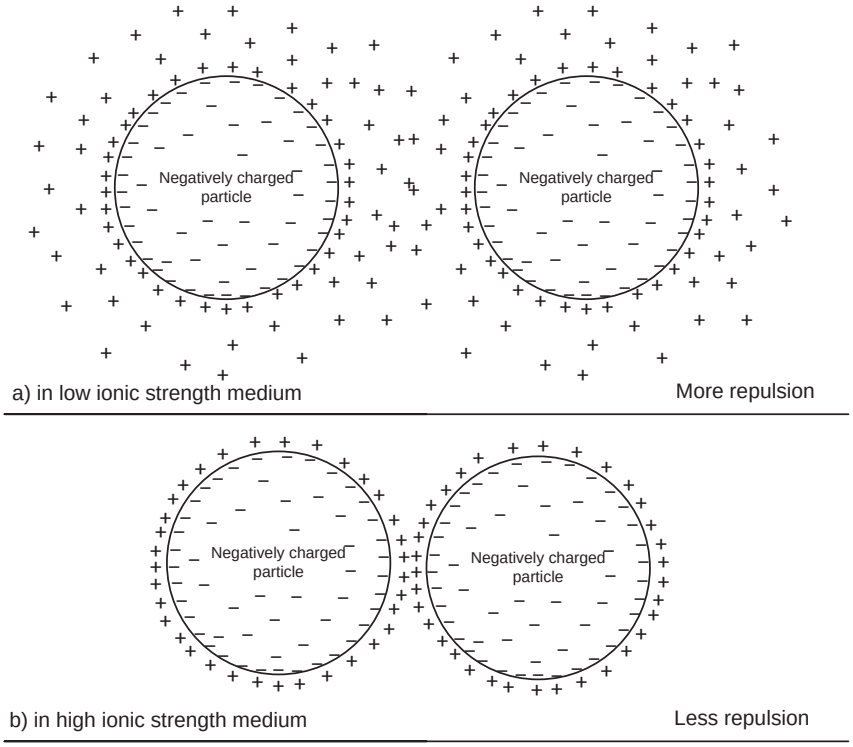


Figure 2.6. The effect of the ionic strength on the magnitude of the double layer repulsion: (a) in low ionic stength; and (b) in high ionic strength. Source: Adapted from Gregory (2006).

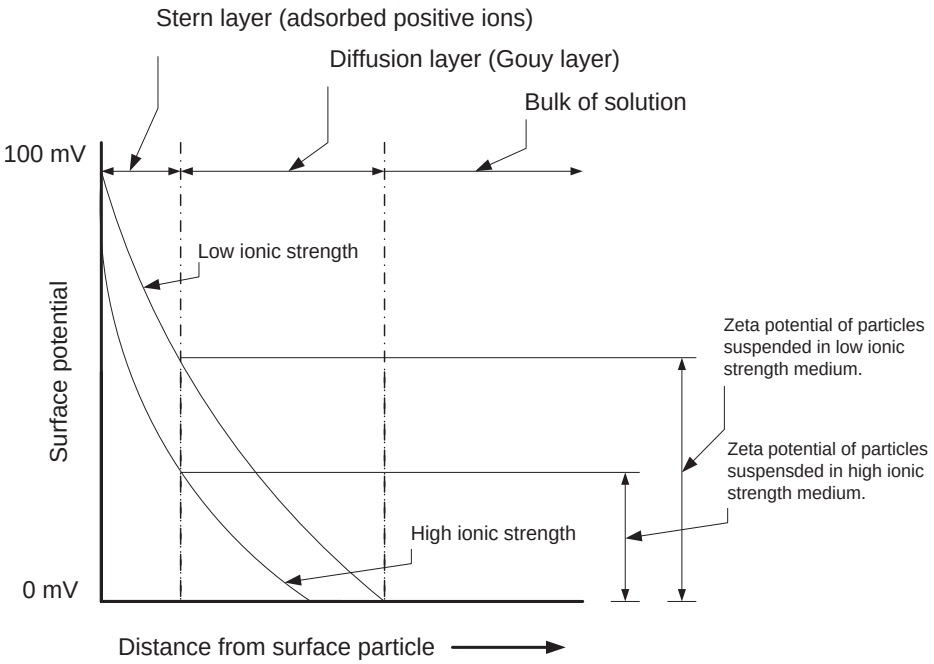


Figure 2.7. Effect of salt concentration or ionic strength on the value of the zeta potential. Source: Adapted from Pahl et al. (2013a).

2.5.2.1 Microbial flocculation (bio-flocculation)

Microbial flocculation or bio-flocculation uses extracellular polymeric substances (EPS) secreted by various microorganisms as flocculation agents for harvesting algal biomass from a culture medium (Lee et al., 2013b; Pahl et al., 2013a; Vandamme et al., 2013; Shen et al., 2015). Some microalgae, bacteria and fungi can be induced to produce ESP which are normally acidic polysaccharides (i.e. polymers of uronic acid or pyruvic acid) (Pahl et al., 2013a). The flocculating microorganisms are grown separately and then add to the target microalgal culture as a co-cultures (Salim et al., 2011). For example, Lee et al. (2009) showed that *Pleurochrysis carterae* biomass could be harvested successfully with over 90% recovery using the flocculating microbes. Flocculating microbes typically require organic carbon for growth during the co-culture with algae (Lee et al., 2009). Under stress conditions relating to nutrients, temperature, or pH, the flocculating microbes secrete EPS and this induces microalgal flocculation (Lee et al., 2009; Pahl et al., 2013a). Salim et al. (2011) reported a high recovery efficiency using the flocculating microalgae *Scenedesmus obliquus*, *Tetraselmis suecica*, and *Ankistrodesmus falcatus* to flocculate the non-flocculating lipid producing microalgae *Chlorella vulgaris* and *Neochloris oleoabundans*. This method does not involve separate addition of chemical flocculants, but remains to be proven commercially.

2.5.2.2 Electrolytic flocculation (electro-coagulation-flocculation)

Electro-coagulation-flocculation is a highly efficient method for harvesting microalgal biomass from the culture media (Gao et al., 2010; Xu et al., 2010; Uduman et al., 2011; Vandamme et al., 2011). In this method, the microalgal negative surface charge is neutralized by aluminum or iron ions which are generated from sacrificial aluminum or iron anodes by applying an electric current. Once microalgal cells lose their surface charge by complexing with the metal cations, they agglomerate to form flocs (Poelman et al., 1997; Vandamme et

al., 2011). This technique consumes relatively low energy of 0.3 kWh kg^{-1} (Poelman et al., 1997) compared to centrifugation which requires almost 2 kWh kg^{-1} (Milledge & Heaven, 2013) of the biomass recovered. Many microalgae have been successfully harvested from the culture broth using electrolytic flocculation. These include *Chlorella vulgaris*, *Phaeodactylum tricornutum* (Vandamme et al., 2011) and *Botryococcus braunii* (Xu et al., 2010). Electro-coagulation-flocculation has been extensively reviewed in the literature (Souza et al., 2014; Barros et al., 2015; Misra et al., 2015). It has been used in pilot scale operations, but not in large scale commercial operations (Milledge & Heaven, 2013). This is because the power requirements increase in direct proportion to scale (Vandamme et al., 2011).

2.5.2.3 Ultrasonic flocculation

This technique uses ultrasound or acoustic waves to force the microalgal cells in the resonator chamber to flocculate (Bosma et al., 2003; Pahl et al., 2013a; Vandamme et al., 2013). Bosma et al. (2003) demonstrated a continuous ultrasonic separation process to harvest *Monodus subterraneus* with more than 90% of biomass recovery from the culture broth. This method has shown a high efficiency of harvesting at the laboratory scale without requiring any chemicals, but it is difficult to scale up to a commercial level (Vandamme et al., 2013). A resonator chamber of 1,000 L day^{-1} capacity, for example, would have a substantial energy consumption (Pahl et al., 2013a).

2.5.2.4 Autoflocculation

In algal cultures that are not continuously fed with carbon dioxide, the pH naturally increases as the dissolved carbon dioxide is consumed. This can lead to autoflocculation or

spontaneous floc formation and settling (Uduman et al., 2010a; Vandamme et al., 2013). Flocculation due to increased pH, i.e. an increased concentration of OH^-, is due to the formation of insoluble metal hydroxides that tend to entrap the cells. Many metal salts are present in the culture media and at high pH, they form gel-like hydroxides. Horiuchi et al. (2003) investigated the harvesting of a halotolerant microalga by increasing the pH of the culture broth using NaOH solution. Suspended *Dunaliella tertiolecta* cells were coagulated and settled within a few minutes of this treatment. The pH values for successful coagulation were between 8.6 and 10.5 and more than 90% of cells could be recovered from the culture broth. In addition to sodium hydroxide, calcium phosphate, magnesium hydroxide and calcium carbonate have been mentioned in the literature for their potential to increase pH for harvesting microalgal biomass (Vandamme et al., 2012; Wu et al., 2012; Pahl et al., 2013a; Vandamme et al., 2013; Barros et al., 2015).

2.5.2.5 Chemical flocculation

Flocculation of suspended particles with chemical flocculants can involves at least two distinct mechanisms that occur concurrently. First, the particles in suspension are destabilized by a change of their surface properties using chemical flocculants. As a result the mutual repulsion is reduced and particles come together to form flocs. Second, the flocs increase their size by attaching more particles and settle from the suspension by gravity (Tambo & Watanabe, 1979). Chemical flocculants are highly effective and widely used in diverse applications. Either inorganic flocculants or polymeric organic flocculants (Shelef et al., 1984; Grima et al., 2003b; Pahl et al., 2013a; Vandamme et al., 2013) may be used. For use with large-scale operations anticipated for production of microalgae for biofuels, a flocculant must meet certain essential requirements: it must be effective, cheap, environmentally benign

and readily available in quantity. Only certain inorganic flocculants (e.g. aluminum sulfate, or alum, and ferric chloride) meet all these criteria. Many other effective organic flocculants have been developed (Uduman et al., 2010b; Vandamme et al., 2010; Granados et al., 2012), but none is as cheap as the commonly used inorganic salts (Vandamme et al., 2010; Schlesinger et al., 2012; Milledge & Heaven, 2013).

2.5.2.5.1 Organic flocculants

Polymeric flocculants or polyelectrolytes may be anionic, cationic or non-ionic polymers. They may be synthetic or natural (Shelef et al., 1984). A combination of cell surface charge neutralization and particle bridging is the mechanism of microalgal flocculation by polymeric flocculants (Tenney et al., 1969). The effectiveness of polyelectrolyte flocculants is affected by the following factors (Chisti, 1999): the molecular mass or chain length of the polymer, the charge density on the molecule, the dose used, the biomass concentration, the ionic strength or pH of the broth, and the extent of mixing in the fluid. High molecular weight polyelectrolytes (i.e. longer chain polymers) are better bridging agents. A high charge density tends to unfold the polymer molecule which improves its ability to neutralize the surface charge on cells and its bridging performance. A high cell concentration in the broth helps flocculation by increasing the frequency of the cell–cell encounters. A certain low level of mixing is useful because it brings the cells together. However, the disruption of flocs occurs if the shear forces are excessive. Cationic polyelectrolytes can induce efficient flocculation of freshwater microalgae at low dosages (Bilanovic et al., 1988). Flocculation efficiency declines in higher salinity media (Sukenik et al., 1988; Knuckey et al., 2006; Uduman et al., 2010b). However, a 100% flocculation efficiency of marine microalgae has been reported at

fairly high concentration (above 40 mg L^{-1}) of a chitosan flocculant without the need for pH control (Morales et al., 1985).

2.5.2.5.2 Inorganic flocculants (multivalent metal salts)

The commercially used inorganic flocculants can be divided into two groups: aluminum based and iron based (Bratby, 2006). The aluminum based group includes aluminum sulfate, aluminum chloride, sodium aluminate, aluminum chlorohydrate, and polyaluminum chloride. The iron based group includes ferric chloride, ferric sulfate, ferrous sulfate, and ferric chloride sulfate (Bratby, 2006; Hendricks, 2006). These flocculants are commonly used for removing colloidal particles in water and wastewater treatment processes due to their efficacy, availability and relatively low cost (Bratby, 2006; Pahl et al., 2013a). Aluminum sulfate or alum ($Al_2(SO_4)_3$), ferric chloride ($FeCl_3$) and ferric sulfate ($Fe(SO_4)_3$) are the most widely used chemical flocculants (Duan & Gregory, 2003; Grima et al., 2003b).

Table 2.3 shows the high flocculation efficiencies of several metal salts used to harvest microalgal biomass from the culture broth. The multivalent metal ions in these salts neutralize the cell surface charge and bridge cells together to facilitate flocculation (Bratby, 2006; Knuckey et al., 2006). Papazi et al. (2010) reported that aluminum salts have been found to be more effective flocculants than ferric and zinc salts because of a high charge (+3) and a lower ionic radius of aluminum. Shelef et al. (1984) also observed that aluminum sulfate had a better flocculating ability than ferric sulfate. Salt dosage for flocculation of marine microalgal cells suspended in a high ionic strength medium (i.e. seawater) is greater than the dose needed for flocculating the cells suspended in a freshwater medium (Sukenik et al., 1988).

Table 2.3 Some inorganic flocculants used for harvesting microalgal biomass from the culture broth

Flocculant	Optimal concentration (mg L^{-1})	Microalgal species	Biomass concentration	Flocculation efficiency (%)	Reference
Aluminum chloride	48	*Nannochloris oculata*	3.0×10^7 cells mL^{-1}	97	Garzon-Sanabria et al. (2012)
Polyaluminum chloride Aluminum sulfate	30	*Phaeodactylum tricornutum*	3.11×10^6 cells mL^{-1}	90	Şirin et al. (2012)
Aluminum nitrate Ferric sulfate	5.4–108	*Nannochloropsis salina*	0.3–3.0 g L^{-1}	84–99	Rwehumbiza et al. (2012a)
Aluminum chloride Ferric sulfate Zinc chloride	750 500 500	*Chlorella minntissima*	2.20×10^8 cells mL^{-1}	>90	Papazi et al. (2010)
Aluminum sulfate	200	*Botryococcus branuii*	-	90	Lee et al. (1998b)
Aluminum sulfate	225±21 140±15 25±4	*Isochrysis galbana* *Chlorella stigmatophora* *Chlorella vulgaris*	1.0×10^6 cells mL^{-1} 1.0×10^6 cells mL^{-1} 1.0×10^6 cells mL^{-1}	>97 >97 >97	Sukenik et al. (1988)
Ferric chloride	120±15 55±9 11±4	*Isochrysis galbana* *Chlorella stigmatophora* *Chlorella vulgaris*	1.0×10^6 cells mL^{-1} 1.0×10^6 cells mL^{-1} 1.0×10^6 cells mL^{-1}	>97 >97 >97	

Sometimes more than one type of flocculant is used. Sukenik et al. (1988) used an organic polyelectrolyte (chitosan) and an inorganic flocculant (ferric chloride) for flocculation of *Isochrysis galbana*. The overall degree of flocculation was improved and the dosage of the inorganic flocculant required was reduced by using the two flocculants in combination. Similarly, Danquah et al. (2009a) used a combination of both a high molecular weight synthetic cationic polyelectrolytic polymer (Zetag 7650) and an inorganic cationic flocculant (aluminum sulfate).

2.5.2.5.3 Mechanisms of action of the inorganic flocculants

The inorganic flocculants undergo two reactions when placed in an aqueous colloidal suspension. First, when metal salts are introduced in water, the metal ions (Al^{3+}, Fe^{3+}) hydrolyze immediately, forming a series of metal hydrolysis species (or metal hydrolysis products or metallic hydroxide complexes). The hydrolysis reaction decreases the positive charge of the metal ions to non-charged hydroxide products (Gregory, 1993). For example, Al^{3+} reacts with a water molecule to produce H^+ and $Al(OH)^{2+}$. The latter reacts with another water molecule to produce H^+ and $Al(OH)_2^+$. Further reaction with water produces $Al(OH)_3^0$. For convenience, ions are often represented as Al^{3+} and Fe^{3+} (Bratby, 2006) even though Al^{3+} and Fe^{3+} per se are not directly involved in flocculation. Then, in a second reaction, the various metal hydrolysis ions interact with the colloidal particles to destabilize them (Bratby, 2006; Hendricks, 2006).

The mechanism of action of the metal hydrolysis products on the colloids involves charge neutralization (also called adsorption destabilization) and sweep flocculation (Gregory, 2006). The charge neutralization occurs when the metal hydrolysis products (i.e. divalent and trivalent ions) adsorbed to the diffusion layer of negatively charged colloid in

suspension. As a result, the potential of the electrical double layer surrounding the colloid decreases until the van de Waals attractive forces overwhelm repulsion to bring the charge-reduced colloids together to form lager particles (Duan & Gregory, 2003; Hendricks, 2006) that are easier to sediment. The earlier mentioned sweep flocculation occurs due to interaction of the metal hydroxide precipitate and the particles. The gel-like precipitate of metal hydroxide entraps and sweeps down the particles to facilitate sedimentation (Duan & Gregory, 2003). As the flocculant dosage is progressively increased the suspension passes through four stages (Duan & Gregory, 2003; Bratby, 2006; Gregory, 2006). At low flocculant dosage the colloids remain in suspension (stage 1). In stage 2, the flocculant dosage increases to a sufficient level for neutralization of the negative charge on the colloid cell surface. In stage 3, a higher flocculant dose than necessary for charge neutralization, causes charge reversal and destabilization. In the last stage, as more flocculant is added to the suspension, hydroxide precipitation and sweep flocculation occur. (In sweep flocculation, the gel-like metal hydroxides entrap cells to form larger flocs and the cells are swept to the bottom by the sedimenting flocs.) If charge neutralization is the main mechanism of flocculation, there is a linear relationship between the particle concentration and the flocculant dose. For example, at low concentration of the particles in suspension, low flocculant dosages are required (Gregory, 1993; Duan & Gregory, 2003; Gregory, 2006). This tends to be the case in flocculation of an algal suspension with a cell concentration of 0.1–3.0 g L^{-1} as discussed in Chapter 4 (Results and Discussion).

2.5.3 Cost of harvesting of algal biomass

Table 2.4 compares the cost of harvesting of algal biomass by various methods. In view of Table 2.4, the cost of flocculation–sedimentation is low. In 2014, the estimated cost of flocculation–sedimentation for harvesting algae were US $1,890/MT of dried biomass (Table

Table 2.4 Cost comparison for different harvesting methods

Harvesting method	Year	US $ per metric ton of dried microalgal biomass		Items included in the process cost	References
		Original	2014*		
Centrifugation (self-cleaning plate separator)	1995	1,710	2,660	Plant depreciation, maintenance and energy	Becker (1995)
Flocculation with sedimentation	1996	1,250	1,890	Plant depreciation, maintenance, flocculant and energy	Benemann & Oswald (1996)
Flocculation with flotation	1995	1,390	2,160	Plant depreciation, maintenance, flocculant and energy	Becker (1995)

* US inflation was calculated from http://www.usinflationcalculator.com/

Source: Modified from Lee et al. (2013a).

2.4), compared to a cost of US $2,160/MT of dried biomass for harvesting by flocculation–flotation (Table 2.4). The most expensive harvesting method was centrifugation (estimated cost of US $2,660/MT of dried biomass) (Becker, 1995) (Table 2.4). Therefore, flocculation–sedimentation has a high potential for use in harvesting of microalgal biomass on a commercial scale (Uduman et al., 2010a; Chen et al., 2011; Milledge & Heaven, 2013; Pahl et al., 2013a; Vandamme et al., 2013; Barros et al., 2015).

2.6 Continuous flocculation process

Almost all published microalgal flocculation studies have focused on batch flocculation processes with various flocculants and different techniques (Morales et al., 1985; Sukenik et al., 1988; Poelman et al., 1997; Oh et al., 2001; Danquah et al., 2009a; Danquah et al., 2009b; Papazi et al., 2010; Vandamme et al., 2010; Salim et al., 2011; Garzon-Sanabria et al., 2012; Rwehumbiza et al., 2012a; Schlesinger et al., 2012; Şirin et al., 2012; Zheng et al., 2012; Chen et al., 2013b; Farid et al., 2013; Rashid et al., 2013; Vandamme et al., 2013; Prochazkova et al., 2015; Shen et al., 2015). Batch flocculation processes are suitable for the selection of a suitable flocculant and the evaluation of its performance, but for large scale flocculation, continuous processes are preferred (Hogg, 2000; Hogg & Rattanakawin, 2002; Hogg, 2005; Riffat, 2013). Ideally in a continuous system, microalgal broth and flocculant are pumped continuously to a rapid mixing tank in which the suspension starts to destabilize and form small flocs. The floc size increases progressively with time in the subsequent mildly agitated flocculation tank. Eventually, large flocs settle to the bottom of a sedimentation tank (Hogg, 2000; Sastry et al., 2000; Hogg, 2005).

The rapid mixing tank is where the suspended particles are mixed with the flocculant. This is an important stage, as it achieves a uniform distribution of the flocculant (Edzwald, 2011). The basic design of this tank is one of a well-mixed stirred reactor. The tank may be

circular or square in cross-section (Bratby, 2006). The geometry of a circular tank is such that the ratio of the depth of water to the diameter of the tank is 1 (Oldshue, 1983; Paul, 2003; Spellman, 2009). The position of the impeller is 0.5 of the water depth from the bottom. The impeller diameter ranges between 0.5–0.8 of the tank diameter. In addition, the recommended retention time in the mixing tank ranges from 9 s to 2.5 min for aluminum sulfate concentrations ranging from 10 to 100 mg L^{-1} (Bratby, 2006; Hendricks, 2006).

The flocculation stage is focused on growth of the flocs. This stage requires gentle mixing to favor floc–floc interactions and agglomeration but not disruption so that the floc size increases for the subsequent step (e.g. sedimentation) (Bratby, 2006; Hendricks, 2006; Spellman, 2009). The flocculation tank can be rectangular or circular. For circular tanks the ratio of depth of water to the diameter of the tank is 0.9–1.1. The position of the turbine is 0.33–0.5 of the water depth above the bottom. The turbine diameter usually ranges between 0.3–0.6 of the tank diameter, and the recommended flocculation times range between 10 and 30 min (Crittenden et al., 2012a).

The sedimentation or clarification tank is where the flocs settle under gravity. This tank can be designed as rectangular, square or circular (Crittenden et al., 2012b). However, long rectangular tanks are more effective compared to the other configurations (Camp, 1946). The rectangular continuous horizontal flow tank consists of four zones according to function (Camp & Estrada, 1953): the inlet zone, the settling zone, the outlet zone and the sludge zone as shown in Figure 2.8. The inlet zone is designed to disperse the suspension over the cross section of the tank. The setting zone is where all settling occurs. The outlet zone in where clarified water is collected over the cross section of the tank. The sludge zone is the zone at the bottom of the tank where the solids accumulate (Camp, 1946; Camp & Estrada, 1953; Hendricks, 2006; Riffat, 2013). Generally, the rectangular sedimentation tank is designed to

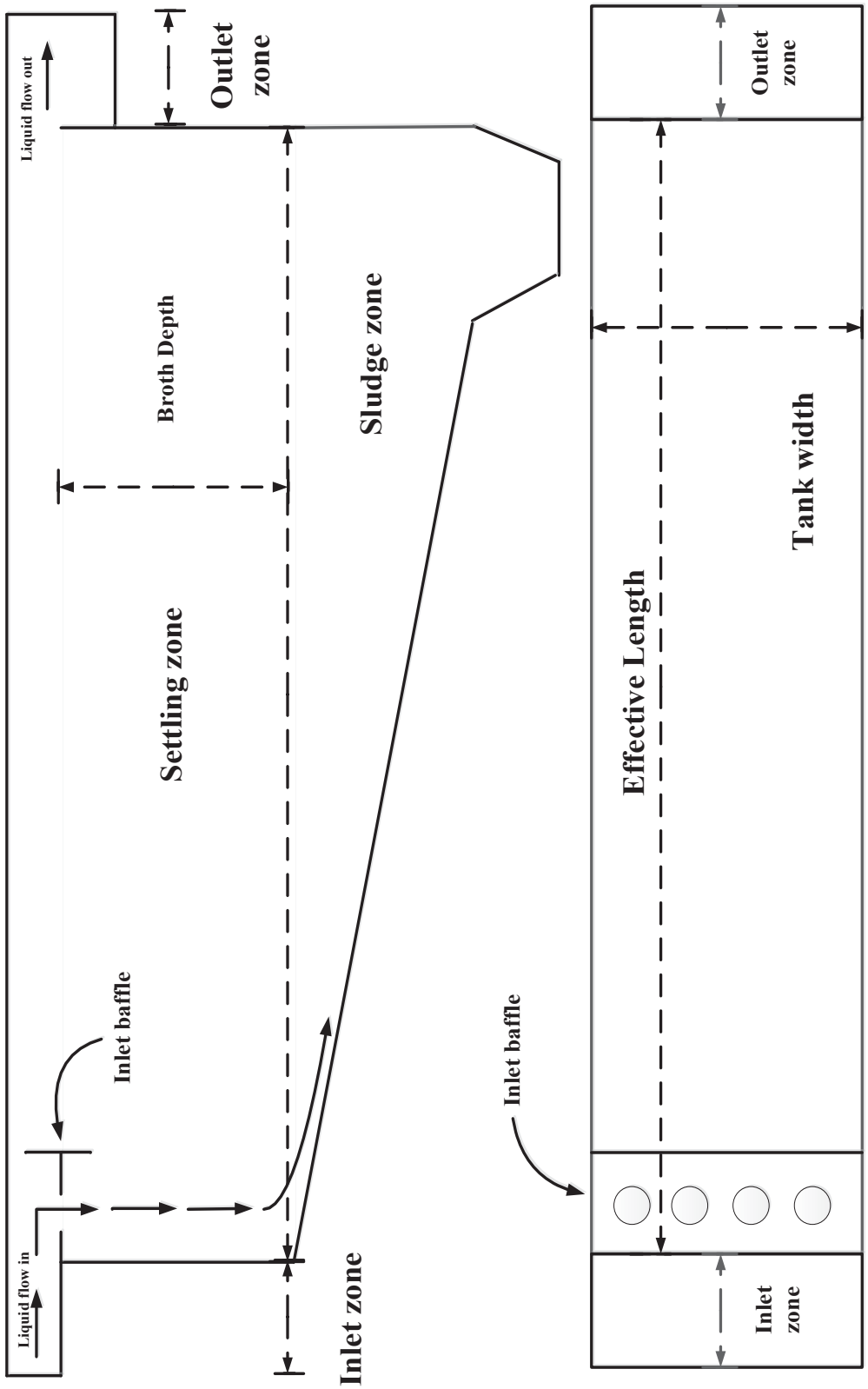


Figure 2.8. Concept of a continuous horizontal flow rectangular settling tank. Source: Adapted from Camp (1946), Hendricks (2006)

be narrow and long with the length-to-width ratio of between 4:1 and 6:1 (Hendricks, 2006; Edzwald, 2011; Riffat, 2013).

2.7 Extraction of oil from microalgal cells

For producing liquid biofuels, the oil must be somehow extracted from the biomass. The main extraction methods used currently are mostly suited to laboratory-scale. These include the accelerated solvent extraction (ASE) (Richter et al., 1996; Halim et al., 2011; Grima et al., 2013a; Li et al., 2014), supercritical fluid extraction (Cheung, 1999; Dufreche et al., 2007; Levine et al., 2010; Halim et al., 2011; Grima et al., 2013a; Taher et al., 2014b), and organic solvent extraction (Folch et al., 1957; Bligh & Dyer, 1959; Axelsson & Gentili, 2014). In addition, there are some assisted techniques to enhance the lipid extractability from microalgal biomass. For example, the microwave-assisted solvent extraction (Lee et al., 2010; Iqbal & Theegala, 2013; Bermúdez Menéndez et al., 2014) and ultrasound-assisted solvent extraction (Ranjan et al., 2010; Araujo et al., 2013; Bermúdez Menéndez et al., 2014; Fast & Gude, 2014). Overall, simple solvent extraction is considered suitable and inexpensive for oil recovery from microalgal biomass (Grima et al., 2013b). This is because bioprocess unit operations for large-scale extraction exist in other industrial sectors (Grima et al., 2013b).

Ideally, an oil extraction process should be useable with the wet biomass paste directly (Chisti, 2008; Lardon et al., 2009; Halim et al., 2011) to avoid an expensive prior drying step. Almost all previously published work has focused on lipid extraction from dry biomass (Fajardo et al., 2007; Mulbry et al., 2009; Lee et al., 2010; Sheng et al., 2011; Soh & Zimmerman, 2011; Horst et al., 2012; Ryckebosch et al., 2012; Iqbal & Theegala, 2013; Jeon et al., 2013; Bermúdez Menéndez et al., 2014; Li et al., 2014; Ryckebosch et al., 2014; Taher

et al., 2014b), and extraction from wet biomass paste has been barely studied (Halim et al., 2011; Dejoye Tanzi et al., 2013; Liu et al., 2013; Yang et al., 2014).

2.7.1 Accelerated solvent extraction (ASE)

Accelerated solvent extraction (ASE) uses organic solvents at a high pressure and a temperature that is above the normal boiling point of the solvent (Richter et al., 1996; Grima et al., 2013a). This of course adds to the cost. In ASE the biomass and the extraction solvent are held in a pressure vessel (500–3000 psi) at an elevated temperature (50–200 °C) for a short period (5–10 min) without mixing. Afterwards, an inert compressed gas is used to purge the extract from the extraction vessel into a collection vessel (Cooney et al., 2009). ASE is efficient if the extracting solvent, the sample–solvent ratio, the extraction temperature, and time have been optimized (Denery et al., 2004). Comparing the performance of ASE extraction to that of the traditional Folch method (Folch et al., 1957) for microalgae grown on dairy manure effluent, the ASE, depending on the solvent, could extract more fatty acids in the first solvent extraction cycle than the Floch treatment (Mulbry et al., 2009). However ASE requires a high input of energy, a substantial capital expense and it is generally applied only to dried biomass (Cooney et al., 2009). ASE is unlikely to be of use in any commercial operation for producing fuel oil from algal biomass.

2.7.2 Supercritical fluid extraction

Supercritical fluid extraction involves the use of an extraction fluid above its critical point (Halim et al., 2011; Taher et al., 2014b). A fluid above its critical temperature and pressure is known as a supercritical fluid and has a greatly enhanced solvating power relative to the

subcritical conditions. A supercritical fluid has gas-like mass transfer properties (i.e. high diffusion coefficient relative to subcritical condition) and liquid-like solvating power (Cooney et al., 2009; Li et al., 2014). A majority of applications have used supercritical carbon dioxide as the extraction solvent because of its low toxicity, chemical inertness and ease of separation from the solute (Herrero et al., 2004; Taher et al., 2014a). Supercritical fluid extraction requires a dry biomass sample that is placed in an extraction cell that is filled with the gas before being pressurized to above its critical point. Lipids have been selectively extracted from microalgae at temperatures of between 40 to 50 °C and pressures of 241 to 379 bar (Cheung, 1999) using supercritical carbon dioxide. Continuous-flow supercritical extraction is possible. Supercritical fluid extraction tends to be quite expensive and also difficult to scale up (Cooney et al., 2009).

2.7.3 Organic solvent extraction

Solvent extraction is recognized as the simplest method for recovery of oil from algal biomass (Lam & Lee, 2012). The basis of solvent extraction is the concept of "like dissolves like" (Bligh & Dyer, 1959). For all lipids recovery from algal biomass, non-polar organic solvents (e.g. hexane, chloroform, ether) mixed with polar organic solvents (e.g. methanol, isopropanol, other alcohols) are used (Halim et al., 2012; Pragya et al., 2013b). A combination of nonpolar and polar solvents facilitates penetration into cell and lipid extraction. After the solvent mixture is added to the algal biomass, it passes through the cell wall and cell membrane into the cytoplasm of the algal cell. The solvents interact with the lipids in the cell. Non-polar organic solvents interact via van der Waals forces with the neutral or non-polar lipids such as triacylglycerol to form a lipid association complex. While polar organic solvents interact via hydrogen bonds, with the polar lipids and lipid-protein

complexes to form other lipid association complexes. These solvent-lipid association complexes diffuse out of the cell into the organic solvent layer (Halim et al., 2012; Grima et al., 2013a; Grima et al., 2013b).

Bligh and Dyer (1959) method is the most widely used method for lipid extraction from algal biomass. It can be applied to a wide range microalgal species and permits essentially complete recovery of lipids compared to the other methods (Lam & Lee, 2012). Bligh and Dyer (1959) method uses a mixture of chloroform and methanol as the extraction solvent. Water is added to facilitate separation of the chloroform from the hydrophillic solvent (Ferrell & Sarisky-Reed, 2010). The lipids are recovered in the chloroform layer. Other suggested combinations of co-solvents for the extraction of lipids are hexane/isopropanal for *Rhizoclonion hiroglyphiom* (Mulbry et al., 2009) and hexane/ methanol/acetone for sewage sludge (Dufreche et al., 2007). The hexane system has been recommended because hexane and alcohol rapidly separate into two separate phases when water is added and this facilitates further oil recovery by evaporation of hexane (Cooney et al., 2009). In addition, there are a number of other effective solvents and solvent combinations for recovering lipids from algal biomass. For example, the hexane (Suganya & Renganathan, 2012), 1,2-dimethoxyethane (Liu et al., 2013) and mixture of methanol/dichloromethane (Jeon et al., 2013).

In addition to the extraction solvent or solvent combination (Liu et al., 2013), other factors influence the lipid recovery (Lam & Lee, 2012). These include the ratio of solvent to biomass (Yang et al., 2014), the sequence of solvents used (Lewis et al., 2000), the moisture content in the biomass (Liu et al., 2013), the extraction temperature, the extraction time, the mixing intensity used in extraction (Suganya & Renganathan, 2012), the extraction pressure

(Halim et al., 2011) and the nature of the biomass. The key solvent extraction factors are discussed in Chapter 4 (Results and Discussion).

2.8 Hypotheses

The hypotheses for this research were the following:

1. Cheap and safe commercial flocculants may be used effectively to flocculate both marine and freshwater microalgae;
2. Flocculant requirements likely depend on algal species, morphology and growth conditions;
3. Superior oil extraction protocols could be found for both dry and wet algal biomass through an optimization approach;
4. Removal of algal biomass is potentially feasible by a continuous flocculation–sedimentation process

2.9 Objectives

This research aimed to establish effective methods of large-scale production of oils from microalgae. Flocculation–sedimentation for harvesting microalgal biomass and solvent-based extraction of oils from the recovered biomass were focused on. The specific objectives of this study were as follows:

1. A preliminary characterization of selected microalgae for the potential to grow and produce lipids in batch photoautotrophic conditions, for subsequent flocculation studies;

2. To determine the optimum batch flocculation dosages of two inexpensive flocculants (i.e. aluminum sulfate, ferric chloride) for recovering the microalgal biomass from the culture broth;

3. To characterize the impact of cell morphology, ionic strength of the culture medium and the microalgal biomass concentration on the flocculant dose for achieving 95% removal the biomass from the culture medium;

4. To design and characterize a process for continuous flocculation–sedimentation of a selected microalga, based on the data obtained from batch flocculation experiments;

5. To assess the effect of the flocculants used and the moisture content in the algal biomass paste on oil recovery by solvent extraction;

6. To identify a suitable solvent system and the optimal extraction conditions for extracting oils from the biomass paste of a selected alga.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

This study focused on flocculation as a method for harvesting the microalgal biomass from the culture broth and an assessment of the possible impact of flocculation treatments on extractability of algal oils. The materials and methods used in the experimental work are explained in this chapter.

The experimental work consisted of four main parts:

1. A characterization of selected microalgae for potential use in biodiesel production in terms of: the attainable biomass concentration; the lipid content; the biomass productivity; the lipid productivity; the specific growth rate; and the cell morphology, size, surface area, and zeta potential.

2. A characterization of flocculation for harvesting the selected algae in terms of the optimal dose of flocculants for removing the biomass from culture media and its dependence on various factors.

3. Design and characterization of a continuous flocculation and sedimentation process, based on the data obtained in 2.

4. An assessment of the impact of flocculation treatments on extractability of algal lipids from the algal biomass. This included an investigation of the suitable solvent systems and the operational protocols for extracting the oils from wet paste of the microalgal cells.

3.2 Materials and methods

3.2.1 Microalgal strains

Five microalgae were used. These are identified in Table 3.1.

3.2.2 Culture media

The BG11 medium or its modifications were used to maintain and grow the microalgae (Stanier et al., 1971; Andersen et al., 2005). For making the medium, the stock solutions 1-4 were made according to the recipes in Tables 3.2-3.5. The solutions were separately sterilized at 121°C, 15 min, cooled and kept at 4°C until further use. For making the working BG11 medium, the specified amounts of stocks 1-4, sodium nitrate and sodium carbonate were combined in the sequence shown in Table 3.6. For preparing the standard BG11, distilled water was added to the mixed stock solutions (Table 3.6) to make up to 1 L. The silicate stock (see Table 3.7) was used for culturing the diatom only and was added at 1 mL per liter of the BG11 seawater medium.

For preparing the BG11 seawater medium, the mixed stock solutions (Table 3.6) were made up to 1 L by adding artificial seawater. Artificial seawater was prepared by dissolving 40 g of sea salt (natural unrefined Southern Pacific Ocean salt; Pacific Natural Fine Salt; Dominion Salt Ltd, Marlborough, New Zealand) in 1 L of distilled water and filtering with Whatman GF-C (0.45 μm) 90 mm microfiber filters before use in the BG11 medium (Table 3.6). The artificial seawater had a salinity of approximately 38.5 parts per thousand (ppt) (EcoSense® EC300 conductivity/salinity meter; YSI Inc., Yellow Springs, OH, USA). The solid medium for maintaining the microalgae was prepared by using agar (Difco™, Agar

Table 3.1 The microalgal strains used

Alga	Algal group	Source	Habitat
Chlorella vulgaris	Green alga (Chlorophyta, Trebouxiophyceae)	Landcare Research, Lincoln, New Zealand	Freshwater
Choricystis minor	Green alga (Chlorophyta, Trebouxiophyceae)	Landcare Research, Lincoln, New Zealand	Freshwater
Neochloris sp.	Green alga (Chlorophyta, Ulvophyceae)	Landcare Research, Lincoln, New Zealand	Freshwater
Nannochloropsis salina, CCAP849/3	Eustigmatophytes (Heterokontophyta, Eustigmatophyceae)	Culture Collection of Algae and Protozoa (CCAP), Argyll, United Kingdom	Marine
Cylindrotheca fusiformis, CCAP1017/2	Diatom (Heterokontophyta, Bacillariophyceae)	CCAP	Marine

Table 3.2 The components of BG11 stock 1

Component	Amount
Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)	3.6 g
Citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$)	0.6 g
Ferric ammonium citrate ($C_6H_{11}FeNO_7 \cdot H_2O$)	0.6 g
Ethylene diamine tetraacetic acid disodium magnesium salt ($Na_2MgEDTA$) or	0.1 g
Ethylene diamine tetraacetic acid disodium salt (Na_2EDTA)	0.084 g
Distilled water	1.0 L

Table 3.3 The components of BG11 stock 2

Component	Amount
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	7.5 g
Distilled water	1.0 L

Table 3.4 The components of BG11 stock 3

Component	Amount
Dipotassium hydrogen phosphate ($K_2HPO_4 \cdot 3H_2O$) or	4.0 g
Dipotassium hydrogen orthophosphate anhydrous (K_2HPO_4)	3.05 g
Distilled water	1.0 L

Table 3.5 The components of BG11 stock 4

Component	Amount
Boric acid (H_2BO_3)	2.86 g
Manganese chloride ($MnCl_2 \cdot 4H_2O$)	1.81 g
Zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	0.222 g
Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$)	0.390 g
Copper sulfate ($CuSO_4 \cdot 5H_2O$)	0.079 g
Cobalt chloride ($CoCl_2 \cdot 6H_2O$)	0.050 g
Distilled water	1.0 L

Table 3.6 The components of the working BG11 medium

Stock solution	Amount
Stock 1	10 mL
Stock 2	10 mL
Stock 3	10 mL
Sodium carbonate (Na_2CO_3)	0.02 g
Stock 4	10 mL
Sodium nitrate ($NaNO_3$)	1.5 g
Silicate stock (for culturing diatoms only)	1 mL
Distilled water or filtered artificial seawater	1 L

Table 3.7 The components of silicate stock

Component	Amount
Sodium metasilicate nonahydrate ($Na_2SiO_3 \cdot 9H_2O$)	30 g
Deionized water	1.0 L

Table 3.8 The components of vitamin stock

Component	Amount
Thiamine HCl (vitamin B1)	0.1 g
Biotin solution (biotin 0.1 g in 1 L distilled water)	5.0 mL
Vitamin B12 solution (vitamin B12 0.5g in 1 L distilled water)	1.0 mL
Distilled water	1.0 L

Noble, Becton, Dickinson and Company, USA) (10 g L^{-1}) and adding 1 mL of a vitamin solution per liter of BG11 medium (Table 3.8). The vitamin stock was sterilized by filtering with a sterile 0.20 micrometer membrane filter (28 mm diameter Minisart® NML, syringe driven filter unit; Sartorius Stedim Biotech GmbH, Gottingen, Germany) and stored in a sterile plastic bottle at 4°C. The pH of all media was adjusted to 7.5 using 1.0 M HCl and 1.0 M NaOH before sterilization in the autoclave.

3.2.3 Stock culture maintenance

Stock cultures were maintained on solid media on Petri diskes and as slants at 4 °C under fluorescent light (~ 20 µmol photons $m^{-2}s^{-1}$). Stock cultures were refreshed every 6-8 weeks. Starter cultures were maintained in Erlenmeyer flasks (100 mL and 250 mL) in an incubator shaker at 130-140 rpm, at 25 °C, under fluorescent light (~ 30 µmol photons $m^{-2}s^{-1}$). These cultures were transferred to a fresh medium every 2-4 weeks. The freshwater algae were maintained in BG11 while the marine algae were maintained in BG11 seawater medium. *Chlorella vulgaris* was maintained separately in both BG11 and BG11 seawater media.

3.2.4 Cultivation of microalgae

The cultivation of microalgae involved two steps: inoculum preparation and microalgae biomass production (Figure 3.1). For the inoculum preparation, microalgal stock cultures in liquid media or solid media were aseptically transferred to 40 mL of BG11 in 250 mL Erlenmeyer flasks. The flasks were held in an incubator shaker at 130-140 rpm, 25 °C, under fluorescent light (~ 30 μmol photons $m^{-2}s^{-1}$), for around 20-30 days. This culture (40 mL) was used to inoculate 360 mL of BG11 in a 1 L Duran bottle (borosilicate glass 3.3, LabServ, Biolab, Auckland, New Zealand). These bottle cultures were incubated at room temperature (~ 25 °C) for approximately 7-14 days. After pre-culturing in 1 L Duran bottle, 400 mL of the pre-culture was inoculated into 1,600 mL of the medium in a 2 L Duran bottle and cultured for around 7-14 days.

Subsequently, 2,000 mL of inoculum was split into 400 mL lots and transferred to 5 bottles of 1,600 mL BG11 in 2 L Duran bottles. Microalgal cultures in 1-2 L Duran bottles were maintained at room temperature (24-26°C) under continuous light (approximately 219 μmol photons $m^{-2}s^{-1}$). The light was provided by a bank of six tubes of fluorescent lamps (Philips TLD 58w/840, cool white, Thailand). All Duran bottle cultures were continuously bubbled (0.375 L min^{-1}) with prehumidified air mixed with 5% (vol/vol) carbon dioxide. The inlet and exhaust gas streams were sterile filtered by passing through 0.2 μm Teflon membrane filter (Midisart® 2000; Sartorius AG, Goettingen, Germany). The cultures were harvested in the stationary phase (30-55 days) and kept at 4°C in the dark. The broth was used in flocculation studies within 7 days of harvest. When necessary, the cultures were diluted with the fresh medium (either BG11 or BG11 made with seawater for marine algae) to obtain suspensions of different cell concentrations. Figure 3.2 shows the production set up.

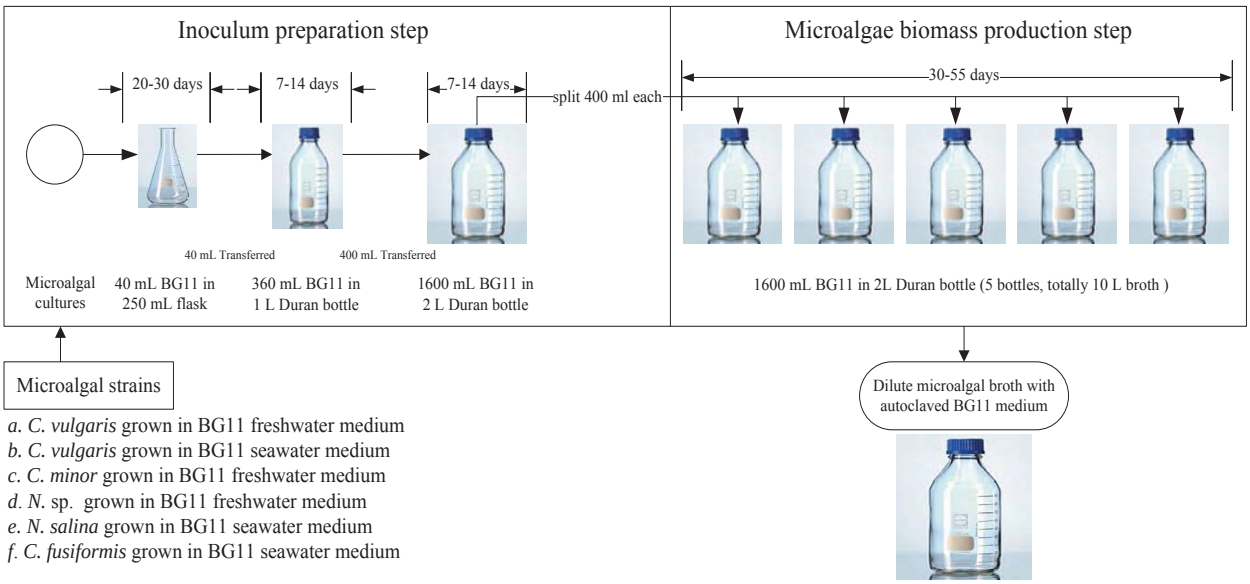


Figure 3.1. Cultivation steps of microalgal biomass production.

Figure 3.2. Batch culture of microalgae in 2 L Duran bottles. The bottles are being bubbled with a prehumidified mixture of air and carbon dioxide.

3.2.5 Measurements

3.2.5.1 Biomass concentration

3.2.5.1.1 Dry cell weight

A 20 mL sample of the algal broth was vacuum filtered using a pre-weighed Whatman GF-C (0.45 μm, 90 mm) microfiber disc filter. The filter disc was washed with 2×20 mL of distilled water (for the cultures grown in BG11 freshwater medium) or with 2×20 mL of 0.5M ammonium formate (for cultures that were grown in the BG11 seawater medium). The filtered biomass samples were dried at 80°C in an oven overnight, cooled in a desiccator and weighed to calculate the dry biomass in 20 mL of the algal broth, as described by Lee & Shen (2004).

3.2.5.1.2 Spectrophotometric determination

A calibration curve was made using measurements of the optical density (680 nm, Ultrospec 2000 spectrophotometer, Pharmacia Biotech, Model 80-2106-00, England) of a serially diluted suspension of algal cells of a precisely known concentration (dry weight). The samples had been serially diluted with the freshly prepared BG 11 culture medium and were measured against a blank of the same medium. At least six dilutions were measured such that the maximum measured optical density did not exceed 0.6. The measured absorbance was plotted against the calculated dry weight to construct a linear calibration curve. Separate calibration curves were made for the different microalgae (Figure 3.3 and Figure 3.4).

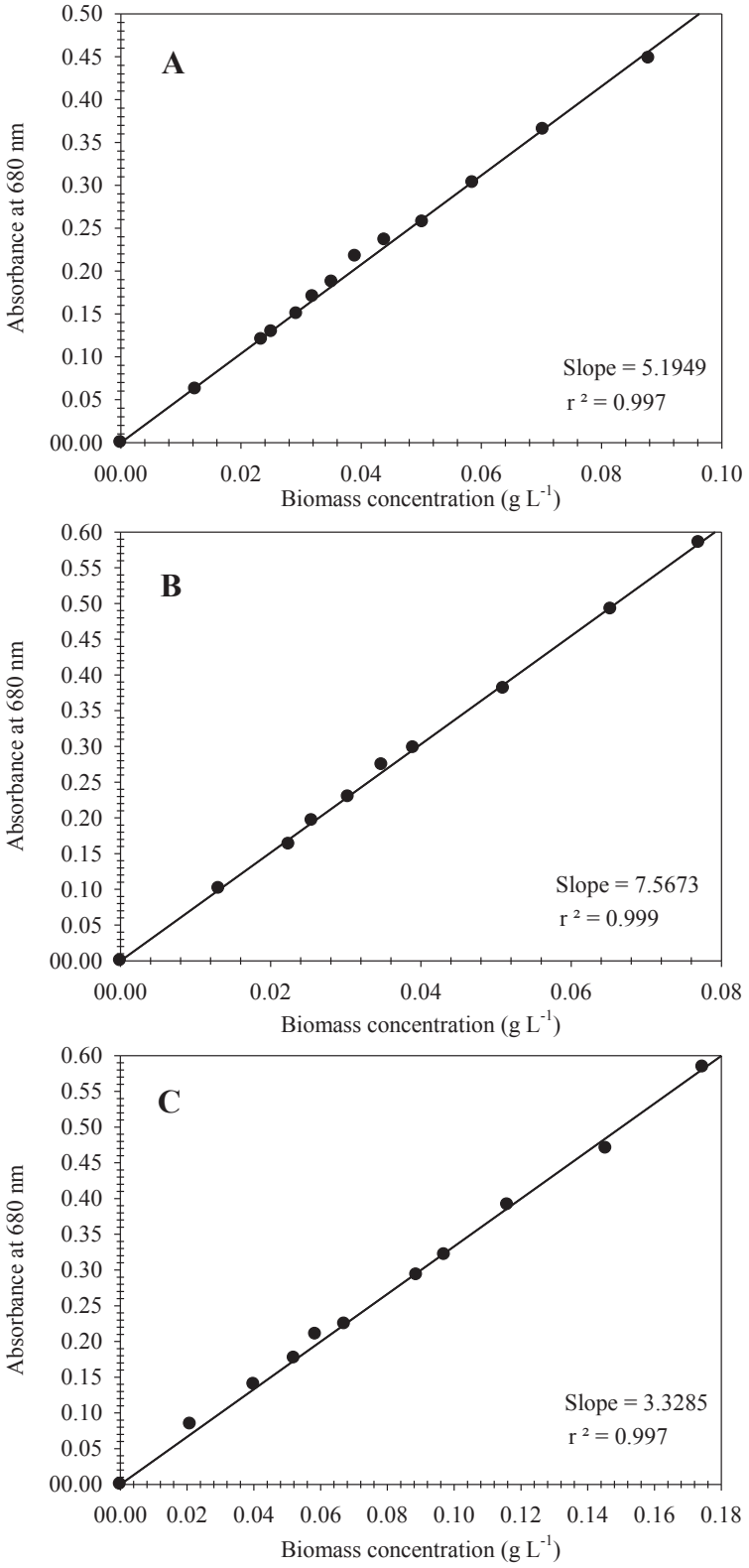


Figure 3.3. Spectrophotometric calibration curves for: *C. vulgaris* grown in BG11 freshwater medium (A); *C. minor* grown in BG11 freshwater medium (B); *Neochloris sp.* grown in BG11 freshwater medium (C).

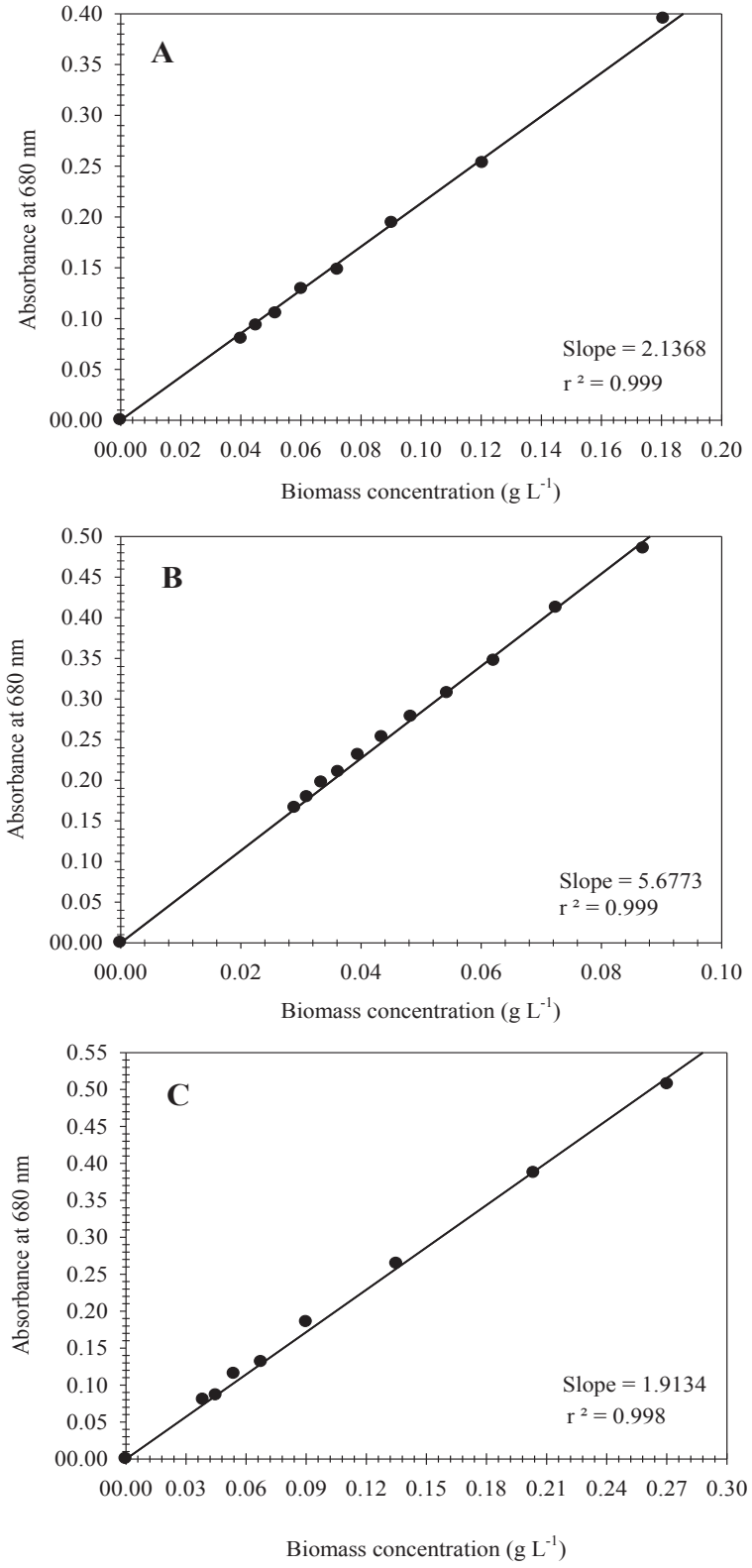


Figure 3.4. Spectrophotometric calibration curves for: *C. vulgaris* grown in BG11 seawater medium (A); *N. salina* grown in BG11 seawater medium (B); *C. fusiformis* grown in BG11 seawater medium (C).

Subsequently, the biomass concentration of an unknown sample was determined by comparing the measured absorbance of an appropriately diluted sample with the appropriate calibration curve. The biomass concentration of the sample was calculated using the equation:

$$\text{Biomass concentration } (\text{g L}^{-1}) = \frac{\text{Absorbance at 680 nm of microalgal culture} \times \text{Dilution factor}}{\text{Slope value of calibration curve}} \quad (3.1)$$

The slope values of the calibration curves for the various microalgae were as in Table 3.9

Table 3.9 Slope values for biomass concentration calibration curve

Microalga	Slope value
C. vulgaris freshwater	5.1949
C. minor	7.5673
Neochloris sp.	3.3285
C. vulgaris seawater	2.1368
N. salina	5.6773
C. fusiformis	1.9134

The slope of the calibration curves (Table 3.9) for different algae is different because of the differences in cell size, morphology and the chlorophyll content per cell. Although, the growth conditions can affect the chlorophyll content in a cell of a given microalga, the growth conditions used in this works were always the same as for the samples grown for making the curves in Figures 3.3-3.4.

The biomass productivity was calculated as follows:

$$\text{Biomass productivity } (\text{g L}^{-1}\ \text{day}^{-1}) = \frac{\text{Final biomass concentration (g L}^{-1}) - \text{Initial biomass concentration } (\text{g L}^{-1})}{\text{Time (days)}} \quad (3.2)$$

The specific growth rate (μ, d^{-1}) was calculated from the slope of the linear regression line of the semilog plot of the cell concentration versus time during the logarithmic phase (Wood et al., 2005), with the following equation:

$$\text{Specific growth rate } (d^{-1}) = \frac{ln\ (X_2 / X_1)}{T_2 - T_1} \quad (3.3)$$

where X_1 and X_2 are the biomass concentrations (g L^{-1}) at times T_1 and T_2, respectively. T_1 and T_2 are the times at the beginning and the end of the logarithmic phase, respectively.

3.2.5.2 Cell morphology and size

Optical microscopy (Zeiss Axiophot compound light microscope) and imaging camera (Leica DFC320) and Leica application suite version 3.3.0 were used to record morphology (1000× magnification) and cell size. Cell size was calculated from the images using Fiji software (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany), an image processing package. This software was calibrated with specific numbers (distance in pixels: 2088, known distance: 145 μm) as per the manual before measurements of cell size. At least 100 microalgal cells of each strain were measured. The microscopic examinations were done by the author at the Manawatu Microscopy and Imaging Centre, Massey University, Palmerston North. The microalgal images are shown in Figures 3.5 - 3.10.

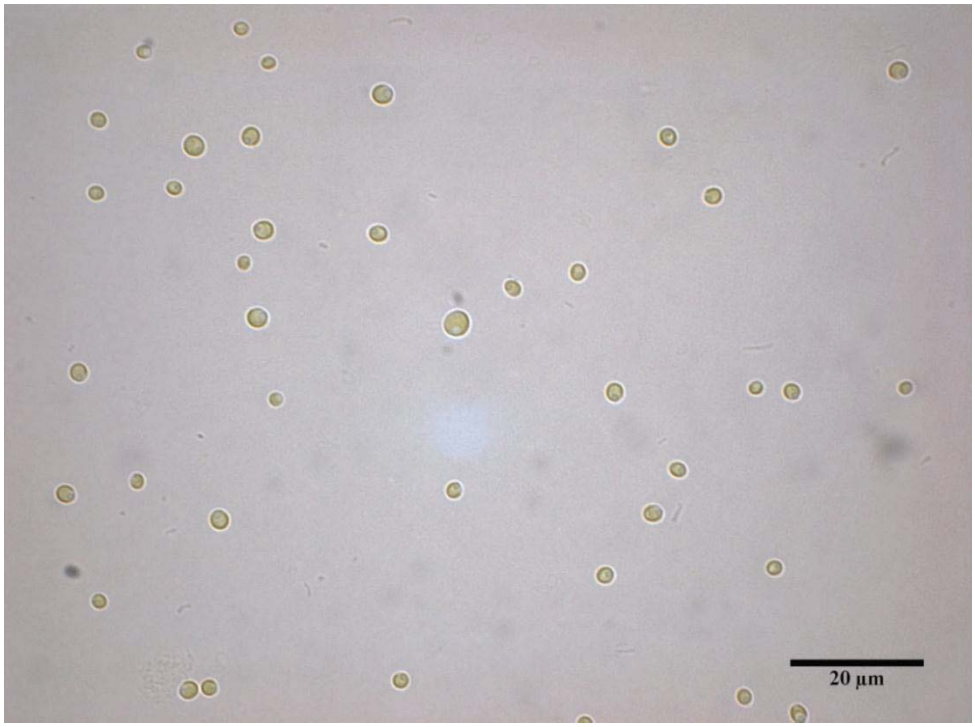


Figure 3.5. Microscopic image (bright-field microscopy) of *C. minor*.

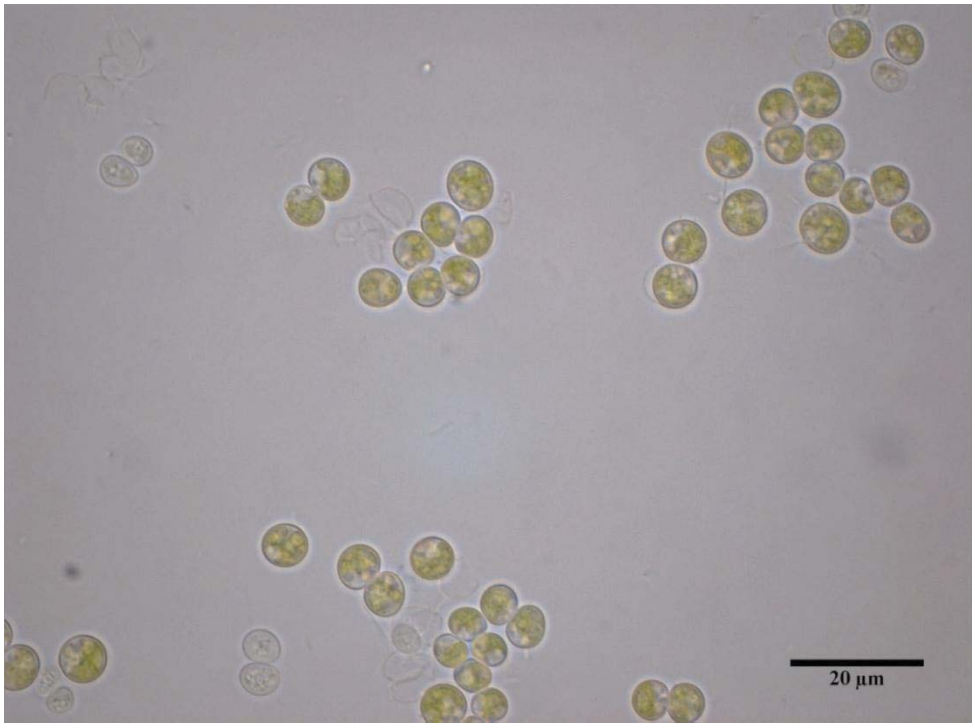


Figure 3.6. Microscopic image (bright-field microscopy) of *Neochloris* sp.

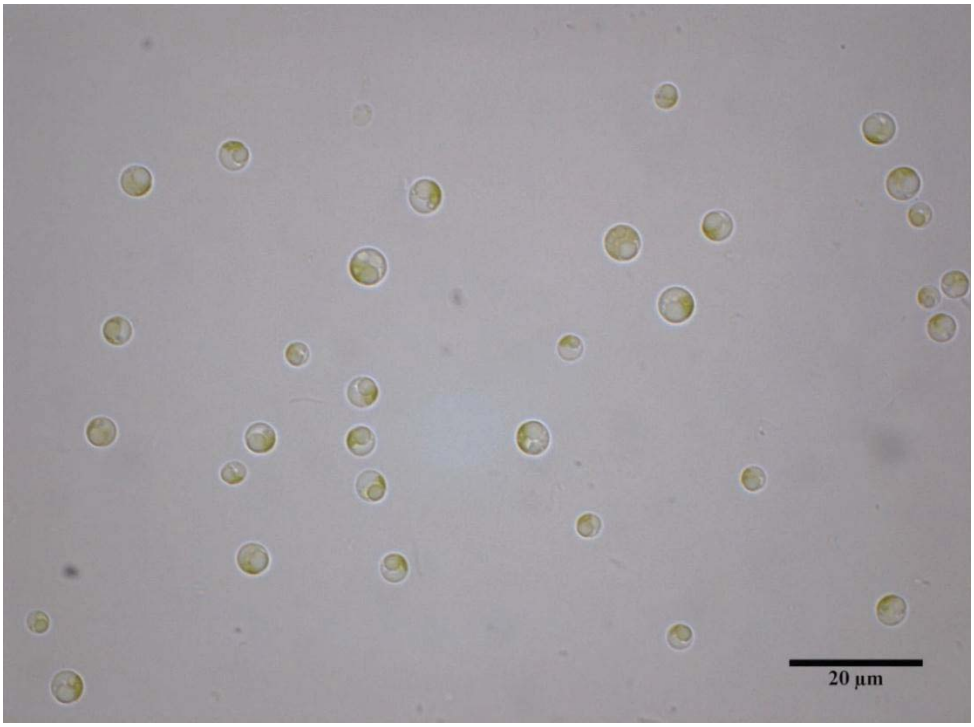


Figure 3.7. Microscopic image (bright-field microscopy) of *C. vulgaris* (freshwater).

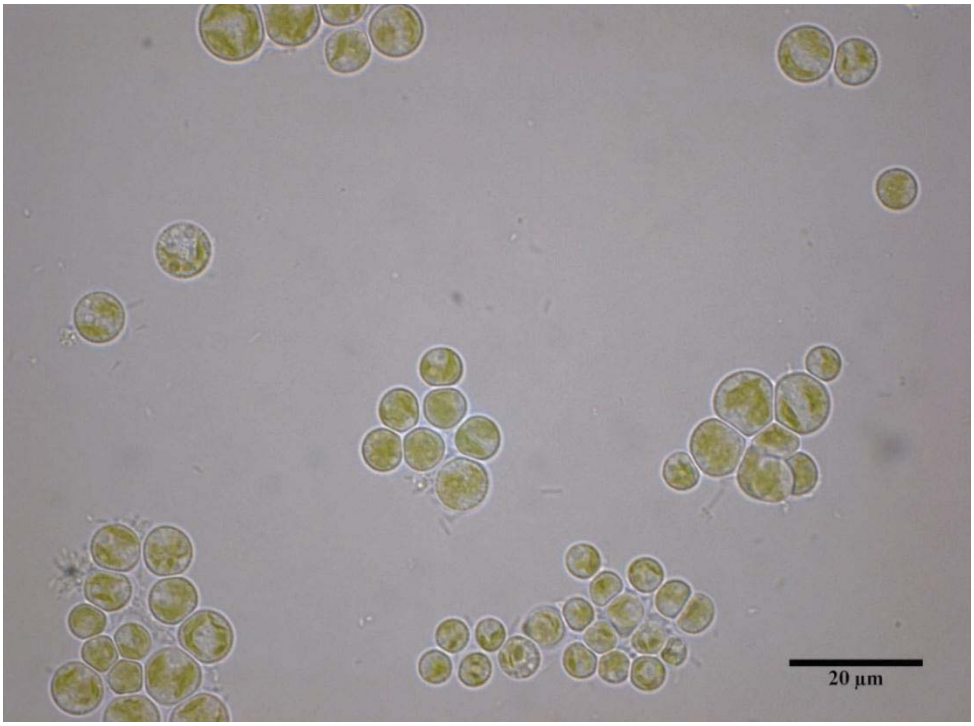


Figure 3.8. Microscopic image (bright-field microscopy) of *C. vulgaris* (seawater).

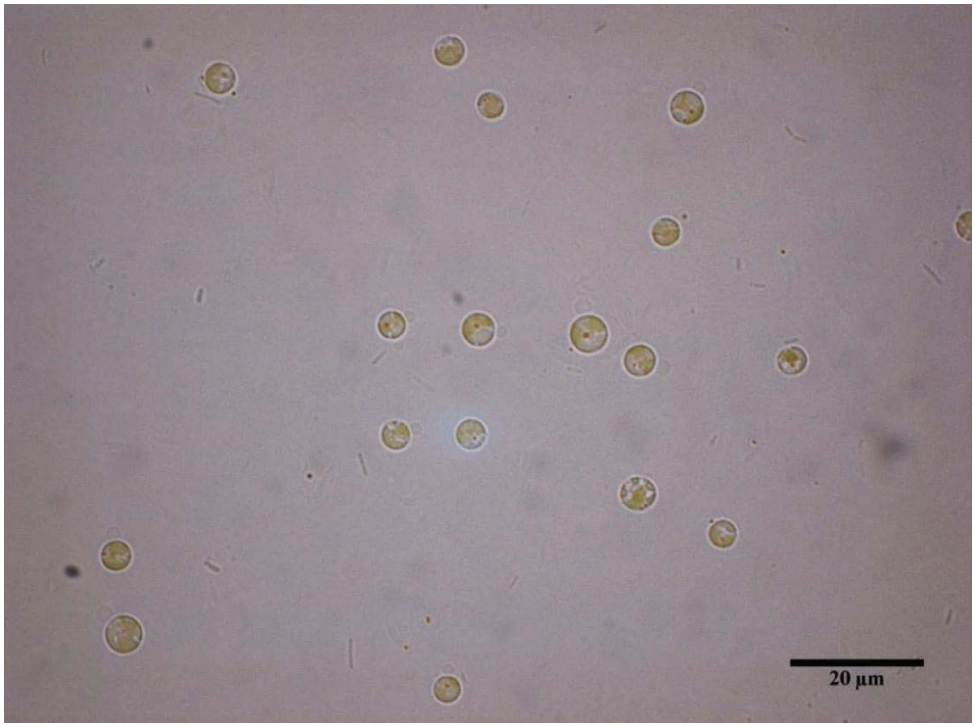


Figure 3.9. Microscopic image (bright-field microscopy) of *N. salina*.

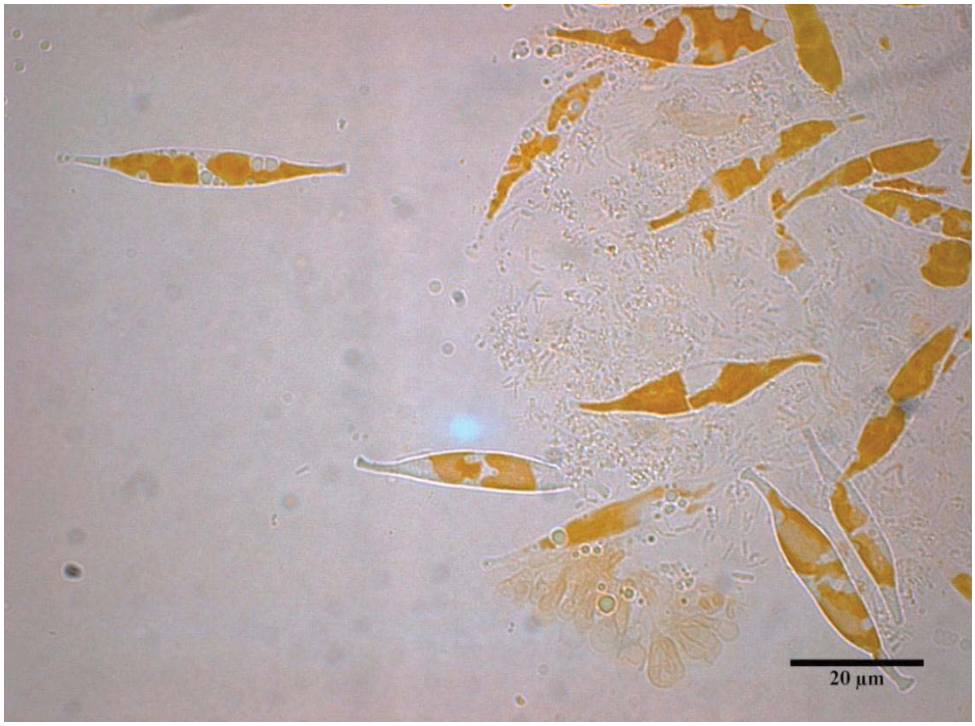


Figure 3.10. Microscopic image (bright-field microscopy) of *C. fusiformis*.

3.2.5.3 Cell surface area

Cell surface area (μm^2) was calculated as follows: *C. vulgaris*, *C. minor*, *Neochloris* sp., and *N. salina* cells were assumed to be spherical with a surface area of $4\pi r^2$ (r is the mean measured radius of the cell) (Henderson et al., 2008b). *C. fusiformis* cell surface area was calculated by assuming the cell to be the equivalent of two cones each with a surface area of πrl (r is half of the distance at midpoint of the shorter dimension of the cell and l is slant height). The equivalent diameter of *C. fusiformis* was calculated as the diameter of a sphere having the same volume as the cell. At least 100 cells of each species were measured.

3.2.5.4 Cell number

An appropriate dilution of microalgal suspension was used to manually count the cells with a haemocytometer under a light microscope. A minimum of four readings were taken for each sample and the mean of these values was used in calculations, as specified by Guillard & Sierachki (2005). Cell counts were used to calculate the concentration as cells/mL.

3.2.5.5 Zeta potential

The zeta potential of the microalgal cells was monitored during cultivation by using a Malvern Nano Zetasizer ZS (model ZEN 3600 with Zetasizer software version 6.20; Malvern Instruments Ltd.). Each microalgal culture sample was uniformly suspended. Approximately 1.0 mL of the optimally diluted sample was transferred to a clear disposable zeta cell and placed in the instrument (Danquah et al., 2009b). Care was taken to ensure that the sample was free of air bubbles. Three measurements were made for each sample. Only the results

meeting the “Malvern quality criteria” were used. The measurements were made by the author at the Riddet Institute, Massey University, Palmerston North.

For the high conductivity samples, that is the samples of marine microalgae grown in BG11 seawater medium, the measurement protocol followed the technical note number MRK1431-01: Measuring high conductivity samples on the zetasizer nano and the technical note number MRK835-01: Automatic settings for zeta potential measurements in version 5.00 software.

3.2.5.6 Total lipids

The lipid contents of the biomass were measured using a modification of Bligh and Dyer (1959) method (Figure 3.11). Microalgal cells were harvested by centrifuging at 8370 × g, 4°C, for 10 min (Hitachi, high speed refrigerated centrifuge (bimac CR22GII), Hitachi Kok Co., Ltd., Tokyo; rotor number R9A). The cells were washed three times with distilled water (for microalgae grown in BG11 freshwater) or with 0.5 M ammonium formate (for microalgae grown in BG11 seawater). Each wash volume was the same as the volume of the broth sample used in recovering the biomass. The washed cells were freeze dried (Laboratory Freeze Dryer, CRYODOS-80, Telstar Industrial, S.L., Terrassa, Spain) and then pulverized in a grinder (Breville Model CG2B, China). Total lipids were extracted from 1 g of lyophilized biomass with a solvent mixture of 5 mL of chloroform, 10 mL of methanol and 4 mL of water. This mixture of solvents and the cells was homogenized for 3 min and stirred for a further 4 h at 760 rpm with a magnetic stirrer (IKA® KMO 2 Basic IKAMAG™, IKA® Werke GmbH & Co. KG, Germany) at room temperature (Figure 3.12). Chloroform (5 mL) was then added and the mixture was blended for 30 s. Distilled water (5 mL) was added

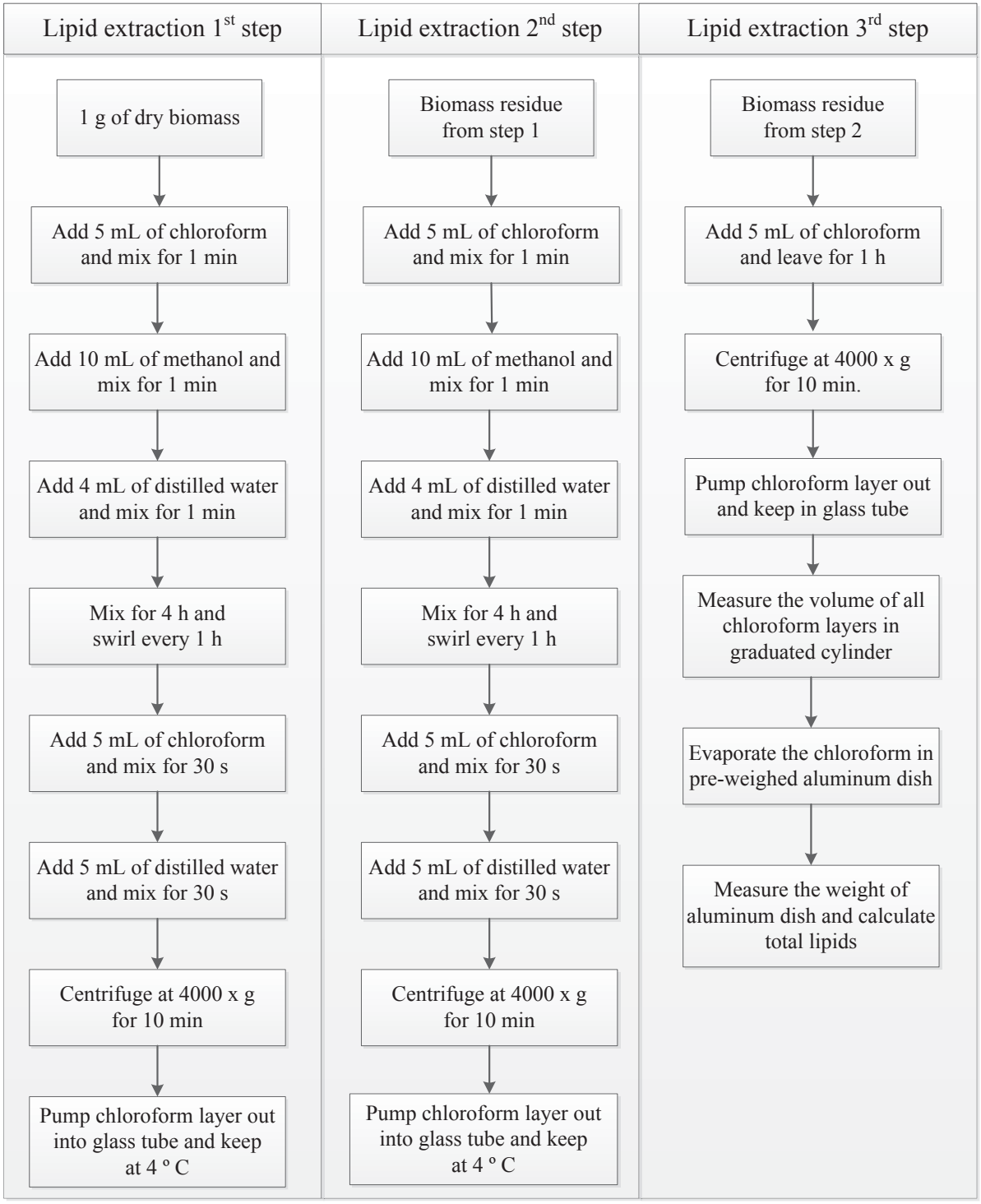


Figure 3.11. Three steps of lipid extraction by the modified Bligh and Dyer (1959) method.

and blending was continued for a further 30 s. The suspension was centrifuged (4000 × g, 4°C, 10 min, rotor number R20A2) and allowed to separate into three layers (Figure 3.13). The top methanol/water layer was discarded. The chloroform layer (the bottom layer) was collected. The middle layer was the residual biomass. This was extracted again as described in Figure 3.11. The three chloroform extracts were combined and left overnight at 4°C.

The volume of the combined chloroform extracts was measured in a graduated cylinder. Total lipids in the chloroform extract were determined gravimetrically by evaporating an aliquot of the extract in a preweighed aluminium dish (Figure 3.14) for 12 h (room temperature in the fume hood) and further drying in a desiccator (12 h, room temperature). Using the measured volume of the pooled chloroform, the total lipid concentration in the extract, and the amount of dry biomass used in extraction, the total lipid content was calculated as a weight percent of dry biomass as follows:

The percentage of total lipids =

$$\frac{\text{Volume of chloroform extract (mL)} \times \text{total lipids concentration in extract (mg mL}^{-1}\text{)}}{\text{Mass of biomass extracted (mg)}} \times 100 \tag{3.4}$$

Lipid productivity was calculated as follows:

$$\text{Lipid productivity } \left(\text{mg L}^{-1}\ \text{day}^{-1}\right) = \text{Biomass productivity } \left(\text{g L}^{-1}\text{day}^{-1}\right) \times \text{Total lipids (\%)} \times 1000 \tag{3.5}$$

Figure 3.12. The extraction unit for lipid extraction.

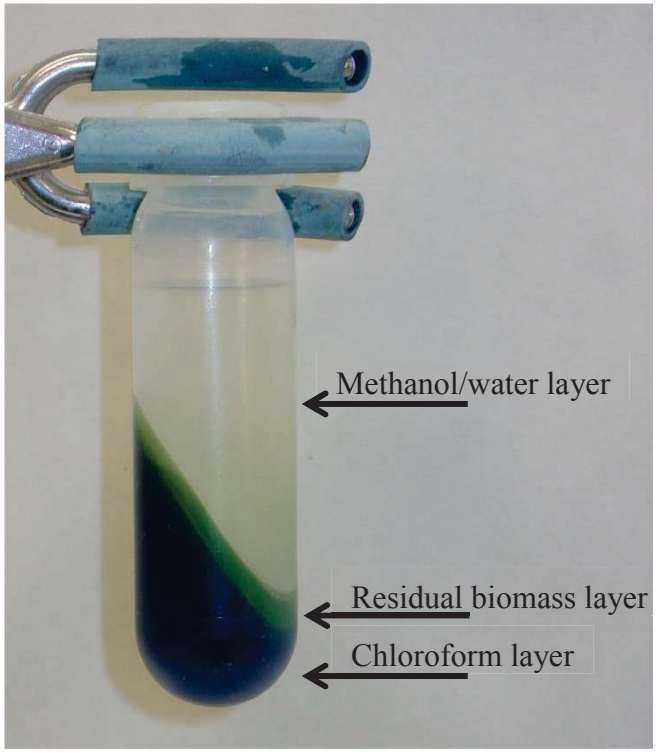


Figure 3.13. The three layers from the extraction process: methanol/water at the top; the middle was layer residual biomass; the bottom was the chloroform layer.

Figure 3.14. The crude oil from microalgal biomass in a preweighed aluminium dish.

Depending on the size of the biomass sample, the above method was scaled in such a way that the volume ratio of chloroform, methanol and water remained at 1:2:0.8 (monophasic step or extraction step) and 2:2:1.8 (biphasic step or separation step). In experiments involving wet microalgae, the microalgal paste obtained after the centrifugation and washing steps was used for lipid extraction. The solids contents of the paste was around 18-30% (g/100 g) depending on the microalgal species. This was determined exactly by drying a portion of the paste and comparing its pre-dried mass to that of the corresponding dried biomass (Section 3.2.5.1.8). The lipids were extracted from a 1 g (dry mass) equivalent of the moist paste. For example, the total lipids were extracted from 5.5556 g the of paste biomass (equivalent to 1 g of dried biomass, if the paste biomass contained 18% solids) with

a solvent mixture of 5 mL of chloroform, 10 mL of methanol and 4 mL of water. The extraction method used was the same as for the other samples.

3.2.5.7 Moisture content (AOAC, 1999)

This method was used to determine the percentage of water in a wet biomass paste by drying the biomass to a constant weight. The moisture content was expressed as the percentage, by weight, of the dry microalgal biomass. The empty moisture can with the lid was dried in the oven at 105°C for 3 hours, transferred to a desiccator to cool (around 15 minutes) and weighed. The paste biomass was weighed accurately and approximately 3 grams was placed in the moisture can. The moisture can with the paste biomass was placed in an oven at 105°C, overnight. After drying, the moisture can was cooled in a desiccator as above and weighed. The moisture content of the paste biomass was calculated using the following equation:

$$\text{Percentage of moisture} = \frac{(W_1 - W_2)}{W_1} \times 100 \quad (3.6)$$

$$\text{Percentage of biomass} = 100 - \text{Percentage of moisture} \quad (3.7)$$

where W_1 is weight (g) of sample before drying and W_2 is weight (g) of sample after drying.

3.2.6 Characterization of microalgae

The objective of these experiments was to study the background characteristics of the selected microalgal species. The important characteristics for evaluating the economic feasibility of microalgae as a source of biofuels are biomass productivity, the total lipid

content and the lipid productivity (Rodolfi et al., 2009; Huerlimann et al., 2010). In addition, microalgal morphology, cell size and surface charge can influence the harvesting of the microalgal biomass by flocculation (Danquah et al., 2009b). Therefore, the growth rate, the final biomass concentration, the biomass productivity, the lipid content, lipid productivity, cell morphology, cell size, cell surface area and the zeta potential were determined.

3.2.6.1 Biomass concentration and lipid contents

Microalgae were grown aseptically in Duran bottles as explained in Section 3.2.4. The biomass concentration was measured daily by the optical density method (Section 3.2.5.1.2). The optical density measurements were correlated to the directly measured biomass dry weights (g L^{-1}). The biomass dry weight is reported as an average of three independent replicate batch cultures. The biomass concentration values (g L^{-1}) were plotted against time (day) to construct the growth curve. The specific growth rate of each microalgae was calculated (Section 3.2.5.1.2). The microalgal biomass harvested in the stationary phase at the end of the culture was used to measure the lipid content (Section 3.2.5.6). The biomass and lipid productivities were calculated as explained in Section 3.2.5.1.2 and Section 3.2.5.6, respectively.

Average values of the biomass productivity, specific growth rate, lipid content, and lipid productivity of three replicates and their standard deviations were calculated. Significant differences were determined using one-way analysis of variance (ANOVA) with 95% confidence (probability limit of $p<0.05$) and a Duncan's Multiple Range Test (DMRT). Data analyses were done by using the SAS (Statistic Analysis System) program (version 9.1, SAS Institute Inc., Cary, NC).

3.2.6.2 Microalgal morphology, size and zeta potential

Light microscopy was used to characterize the algae cell morphology and size (Section 3.2.5.2) while the zeta potential of cells was measured by using Malvern Nano Zetasizer ZS (Section 3.2.5.5). These measurements were made periodically during the cultivation process until the stationary phase was reached.

3.2.7 Microalgae harvesting by flocculation

Batch flocculation experiments were performed as explained in the following sections.

3.2.7.1 Flocculants

Two relatively nontoxic, readily available and inexpensive flocculants were used in separate experiments. These were aluminum sulfate ($Al_2(SO_4)_3.18H_2O$; Riedel-de Haen, Hanover, Germany) and ferric chloride ($FeCl_3.6H_2O$; ACROS ORGANICS, Geel, Belgium). Stock solutions of the inorganic flocculants were made at 20 g L^{-1} concentration. The 20 g of salt (calculated as $FeCl_3$ or $Al_2(SO_4)_3$) was dissolved in deionized water and the volume was made up to 1 L. The stock solutions were kept at room temperature (24-26 °C) and used within 14 days.

3.2.7.2 Flocculation conditions

For flocculation tests, the microalgal broth were harvested in the stationary phase. Batch flocculation-sedimentation tests were conducted using a 200 mL suspension of the microalgal cells in each of the six 250 mL beakers (Figure 3.15-3.16). Tests were performed in a jar test

apparatus (six stirrer units, Figure 3.16; VELP Scientifica, model JLT6, Europe) at room temperature (25°C). The geometric details of the beakers and stirrers are shown in (Figure 3.17A and B). The beakers had an internal diameter of 6.3 cm (D_b); the broth depth (H_b) was 6.4 cm; the impeller diameter (d_b) was 5 cm. The impeller clearance (C_b) from the bottom of the beaker was 2.5 cm. The impeller blade width (W_b) was 1.1 cm and the blades were inclined at 25° angle from the horizontal.

The initial microalgae biomass concentration in the suspension was estimated from the optical density measured at 680 nm. Each 200 mL sample was dosed with a predetermined concentration of the flocculant using a freshly prepared stock solution (Section 3.2.7.1). The flocculation test methodology was adopted from Sukenik et al. (1988). All beakers were simultaneously treated as follow: 1. rapid mixing at 80 rpm for 2 min to disperse the flocculant; 2. gentle mixing at 20 rpm for 30 min (the flocculation period) to flocculate the cells; and 3. no agitation for 30 min (the settling period) to allow the flocs to settle.

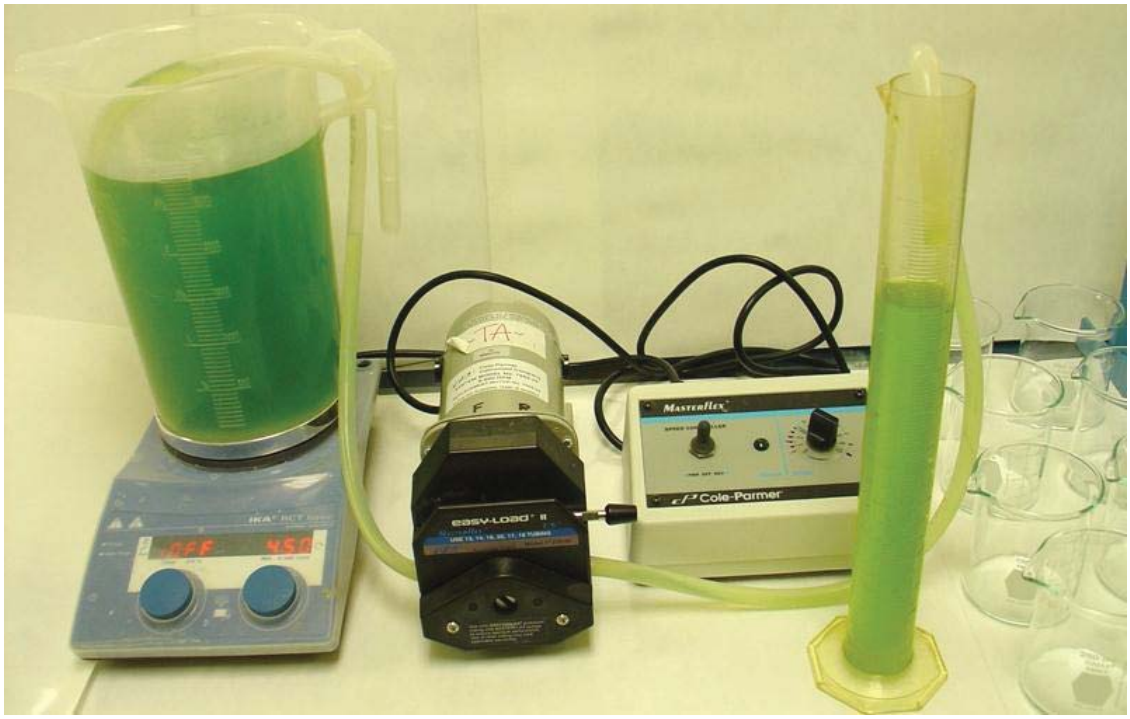


Figure 3.15. Preparation and dispensing microalgal broth in 250 mL beakers for batch flocculation experiments.

Figure 3.16. A jar test unit for flocculation experiments. The microalgal broth samples (200 mL) in the six beakers were identical but had been dosed with different quantities of the flocculant.

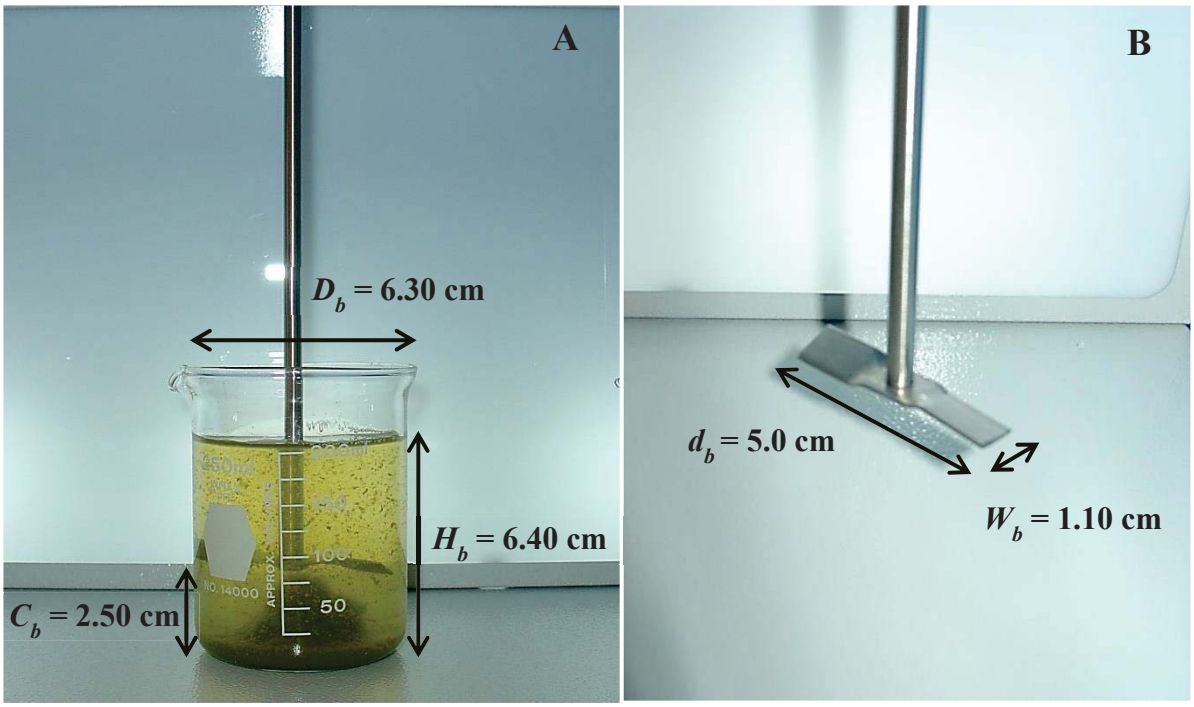


Figure 3.17. Geometric details of batch flocculation system: flocculation beaker (A) and 2-bladed impeller (B).

At the end of the 62 min test, a 5 mL sample of the suspension was withdrawn from the 100 mL level of the beaker for measurement of the suspended cell concentration by the optical density (680 nm) method. The percentage of the microalgal biomass remaining in the broth was estimated as follows:

The percentage of microalgal cells remaining in the broth

$$= \frac{\text{Final cell concentration (g L}^{-1}\text{, after flocculation)}}{\text{Initial cell concentration (g L}^{-1}\text{, before flocculation)}} \times 100 \qquad (3.8)$$

The percentage of the microalgal cells removed from the broth was then 100 minus the percentage of the cells remaining in the broth.

3.2.7.3 Data collection

Five microalgae species were used for flocculation experiments at different biomass concentrations. Various doses of the two flocculants were investigated under the standardized test regimen (Section 3.2.7.2) for identifying the optimal flocculant dosage. The optimal dose was defined as the lowest flocculant dosage for achieving a 95% removal of the microalgal cells from a culture broth. The experiments were repeated in triplicate. The dependence of flocculant dose required to achieve 95% flocculation, on the initial biomass concentration was explored. Correlations between the algal morphological parameter of surface-to-volume ratio and the flocculant dosage required for a given efficiency of recovery were established. The cost of harvesting of algal biomass from the culture medium by flocculation was calculated based on the flocculant dosage required. Only the costs of flocculants based on dosage were compared, as the basic flocculation operation, the hardware and the labor expense are expected to be the same irrespective of which flocculant is used.

3.2.8 Design and characterization of a continuous flocculation/sedimentation process

Batch flocculation is not suitable for large-scale commercial use. Most industrial flocculations are carried out as continuous operations (Hogg, 2000; Hogg & Rattanakawin, 2002; Riffat, 2013). Therefore, a continuous flocculation-sedimentation process was designed and tested, based on the data obtained from the batch flocculation experiments. The aim was to improve the understanding of continuous flocculation and sedimentation for use with microalgae and establish a basis for possible future work outside this thesis.

3.2.8.1 Setup of continuous flocculation and sedimentation process

The schematic diagram of the continuous flocculation-sedimentation system used in this study is shown in Figure 3.18. The system consisted of a 20 L storage tank (1) for culture broth (Figure 3.18); a 5 L storage tank (2) for the flocculant; two variable speed slurry transfer pumps (3, 4); a rapid mixing vessel with a 2-blade impeller (5); a flocculation vessel with a 2-blade impeller (6); and a sedimentation tank (7). Details of the rapid mixing vessel, the flocculation vessel and the sedimentation tank are described in following section.

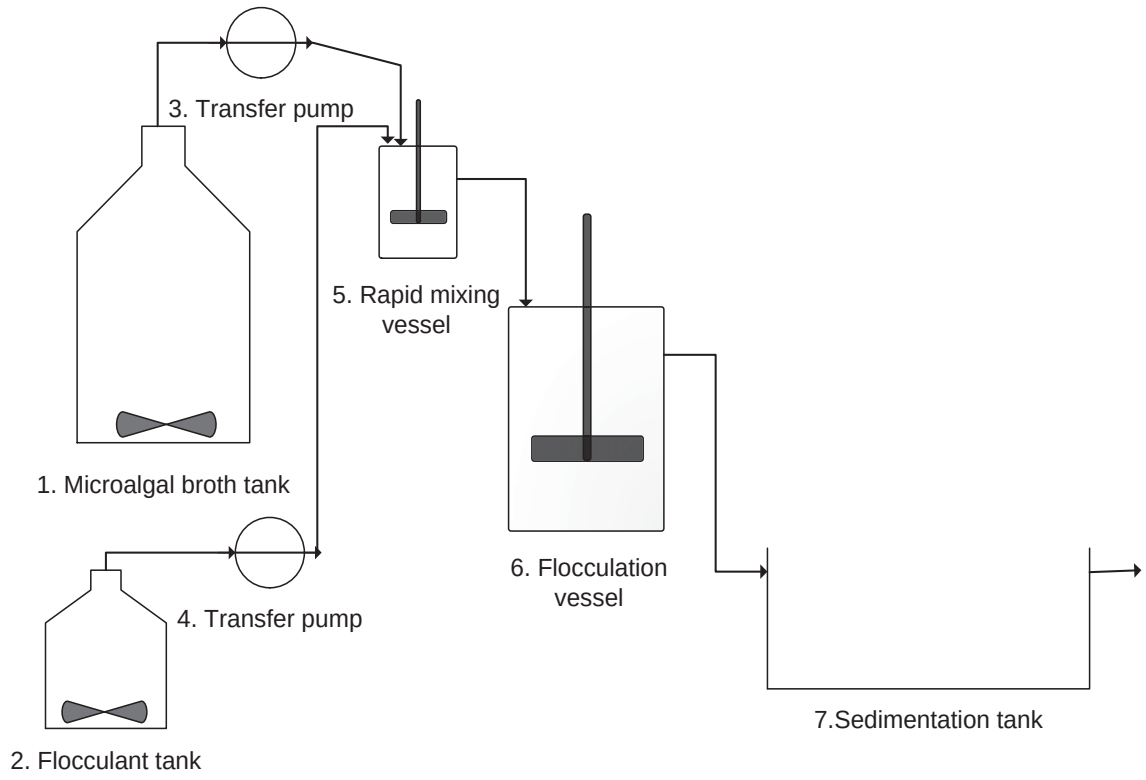


Figure 3.18. The continuous flocculation–sedimentation setup.

3.2.8.2 Design of continuous rapid mixing vessel and impeller

Rapid or flash mixing is required to mix the flocculant with the algal broth to initiate the particle aggregation process (Edzwald, 2011). Rapid mixing is usually carried out with an impeller in a small mixing vessel.

In scaling up a mixing system the relevant geometric ratios of the small system are kept the same at the larger scale. The suggested geometric proportions for a circular mixing tank are a broth depth (*H)* to vessel internal diameter (*D*) ratio of 1 (Oldshue, 1983; Paul, 2003; Spellman, 2009). Based on this, a suitable vessel which was available in the laboratory was used. It had a 5.2 cm internal diameter and could accommodate a 5.3 cm broth depth. The broth depth to vessel internal diameter ratio was 1.02, or close to the recommended value and nearly the same as in the jar test batch vessels.

The volume of microalgal broth in the vessel (*V*, mL) could be calculated as follows:

$$V = \frac{\pi D^2}{4} \cdot H \tag{3.9}$$

The calculated volume of the broth was 112.57 mL, or ~113 mL

The flow rate (*Q*, mL min^{-1}), required for a given residence time (*t*) in the vessel was estimated as:

$$Q = \frac{V}{t} \tag{3.10}$$

The value of the residence time was set at 2 min to correspond to the 2 min mixing time of the batch experiments. Thus, the microalgal broth flow rate through the continuous rapid mixing vessel was 56.5 mL min^{-1}.

For geometric similarity with the small batch impeller, the diameter the larger impeller was calculated as follow:

$$\frac{d_b}{D_b} = \frac{d_I}{D_I} \tag{3.11}$$

where d_b is impeller diameter of the small batch vessel (5 cm); D_b is the internal diameter of the small batch vessel (6.3 cm); d_l is impeller diameter of the continuous rapid mixing vessel (cm); D_l is internal diameter of the continuous rapid mixing vessel (5.2 cm). Based on this, the impeller diameter for the large mixing vessel was 4.13 cm.

The vertical blade height (W_l, cm), of the large impeller was calculated using

$$\frac{W_b}{d_b} = \frac{W_I}{d_I} \tag{3.12}$$

where W_b is vertical blade height of the small impeller (1.10 cm); d_b is impeller diameter at the small impeller (5.0 cm); W_l is vertical blade height of the large impeller (cm); and d_l is diameter of the large impeller (4.13 cm). Thus, the vertical blade height of the larger impeller was 0.91 cm.

The impeller clearance (C_l, cm), from the bottom of the tank at the two scales had to satisfy the following relationship for geometric similarity:

$$\frac{D_b}{C_b} = \frac{D_l}{C_l} \tag{3.13}$$

where C_b is the clearance at the small scale and C_l is the clearance at the large scale. As the internal diameter of the batch vessel (D_b) was 6.3 cm and C_b was 2.5 cm, a C_l value of 2.1 cm was calculated.

For similar levels of mixing in mixing tanks of two different scales, the tip speed of the impeller at the two scales has to be identical. The impeller tip speed (T) of any scale was calculated as follows:

$$T = \frac{\pi N d}{60} \tag{3.14}$$

where T (cm s^{-1}) is the tip speed; N is impeller rotational speed (rpm); and d is impeller diameter. In the small batch mixing vessel the tip speed was 20.95 cm s^{-1} (80 rpm). Thus, the impeller rotational speed in the larger vessel needed to be 96.87 rpm (97 rpm).

3.2.8.3 Design of continuous flocculation vessel and impeller

Continuous flocculation vessel and impeller were geometrically identical to the system used in small batch operations. Continuous flocculation was performed in a vessel which had an internal diameter of 11 cm and a broth depth of 11.2 cm. The broth volume in the vessel was 1064.51 mL, or ~1065 mL.

Based on the previously calculated (Section 3.2.8.2) feed flow rate of 56.5 mL min^{-1} (from Equation 3.10), the retention time in the continuous flocculation vessel was 19 min.

This was deemed sufficient based on prior experience although it was less than the 30 min used in the batch operations.

Using the previously described methods, the impeller diameter for the continuous flocculation vessel was calculated to be 8.73 cm. The calculated vertical blade height was 1.92 cm. The calculated impeller clearance from the bottom was 4.36 cm, or 4.4 cm. Based on the impeller tip speed used in the small batch flocculation beaker, an identical tip speed of 5.24 cm s^{-1} was used at the larger scale. At the larger scale this was equivalent to an impeller rotational speed of 11.46 rpm, or 11.5 rpm. The results from the above calculations are summarised in Table 3.10.

Table 3.10 Design summary of vessels, impellers and operating conditions for batch flocculation and continuous flocculation systems

Detail	Batch vessel	Continuous vessels	
		Rapid mixing vessel	Flocculation vessel
Vessel			
1. Internal diameter of vessel (D, cm)	6.3	5.2	11
2. Broth depth in vessel (H, cm)	6.4	5.3	11.2
3. H to D ratio	1.02	1.02	1.02
4. Working volume of vessel (V, mL)	200	113	1065
Impeller			
1. Impeller diameter (d, cm)	5	4.13	8.73
2. Vertical blade height (W, cm)	1.1	0.91	1.92
3. Impeller clearance from bottom (C, cm)	2.5	2.1	4.37
4. Tip speed in rapid mixing vessel (T, cm s^{-1})	20.95	20.95	-
5. Tip speed in flocculation vessel (T, cm s^{-1})	5.24	-	5.24
Conditions			
1. Speed of impeller for rapid mixing (rpm)	80	97	-
2. Speed of impeller for flocculation (rpm)	20	-	11.5
3. Flow rate (mL min^{-1})	-	56.5	56.5
4. Retention time of rapid mixing (min)	2	2	-
5. Retention time of flocculation (min)	30	-	19

3.2.8.4 Design of rectangular sedimentation tank (Hendricks, 2006; Kaira & Christian, 2006)

Both rectangular and circular sedimentation tanks are commonly used in continuous large scale water treatment processes (Joint Task Force of the Water Environment Federation and the American Society of Civil Engineers, 1992; Lin, 2007). Rectangular tanks have several advantages compared to circular tanks (Vesilind, 2003). A rectangular sedimentation tank (Figure 3.19) was designed for this work. The relevant calculations and operation are described below.

Based on batch sedimentation experiments, at least 25 min were required to settle the flocculated biomass in a 6.4 cm deep tank. Therefore, the settling velocity of microalgal biomass was 0.256 cm min^{-1}. In concept, in a sedimentation tank a particle with a settling velocity (v_s) greater than or equal to the overflow velocity (v_o) in the tank will settle out (Hendricks, 2006). Therefore the maximum overflow velocity (v_o) had to be 0.256 cm min^{-1}.

The tank surface area for the maximum overflow velocity was calculated using the following equation:

$$v_o = \frac{Q}{W \times L} \tag{3.15}$$

where v_o is the overflow velocity (0.256 cm min^{-1}), Q is the flow rate of microalgal broth through the sedimentation tank (the same flow rate as in the mixing and flocculation tanks, or 56.5 mL min^{-1}), W is the width of the tank (cm) and L is the length of the settling tank (cm). The required surface area of the tank was 220.7 cm^2, or 221 cm^2.

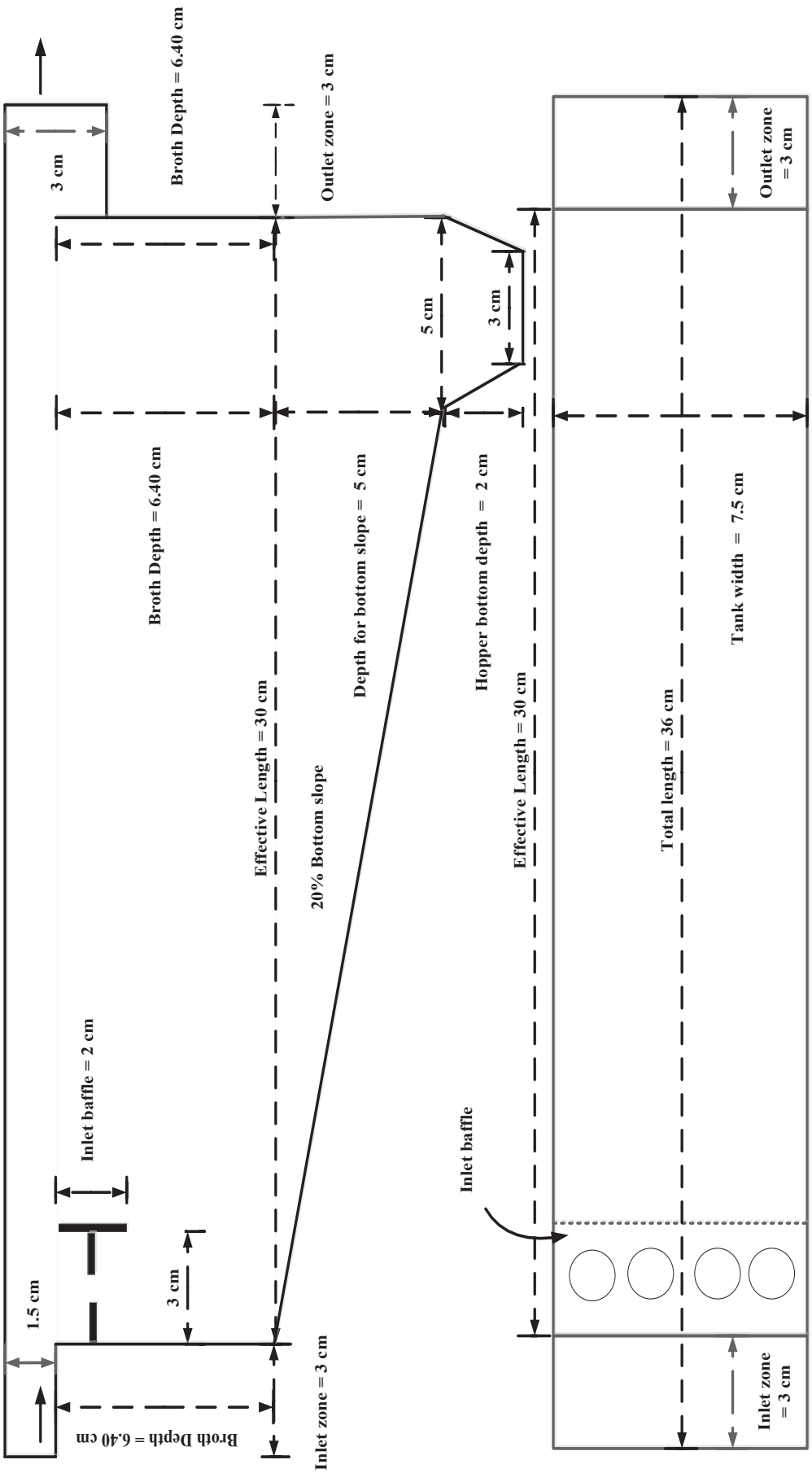


Figure 3.19. Schematic diagram of the designed sedimentation tank.

Hendricks (2006), Edzwald (2011) and Riffat (2013) recommended an economically acceptable length (L) to width (W) ratio (L:W) of 4 to 6. Therefore, a ratio of 4 was used for a tank with an area of $L \cdot W$. Thus,

$$4W \times W = 221 \text{ cm}^2, \text{ or} \tag{3.16}$$

W = 7.43 cm (or 7.5 cm) and therefore L = was 30 cm. Based on the above, the tank volume was $W \times L \times H$, or 1440 mL for a broth depth H of 6.4 cm.

The dimensions of the hopper at the end of the sedimentation tank (Figure 3.19) were then calculated using an estimate of the generation rate of the flocculated biomass. The microalgal biomass production rate (M_b, g min^{-1}) was estimated for 95% removal of the biomass from the broth on a dry weight basis:

$$M_b = P_r \times B_c \times Q \tag{3.17}$$

where P_r (95%) is the percentage of the microalgal biomass removed from the broth; B_c (1 g L^{-1}) is microalgal biomass concentration in the broth; and Q is flow rate of the microalgal broth through the sedimentation tank (56.5 mL min^{-1}). Thus, the flocculated biomass production rate was 0.054 g min^{-1}.

The volume of the microalgal biomass produced (V_b, mL min^{-1}) was computed as follows:

$$V_b = \frac{M_b}{\rho_w \times S_b \times P_s} \tag{3.18}$$

where ρ_w is the density of water (0.997 g mL^{-1} at 25°C); S_b is the specific gravity of the microalgae, 1.10 (Fogg, 1975); and P_s is the percentage of solids in the harvested microalgal

biomass expressed as a decimal fraction, 0.20 (about 80% moisture content in the harvested microalgal biomass, the result from preliminary experiments). Therefore, the volume flow rate of microalgal biomass was 0.25 mL min^{-1}.

The hopper capacity was calculated assuming that the biomass was removed from the hopper every four hours by pumping. Therefore, the hopper had to have biomass storage capacity for 4 hours (240 min). Hence, the capacity of the hopper needed to be 60 mL (0.25 mL min^{-1}×240 min).

Commonly, the hopper bottoms are designed with a trapezoidal shape (Kaira & Christian, 2006) as shown in Figure 3.20. The volume (V_{hb}) of such a shape with a channel width W is given by the following equation.

$$V_{hb} = \frac{1}{2} \times H_h(a+b) \times W \tag{3.19}$$

For the specified volume of 60 mL and a tank width W of 7.5 cm, suitable values of H_b, a and b were 2 cm, 5 cm and 3 cm, respectively.

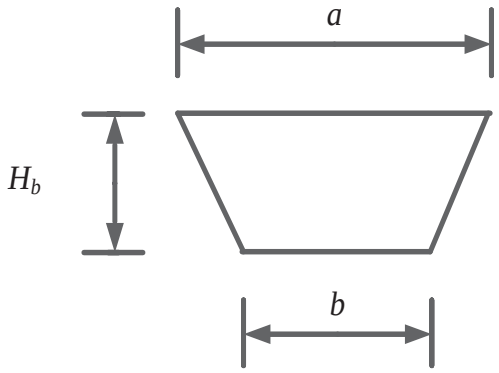


Figure 3.20. Dimensions of the hopper bottom.

The sedimentation tank was designed with a slope of 20% based on typical values used in the literature (Kaira & Christian, 2006) and the preliminary experiments. For this slope, the broth depth (H_s) at the bottom of the slope was calculated as follows:

$$H_s = (L - a) \times 0.20 \quad (3.20)$$

where L (30 cm) is the length of the settling tank and a is width of the hopper bottom (5 cm) (Figure 3.20). The slope depth was 5.0 cm. Therefore, the overall depth of the settling tank was 13.40 cm ($H+H_s+H_h$). For the overall length of the settling tank, a 10% length each was added at the inlet and the outlet zones. Therefore, the overall length of the settling tank was 36 cm.

The overall or average retention time of the designed sedimentation tank for a flow rate of 56.5 mL min^{-1} was 38.2 min.

3.2.8.5 Continuous flocculation and sedimentation procedure

The microalgae were cultured until they reached the stationary phase and were then diluted with the appropriate fresh medium to a biomass concentration of 0.5 g L^{-1}. This corresponded to a biomass concentration that would typically occur in algal broth produced in commercial raceway systems (Borowitzka, 2005; Chisti, 2012, 2013). The flocculant solution was prepared one day in advance. The flocculant addition rate was based on the optimal dose for a given microalgal species obtained from the batch experiments (Section 3.2.7).

The start-up procedure consisted of establishing the flows of the microalgal broth and the flocculant solution at the desired rates. After the flow rates had been properly adjusted,

the continuous flocculation-sedimentation experiment was initiated by beginning the pumping of the microalgal broth and the flocculant solution to the rapid mixing tank. Here the microalgal broth and the flocculant were rapidly mixed (impeller speed of 97 rpm). Then, the suspension flowed by gravity to the flocculation tank with a low speed mixing rate of 11.5 rpm and subsequently it moved by gravity into the sedimentation tank. Samples (5 mL) were taken from the outlet zone of the sedimentation tank and the biomass concentration (g L^{-1}) remaining in the samples was determined by a spectrophotometer (680 nm). The samples were taken every 30 min until the process reached steady state (equivalent to four residence times of operation).

3.2.9 Solvent extraction of biomass paste

Solvent extraction is the traditional method for extracting lipids from food (Halim et al., 2011). This method is potentially suitable for recovering lipids from wet algal biomass paste without a prior drying step. Elimination of a drying step would lead to significant energy and cost savings (Liu et al., 2013). Most earlier studies of solvent extraction of algal biomass have used dry biomass. This study aimed to investigate the effects of the flocculants used on the ability to recover the lipids from the biomass using a suitable solvent system and without involving prior drying of the biomass.

3.2.9.1 Effect of flocculants used on lipid extraction

The microalgae were cultured until they reached the stationary phase (Section 3.2.4). The broth was then divided into three equal parts. The first portion of the broth was used in a control treatment (treatment 1). Thus, the biomass was recovered by centrifuging at 8370 × g,

4°C for 10 min (Hitachi, high speed refrigerated centrifuge (bimac CR22GII), Hitachi Kok Co., Ltd., Tokyo; rotor number R9A). The second portion of the broth was subjected to treatment 2: the flocculation treatment using aluminum sulfate at the optimal dose. The third portion of the broth was subjected to harvest treatment 3 involving flocculation with ferric chloride at the optimal dose.

The flocculated microalgal biomass was separated from the supernatant by centrifuging at 8370 × g, 4°C for 10 min. Then, the biomass from all treatments was washed separately three times with distilled water (for microalgae grown in BG11 freshwater) or with 0.5 M ammonium formate (for microalgae grown in BG11 seawater). Each wash volume was the same as the volume of the original broth sample used in recovering the biomass. The washed biomass samples were freeze dried (Laboratory Freeze Dryer, CRYODOS-80, Telstar Industrial, S.L., Terrassa, Spain) and then pulverized in a grinder (Breville Model CG2B, China). The dried microalgal biomass samples were weighed. The paste biomass samples from the three treatments were taken such that each sample contained the equivalent of 1 g of actual dried microalgal biomass (i.e. not including the mass of the adsorbed flocculant). Total lipids were extracted from these samples in triplicate (see Section 3.2.5.6).

The average values of the lipid content of three replicates and their standard deviations were calculated. Mean values of the control and treatments were compared using *t*-tests. Data analysis was done by using the SAS (Statistic Analysis System) program (version 9.1, SAS Institute Inc., Cary, NC).

3.2.9.2 Comparison of solvent extraction of dry and paste biomass

The objective of this experiment was to establish the efficacy of the standard extraction method (the modified Bligh & Dyer (1959), Section 3.2.5.6) in extracting lipids from a biomass paste as opposed to using the same biomass for extraction after freeze drying.

The microalgae were harvested by centrifuging (8370 × g , 4°C, for 10 min) after they had reached the stationary phase. The recovered biomasses was washed three times with distilled water (for microalgae grown in BG11 freshwater) or with 0.5 M ammonium formate (for microalgae grown in BG11 seawater). Each wash volume was the same as the volume of the broth sample used in recovering the biomass. Wet biomass paste obtained after centrifugation contained approximately 70-80% of water (from preliminary experiments). The exact moisture content were determined experimentally for a small sample. An amount of the wet biomass paste equalling 1 g dry biomass was extracted in triplicate with the solvent system as explained in Section 3.2.5.6. The total lipid amount recovered from the 1 g equivalent biomass paste was compared to the total lipid amount recovered from 1 g of dry biomass.

3.2.9.3 Optimization of solvent composition for extraction of total lipids from biomass paste

The aim of this study was to identify the best solvent combination (including chloroform/methanol/water) for a single step extraction of the total lipids from the microalgal biomass paste. Microalgal biomass was recovered by continuous flow centrifugation at 11,800 × g, 4°C, after it had reached the stationary phase. The harvested biomass (Figure 3.21) was re-suspended in l L of 0.5 M ammonium formate (for microalgae grown in BG11

seawater) for washing. This microalgal suspension was centrifuged in a batch centrifuge at 8370 × g, 4°C, for 10 min. The microalgal biomass was washed three times. A small portion of the washed biomass was used to determine the moisture content. The fresh biomass paste (equivalent to 1 g of dried biomass) was weighed in the extraction vessel (100 mL Duran bottle) and kept in the freezer in the dark for further use.

The single step extraction (Figure 3.22) was performed by adapting the procedure from Section 3.2.5.6. The biomass paste (equivalent to 1 g dry biomass) was homogenized with the specified volumes of chloroform and methanol in 100 mL Duran bottle (see Table 3.11). This mixture (monophasic system) was stirred for 4 h at 760 rpm with magnetic stirrer at room temperature (25°C). The specified volume of water (Table 3.11) was then added and mixed for 1 min for forming the biphasic system. The mixture was centrifuged and the chloroform layer was collected to determine the lipid content as described in Section 3.2.5.6.

A simplex-centroid mixture design was used to optimize the solvent mixture components for the total lipid extraction from the biomass paste. Extraction solvents were mixtures of chloroform, methanol, and water. The three components of the mixed solvent were varied according to the simplex-centroid design, based on the solvent formulation of the standard extraction method (a modification of Bligh and Dyer (1959) method, Section 3.2.5.6) which used 10 mL chloroform, 10 mL methanol, and 9 mL of water. The volumes of chloroform and methanol were varied from 7 to 19 mL for each. Water was varied from 3 to 15 mL. The sum of the solvent volumes in each mixture was always 29 mL. All other factors including the extraction time (4 h), the extraction temperature (25°C), the total volume of solvent mixture (29 mL), and the amount of biomass paste (equal to 1 g of dry biomass) remained constant.

Figure 3.23 shows the design plot for the composition of the solvent mixtures. The experimental points were located at the vertices (three points) of the triangle, the three mid points of the edges of the triangle, the center point, and three axial points at halfway between the vertices and the center point of the triangle. This design generated a total of 10 points corresponding to 10 experimental solvent compositions. The experimental run order was randomized within the two separate blocks (2×10 = 20 runs in total) as shown in Table 3.11.

Figure 3.21. The wet microalgal biomass of *Nannochloropsis salina* after continuous flow centrifuging steps.

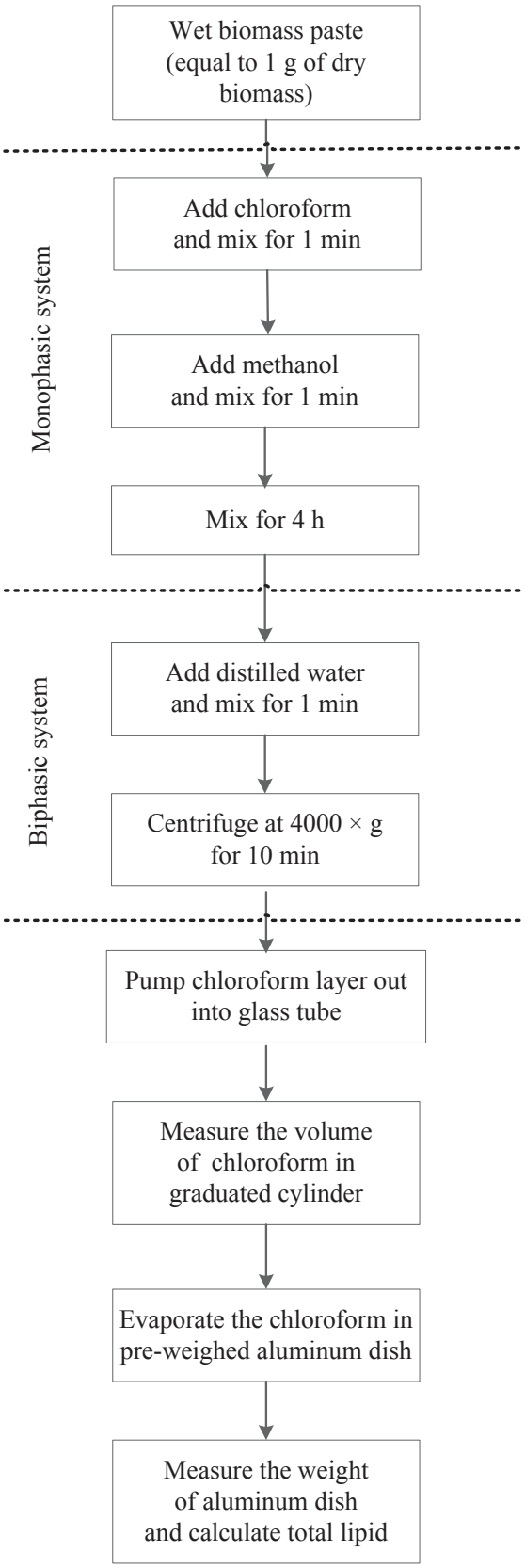


Figure 3.22. The single step extraction procedure (adapted from Section 3.2.5.6, Figure 3.11) for total lipid extraction from the biomass paste.

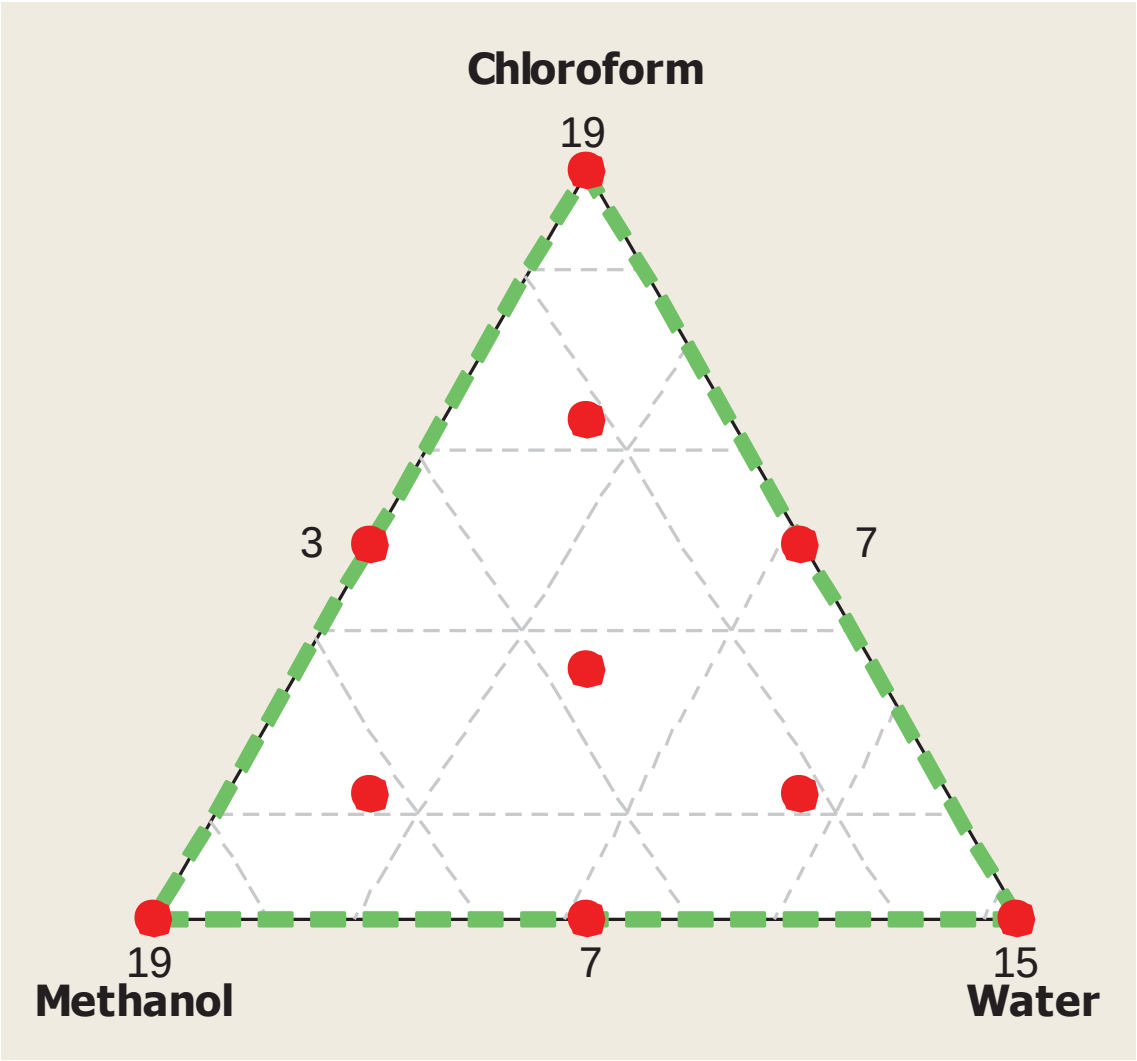


Figure 3.23. An augmented simplex-centroid design plot for amounts (mL) of the three solvents in the ternary mixture. At the top corner of the above triangle, the solvent composition is: 19 mL chloroform, 7 mL methanol, 3 mL water; at the bottom left corner, the solvent composition is: 7 mL chloroform, 19 mL methanol, 3 mL water; at the bottom right corner, the solvent composition is: 7 mL chloroform, 7 mL methanol, 15 mL water.

Table 3.11 Compositions of the solvent mixtures (mL) for the lipid extraction experiment

Block 1				Block 2			
Order	Chloroform	Methanol	Water	Order	Chloroform	Methanol	Water
A	13	13	3	K	9	9	11
B	7	7	15	L	13	7	9
C	9	9	11	M	7	7	15
D	19	7	3	N	9	15	5
E	7	19	3	O	7	13	9
F	11	11	7	P	13	13	3
G	9	15	5	Q	11	11	7
H	7	13	9	R	15	9	5
I	15	9	5	S	7	19	3
J	13	7	9	T	19	7	3

The data of the total lipid extracted (Y, % of dry biomass) were fitted to the following polynomial equation:

$$Y = b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3 \quad (3.21)$$

In this equation, b_1, b_2, b_3 are the linear coefficients estimated by the least-squares method; b_{12}, b_{13}, b_{23} are the quadratic coefficients estimated by the least-squares method; b_{123} is the

cubic coefficient estimated by the least-squares method; and x_1, x_2, x_3 are the coded values of the volumes of chloroform (mL), methanol (mL) and water (mL), respectively. The response surface graph and desirability parameters were generated for the response function to determine the optimal solvent composition for the lipid extraction. The analysis of variance (ANOVA) of the regression and the coefficient of determination (R^2) were used to compare the fit of the model to the experimental data. The replicates provided the degrees of freedom for calculation of the pure error and the lack of fit. For validation of the model, a new lipid extraction was carried out using the best identified solvent composition. The predicted and the observed values were compared using a *t*-test at a 5% significance level. The Minitab software (version 16.1.0) was used for the experimental design and all data analysis.

3.2.9.4 Optimization of the conditions for the extraction of total lipids from the biomass paste

Based on the results of the previous experiment, a solvent mixture of a suitable composition was used for total lipids extraction from the biomass paste. The following three factors were varied: the volume of the solvent mixture; the extraction time; and the extraction temperature. The biomass paste used for the extraction was a portion of the same batch as was used in the previous section. The extraction procedure was as described in Section 3.2.9.3 (Figure 3.22).

A 2^3 factorial design with center point was used to explore the influence of the three extraction factors on the total lipid extraction yield and to determine the optimum extraction conditions. Table 3.12 illustrates the extraction factors and levels including the volume of the solvent mixture (X_1), the extraction temperature (X_2), and the extraction time (X_3). These factors were varied at low, high, and center point levels. These three factors were arranged in a replicated, randomized 2^3 factorial structure with one center point added. The order of the

18 runs was completely randomised as shown in Table 3.13. Analysis of variance (ANOVA) was used to calculate the main effects and the interaction effects of factors. The coefficients of determination (R^2) were used to compare the fit of the model to the experimental data. The experimental data were fitted to the following model:

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \qquad (3.22)$$

In this equation, Y is the total lipid extracted (% of the dry weight); b_1, b_2, b_3 are the linear coefficients estimated by the least-squares method; b_{12}, b_{13}, b_{23} are the quadratic coefficients estimated by the least-squares method; b_{123} is the cubic coefficient estimated by the least-squares method; X_1, X_2, X_3 are the coded values of volume of the solvent mixture (mL), the extraction temperature (°C), and the extraction time (h), respectively. This equation allowed the determination of the optimum extraction conditions. For validation of the model, a new lipid extraction was carried out at the identified optimum conditions. The predicted and the observed values were compared using a *t*-test at a 5% significance level. The Minitab software (version 16.1.0) was used for the experimental design and the data analysis.

Table 3.12 Experimental factors and their levels in the total lipid extraction experiment

Factors	Levels		
	Low	Center point	High
X_1: Volume of the solvent mixture (mL)	25	29	33
X_2: Extraction time (h)	2	4	6
X_3: Extraction temperature (°C)	25	35	45

Table 3.13 The experimental layout of the replicated, randomized 2^3 factorial design with center point

Number	Run order	Total volume of solvent mixtures (mL)	Temperature (°C)	Time (h)
1	10	33	25	2
2	2	33	25	2
3	14	33	25	6
4	6	33	25	6
5	4	33	45	2
6	12	33	45	2
7	16	33	45	6
8	8	33	45	6
9	18	29	35	4
10	17	29	35	4
11	1	25	25	2
12	9	25	25	2
13	5	25	25	6
14	13	25	25	6
15	3	25	45	2
16	11	25	45	2
17	15	25	45	6
18	7	25	45	6

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter is divided into four major sections: (1) a characterization of growth and lipid production of the relevant microalgal species (Section 4.2); (2) batch flocculation of microalgae (Section 4.3); (3) design and characterization of the continuous flocculation–sedimentation process (Section 4.4); and (4) extraction of lipids from the wet biomass paste using solvents (Section 4.5).

4.2 Characterization of microalgae

The objective of this work was a basic characterization of growth and oil production of the selected microalgal species in photoautotrophic conditions to establish biomass and oil productivities and the suitable time to harvest the broth for flocculation work.

4.2.1 Biomass growth and lipid contents

Algae were grown photoautotrophically in 2 L Duran bottles (2 L working volume) using the normal BG11 medium or the same medium made in seawater, depending on the alga (Section 3.2.2). The typical biomass concentration growth curves starting from an initial biomass concentration of approximately 0.5 g L^{-1} are shown in Figure 4.1. The growth of all algae was consistent with the expected pattern: an initial lag phase followed by exponential growth

and eventually a stationary phase. The biomass concentration increased to approximately four to eight times the initial concentration. Depending on the microalga and the growth medium, the stationary phase was reached at different times and the final biomass concentration was different. For example, *C. vulgaris* grown in BG11 freshwater medium attained a biomass concentration of nearly 4.1±0.17 g L^{-1} by day 53 whereas, the same microalga adapted to grow in BG11 seawater medium attained a lower stationary phase biomass concentration of ~3.3±0.16 g L^{-1} by day 43 (Figure 4.1).

The batch kinetic parameters based on the data of Figure 4.1 are shown in Table 4.1. The freshwater microalga *Neochloris* sp. exhibited the highest biomass productivity of 0.08±0.02 g L^{-1} d^{-1}. *C. vulgaris* grown both in freshwater and seawater had the second highest biomass productivity (0.07±0.00 g L^{-1} d^{-1}) of the algae tested. Significantly, the biomass production of *C. vulgaris*, normally a freshwater alga, was not affected by growth in full strength seawater. For the freshwater microalgae the specific growth rates (i.e. during exponential growth phase) were generally higher than for the marine microalgae (Table 4.1). The specific growth rate of *C. vulgaris* grown in freshwater was ~50% greater than the growth rate in full strength seawater. From the perspective of oil production, the lipid productivity is of course more important than the biomass productivity (Griffiths & Harrison, 2009; Rodolfi et al., 2009; Huerlimann et al., 2010). The lipid productivity of the algae harvested at the end of the batch culture (Figure 4.1) is shown in Table 4.1. The highest lipid productivity (31.4±0.6 mg L^{-1} d^{-1}) was found for the marine alga *N. salina*. (Table 4.1). The lipid productivity of *C. vulgaris* was relatively high (Table 4.1) and was not significantly affected by whether the alga was grown in seawater or freshwater (Table 4.1).

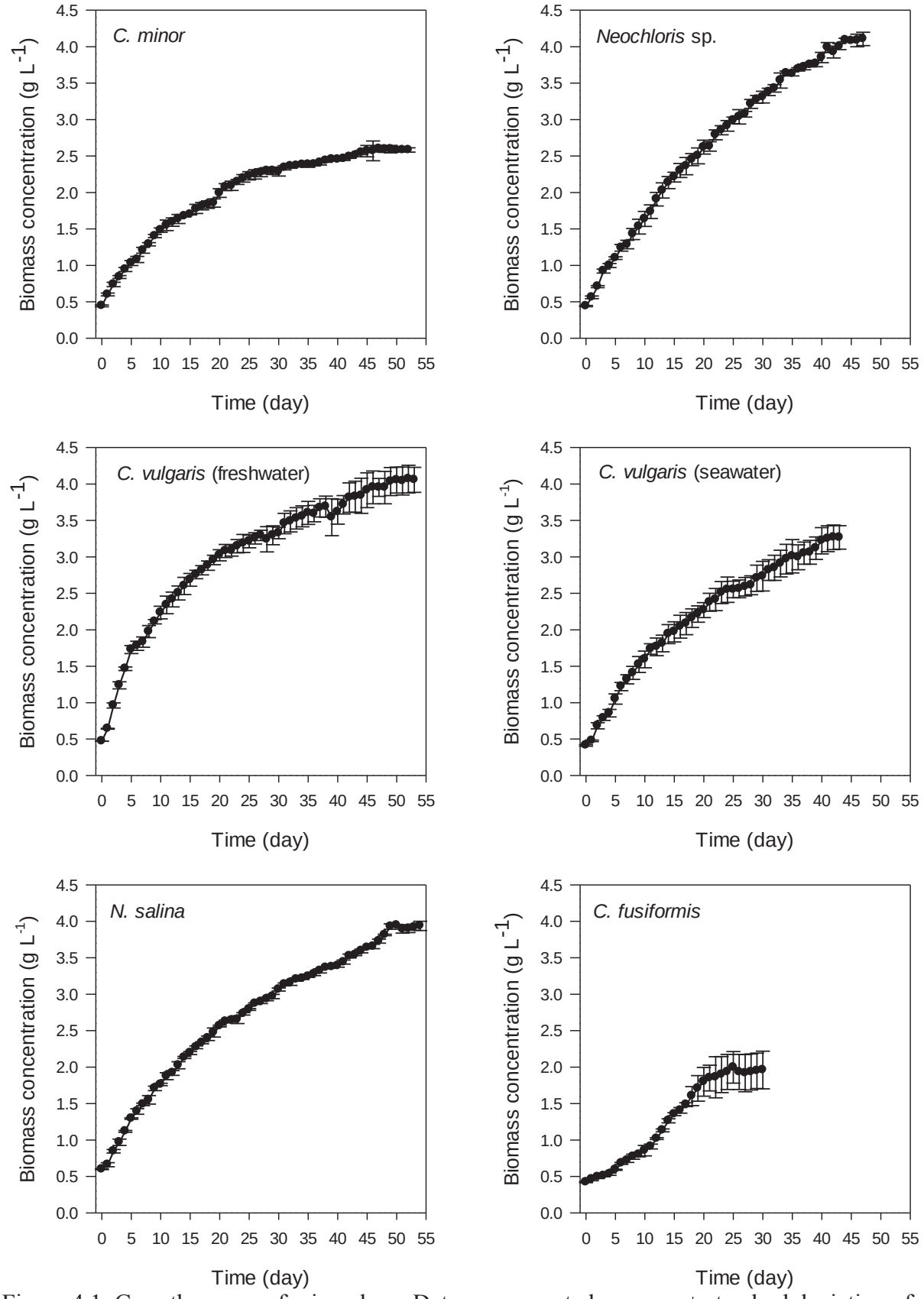


Figure 4.1. Growth curves of microalgae. Data are presented as mean ± standard deviation of triplicate runs.

Table 4.1. The productivity of biomass and lipids in 2 L Duran bottles.

Species	Harvesting day (day)	Biomass productivity* ($g\ L^{-1}\ d^{-1}$)	Specific growth rate* (μ, d^{-1})	Lipid content* (% w/w)	Lipid productivity* ($mg\ L^{-1}\ d^{-1}$)
Grown in the BG11 freshwater medium					
Choricystis minor	52	0.04±0.00 ^{d}	0.208±0.002 ^{bc}	30.1±1.8 ^{d}	11.6±0.7 ^{c}
Neochloris sp.	47	0.08±0.02 ^{a}	0.222±0.003 ^{b}	17.5±1.1 ^{f}	13.7±1.2 ^{c}
Chlorella vulgaris	53	0.07±0.00 ^{b}	0.306±0.007 ^{a}	33.2±1.7 ^{c}	22.4±1.2 ^{b}
Grown in the BG11 seawater medium					
Chlorella vulgaris	43	0.07±0.00 ^{b}	0.201±0.008 ^{c}	36.1±1.1 ^{b}	23.9±0.6 ^{b}
Nannochloropsis salina	54	0.06±0.00 ^{b}	0.176±0.013 ^{d}	50.9±0.1 ^{a}	31.4±0.6 ^{a}
Cylindrotheca fusiformis	30	0.05±0.01 ^{c}	0.104±0.014 ^{e}	23.9±2.4 ^{e}	11.4±2.2 ^{c}

* Data are the means ± SD of triplicate experiments.

The mean value followed by different letters in a given column differ significantly ($p < 0.05$).

In addition to lipid productivity, the lipid weight fraction in the dry biomass is important. This is because less biomass needs to be extracted for a given amount of lipid if the biomass has a high lipid content. The lipid content of the microalgae at harvest ranged from 17.5% to 50.9% by dry weight, depending on the alga (Table 4.1). The marine alga *N. salina* had the highest lipid content of 50.9±0.1% by dry weight. The microalga *C. vulgaris* had a relatively high lipid content of >30% by dry weight (Table 4.1). Although the specific growth rate of *C. vulgaris*, a freshwater alga, was reduced in the seawater medium (Table 4.1) the biomass grown in seawater had a significantly higher lipid content (36.1±1.1%) compared to the biomass grown in freshwater (lipid content of 33.2±1.7%; Table 4.1). As a consequence, the lipid productivity in seawater was essentially the same as in freshwater growth (Table 4.1).

In view of a high lipid productivity and a high lipid content in the biomass, the two algae of greatest interest were *N. salina* and *C. vulgaris* (Table 4.1). In other studies *N. salina* and *C. vulgaris* have also been reported to have high lipid productivity and lipid content (Rodolfi et al., 2009; Doan et al., 2011; Yeh & Chang, 2012). Rodolfi et al. (2009) have suggested *Nannochloropsis* as being the best lipid producer of the several species tested. Of course, for all microalgae the lipid content of the biomass depends on the specific strain, the growth conditions (light, temperature, salinity), the growth phase, and the levels of certain nutrients (Hu et al., 2008; Mata et al., 2010).

4.2.2 Microalgal morphology and zeta potential

The morphology (cell size and shape) of the algal cell and its surface charge (zeta potential) affect flocculation-sedimentation behavior of the biomass. Cell morphology and charge depend on the microalgal species, the cultivation conditions and the age of the cell (Pahl et

al., 2013b). For this study, light microscopy was used to characterize the cell morphology and size at various stages of the cultivation process. Figure 4.2 and Figure 4.3 show the images of the various algae on day 7 (exponential phase) of culture and on the day of harvest (i.e. the stationary phase). Five of the studied microalgae species comprised of single spheroidal cells that were grass-green in color mainly due to the presence of chlorophyll (Henderson et al., 2008b). The marine diatom *C. fusiformis* had the shape shown in Figure 4.3 and had an orange-brown color likely because of a high concentration of carotenoids in the cell (Friedl et al., 2012).

The cell size and the cell surface area depended on the microalgal species, the growth medium and the stage of cultivation (Table 4.2). On the harvesting day, *C. minor* cells were the smallest with an average diameter of 2.9±0.2 µm. *C. fusiformis* cells were the biggest with an average length of 43.3±4.9 µm and width of 6.2±1.2 µm. For *C. vulgaris*, the average cell diameter for freshwater growth was 5.1±0.5 µm whereas for growth in seawater medium the diameter was 8.1±1.3 µm. The larger cell size of *C. vulgaris* in a hypersaline medium is likely explained by synthesis and accumulation of carbohydrates such as glycerol. An increased concentration of such materials within a cell increases the intracellular osmotic pressure to prevent dehydration of the cell in a hypersaline medium (Becker, 1995).

For all algae the average cell diameter and the cell surface area were larger in the stationary phase in comparison with the exponential phase. Zhang et al. (2012) reported that the cell size of *Chlorella zofingiensis* grown in modified BG-11 medium was larger in the declining phase compared with the size in the exponential growth phase. During exponential growth a cell increases in size though the synthesis of cell components but then soon divides into smaller daughter cells (Becker, 1995). This limits the average cell size during exponential growth. Cell concentration in a broth increases progressively as growth occurs.

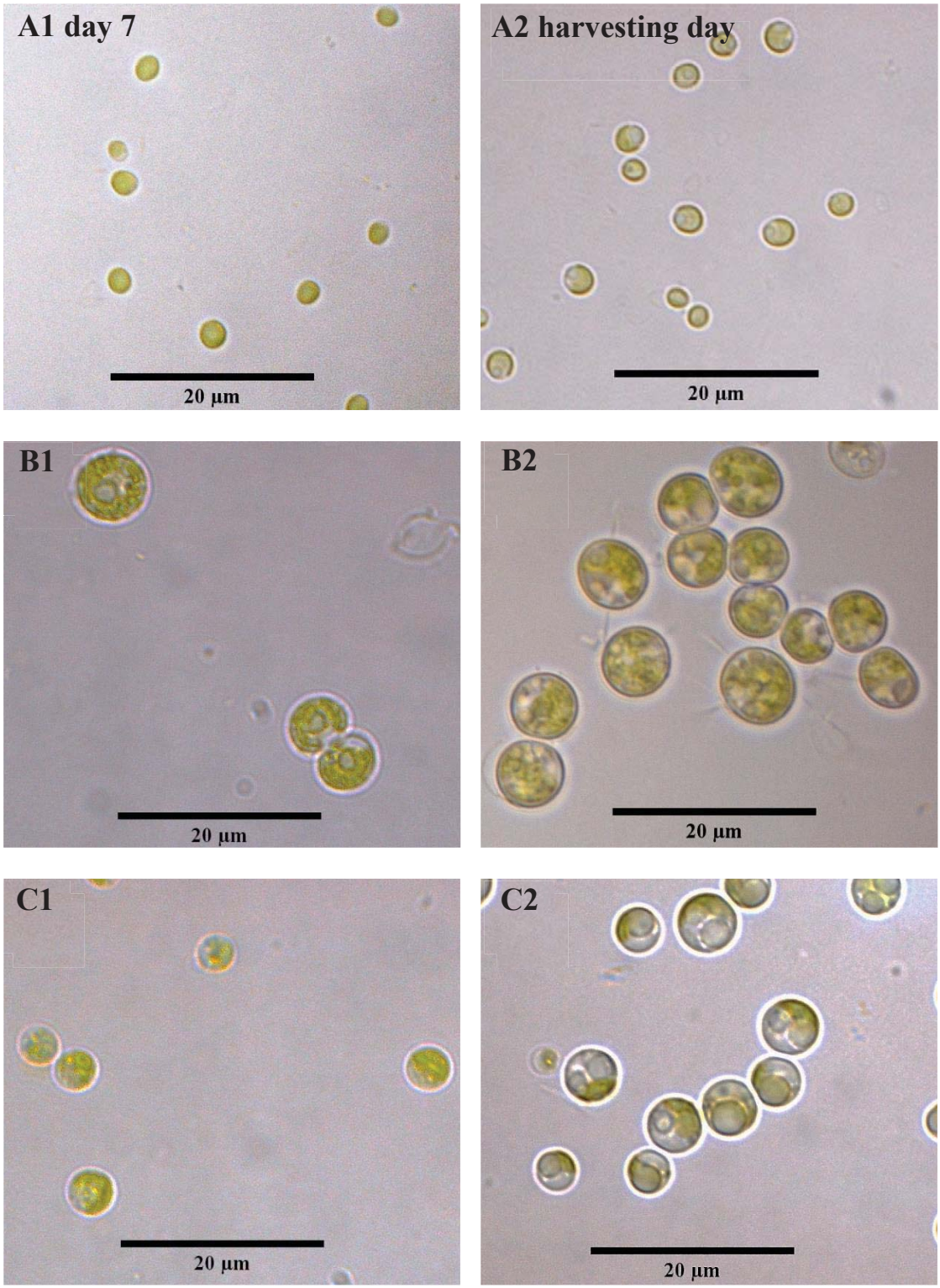


Figure 4.2. Microscopic images of microalgae on day 7 (A1, B1, C1) and harvesting day (A2, B2, C2; Figure 4.1): *C. minor* (A1 and A2), *Neochloris* sp. (B1 and B2), *C. vulgaris* (freshwater) (C1and C2).

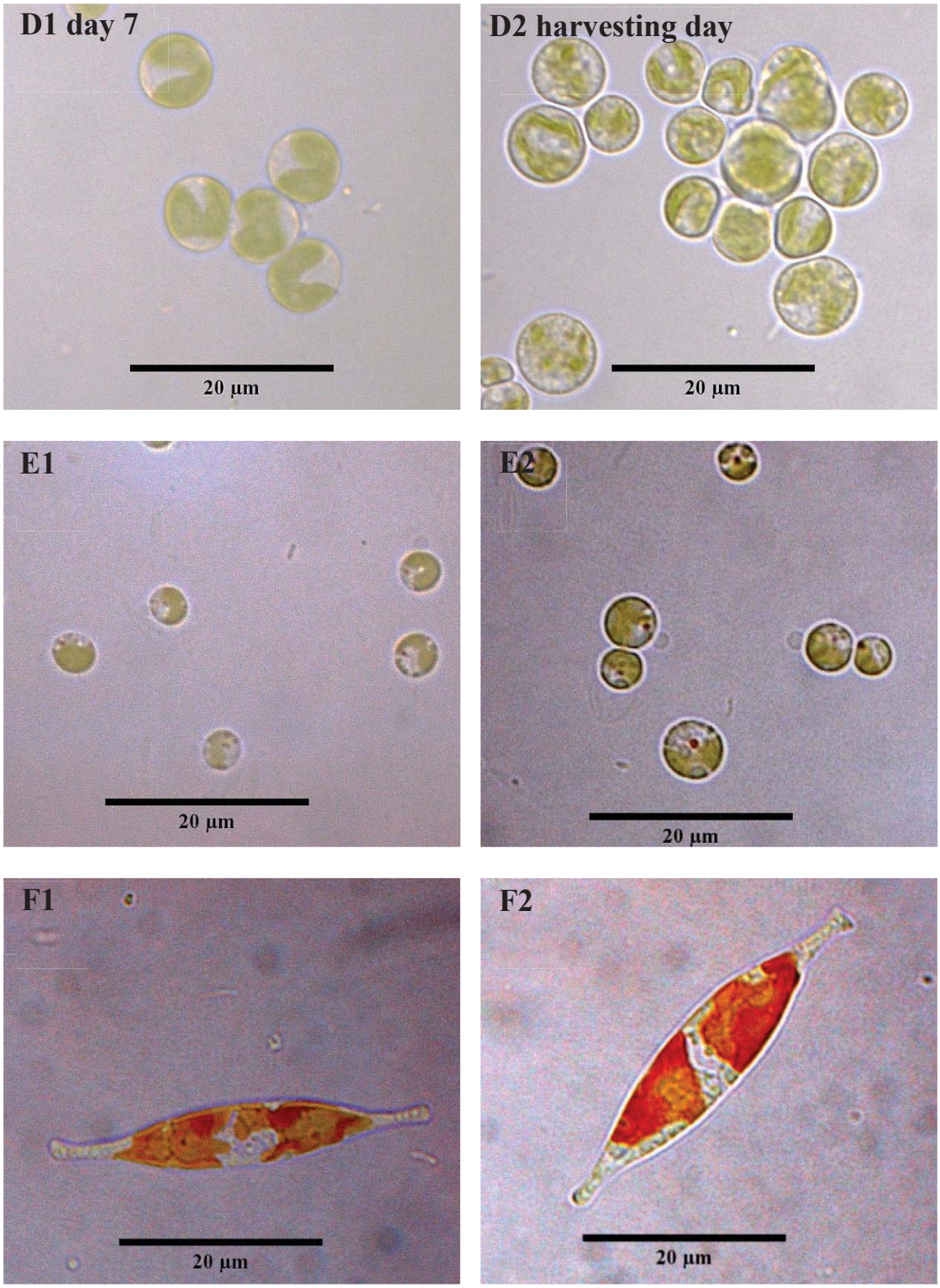


Figure 4.3. Microscopic images of microalgae on day 7 (D1, E1, F1) and harvesting day (D2, E2, F2; Figure 4.1): *C. vulgaris* (seawater) (D1 and D2), *N. salina* (E1 and E2) *C. fusiformis* (F1and F2).

Table 4.2 Characteristics of microalgae

Species	Harvesting day (day)	Cell size [a] (μm)		Cell surface area [a] (μm^2 $cell^{-1}$)		Zeta potential [b] (mV) at pH 7.5	
		Day 7	Harvesting day	Day 7	Harvesting day	Day 7	Harvesting day
BG11 freshwater medium							
Choricystis minor	52	Diameter 2.3±0.2	Diameter 2.9±0.2	16.4±3.1	25.9±3.6	–28.9±0.2	–23.2±0.9
Neochloris sp.	47	Diameter 6.4±0.8	Diameter 6.9±0.6	129.3±30.7	151.9±24.6	–16.5±1.5	–15.3±1.6
Chlorella vulgaris	53	Diameter 3.8±0.5	Diameter 5.1±0.5	47.3±13.2	83.3±15.0	–26.6±1.4	–15.2±0.6
BG11 seawater medium							
Chlorella vulgaris	43	Diameter 6.8±1.3	Diameter 8.1±1.3	148.3±54.7	211.2±75.6	–16.2±0.5	–15.3±1.2
Nannochloropsis salina	54	Diameter 4.4±0.4	Diameter 4.8±0.4	60.2±11.5	73.4±11.4	–14.5±0.8	–13.3±1.2
Cylindrotheca fusiformis	30	Length 39.7±4.6 Width 5.6±1.0	Length 43.3±4.9 Width 6.2±1.1	350.4±89.3	447.5±83.9	–16.8±0.3	–15.6±0.4

[a] Data are mean values ± SD of measurements on 100 distinct cells.

[b] Data are mean values ± SD of triplicate measurements.

At a high concentration, cells of some algae have a tendency to clump (Danquah et al., 2009b). This was seen in the stationary phase (Figure 4.2, Figure 4.3) for *Neochloris* sp., *C. vulgaris* (freshwater), *C. vulgaris* (seawater) and *C. fusiformis*. The stationary phase is often associated with the production of extracellular polymers and these tend to cause the cells to adhere (Henderson et al., 2008a; Henderson et al., 2008c).

Microalgal cell surface charge affects the stability of a cell suspension. The surface charge also affects the dosage of an ionic flocculant required to produce effective flocculation. The microalgal cell surface charge results in a mutual repulsion force that keeps the cells apart. A small cell is harder to settle under gravity and tends to remain in suspension. The magnitude of this surface charge is a function of the microalgal species, the ionic strength of the medium, the pH, and possible other environmental conditions (Shelef et al., 1984). For all algae the zeta potential was negative (Table 4.2) indicative of a negative surface charge. The surface charge at harvesting day varied between −13.3 to −23.2 mV (Table 4.2). Microalgae cells are known to have a net negative surface charge (Bilanovic et al., 1988; Henderson et al., 2008a; Lee et al., 2009a; Sukenik et al., 1988; Tenney et al., 1969; Uduman et al., 2010) at neutral and alkaline pH values. The source of the cell surface charge is the ionization of functional groups such as -COOH (Shelef et al., 1984; Henderson et al., 2008c) that occur with a high frequency in carbohydrate-based cell walls. Therefore, the negative measured zeta potential values were consistent with expectation.

The stage of the life cycle of a microalgal cell also appears to influence its zeta potential (Henderson et al., 2008c). This is likely because the composition of the cell wall can vary with age. As shown in Table 4.2, the zeta potential values of the cells of some microalgal species were substantially more negative during exponential growth compared to the zeta potential values during the stationary phase. Therefore, at least for some algae, the

intercellular repulsion forces were lower for aged cells and the cell suspension may have been somewhat more unstable at harvest compared to during exponential growth. Danquah et al. (2009b) reported that the zeta potential values of the microalgal cells were –43.2±0.7 mV during rapid growth compared to values of –34.5±0.4 mV in the slower growth rate phase for a mixed culture of the algae *Tetraselmis suecica* and *Chlorococum* sp. No clear explanation was advanced for these observations. Zhang et al. (2012) associated the change in the zeta potential values during growth with change in the surface density of ionizable functional groups. The microalgal cell suspension in the broth was more stable during exponential growth than in the stationary and declining phases (Zhang et al., 2012) in keeping with some of the observations of the present work.

4.3 Microalgal flocculation

Five microalgal species were used for flocculation experiments at different biomass concentrations (Section 3.2.7). Various doses of two flocculants (aluminum sulfate, ferric chloride) were investigated under the standardized treatment regimen (Section 3.2.7) for identifying the optimal flocculant dosage. The optimal dose was defined as the lowest flocculant dosage for achieving a 95% removal of the microalgal cells from the culture broth. The two flocculants tested are widely used commercially in large scale water treatment processes where they have been found to be effective and relatively inexpensive (Grima et al., 2003a; Bratby, 2006; Knuckey et al., 2006). Also, these flocculants are safe and have a low environmental impact.

The addition of multivalent metal ion flocculants to a microalgal suspension tends to reduce or neutralize the negative surface charge that prevents the cells from coming together (Becker, 1995; Grima et al., 2003a; Grima et al., 2003b). The metal cations (e.g. Al^{3+}, Fe^{3+}) may also bridge cells which results in aggregation into larger particles that settle out of

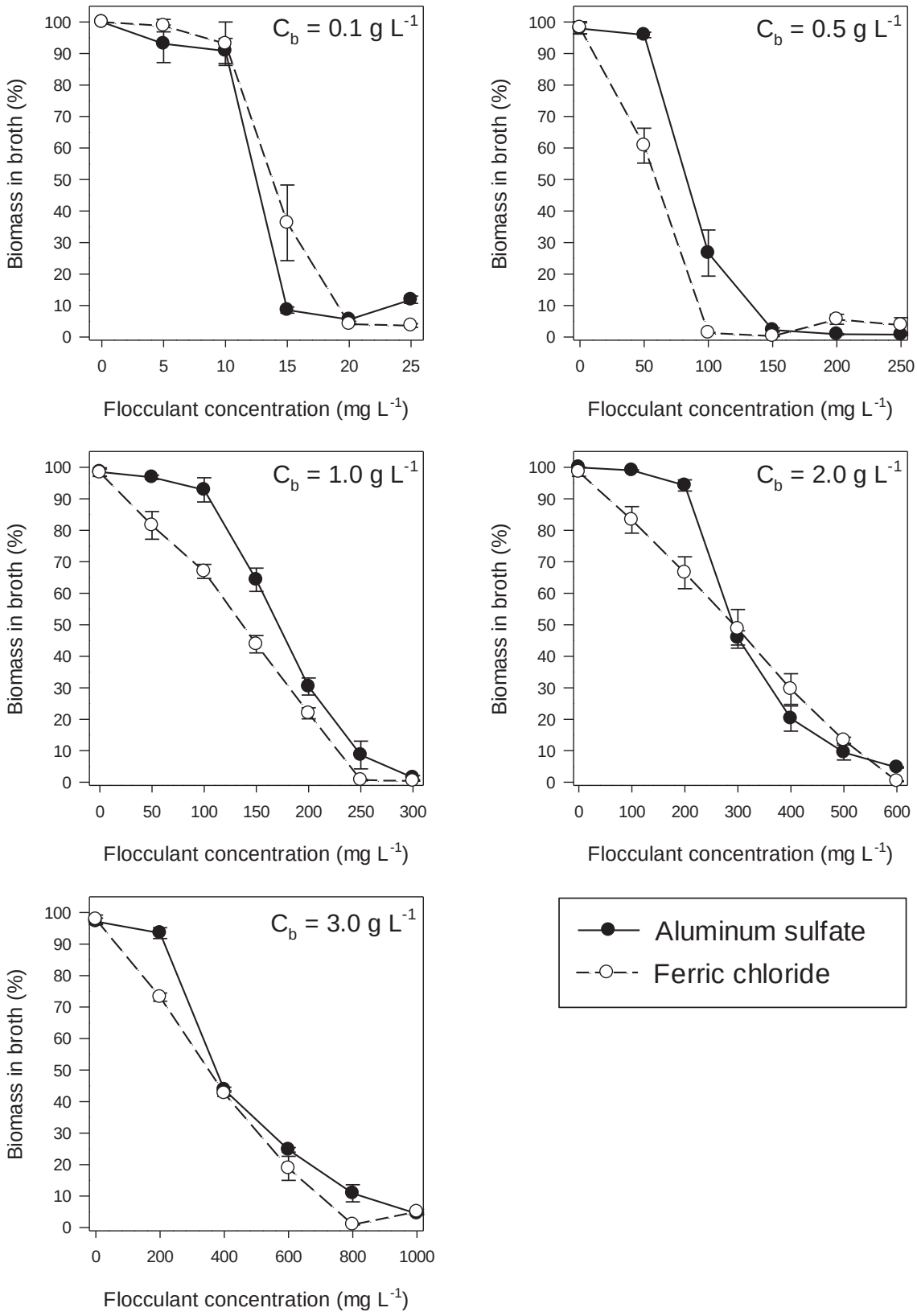


Figure 4.4. Flocculation of *C. minor* with aluminum sulfate and ferric chloride at various initial biomass concentrations (C_b). Data are mean values ± SD of triplicate experiments.

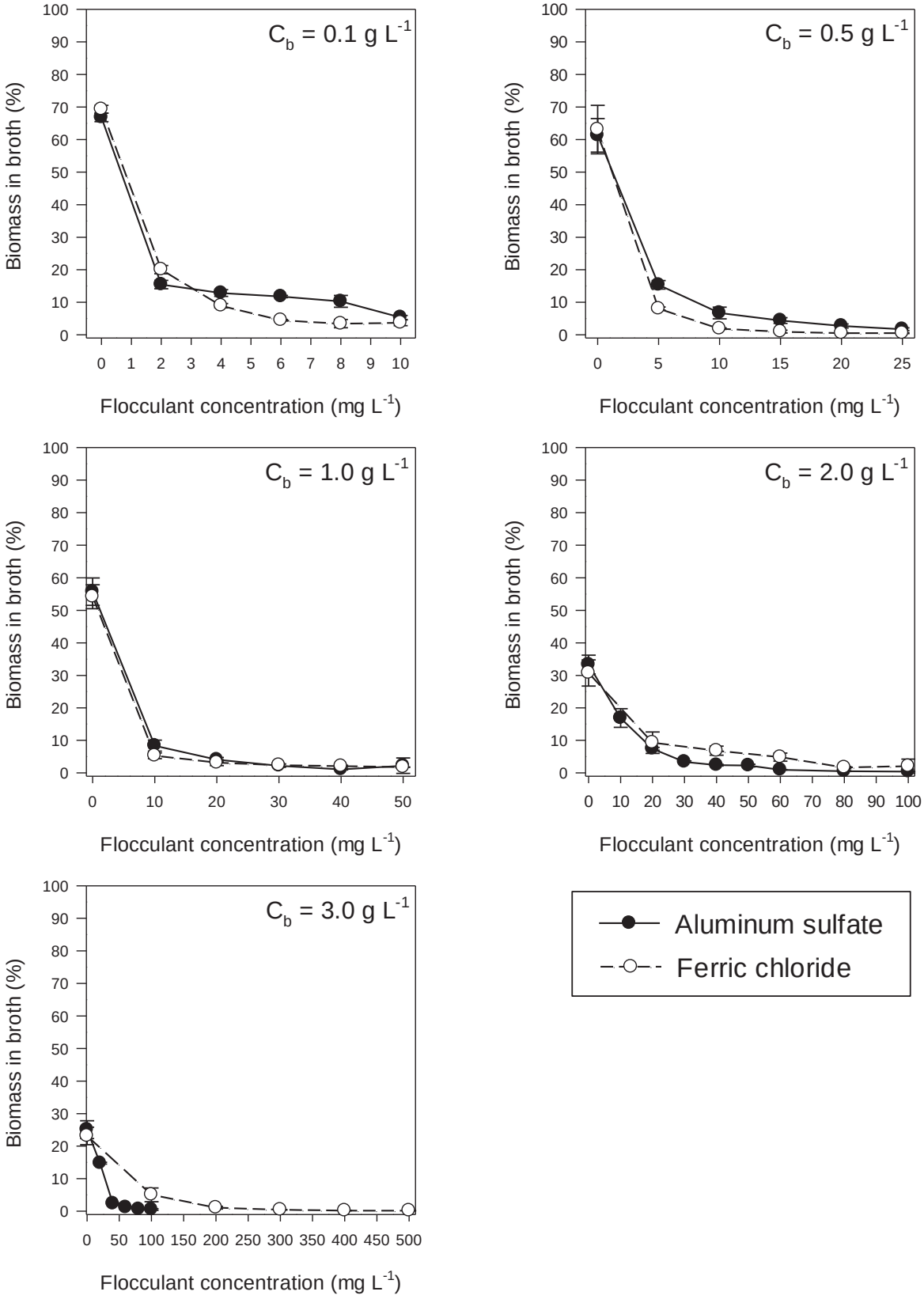


Figure 4.5. Flocculation of *Neochloris sp.* with aluminum sulfate and ferric chloride at various initial biomass concentrations (C_b). Data are mean values ± SD of triplicate experiments.

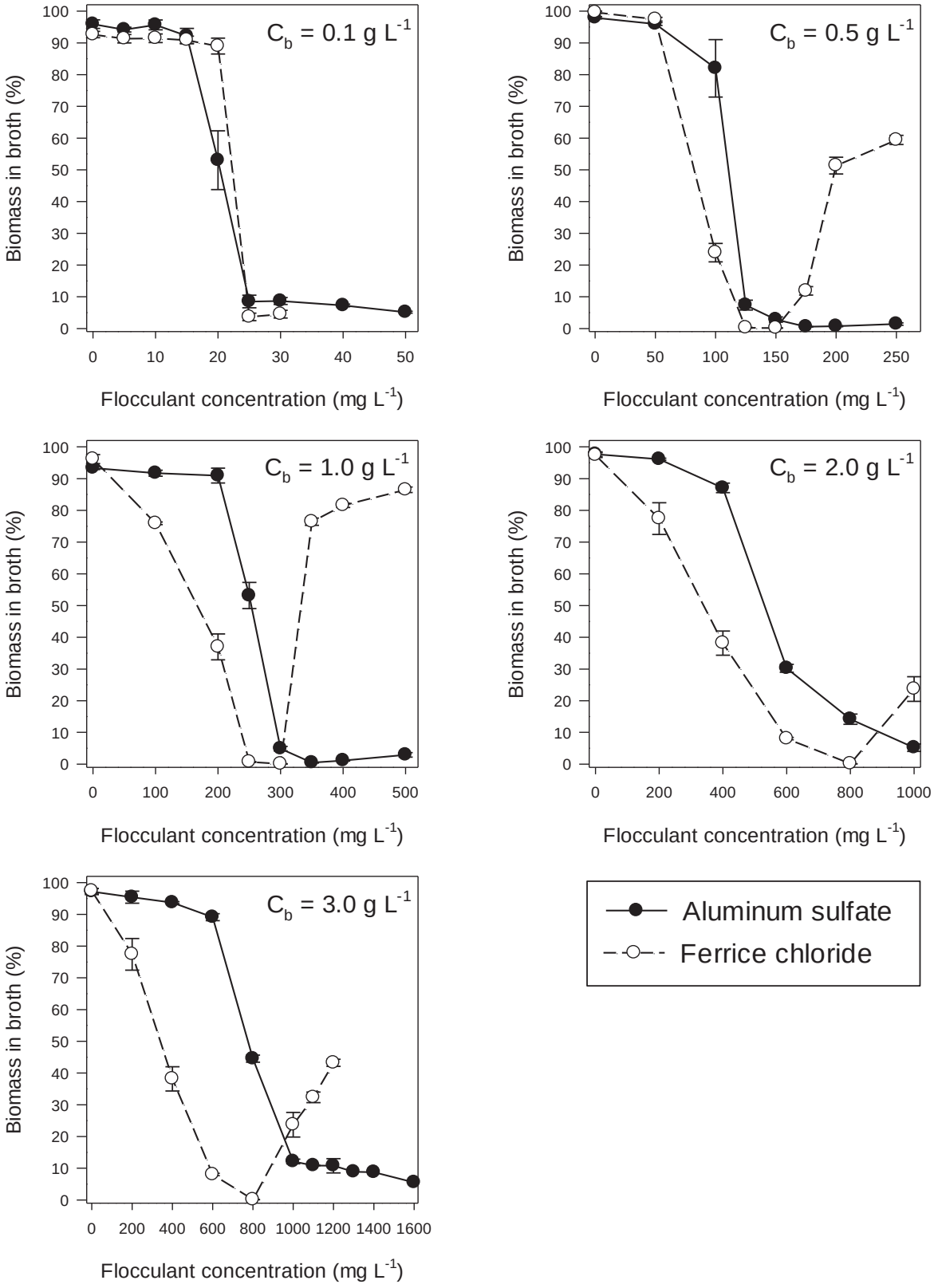


Figure 4.6. Flocculation of *C. vulgaris* (freshwater) with aluminum sulfate and ferric chloride at various initial biomass concentrations (C_b). Data are mean values ± SD of triplicate experiments.

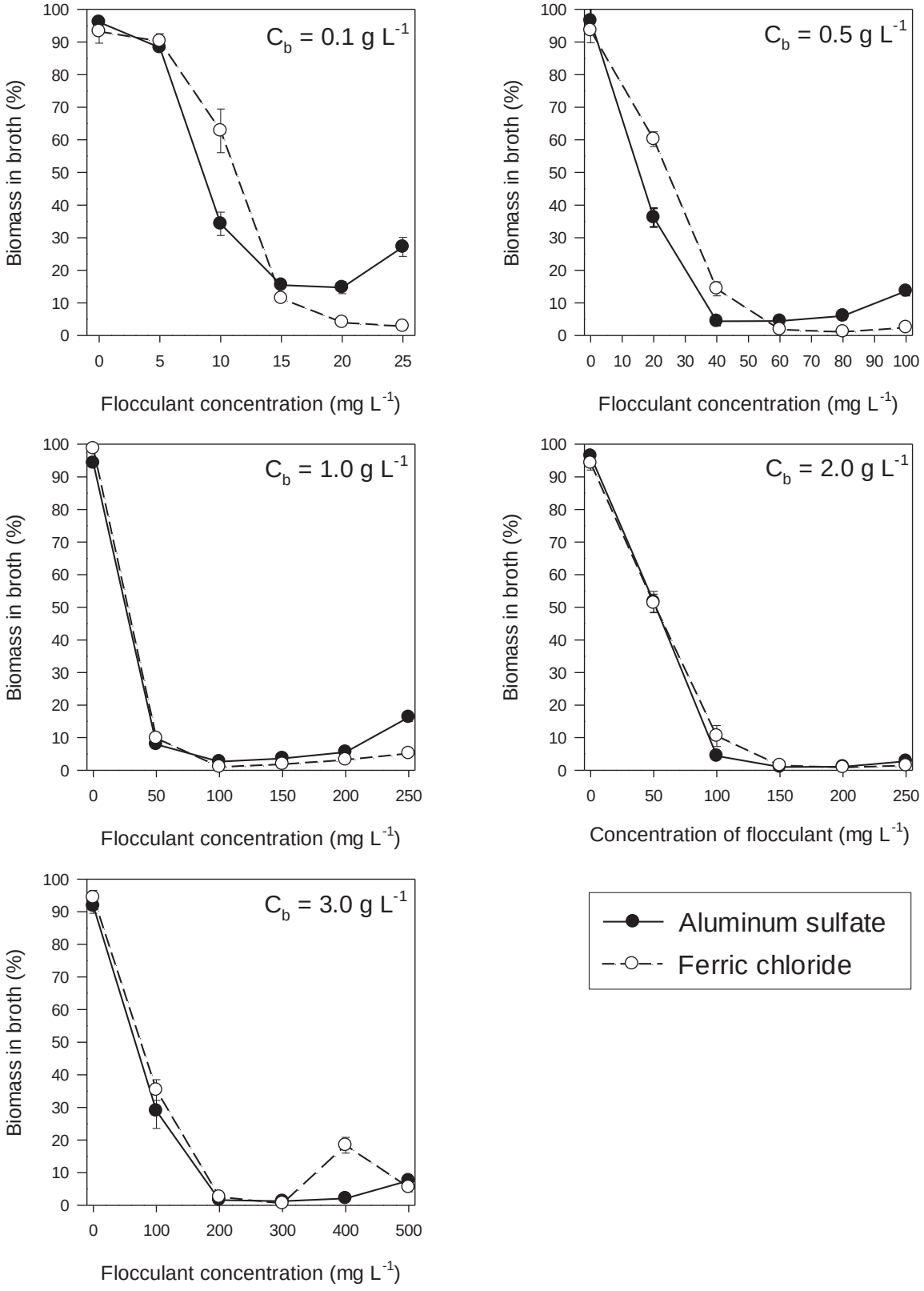


Figure 4.7. Flocculation of *C. vulgaris* (seawater) with aluminum sulfate and ferric chloride at initial various biomass concentrations (C_b). Data are mean values ± SD of triplicate experiments.

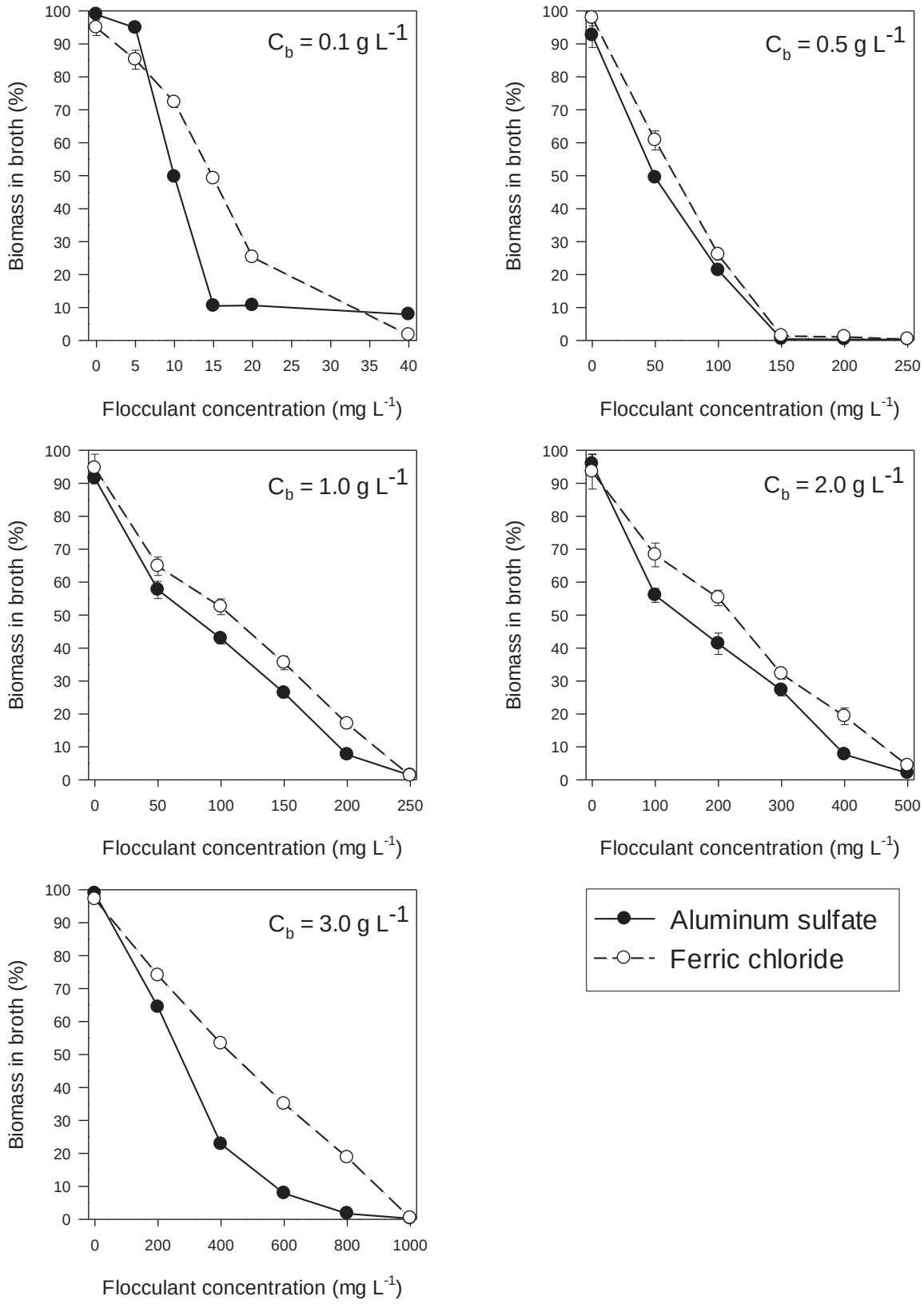


Figure 4.8. Flocculation of *N. salina* with aluminum sulfate and ferric chloride at various initial biomass concentrations (C_b). Data are mean values ± SD of triplicate experiments.

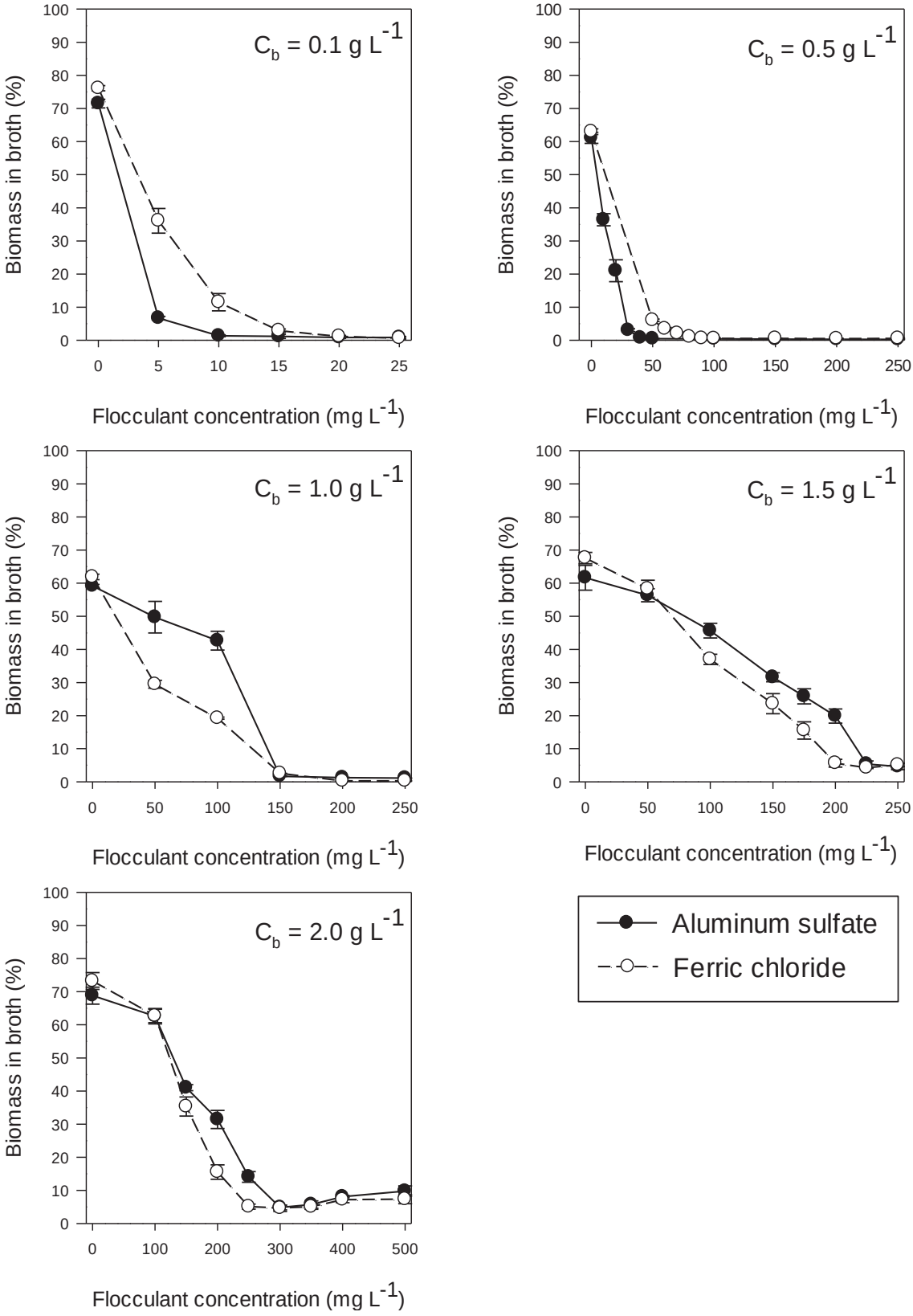


Figure 4.9. Flocculation of *C. fusiformis* with aluminum sulfate and ferric chloride at various initial biomass concentrations (C_b). Data are mean values ± SD of triplicate experiments.

suspension (Knuckey et al., 2006; Papazi et al., 2010; Salim et al., 2011) relatively easily. Figure 4.4 to Figure 4.9 show the percentage of the microalgal cells remaining in the suspension after the standardized treatment (Section 3.2.7.2) with various doses of the flocculants applied to algal broth with different initial biomass concentrations.

4.3.1 Effect of flocculant dose on microalgal flocculation

Both the flocculants tested were able to flocculate and remove nearly all the biomass of all the algae from the culture media (Figure 4.4 to Figure 4.9), but the flocculants had different efficacies in different cases. For a given initial biomass concentration, an increasing amount of the biomass was removed as the flocculant dosage increased. For example, for the freshwater microalga *C. minor*, at an initial biomass concentration of 1.0 g L^{-1} (Figure 4.4), >95% of the biomass could be removed from suspension by flocculation with 250 mg L^{-1} of ferric chloride or 275 mg L^{-1} of aluminum sulfate.

For the same alga, only ~50% of the biomass could be removed from the culture broth by flocculation with 140 mg L^{-1} of ferric chloride or 180 mg L^{-1} of aluminum sulfate. An increasing concentration of the algal biomass in the broth increased the flocculant dosage for a given extent of removal because more surface charge needed to be neutralized at the higher biomass concentration (Granados et al., 2012). Therefore, at a given initial biomass concentration, a certain minimum dose was required to achieve a complete removal of the biomass (Lee et al., 1998a). In addition, the minimum required dose depended on the algal species and the flocculant.

4.3.2 Effect of type of flocculant on microalgal flocculation

The type of flocculant used for flocculation also affected the biomass recovery. For *C. vulgaris* (seawater) (Figure 4.7) and *N. salina* (Figure 4.8), aluminum sulfate was a more efficient flocculant than ferric chloride at all biomass concentrations (Figure 4.7 and 4.8), as a lower dose of aluminum sulfate was required for 95% removal of the biomass following the standardized flocculation treatment (Section 3.2.7.2). On the order hand, for *C. minor* (Figure 4.4) and *C. vulgaris* (freshwater) (Figure 4.6), ferric chloride was more efficient flocculant than aluminum sulfate for the entire range of biomass concentrations. For *Neochloris* sp. (Figure 4.5), ferric chloride was more efficient than aluminum sulfate at biomass concentrations of 0.1 g $L^{-1}$and 0.5 g L^{-1}, but at biomass concentrations of 1.0 g L^{-1}, 2.0 g L^{-1} and 3.0 g L^{-1}, lower doses of aluminum sulfate were needed. For *C. fusiformis* (Figure 4.9), at low biomass concentrations (≤0.5 g L^{-1}), aluminum sulfate was more efficient than ferric chloride but the situation was reversed at biomass concentrations of ≥1.0 g L^{-1}. Therefore, the type of flocculant, the type of alga, the ionic strength of medium and the biomass concentration in the slurry all affected the efficacy of flocculation.

In cases where aluminum sulfate appeared to be a somewhat better flocculant compared to ferric chloride (Figures 4.5, 4.7, 4.8), it was actually much better than the data in the relevant figures suggest. This was because for an equal mass of the two salts (mass concentration for aluminum sulfate is based on the formula $Al_2(SO_4)_3$; mass concentration of ferric chloride is based on the formula $FeCl_3$), aluminum sulfate provided a slightly lower molar concentration of the cation (i.e. Al^{3+}) than did ferric chloride. Yet, a lower concentration of aluminum sulfate was needed compared to the concentration of ferric chloride.

This suggests that in some cases at least, Al^{3+} is a better flocculant than Fe^{3+}. The ionic radii of Al^{3+} and Fe^{3+} are 0.050 nm and 0.064 nm, respectively. This means that Al^{3+} has a higher surface charge density than Fe^{3+} and this likely explains the better flocculation performance of Al^{3+} compared to Fe^{3+}. The surface charge density of Al^{3+} and Fe^{3+} are 95.5 nm^{-2} and 58.0 nm^{-2}, respectively. The difference in the flocculation performance of the two salts may also relate to their different counterions. The high charge density of Al^{3+} likely improves its ability to bridge cells and neutralize the surface charge. In the cases in which ferric chloride was a better flocculant than aluminum sulfate (Figures 4.4, 4.6 and 4.9), a better performance may be linked to the counterion. Papazi, et al. (2010) noted that chloride salts were more efficient in flocculation than the corresponding sulfate salts. This is because chloride anions are more soluble in water than the sulfate anions, and chloride salts also have a good solubility over a wider concentration range when compared to the sulfate salts.

Although the flocculation efficiency is often expressed in terms of mass (e.g. g L^{-1} or mg L^{-1}) of flocculants (Sukenik et al., 1988; Henderson et al., 2008a; Danquah et al., 2009a; Papazi et al., 2010; Vandamme et al., 2010; Granados et al., 2012), it ought to be expressed in terms molar concentrations (mole of M^{+3} L^{-1}) as shown in Figures 4.10-4.15 for the same data as in Figures 4.4 to 4.9. In comparing different flocculants, expressing the dosage in terms of the molar concentration of the metal ion is essential in order to clearly identify a superior flocculant. The graphs in Figures 4.10-4.15 confirm that in almost all cases aluminum sulfate is a more efficient flocculant than ferric chloride in view of the higher surface charge density of Al^{3+}. When flocculation is performed with aluminum sulfate, fewer moles of Al^{3+} are required compared to the moles of Fe^{3+}.

In Figure 4.6 and Figure 4.12, the data for ferric chloride show an apparent increase in the percentage of the biomass in the broth with an increase in ferric chloride concentration

after the point at which all the biomass had been flocculated. This is an artefact. Addition of ferric chloride to the algal suspension resulted in adsorption of Fe^{3+} to the biomass until essentially all the biomass was removed. Up to this point the supernatant was colorless. However, once all the biomass had flocculated and the metal adsorption sites on the biomass had been occupied by Fe^{3+}, a further addition of ferric chloride led to the Fe^{3+} appearing in the supernatant and imparting an increasingly brown ting to it. As a consequence, the spectrophotometric absorbance increased even though there was no suspended biomass in the broth.

4.3.3 Effect of microalgal species on flocculation

Flocculant dose required for optimal flocculation (i.e. 95% biomass removal after the standardized flocculation treatment, Section 3.2.7.2) was different for different microalgal species. Both the flocculants were more efficient in harvesting of *Neochloris* sp. (Figure 4.5), *C. vulgaris* (seawater) (Figure 4.7) and *C. fusiformis* (Figure 4.9) than in harvesting the other species. For example, at a biomass concentration of 1.0 g L^{-1} of *Neochloris* sp., the optimal flocculant doses were 25 mg L^{-1} for aluminum sulfate and 55 mg L^{-1} for ferric chloride. At the same biomass concentration, the optimum flocculant doses for *C. vulgaris* (seawater) and *C. fusiformis* were 75 mg L^{-1} of aluminum sulfate, or ferric chloride, and 150 mg L^{-1} of aluminum sulfate, or ferric chloride.

In contrast, the other microalgae required >200 mg L^{-1} of a flocculant to achieve a 95% removal of the microalgal cells from the culture broth (Figures 4.4, 4.6, 4.8). A lower requirement of a flocculant for the microalgae *Neochloris* sp., *C. vulgaris* (seawater) and *C. fusiformis* may have been because the cells of these algae were larger compared to the cells of the others (Table 4.2). For algae with larger cells, the control treatment (no flocculant added)

could sediment a certain fraction (e.g. 30%) of the biomass initially in the broth. This was especially so for the microalgae *Neochloris* sp. (Figure 4.11) and *C. fusiformis* (Figure 4.15).

Furthermore, the microscopic images (Figures 4.2, 4.3) showed clearly that in the stationary phase the cells of these algae were already close together or clumped. Consequently, these microalgal species required a lower concentration of flocculant than did *C. minor*, *C. vulgaris* (freshwater) and *N. salina*. In a similar vein, Vandamme et al. (2010) noted that *Scenedesmus* sp., an alga with relatively large cells, required a lower dosage of a polymeric flocculant for effective flocculation compared to the dose needed for the smaller cells of the alga *Parachlorella* sp. In addition, the differences in the chemical composition of the cell envelopes can be a factor in affecting the flocculant dose requirement for flocculation (Bratby, 2006).

For *C. vulgaris*, the only microalga that could be grown both in freshwater and seawater, the flocculant dose was much lower in seawater than in freshwater at any given initial concentration of the biomass in the broth (Figures 4.6 and 4.7). The presence of other multivalent metal ions (e.g. Ca^{2+}, Mg^{2+}) in seawater may have reduced the net negative charge on the surface of the microalgal cells suspended in seawater and this potentially explains the lower flocculant dosage requirement in seawater.

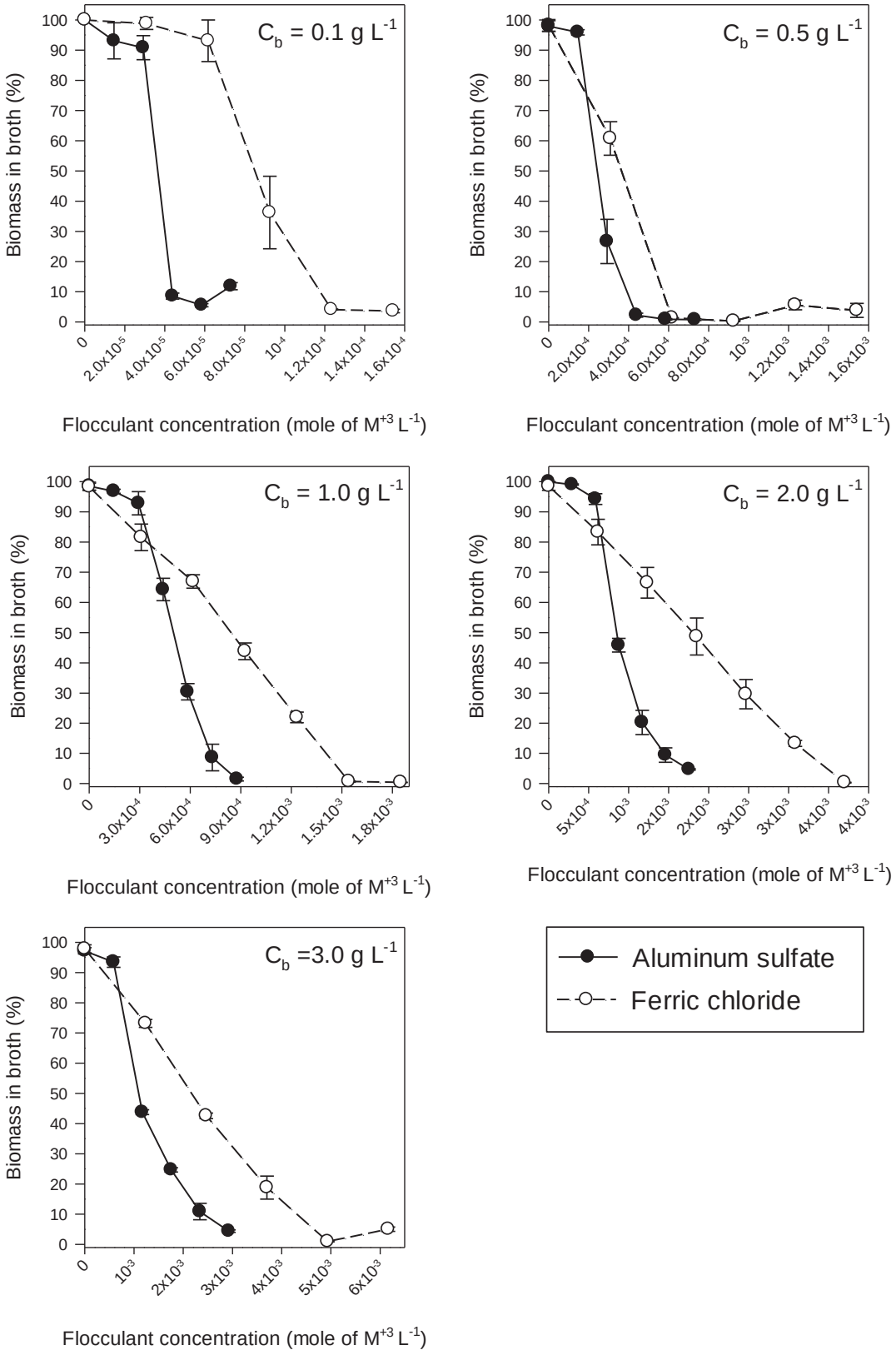


Figure 4.10. Flocculation of *C. minor* with aluminum sulfate and ferric chloride in terms of molar concentration (M^{3+} L^{-1}) of the metal ion (M^{3+}) at various initial biomass concentrations (C_b). Data are mean values ± SD of triplicate experiments.

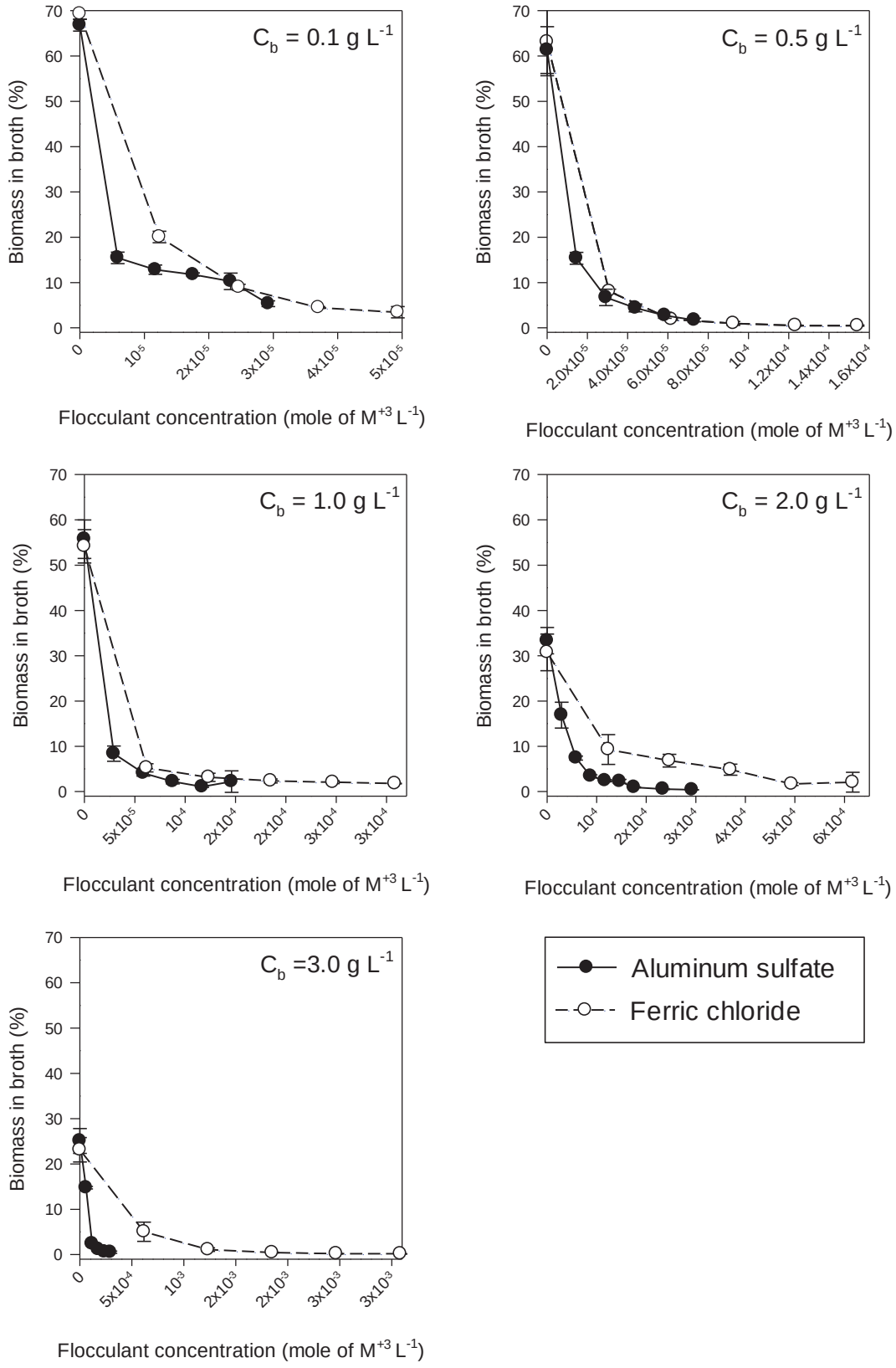


Figure 4.11. Flocculation of *Neochloris* sp. with aluminum sulfate and ferric chloride in terms of molar concentration (M^{3+} L^{-1}) of the metal ion (M^{3+}) at various initial biomass concentrations (C_b). Data are mean values ± SD of triplicate experiments.

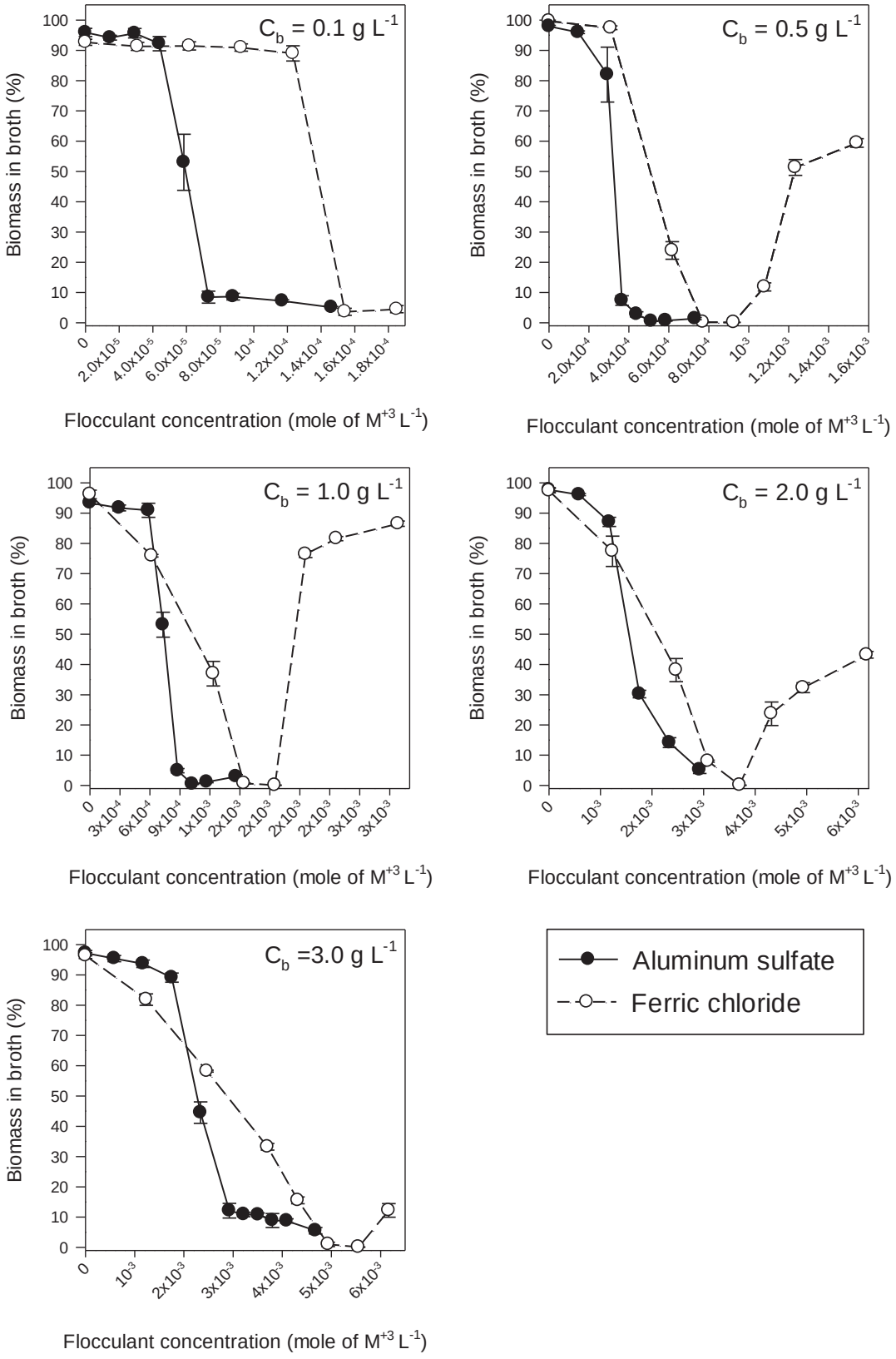


Figure 4.12. Flocculation of *C. vulgaris* (freshwater) with aluminum sulfate and ferric chloride in terms of molar concentration (M^{3+} L^{-1}) of the metal ion (M^{3+}) at various initial biomass concentrations (C_b). Data are mean values ± SD of triplicate experiments.

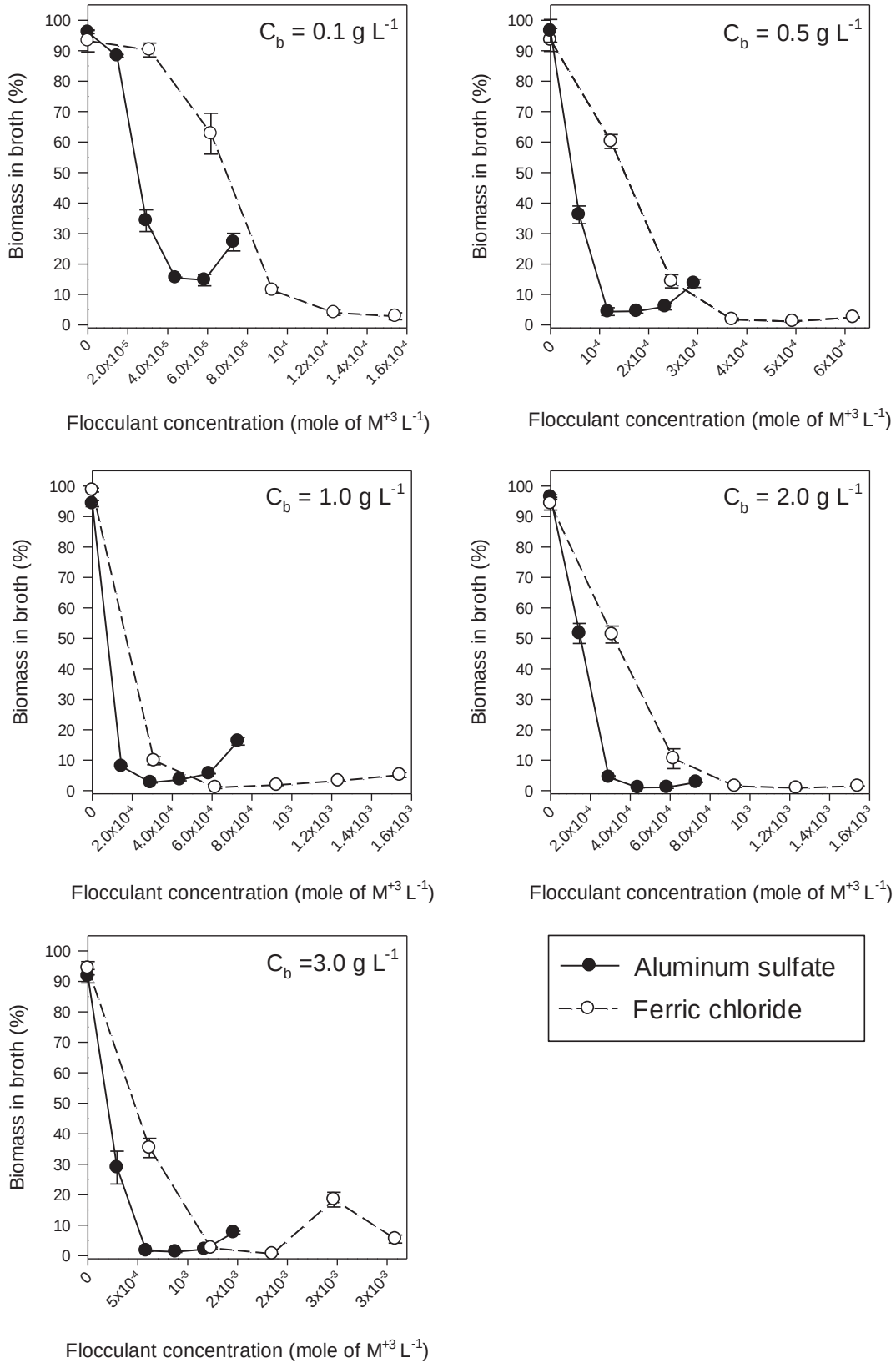


Figure 4.13. Flocculation of *C. vulgaris* (seawater) with aluminum sulfate and ferric chloride in terms of molar concentration (M^{3+} L^{-1}) of the metal ion (M^{3+}) at various initial biomass concentrations (C_b). Data are mean values ± SD of triplicate experiments.

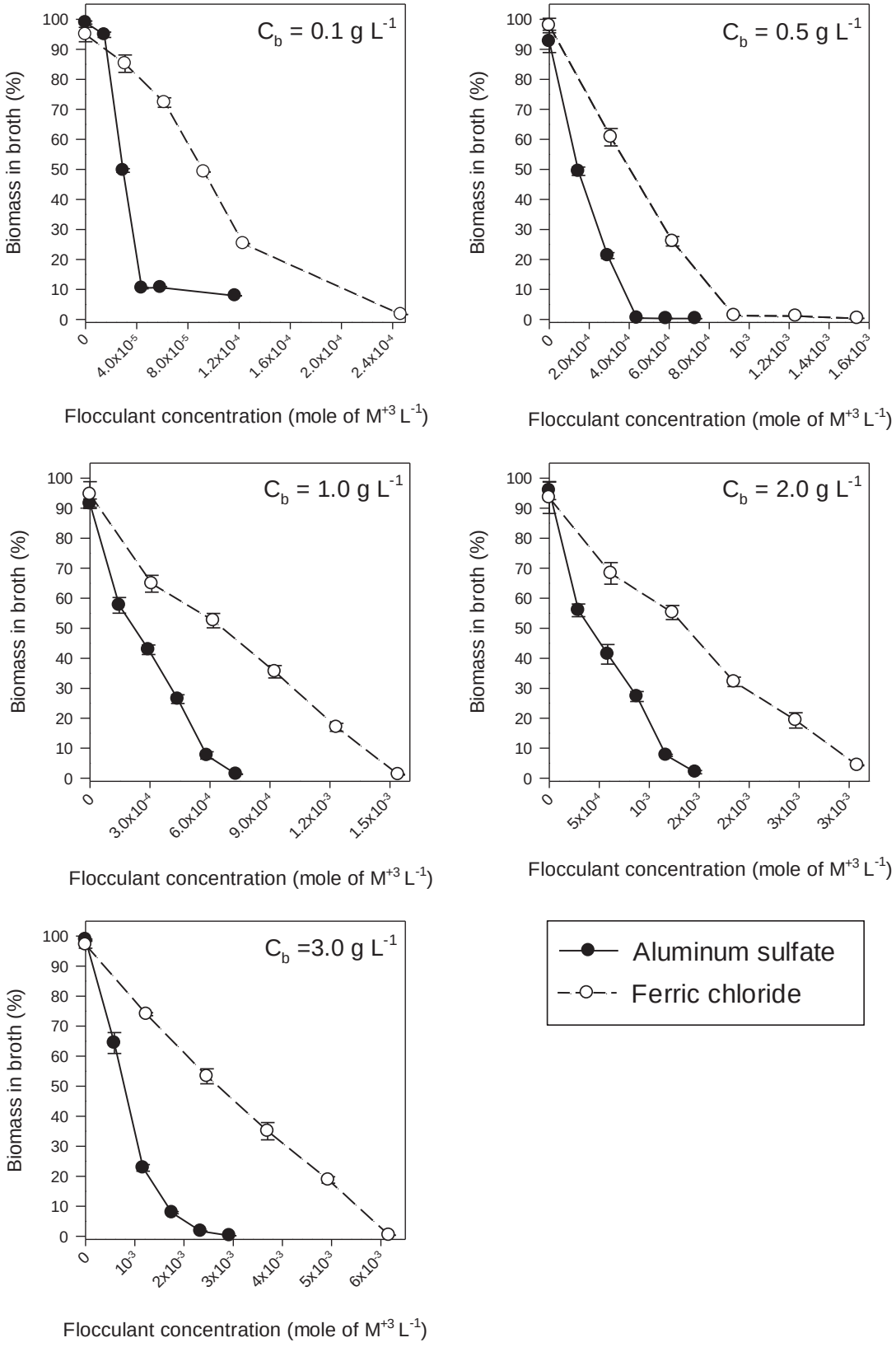


Figure 4.14. Flocculation of *N. salina* with aluminum sulfate and ferric chloride in terms of molar concentration (M^{3+} L^{-1}) of the metal ion (M^{3+}) at various initial biomass concentrations (C_b). Data are mean values ± SD of triplicate experiments.

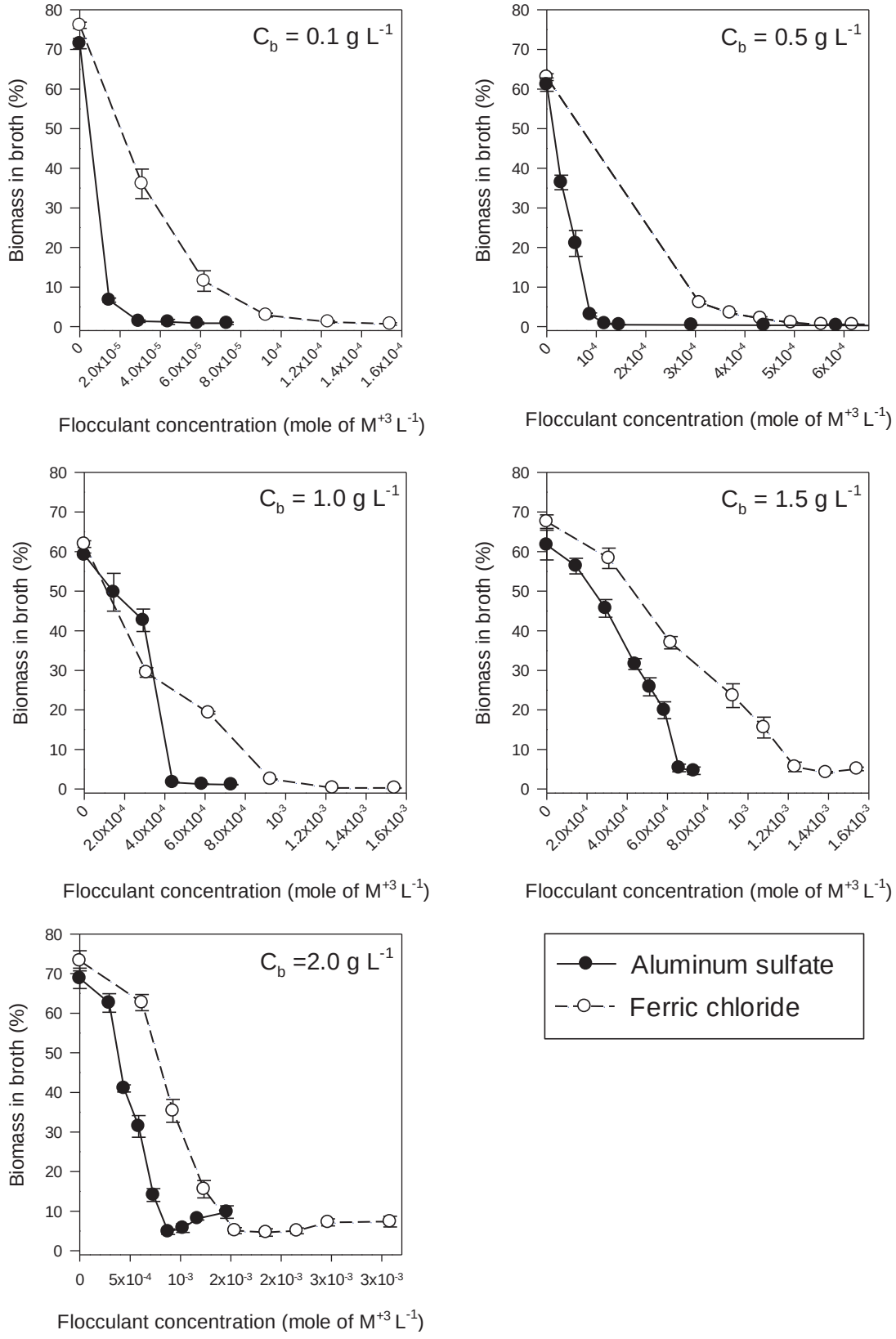


Figure 4.15. Flocculation of *C. fusiformis* with aluminum sulfate and ferric chloride in terms of molar concentration (M^{3+} L^{-1}) of the metal ion (M^{3+}) at various initial biomass concentrations (C_b). Data are mean values ± SD of triplicate experiments.

4.3.4 Effect of initial biomass concentration on flocculation

The concentration of a flocculant needed to achieve to a given level of cell removal also depends on the biomass concentration in suspension (Papazi et al., 2010; Vandamme et al., 2010; Granados et al., 2012). In this study, for all cases, the optimal flocculant dose increased essentially linearly as the concentration of the biomass in the broth increased (Figure 4.16). Linear relationships existed between the flocculant dose and the initial biomass concentration as shown in Table 4.3. With more biomass in suspension a large dose of the flocculant was needed for charge neutralization and flocculation. For a given surface charge density on an algal cell, the concentration of the cations required for charge neutralization is expected to increase linearly with increasing concentration of the biomass in the cell slurry (Duan & Gregory, 2003). The data in Figure 4.16 show that the effectiveness of a given flocculant for a given microalgal species depended also on the ionic strength of the fluid in which the cells were suspended.

From the equations in Table 4.3, the optimal flocculant dosage (mg L^{-1}) for 95% removal of the biomass can be calculated for any given initial concentration of an algal biomass in the slurry. For any given flocculant and ionic strength (freshwater or seawater), the constant multiplier (ϕ) of the linear equations in Table 4.3 could be correlated with the microalgal cell diameter (μm) in the following form:

$$\phi = \alpha + \beta\, d + \gamma\, d^2 \qquad (4.1)$$

In this equation, α, β, and γ are constants and d is the microalgal cell diameter (μm). Table 4.4 provides the best fit value of α, β, and γ for the algae grown in a given medium and

Table 4.3 The flocculant dose dependence on the microalgal biomass concentration (C_b) for 95% removal of the biomass from the culture broth

Species	Equations	Correlation coefficient
BG11 freshwater medium		
Choricystis minor	Aluminum sulfate (mg L^{-1}) = 318.980 C_b (g L^{-1})	0.995
	Ferric chloride (mg L^{-1}) = 257.815 C_b (g L^{-1})	0.991
Neochloris sp.	Aluminum sulfate (mg L^{-1}) = 11.796 C_b (g L^{-1})	0.999
	Ferric chloride (mg L^{-1}) = 32.228 C_b (g L^{-1})	0.970
Chlorella vulgaris	Aluminum sulfate (mg L^{-1}) = 503.156 C_b (g L^{-1})	0.980
	Ferric chloride (mg L^{-1}) = 265.252 C_b (g L^{-1})	0.905
BG11 seawater medium		
Chlorella vulgaris	Aluminum sulfate (mg L^{-1}) = 62.903 C_b (g L^{-1})	0.956
	Ferric chloride (mg L^{-1}) = 67.742 C_b (g L^{-1})	0.990
Nannochloropsis salina	Aluminum sulfate (mg L^{-1}) = 229.576 C_b (g L^{-1})	0.996
	Ferric chloride (mg L^{-1}) = 290.060 C_b (g L^{-1})	0.976
Cylindrotheca fusiformis	Aluminum sulfate (mg L^{-1}) = 154.594 C_b (g L^{-1})	0.976
	Ferric chloride (mg L^{-1}) = 132.690 C_b (g L^{-1})	0.983

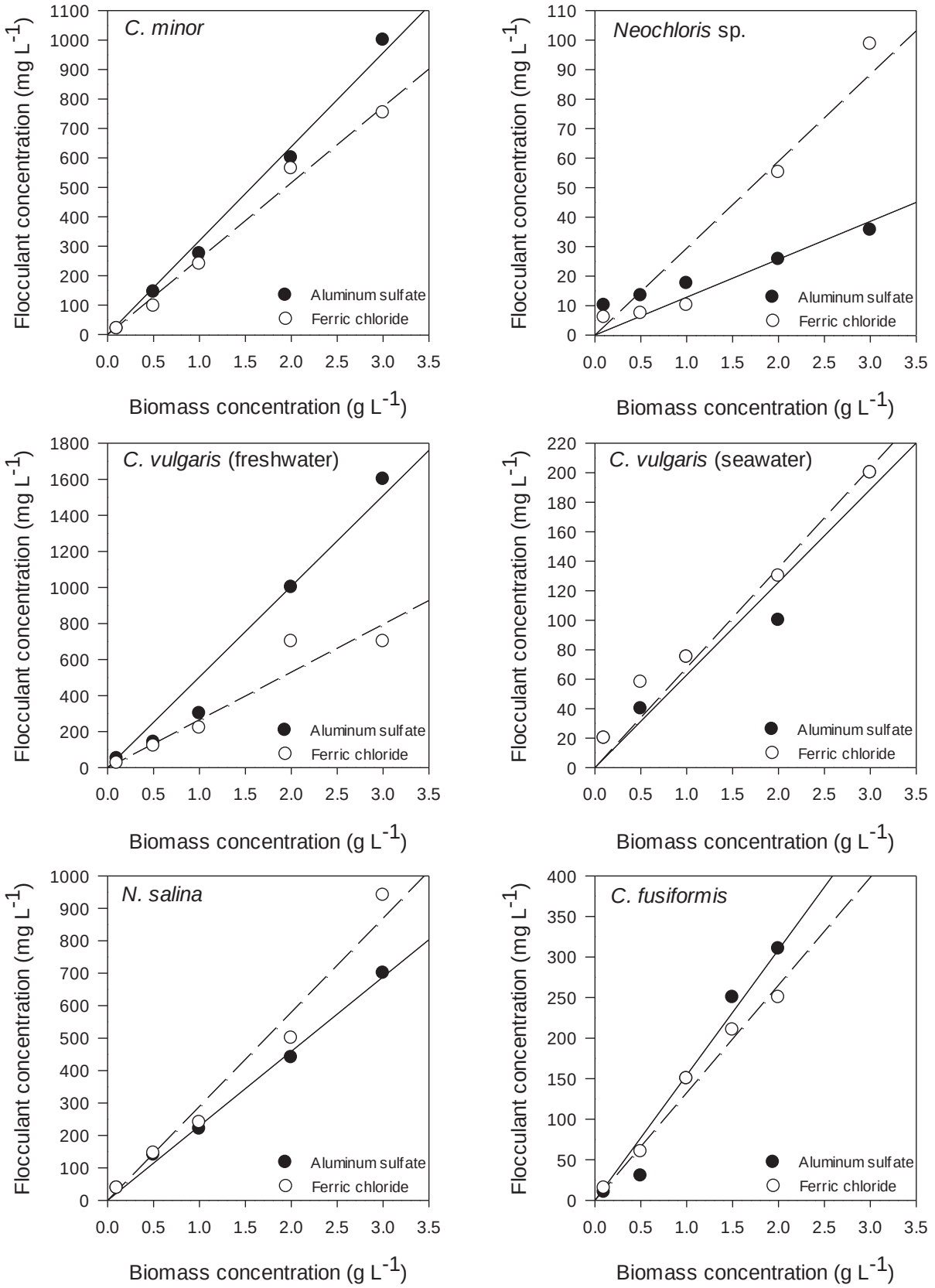


Figure 4.16. The relationship between microalgal biomass concentration and the flocculant dose required to achieve 95% removal of the cells from the culture broth following the standard flocculation treatment (Section 3.2.7.2).

Table 4.4 The estimated coefficients for Equation 4.1

Flocculant	Culture medium	α	β	γ
Aluminum sulfate	Freshwater	–1200	780	–87.3
	Seawater	65.5	229.13	154.28
Ferric chloride	Freshwater	–233	264.9	–32.7
	Seawater	1744	–440.8	28.9

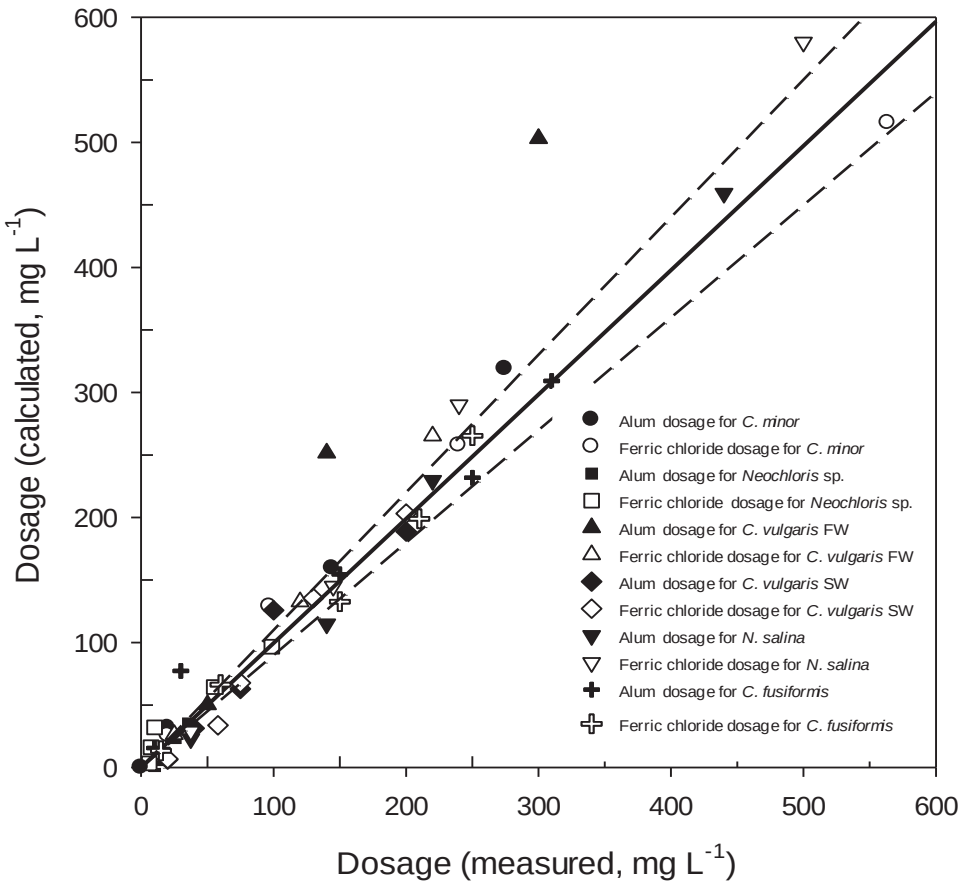


Figure 4.17. The parity plot of the measured flocculant dosage and the dosage calculated using Equation 4.2 with the α, β, and γ value shown in Table 4.4. The dashed lines denote ±10% of the exact agreement (solid line) between the measured and the calculated data.

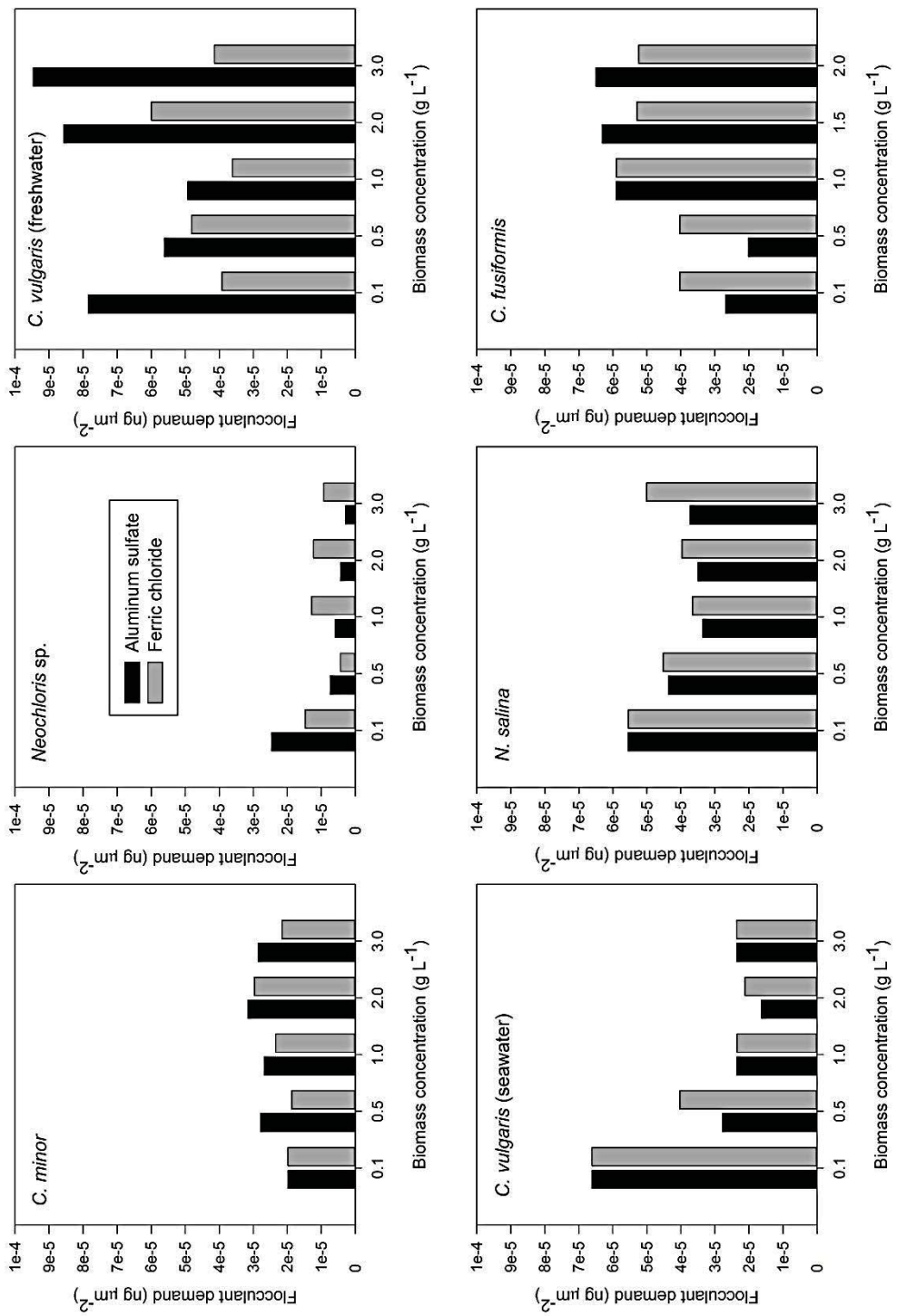


Figure 4.18. Flocculant demand (ng) per unit cell surface area (μm^2) at various initial biomass concentrations for achieving 95% removal of the microalgal cells from the culture broth.

flocculated with a given flocculant. Therefore, the equations in Table 4.3 could be rewritten in the following general form:

$$\text{Flocculant dose (mg L}^{-1}) = (\alpha+\beta\, d + \gamma\, d^2)\, C_b \qquad (4.2)$$

where C_b is microalgal biomass concentration in the culture broth (g L^{-1}). Equation 4.2 spanned cell diameter range of 2.9 – 9.2 μm and a biomass concentration range of 0.1 – 3.0 g L^{-1}. For validation of the Equation 4.2, the measured flocculant dose for 95% biomass removal and the dose calculated using the equation were plotted in the form of a parity plot as in Figure 4.17. As shown in Figure 4.17, Equation 4.2 agreed with most of the measured data within ±10% of the measured value.

The flocculant dose per unit surface area of the cell for 95% cell removal after the standard flocculation treatment (Section 3.2.7.2) is shown in Figure 4.18 for different initial concentrations of the biomass in the slurry. The area specific flocculant dosage was largely independent of the cell concentration, as anticipated, but seemed to depend on the algal species (Figure 4.18). In some cases, at a low biomass concentration (e.g. 0.1 g L^{-1}), the flocculant demand per unit cell surface area was relatively high (Figure 4.11). This is because at extremely low biomass concentration, the flocs can be difficult to form and grow as the probability of contact between widely dispersed particles is small. On the other hand, at too high a concentration of cells the flocs can be too large and interact together to make settling difficult (Granados et al., 2012). Granados, et al. (2012) suggested that the optimum biomass concentration for flocculation to be in the range from 0.5 g L^{-1} to 2.5 g L^{-1}.

4.3.5 Costs of the flocculants for harvesting microalgal biomass

The cost associated with harvesting of microalgal biomass from the culture broth by flocculation with aluminum sulfate or ferric chloride is an important consideration. The bulk prices of flocculants were obtained from the literature (Wastewater Innovations in Asia and the Pacific, 2014; Wastewater Technology Fact Sheet, EPA, 2000). The prices of aluminum sulfate ($Al_2(SO_4)_3.14H_2O$) and ferric chloride ($FeCl_3$) in 2013 were US $318.90 and US $354.33 per metric ton (MT), respectively. For aluminum sulfate the price was corrected to the unhydrated form ($Al_2(SO_4)_3$). Thus, the price was US $440.39 per MT. The relevant amounts (mg) of each flocculant required to harvest a metric ton (dry basis) of microalgal biomass were calculated, based on the optimal flocculant dosage of the batch flocculation experiments (Table 4.3), as follows:

$$\text{Salt required (mg)} = \text{Constant (from Table 4.3)} \times \text{microalgal biomass } (10^6) \qquad (4.3)$$

Therefore, the cost (US $) of flocculant required to harvest one MT of the dry microalgal biomass was as follows:

$$\text{Cost}_{\text{salt}}(\text{US \$/MT biomass}) = \frac{(\text{salt required (mg)} \times 10^6)}{10^9} \times \text{price of salt (in US \$ per MT of salt)} \qquad (4.4)$$

Table 4.5 provides the relevant costs. The cost of the flocculants depended on the dosage and varied between US $5.19 and 221.58 per MT of dry biomass. For three microalgae species, ferric chloride proved to be cheaper than aluminum sulfate. For example,

Table 4.5 Cost of flocculant for harvesting microalgal biomass from the culture broth

Alga	US $ per metric ton of dried microalgal biomass	
	Aluminum sulfate ($Al_2(SO_4)_3$)	Ferric chloride ($FeCl_3$)
Grown in the BG11 freshwater medium		
Choricystis minor	140.44	91.35
Neochloris sp.	5.19	11.42
Chlorella vulgaris	221.58	93.99
Grown in the BG11 seawater medium		
Chlorella vulgaris	27.70	24.00
Nannochloropsis salina	101.10	102.78
Cylindrotheca fusiformis	68.08	47.02

in the case of *C. minor*, the cost of harvesting with ferric chloride was US $91.35/MT, whereas it was US $140.44/MT for harvesting with aluminum sulfate. The lower cost of harvesting with ferric chloride is due to its lower price of US $354.33/MT compared to aluminum sulfate (US $440.39 /MT). Also, in some cases (Table 4.3), the dosage required for ferric chloride was less than the dosage needed for aluminum sulfate. In contrast, for *Neochloris* sp., the cost of harvesting with aluminum sulfate (US $5.15 /MT) was lower than the cost of harvesting with ferric chloride (US $11.42/MT). In relative terms, Udom et al. (2013) reported that the cost of harvesting with ferric chloride was US $130/MT of dried algae compared to the cost of US $65/MT of dried algae for harvesting with aluminum sulfate. In addition, the cases of *C. vulgaris* seawater and *N. salina*, the cost of harvesting with aluminum sulfate was close to the cost of harvesting with ferric chloride. In view of Table 4.5, generalizations cannot be made about which flocculant is cheaper and the cost needs to be evaluated on a case-by-case basis.

In conclusion, all the microalgae tested could be effectively harvested by flocculation-sedimentation. Flocculation with multivalent salts (aluminum sulfate and ferric chloride) is potentially useful for microalgal biomass recovery, but the efficacy of a flocculant depends on several factors as discussed in this chapter. Major influencing factors were the surface charge of the cells (e.g. algal species) and their size. At equal mass concentrations of the algae in the broth, the cell number concentrations varied with the species because of the differences in cell size. The total cell surface area per unit of culture volume therefore differed among the species for identical values of the initial mass concentration. The flocculation efficiency depended on the flocculant dose, the type of flocculant, the biomass concentration in culture broth, the microalgal species and the ionic strength of broth. The optimal dose for flocculation of each alga was identified.

4.4 Continuous flocculation–sedimentation for harvesting microalgal biomass

This section discusses the design and characterization of the continuous flocculation-sedimentation process for the marine microalga *N. salina*. *N. salina* was selected for this study because it has a high lipid productivity even though its small cell size (4.82±0.36 μm) means that it requires a higher dosage of flocculant for harvesting. *N. salina* was the best among the studied microalgae in terms of lipid productivity (Table 4.1). For these experiments, *N. salina* was cultured until it reached the stationary phase and was then diluted with fresh medium to a biomass concentration of 0.5 g L^{-1} as this is the concentration typically attained in large-scale production processes in commercial raceways (Grima et al., 2003b; Borowitzka, 2005; Chisti, 2012, 2013). Aluminum sulfate was used as the flocculant because it was found to be more efficient than ferric chloride for flocculation of *N. salina* as reported in Section 4.3.

The continuous flocculation-sedimentation equipment was set up as shown in Figure 4.19. The process consisted of a 20 L microalgal broth storage tank; a 5 L flocculant storage tank; two variable speed slurry transfer pumps; a rapid mixing vessel with a 2-blade impeller; a flocculation vessel with a 2-blade impeller; and a rectangular sedimentation tank (Figure 4.19). Details of the process design were explained in Section 3.3.3.1 and Section 3.3.3.2. For this work, the microalgal broth was pumped from the storage tank using a peristaltic pump (Materflex® L/S Model: 7523-80 with tube Masterflex® 96400 size 17) at a constant flow rate (90% of the total flow rate) to the rapid mixing tank. The aluminum sulfate solution (concentration 885 mg L^{-1}) was delivered using a second peristaltic pump (INTERGRA Biosciences AG Model: DOSE IT P910 with tube Masterflex® 06429 size 14) at a constant flow rate (10% of the total flow rate) to the rapid mixing tank. The initial total flow rate of the microalgal broth and the aluminum sulfate solution were set at 56.5 mL min^{-1} as explained in Section 3.2.8.2. The flow rate had been calculated by using Equation 3.10.

The microalgae broth and aluminum sulfate solution were pumped continuously and mixed rapidly in the rapid mixing tank (Figure 4.19). At this stage, when the aluminum sulfate mixed with microalgal cells, the destabilization of microalgal suspension began as shown Figure 4.20A. Small aggregates or flocs formed very rapidly and dispersed in the tank. From the mixing tank, the algal suspension flowed by gravity to the flocculation tank. Here the small flocs developed into larger flocs by agglomeration caused by the low speed mixing (Figure 4.20B). The large flocs from the flocculation tank flowed to the sedimentation tank and settled by gravity. The clarified broth flowed to the exit of the sedimentation tank. Once all tanks had been filled to the required levels, samples (5 mL) were taken at the outlet zone of the rectangular sedimentation tank for measuring the flocculation efficiency. Samples were taken every 30 min until the process attained a steady state (equivalent to four residence times of operation). The operating conditions of the process are summarized in Table 4.6.

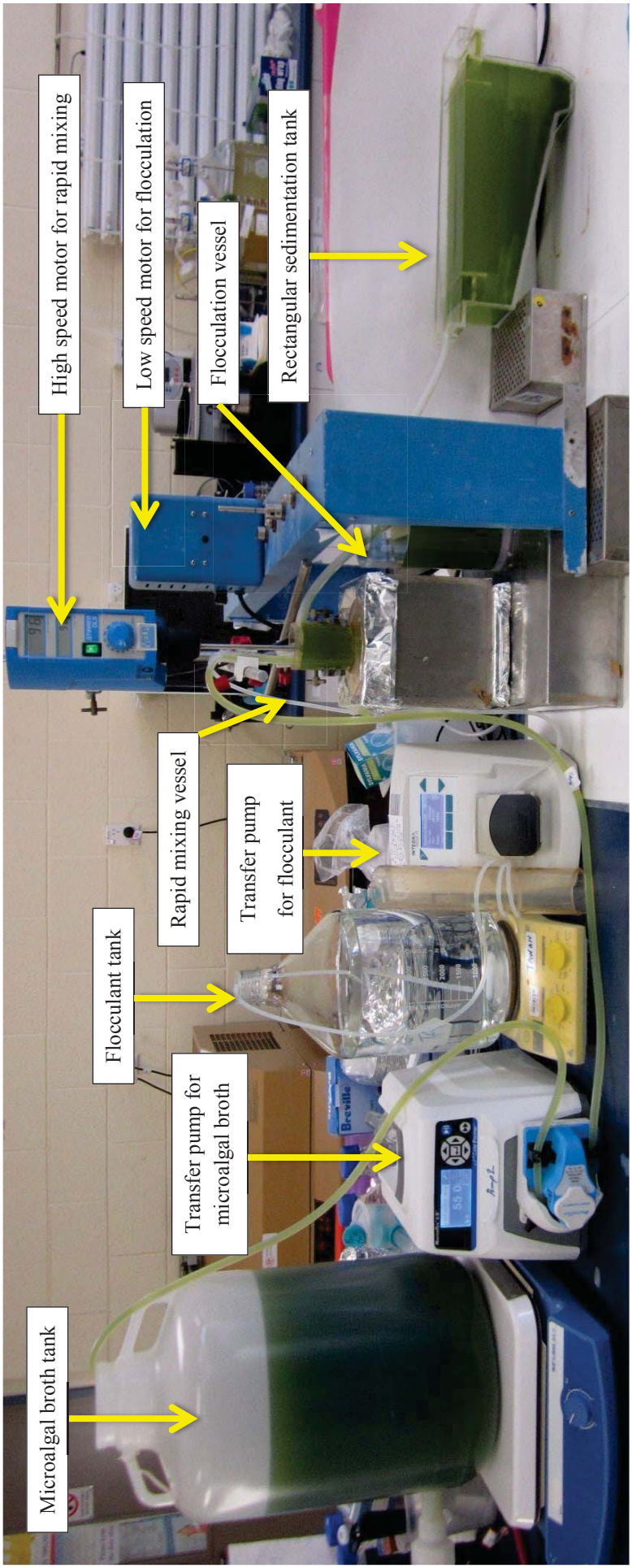


Figure 4.19. Continuous flocculation–sedimentation processes for harvesting microalgal biomass from the culture medium.

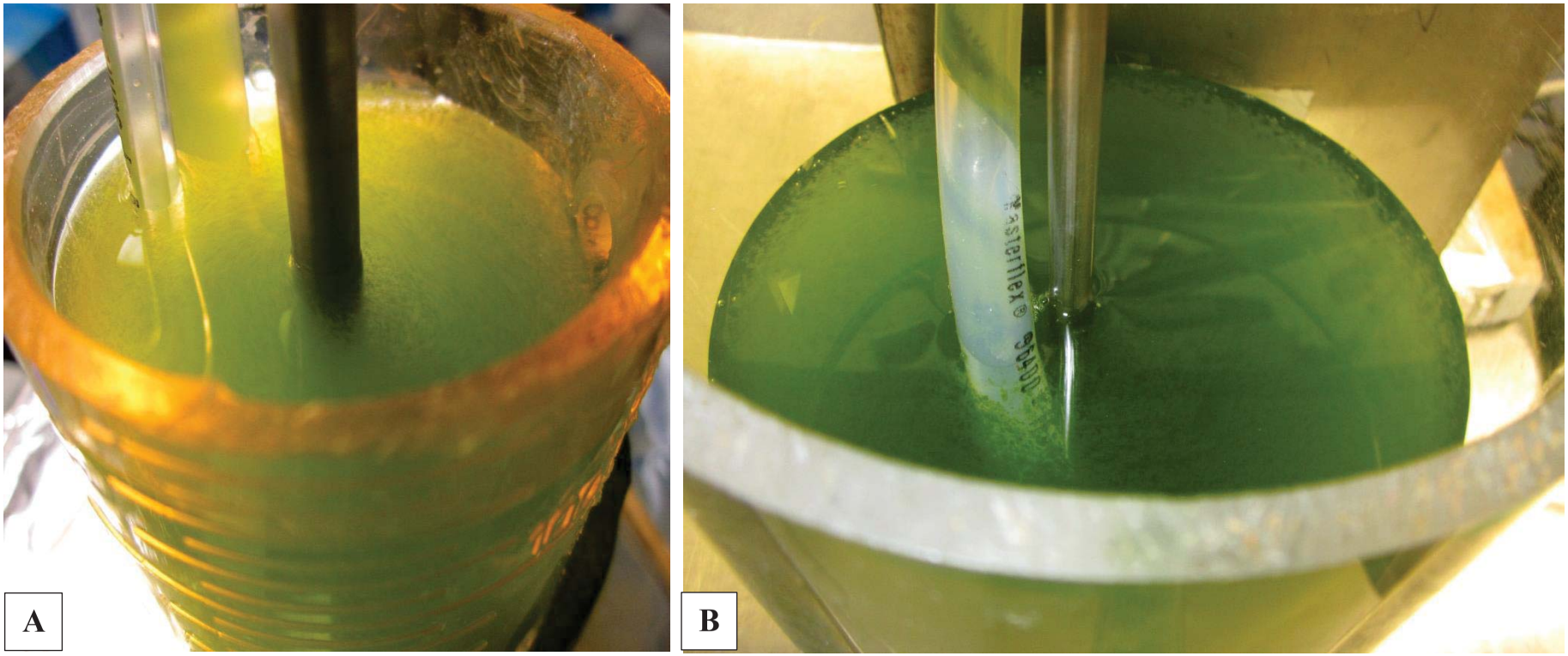


Figure 4.20. Destabilization of microalgal broth in continuous rapid mixing vessel (A) and floc growth of microalgal biomass in continuous flocculation vessel (B).

Table 4.6 The operational conditions of the continuous flocculation–sedimentation

Operation conditions	Value
Residence time of rapid mixing tank (min)	2
Residence time of flocculation tank (min)	18.85
Residence time of sedimentation tank (min)	38.16
Total residence time of the broth (min)	59.01
Flow rate of microalgal broth (mL min^{-1}) (90% of the total flow rate)	50.9
Flow rate of aluminum sulfate solution (mL min^{-1}) (10% of the total flow rate)	5.6
Total flow rate (mL min^{-1})	56.5
Rapid mixing impeller speed (rpm)	97
Flocculation impeller speed (rpm)	11.5

4.4.1 Effect of aluminum sulfate dosage on continuous harvesting

The flocculant dosage is the most important factor in a flocculation process as it influences both the extent and the rate of flocculation (Lee et al., 1998) . This experiment focused on identifying the optimal aluminum sulfate dosage for the recovery of the *N. salina* biomass in the continuous flocculation-sedimentation process. Several dosages of aluminum sulfate were trialled. The optimal aluminum sulfate dosage identified in the batch flocculation process (Section 4.3.4) was initially used in the continuous flocculation-sedimentation process. From the equation in Table 4.3, the optimal dosage for batch flocculation was 114.5 mg L^{-1} at biomass concentration of 0.5 g L^{-1}. Thus, the dosages of aluminum sulfate trialled were: 114.5 mg L^{-1} (control), 171.7 mg L^{-1} (×1.5 of control), 229.0 mg L^{-1} (×2 of control), and 343.5 mg L^{-1} (×3 of control).

Figure 4.21 shows the percentage of the microalgal biomass removed from the broth by the different dosages of aluminum sulfate. The results showed that aluminum sulfate concentration of 114.5 mg L^{-1} exhibited low flocculation efficiency with about 48% of the microalgal cells removed from the broth at steady state (~4 h). The flocculation efficiency greatly increased from approximately 48% to 70% when the dosage increased from 114.5 mg L^{-1} to 171.7 mg L^{-1}. There was no significant change in the flocculation efficiency when aluminum sulfate dosage was further increased to 229.0 mg L^{-1} and higher.

Figure 4.22 and Figure 4.23 show the sedimentation behaviour with different concentrations of aluminum sulfate. As the aluminum sulfate dosage increased, a greater amount of microalgal biomass was harvested from the culture broth at 4 h, as indicated by the volume of the biomass settled in the bottom of the tank. Figure 4.22A compares the broth clarification achieved in the continuous and batch processes at the optimal

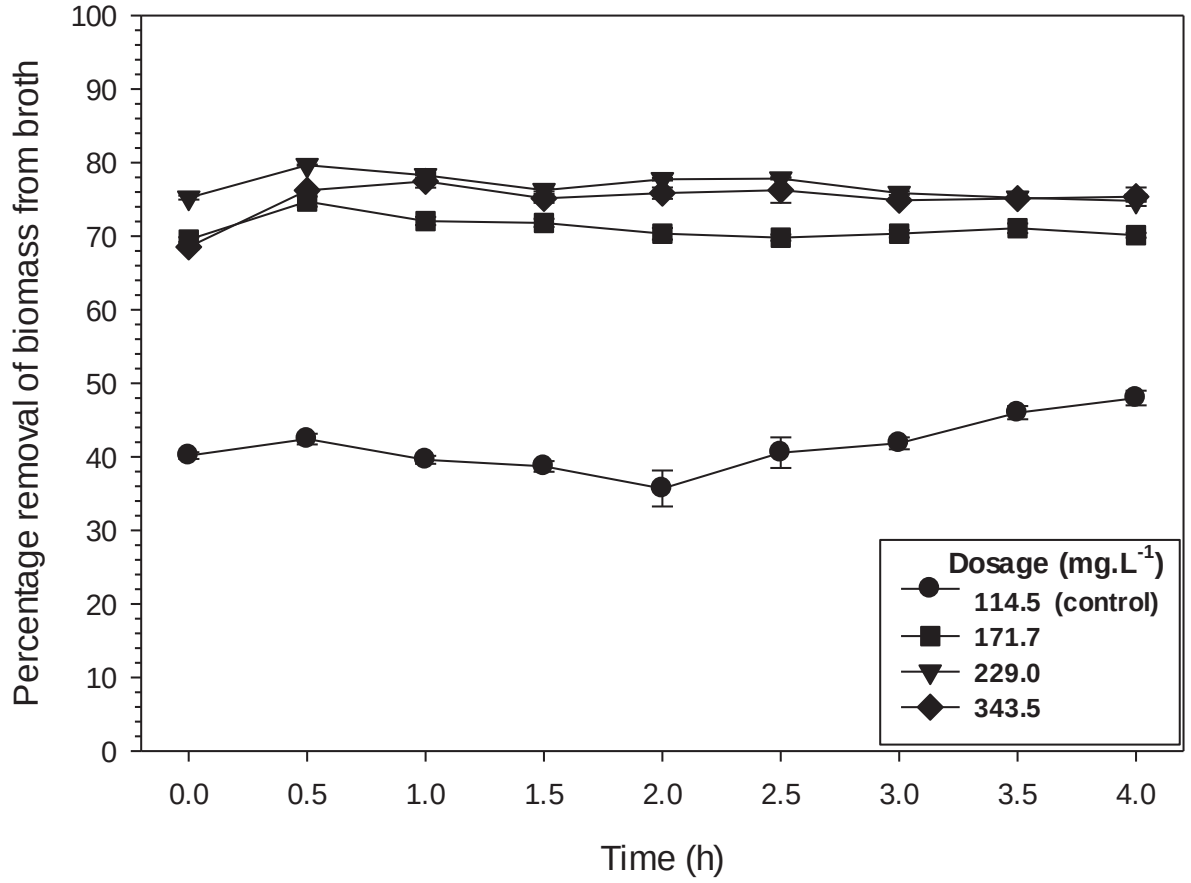


Figure 4.21. The flocculation efficiency of *N. salina* biomass in the continuous flocculation-sedimentation process at various dosages of aluminum sulfate. All measurements were made from instance of the sedimentation tank being filled to the operational level (i.e. 59 min from start of flow, shown as 0 h in the above figure).

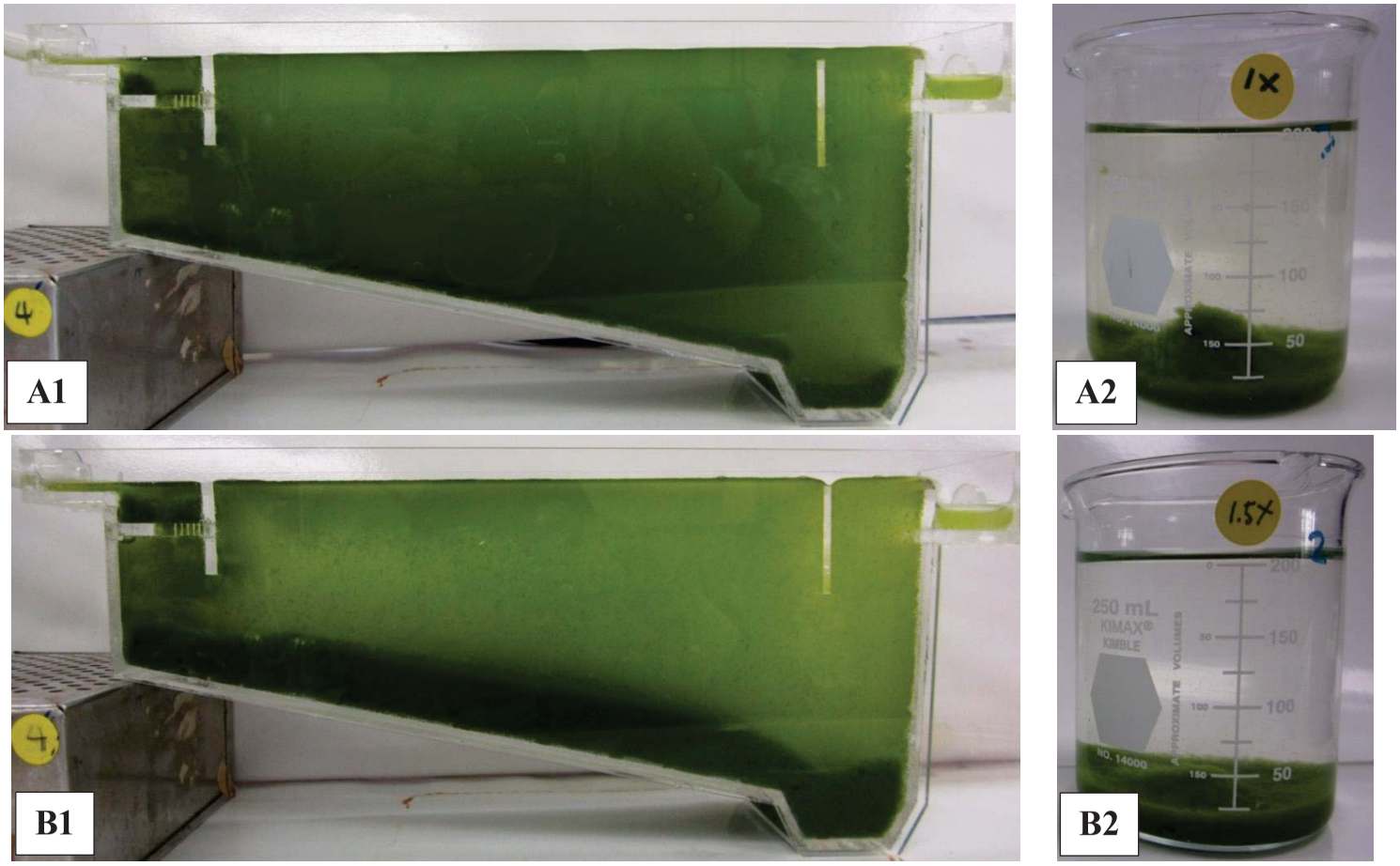


Figure 4.22. Flocculation using: the optimal flocculant dosage in continuous sedimentation tank (A1); the optimal flocculant dosage in the batch vessel (A2); 1.5 × optimal flocculant dosage in continuous sedimentation tank (B1); 1.5 × optimal flocculant dosage in batch vessel (B2). The residence times in A1 and B1 was 4 h. The residence times in A2 and B2 was 30 min (settling period of batch flocculation process).

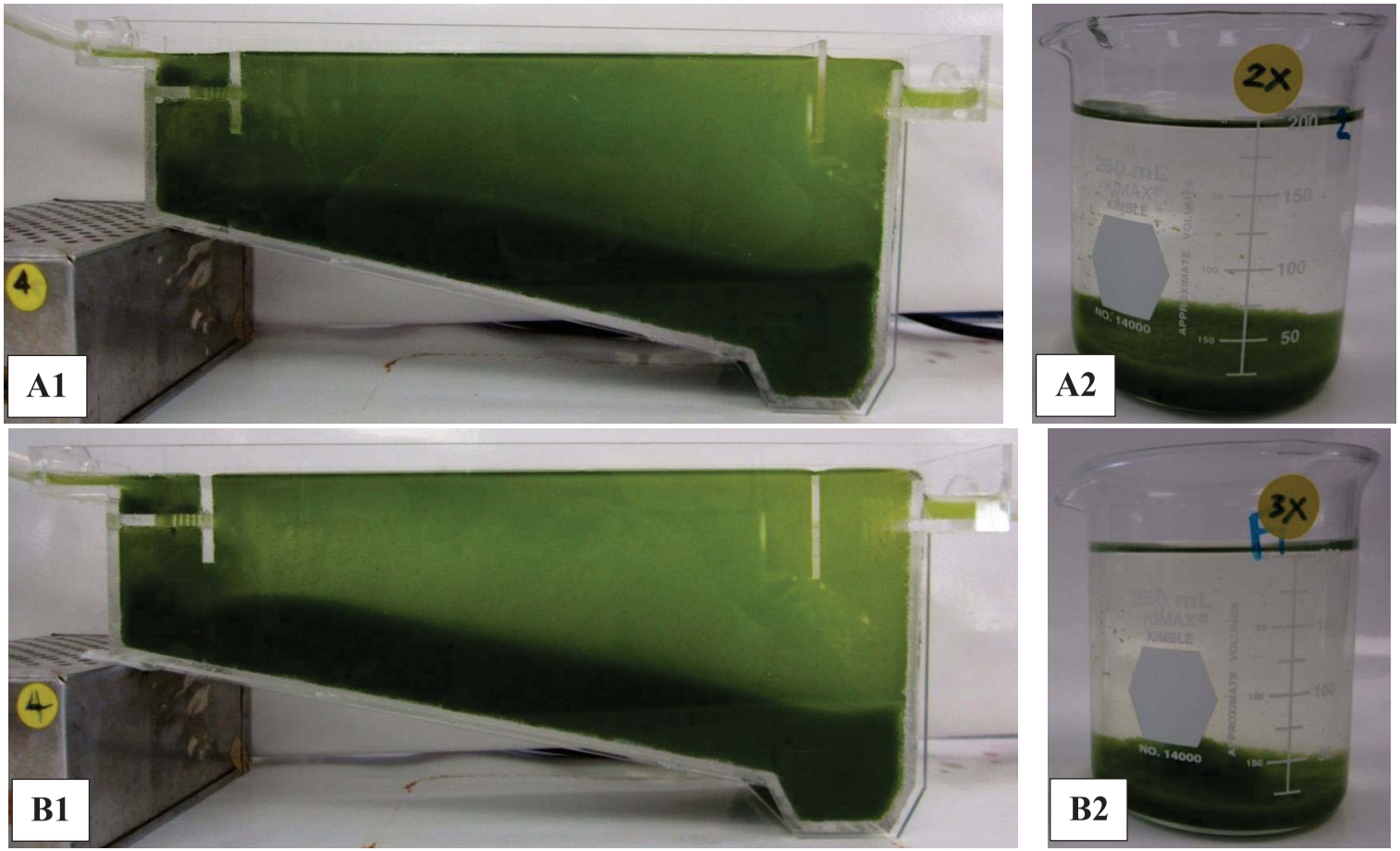


Figure 4.23. Flocculation using: 2 × optimal flocculant dosage in continuous sedimentation tank (A1); 2 × optimal flocculant dosage in batch vessel (A2); 3 × optimal flocculant dosage in continuous sedimentation tank (B1); 3 × optimal flocculant dosage in batch vessel (B2). The residence times in A1 and B1 was 4 h. The residence times in B1 and B2 was 30 min (settling period of batch flocculation process).

aluminum sulfate dosage (114.5 mg L^{-1}) of the batch process. Batch flocculation removed >95% (Figure 4.22 A2) of the biomass, while continuous flocculation removed only 48% (Figure 4.22 A1) of the biomass. Figure 4.22 and Figure 4.23 also show a similar pattern of results at higher flocculant concentrations. The result demonstrated that the optimal aluminum sulfate dosage (114.5 mg L^{-1}) found for the batch flocculation was insufficient for use in the continuous flocculation-sedimentation process. The optimal aluminum sulfate dosage for the continuous flocculation–sedimentation process was 229.0 mg L^{-1} (Figure 4.21) or twice the optimal dosage of the batch flocculation process. At this dosage 75% of the biomass was removed from the broth. This difference in performance relates to the differences in the flocculation conditions in the batch and continuous systems. The very high flocculation efficiency of the batch operation is because all the particles have exactly the same residence time and mixing time. However, in continuous operation, there is a distribution of residence times in the sedimentation tank as well as the mixing tank. The floc size in a batch flocculation system increases progressively with time but in the continuous system the size of the flocs has a broader range (Hogg, 2000; Rattanakawin & Hogg, 2000). Smallest of the flocs washed out of the system at the operating conditions used. Most previously published work only investigated batch flocculation to successfully remove the microalgal biomass from the culture broth (Morales et al., 1985; Sukenik et al., 1988; Poelman et al., 1997; Oh et al., 2001; Danquah et al., 2009a; Danquah et al., 2009b; Papazi et al., 2010; Vandamme et al., 2010; Salim et al., 2011; Garzon-Sanabria et al., 2012; Rwehumbiza et al., 2012b; Schlesinger et al., 2012; Şirin et al., 2012; Zheng et al., 2012; Chen et al., 2013b; Farid et al., 2013; Rashid et al., 2013; Vandamme et al., 2013; Prochazkova et al., 2015; Shen et al., 2015).

4.4.2 Effect of the flow rate of the continuous flocculation-sedimentation process on the flocculation efficiency

The purpose of this study was to improve broth clarification by adjusting the flow rate (and therefore the residence time) at a flocculant concentration of 229.0 mg L^{-1} (Section 4.4.1). Table 4.7 and Figure 4.24 show the effect of varying the total flow rate in the range 20-68 mL min^{-1} on flocculation efficiency. This corresponded to a total residence time variation in the range of 49-148 min. Increasing the residence time by decreasing the flow rate led to an enhancing of the flocculation efficiency. At the initial flow rate of 56.5 mL min^{-1} (residence time of 59.0 min) the flocculation efficiency at steady state was 74.8±0.1%. When the flow rate was increased by 20% to 67.8 mL min^{-1} (residence time of 49.2 min), the flocculation efficiency at steady state was 75.1±0.3% (Figure 4.25 A) which was not significantly different compared to the operation at the lower flow rate. In contrast, when the flow rate was reduced by 20%, 40% and 60% of the initial flow rate, the residence time increased to 73.8, 98.8, and 147.5 min, respectively, and the flocculation efficiency at steady state improved significantly to 80.0±0.4% (Figure 4.25 B), 83.1±0.2% (Figure 4.26 A) and 86.1±0.1% (Figure 4.26 B), respectively. This was because the flocs had more time to settle in the sedimentation tank as the residence time increased.

Whilst changes in flow rate did improve sedimentation, it may be possible to also improve harvesting by changes to capacity of the sedimentation tank. In a continuous flow system the residence time is controlled by the flow rate and the size of the sedimentation tank. At a constant flow rate, increasing the size of the tank should allow more microalgal biomass to be harvested.

Another modification in the continuous process that has been suggested by Sastry et al. (2000) and Hogg (2000) for improving the flocculation performance is to reduce the

Table 4.7 The operation conditions of the continuous flocculation–sedimentation process at various flow rates

Operation conditions	Normal flow rate	20% higher flow rate	20% lower flow rate	40% lower flow rate	60% lower flow rate
Total flow rate (mL min^{-1})	56.5	67.8	45.2	33.9	22.6
Total residence time (min)	59.0	49.2	73.8	98.8	147.5
Residence time in rapid mixing tank (min)	2	1.67	2.5	3.33	5
Residence time in flocculation tank (min)	18.85	15.70	23.56	31.42	47.12
Residence time in sedimentation tank (min)	38.16	31.80	47.70	64	95.4
Flocculation efficiency (%) at steady state (at 4× the total residence time)	74.8±0.1	75.1±0.3	80.0±0.4	83.1±0.2	86.1±0.1

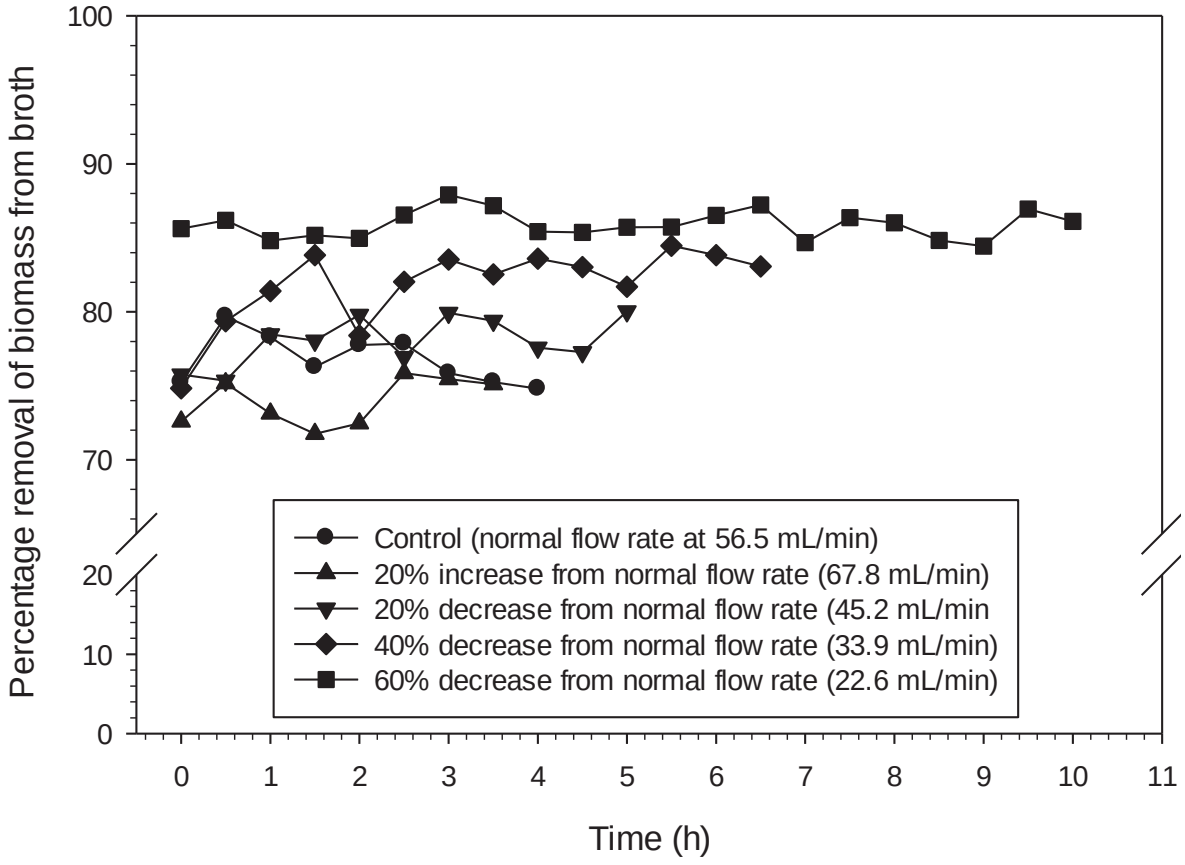
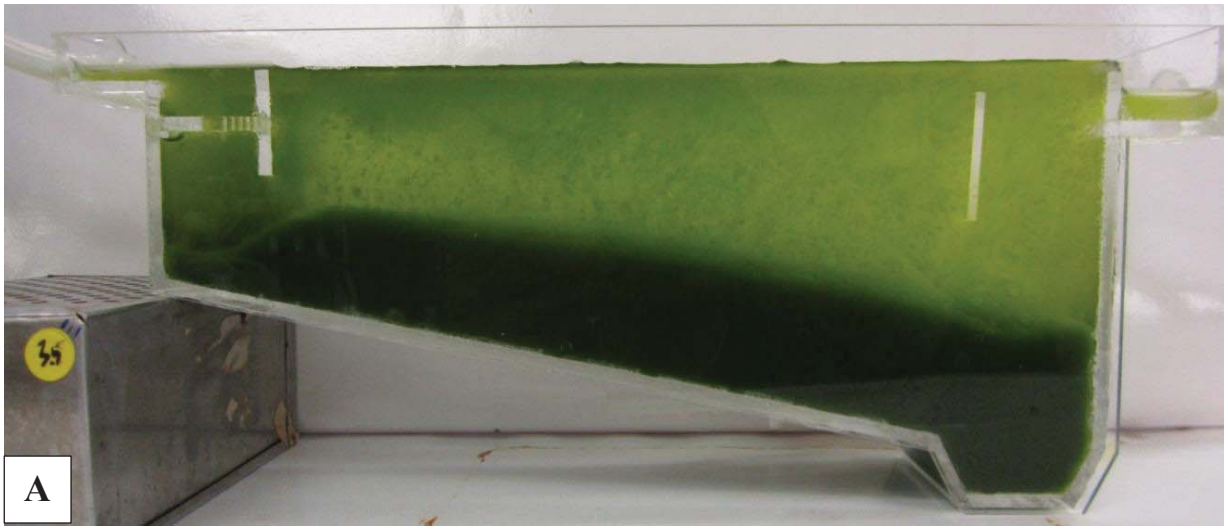


Figure 4.24. The flocculation efficiency of *N. salina* biomass in the continuous flocculation-sedimentation process at various dosages of aluminum sulfate. All measurements were made from instances of the sedimentation tank being filled at operation level (59 min = 0 h for control; 49 min = 0 h for 20% higher flow rate; 74 min = 0 h for for 20% lower flow rate; 99 min = 0 h for 40% lower flow rate; 147.5 min for 60% flow rate).



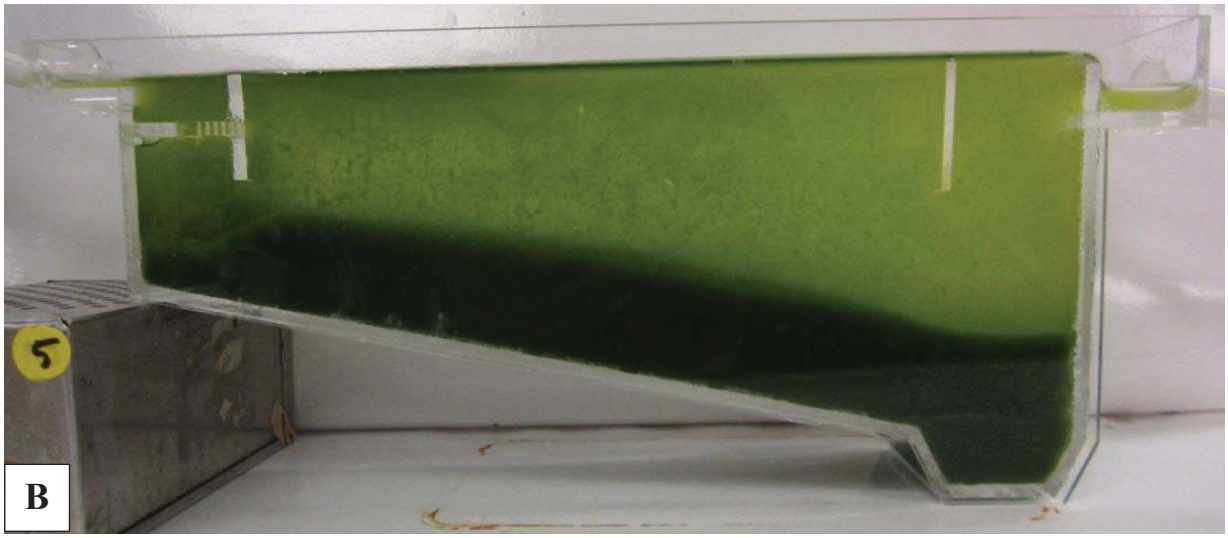


Figure 4.25. The sedimentation of microalgal biomass in the rectangular sedimentation tank at different total flow rates: A) 20% increased flow rate (67.8 mL min^{-1}) relative to the normal flow rate (56.5 mL min^{-1}) at steady state (3.5 h); B) 20% decreased flow rate (45.2 mL min^{-1}) relative to the normal flow rate (56.5 mL min^{-1}) at steady state (5 h).

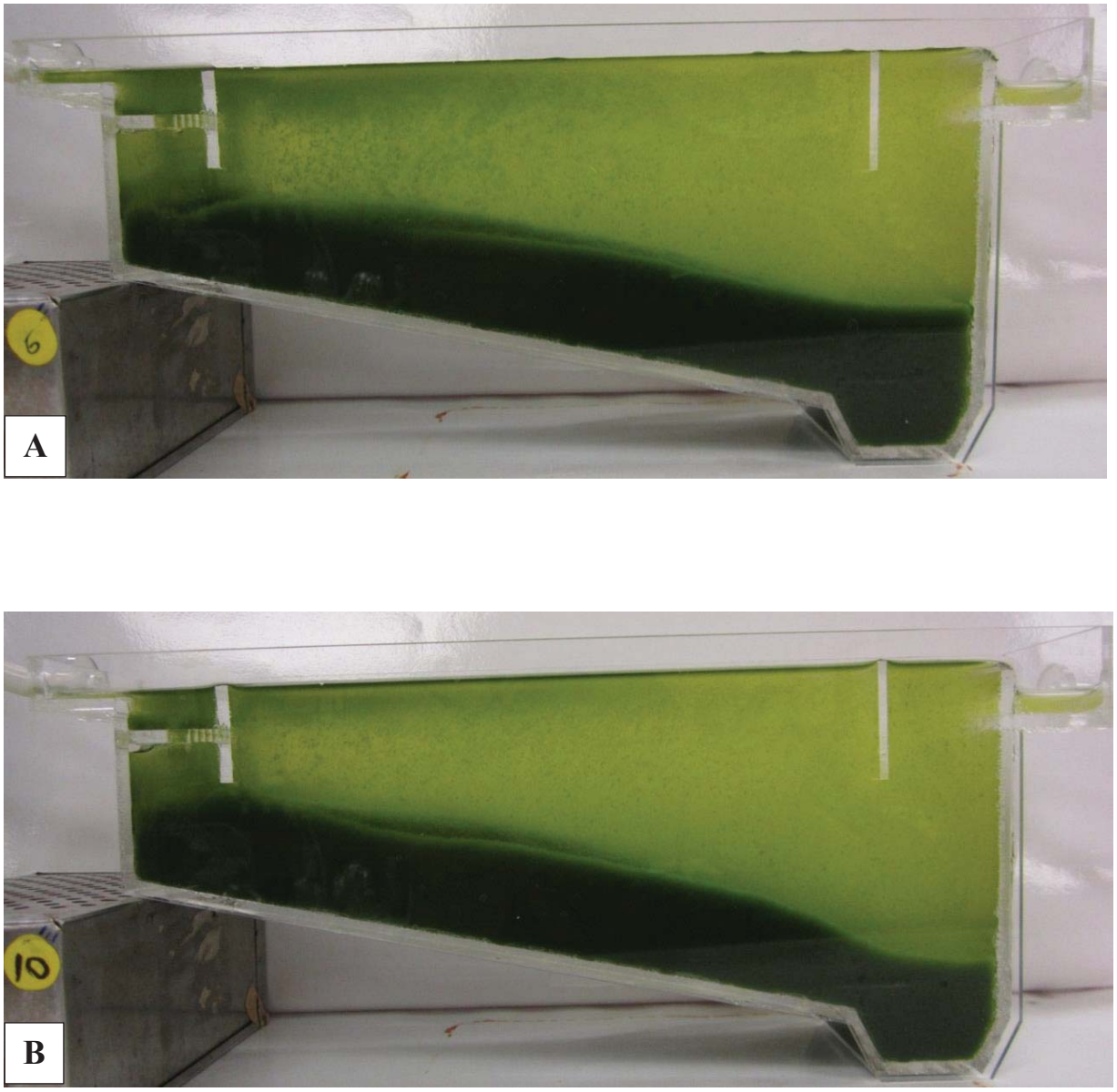


Figure 4.26. The sedimentation of microalgal biomass in the rectangular sedimentation tank at different total flow rates: A) 40% decrease of the total flow rate (33.9 mL min^{-1}) relative to the normal flow rate (56.5 mL min^{-1}) at steady state (6.5 h); B) 60% decrease of the total flow rate (22.6 mL min^{-1}) relative to the normal flow rate (56.5 mL min^{-1}) at steady state (10 h).

agitation speed and the residence time in the flocculation vessel. At a low mixing speed the flocs are larger compared to those at the higher mixing speed. Also, a short residence time in the flocculation vessel reduces the tendency of the flocs to disintegrate. Use of polymeric flocculants in conjunction with aluminum sulfate can be beneficial as polymers increase the resistance of a floc to breakup during mixing (Rebhun, 1990). However, polymeric flocculants are too expensive for use in an operation which is attempting to minimize the expense and is intended for extremely large scale use as would be needed in an algal fuel process.

In summary, in continuous flocculation-sedimentation, the flocculant dosage and the total flow rate are important factors that influence harvesting of the algal biomass from the culture broth. The highest microalgal biomass recovery efficiency was 86% for an aluminum sulfate dosage of 229 mg L^{-1} and a total flow rate of 22.6 mL min^{-1} (residence time of 147.5 min). As the results show, continuous flocculation-sedimentation can be used to effectively recover the biomass but a redesigning of the sedimentation tank may be necessary to further improve performance.

4.5 Solvent extraction of biomass paste

Several extraction techniques are being developed for recovering oil from microalgal biomass (Samarasinghe et al., 2012; Sathish & Sims, 2012; Cheng et al., 2013; Liu et al., 2013; Neto et al., 2013). One of these methods is solvent extraction. Solvent extraction is recognized as being a simple and effective technique (Mercer & Armenta, 2011; Halim et al., 2012; Grima et al., 2013b). The solvent extraction studies in this work focused on three aspects. First an elucidation of the effect of the flocculants used on lipid extraction; secondly, a comparison of efficiency the solvent extraction of dry and wet paste biomass; and third identification of an optimal solvent composition and the conditions for the extraction of the paste biomass. The experiments relating to the first two objectives were performed using a modified Bligh and Dryer method (1959) as the standard extraction method (Section 3.2.5.6). The solvent optimization work focused on only *Nannochloropsis salina* as this alga had the highest lipid productivity (Table 4.1) of all the studied microalgal species.

4.5.1 Effect of flocculants on lipid extraction

The inorganic metal salt flocculant adhere to the microalgal biomass (Henderson et al., 2010) and have the potential to interfere with lipid extraction. Therefore, experiments were performed to quantify the possible effects of flocculants, if any. The microalgal biomass harvesting procedure and the lipid extraction method used were described in Section 3.2.9.1. As shown in Figure 4.27, a certain fraction of the flocculant used at the optimum dose (Table 4.3) was retained on the microalgal biomass. The washing step after the biomass recovery (Section 3.2.9.1) could not remove the adsorbed flocculant from the microalgal biomass. The amount of the flocculant adhering on the biomass depended on the flocculant dose and the microalgal species. The highest retention of flocculant was found with *C. minor* where

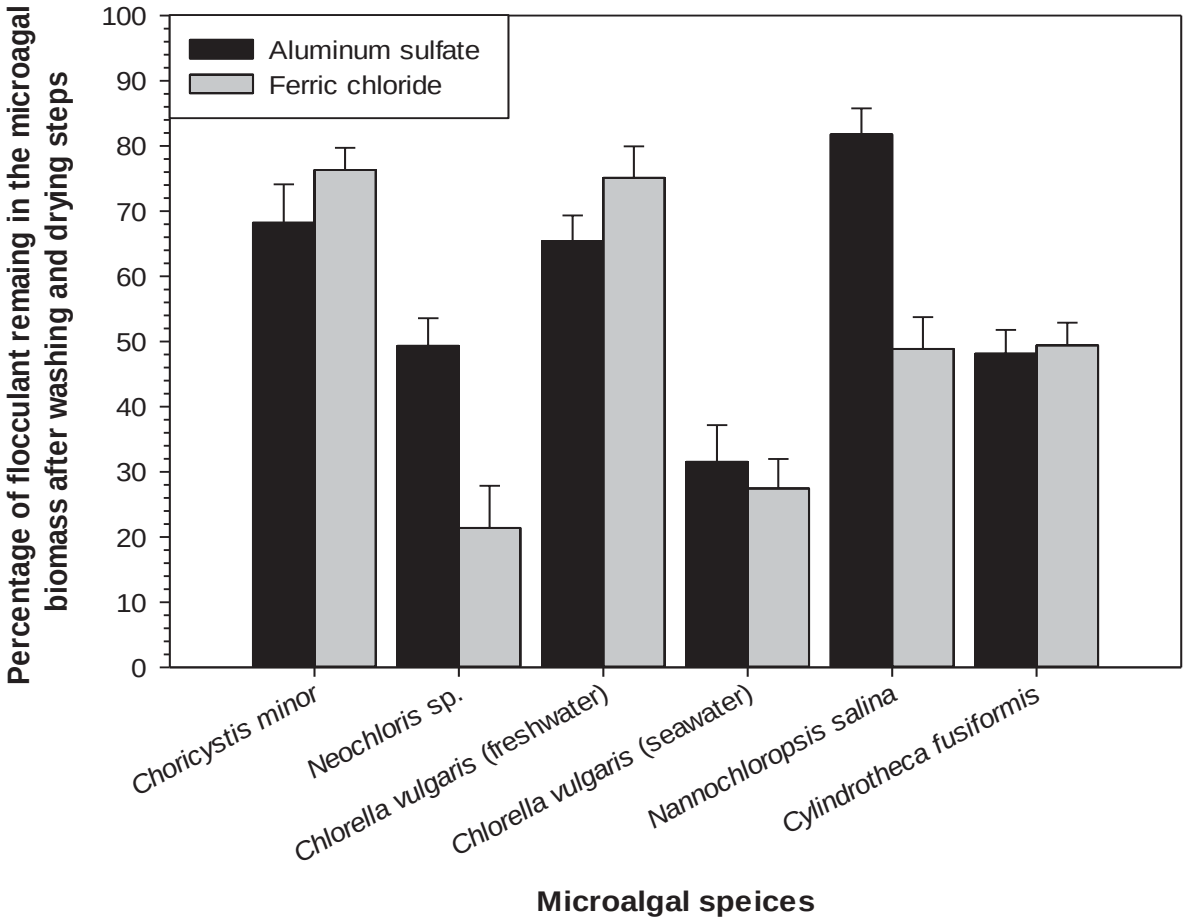


Figure 4.27. Percentage of the flocculant dose remaining in the microalgal biomass after washing and drying.

68.3±5.9% of the aluminum sulfate dosage used was retained. Similarly, *C. minor* retained 76.3±3.4% of the ferric chloride used. *C. vulgaris* (seawater) generally retained the least flocculant (Figure 4.27). In general, the algae that had a higher value of the optimal flocculant dose also retained a high fraction of that dose.

The presence of the flocculants in the algal biomass was found to not significantly affect the total lipid recovery by extraction. All biomass samples were extracted as paste biomass. Table 4.8 presents the results for all the algae. The total lipid contents in the same biomass batch recovered with flocculants and without any flocculants were statistically not significantly different. This meant that the flocculant attached to the biomass had no effect on

Table 4.8 Comparison of the total lipids content in the biomass recovered using flocculants and the control biomass recovered by centrifugation (no flocculants)

Species	The total lipids content in microalgal biomass*		
	Control	Flocculated biomass with aluminum sulfate	Flocculated biomass with ferric chloride
Grown in the BG11 freshwater medium			
Choricystis minor	30.0±0.4[a]	28.3±0.2[ab]	29.5±0.4[a]
Neochloris sp.	16.7±0.4[a]	16.3±1.6[a]	15.3±0.8[ab]
Chlorella vulgaris	35.2±1.8[a]	36.3±1.2[a]	36.1±1.5[a]
Grown in the BG11seawater medium			
Chlorella vulgaris	35.7±0.8[a]	34.8±0.7[a]	34.9±1.0[a]
Nannochloropsis salina	49.1±0.2[a]	48.9±0.9[a]	47.2±0.2[a]
Cylindrotheca fusiformis	23.6±0.8[a]	22.1±0.8[a]	21.9±0.5[ab]

* Data are represented as mean ± SD of triplicate runs.

Means followed by different superscript letters in the same row differ significantly ($p < 0.05$).

the extractability of the total lipids via the standard solvent extraction process. A similar lack of any effect of the presence of flocculants on the percentage of lipids extracted from microalgae by a solvent mix was reported by Borges et al. (2011) who used a 2:1 by volume mixtures of chloroform and methanol for extracting *Nannochloropsis aculata* and *Thalassiosira weissflogii* flocculated by anionic polyacrylamide flocculant and cationic polyacrylamide flocculant. No prior work has assessed the effect of inorganic flocculants on extractability of the biomass.

During extraction with mixed solvents, the solvents (i.e. chloroform and methanol) penetrate the cell membrane and into the cytoplasm. Chloroform interacts with the neutral

lipids to form the organic solvent-lipid complexes (Grima et al., 2013b). Methanol interacts with lipid–protein associations. The lipids complexed in solvents diffuse across the cell membrane into the extranal bulk solvent. All neutral lipids present both as free globules and membrane– associated complexes, are completely extracted using a mixture of non-polar and polar solvents (Halim et al., 2012). Water–methanol solvent layer contains the non-lipid contaminants including proteins and carbohydrates. The bottom solvent layer (the chloroform layer) contains neutral lipids and polar lipids. Evaporation of the chloroform leaves behind the crude lipids (total lipids), which need to be purified before further use in producing biodiesel (Halim et al., 2011; Halim et al., 2012; Grima et al., 2013b).

4.5.2 Comparison of solvent extraction of dry and wet paste biomass

The aim was to compare the total lipids extraction capability of the standard extraction method (modified Bligh and Dyer, Section 3.2.5.6) normally used with the dry biomass, for extraction of biomass paste. This was because any extraction of algal oils for a fuel process will require the use of paste biomass as drying is expensive and energy consuming (Suganya & Renganathan, 2012; Dejoye Tanzi et al., 2013; Liu et al., 2013). The Bligh and Dyer extraction method is a recognized standard solvent extraction method (Mercer & Armenta, 2011). It can be applied to extract wet materials containing nearly 80% water (Iverson et al., 2001), but is mostly used for predried biomass. The energy requirement for recovering microalgal biomass with a 75–82% typical moisture content using a belt filter is between 1161–1786 MJ for processing 100 m^3 of the culture broth with a cell concentration of 1.2 g L^{-1} (Liu et al., 2013). Drying adds to this requirement as about 2575 kJ of energy is needed to evaporate 1 kg of water at 25°C.

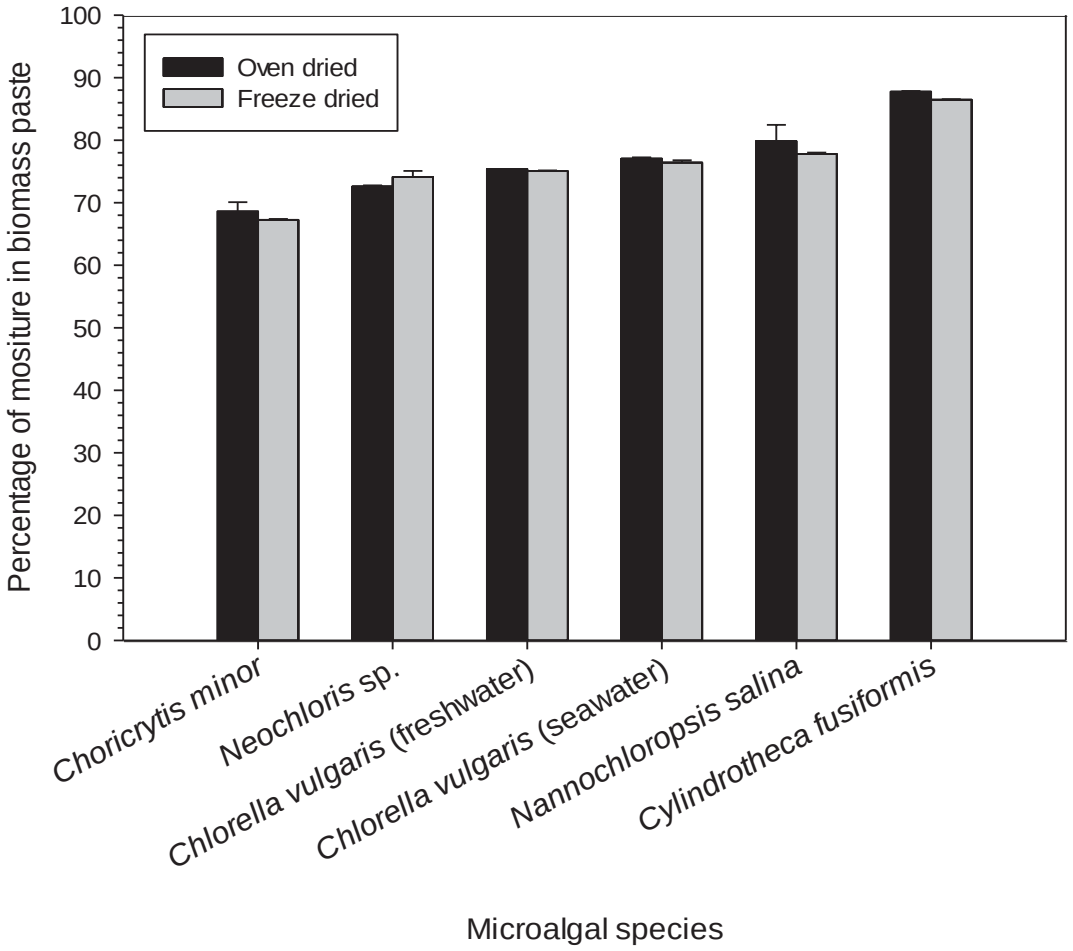


Figure 4.28. A comparison of the moisture content of the microalgal biomass paste obtained after centrifugation (no flocculant) and measured by oven drying and freeze drying.

Figure 4.28 compares the moisture content in the microalgal biomass paste obtained after centrifugation (Section 3.2.9.2) of different algae. Measurements using oven drying and freeze drying are compared. The percentage moisture content in the biomass paste ranged from 67% to 88%. This is similar to approximately 80% moisture content reported for *Nannochloropsis oculata* and *Dunaliella salina* biomass recovered using centrifugation (Dejoye Tanzi et al., 2013). Clearly, oven drying and freeze drying are equally effective in dehydration of algal biomass (Figure 4.28). Figure 4.29 shows the total lipids content of the biomass as determined by dry and wet paste extraction of the same biomass of each alga.

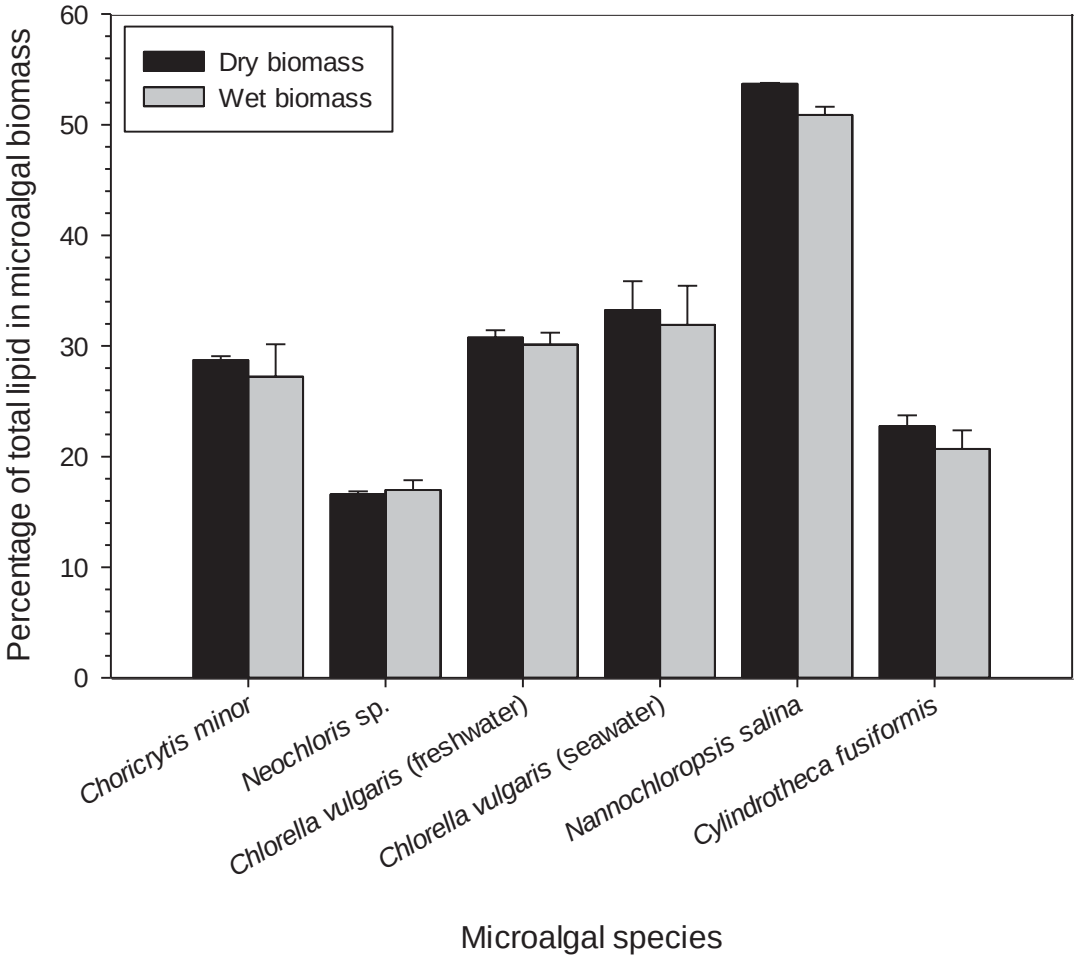


Figure 4.29. Comparison of the total lipids content of the biomass determined by standard solvent extraction (Section 3.2.5.6) of dry biomass (freeze-dried) and wet paste biomass.

No significant differences in the total lipids content (% of dry biomass) as determined by the two methods were found. Therefore, the standard extraction method (Section 3.2.5.6) is equally applicable for total lipid extraction from both the dry and the paste biomass of the various algae.

According to Liu et al. (2013) high water content in biomass paste interferes with extraction using the solvent 1,2-dimethoxyethane because water in the biomass forms a barrier around the lipids (Cooney et al., 2009). A high water content can also restrict the effective contact of solvent with algal cells by acting as a barrier between the solvent and the

cells (Mercer & Armenta, 2011; Suganya & Renganathan, 2012). To counter this, Cooney et al. (2009) and Lewis et al. (2000) suggested adding a solvent of higher polarity to the main extraction solvent. For example, methanol may be added to chloroform. In addition to solvent composition, the volume used, the extraction time and extraction temperature also influence the lipid extraction (Cooney et al., 2009; Mercer & Armenta, 2011; Halim et al., 2012). Therefore, experiments were performed to identify the optimal solvent composition and the extraction conditions for the one selected microalga, i.e. *N. salina*

4.5.3 Optimization of solvent composition for extraction of total lipids from biomass paste

Although the Bligh and Dyer (1959) solvent mixture of chloroform, methanol and water is well established for extraction of lipids from microalgae and other sources, there is no evidence in the literature of the composition of the mixture having ever been optimized especially for extraction of algal lipids. Therefore, this work focused on identifying the optimal solvent composition (using the established solvents of chloroform, methanol and water), for a single step extraction of *N. salina* biomass paste. The biomass harvesting procedure, the lipid extraction method, and the design of the experiment were fully explained in Chapter 3, Section 3.2.9.3. The single step extraction method of Figure 3.22 was adapted from the standard extraction method (Section 3.2.5.6, Figure 3.11) by reducing the number of the extraction steps from three with a total time of 9 h to a single step (4 h). The optimal composition of the solvent was determined for this modified extraction protocol. In the modified protocol, all the chloroform and methanol were added to the sample at the beginning of the extraction step (the monophasic step), while all the water was added at the

separation step (the biphasic step). Adding water to the monophasic step was considered unnecessary as moist paste biomass was being extracted.

In the standard extraction method (Figure 3.11), the chloroform and water are divided into two parts and one part is added to the sample at the extraction step and the second part is added at the separation step. The standard extraction method was considered as the reference method which extracted all the lipids present in the dry biomass (i.e. 100% recovery). The total lipid content of the biomass measured using this extraction method was 49.5±1.7% on dry basis (100% recovery).

The results in Table 4.9 show the effects of the solvent compositions on the total lipids extraction from *N. salina* biomass paste. The total lipids extracted from the same biomass sample as above ranged from approximately 23 to 47% of the dry biomass. The total lipids extraction increased significantly with increasing volume of chloroform as shown in Table 4.10. The highest total lipids extracted was 47.0±1.3% of dry biomass (95% recovery). This was extracted with a solvent composition of 19 mL chloroform, 7 mL methanol, and 3 mL water for 1 g (dry basis) equivalent of paste biomass. The lowest total lipids extraction was 23.6±1.4% of dry biomass (48% recovery) with a solvent mixture of 7 mL chloroform, 19 mL methanol, and 3 mL water (Table 4.11). The measured results from Table 4.9 were analysed for variance (ANOVA).

Table 4.9 Compositions of the solvent mixtures from the experimental design and the amount of total lipids extracted from *N. salina* biomass paste

Run order	Volume of solvent (mL)			Total lipids extracted (% of dry biomass)	
	Chloroform	Methanol	Water	Measured[a]	Predicted
Block 1					
A	13	13	3	42.4	43.3
B	7	7	15	39.3	39.7
C	9	9	11	38.1	38.7
D	19	7	3	46.1	46.7
E	7	19	3	24.6	23.8
F	11	11	7	40.7	39.9
G	9	15	5	36.8	34.6
H	7	13	9	32.4	34.2
I	15	9	5	43.8	44.1
J	13	7	9	38.8	39.9
Block 2					
K	9	9	11	40.1	38.7
L	13	7	9	40.0	39.9
M	7	7	15	40.3	39.7
N	9	15	5	35.6	34.6
O	7	13	9	33.7	34.2
P	13	13	3	43.3	43.3
Q	11	11	7	40.3	39.9
R	15	9	5	44.4	44.1
S	7	19	3	22.6	23.8
T	19	7	3	47.9	46.7

[a] Extracted using the single step extraction method (Figure 3.22); the total lipids content of this biomass was 49.5±1.7% on dry basis (100% recovery) measured using the standard extraction method (Figure 3.11).

Table 4.10 Influence of the solvent composition on total lipids extracted.

Volume of solvent (mL)			Total lipids extracted (% of dry biomass)*[a]
Chloroform	Methanol	Water	
19	7	3	47.0±1.3
15	9	5	44.1±0.5
13	13	3	42.9±0.6
13	7	9	39.4±0.8
11	11	7	40.5±0.3
9	15	5	36.2±0.9
9	9	11	39.1±1.4
7	19	3	23.6±1.4
7	13	9	33.1±0.9
7	7	15	39.8±0.7

* Data are presented as mean ± standard deviation of the duplicate sets of data in Table 4.9.

[a] The average of the duplicate runs shown in Table 4.10.

Table 4.11 Analysis of variance (ANOVA) for the percentage of total lipids

Source	DF	Seq SS	Adj SS	Adj MS	*F* value	*P* value
Regression	5	755.680	755.680	151.136	105.66	0.000
Linear	2	623.615	311.702	155.851	108.95	0.000
Quadratic	3	132.065	132.065	44.022	30.77	0.000
Chloroform*Methanol	1	105.225	105.084	105.084	73.46	0.000
Chloroform*Water	1	17.326	17.168	17.168	12.00	0.004
Methanol*water	1	9.515	9.515	9.515	6.65	0.022
Residual Error	14	20.026	20.026	1.430		
Lack-of-Fit	4	11.104	11.104	2.776	3.11	0.066
Pure Error	10	8.922	8.922	0.892		
Total	19	775.707				

R^2 = 97.42%, R^2(pred) = 94.34%, R^2(adj) = 96.50%

(DF = degrees of freedom; Seq SS = sequence sum of squares; Adj SS = adjusted sum of squares; Adj MS = adjusted mean square; *F* value = the test statistic used to determine whether a term is associated with the response; *P* value = probability that measures the evidence against the null hypothesis; R^2 = coefficient of determination which is used to determine how well the model fits the data; R^2(pred) = a parameter used to determine how well the model predicts the response for new observations; R^2(adj) = a parameter used to compare models that have different number of predictors)

The ANOVA (Table 4.11) shows that the volume of each solvent (linear) affected the total lipid extraction significantly (p<0.05). The synergic interaction of any two solvents (quadratic) also affected the total lipid extraction significantly (p<0.05). ANOVA was

applied to the linear/quadratic models and showed no significant lack of fit (p>0.05) for the total lipids extraction (% of dry biomass). This suggested that the model adequately fitted to the experimental data. Therefore, the quadratic model with a high coefficient of determination (R^2) of 0.97 can be used to predict the total lipids extraction (% of dry biomass) capability of the various solvent compositions from *N. salina* biomass paste. The quadratic model is as follow:

$$Y = 34.76X_1 - 34.31X_2 + 59.96X_3 + 188.17X_1X_2 - 76.06X_1X_3 + 56.62X_2X_3 \quad (4.5)$$

In this equation, Y is the predicted response of the total lipid extraction (% by dry biomass); 34.76, –34.31, and 59.96 are the linear coefficients estimated by the least-squares method; 188.17, –76.06, and 56.62 are the quadratic coefficients estimated by the least-squares method; X_1, X_2, and X_3 are the coded values of the volumes of chloroform (mL), methanol (mL) and water (mL), respectively.

The contour plot and the response surface plot of the quadratic model (Equation 4.5) are shown in Figure 4.30 and Figure 4.31, respectively. The predicted responses of the total lipids extraction (% of dry biomass), calculated using Equation 4.5, are shown in Table 4.9 as predicted values. The dark-green region of Figure 4.30 indicates the high percentage of total lipids extraction (i.e. a measured lipid of >45% of dry biomass) when performed with high proportion of chloroform, and low proportions of methanol and water.

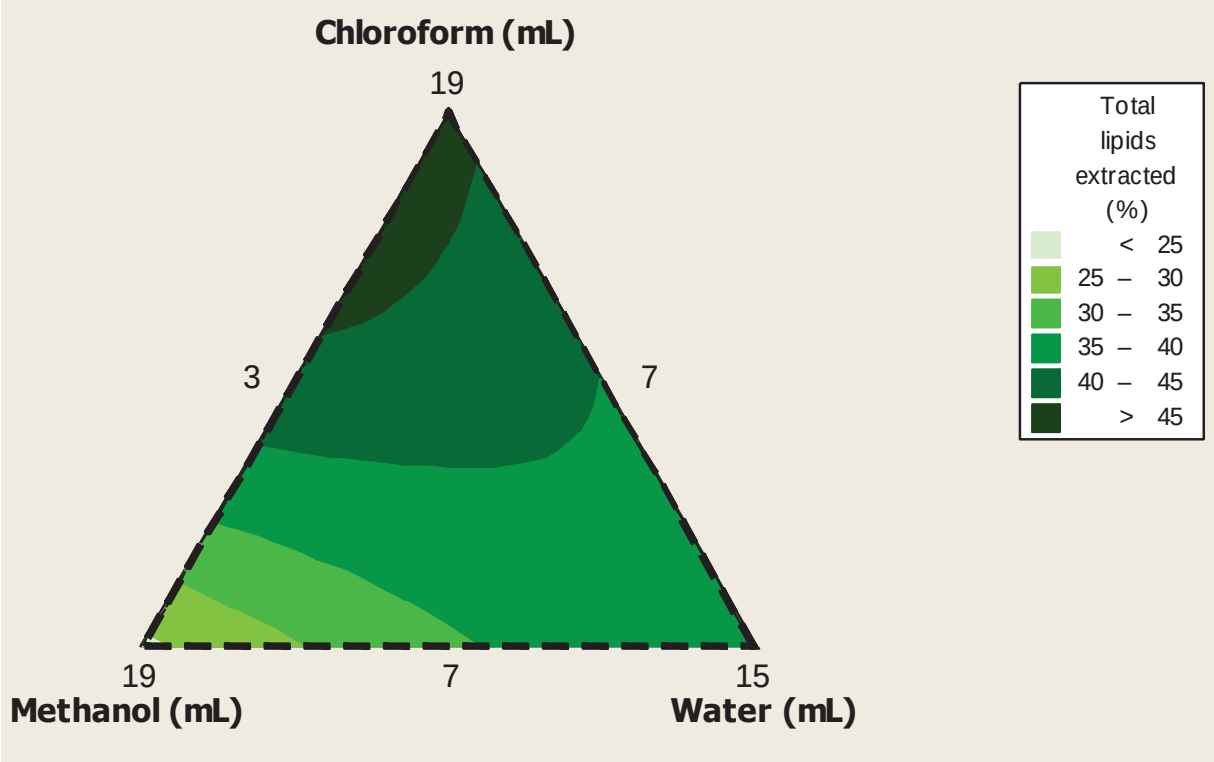


Figure 4.30. Mixture contour plot of the quadratic model for the total lipids extraction (% of dry biomass), as a function of the volume (mL) of chloroform, methanol and water. At the top corner of the above triangle, the solvent composition is: 19 mL chloroform, 7 mL methanol, 3 mL water; at the bottom left corner, the solvent composition is: 7 mL chloroform, 19 mL methanol, 3 mL water; at the bottom right corner, the solvent composition is: 7 mL chloroform, 7 mL methanol, 15 mL water.

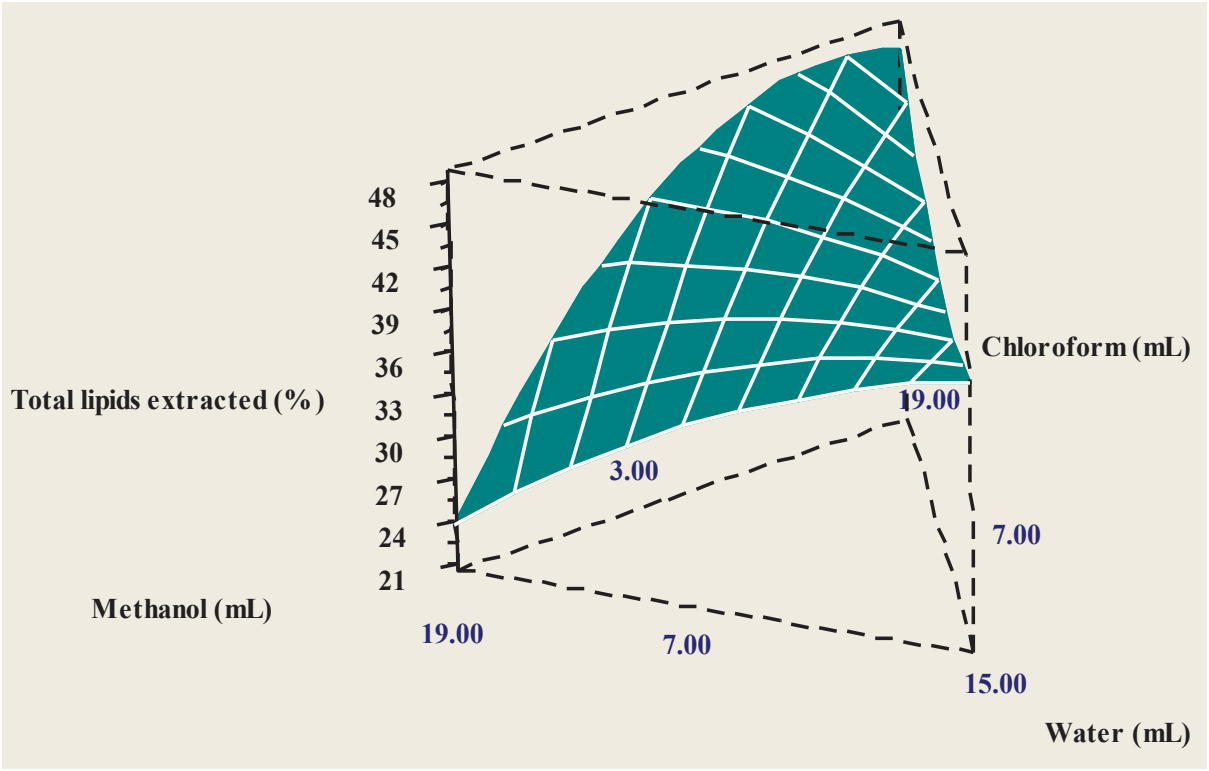


Figure 4.31. Response surface plot of the quadratic model for the total lipids extracted (% of dry biomass), as a function of the volume (mL) of chloroform, methanol, and water.

Based on the response surface, the optimized composition of the solvent mixtures was 17 mL chloroform, 9 mL methanol, and 3 mL water, or a volume ratio of 5.7:3:1 of chloroform:methanol:water. For the validation, an additional experiment using the optimal solvent mixture was performed in duplicate. The total lipids content (% of dry biomass) obtained experimentally was 45.68±0.60% (92% recovery) compared to a value of 47.39% predicted by the model. Thus, there was no significant difference between the predicted and the measured data.

Ryckebosch et al. (2012) reported that extraction with the solvent mixtures of polar and non-polar solvents showed a higher lipid recovery compared to using a single extraction solvent. Chloroform/methanol (1:1, v/v) were recommended for extraction of the lipids from

Chlorella vulgaris SAG 211-11b dry biomass at a solvent: biomass ratio of 100:1 (v/w; mL/g) (Ryckebosch et al., 2012). Sheng et al. (2011) compared the lipid extraction of *Synechocystis* PCC 6803 dry biomass with 14 different solvents and solvent mixtures. The results showed that the solvent mixtures based on Folch (chloroform/methanol, 2:1, v/v) and Bligh & Dyer (chloroform/methanol/water, 1:2:0.8, v/v) methods showed the highest lipid recoveries (Sheng et al., 2011). Lee et al. (1998c) also confirmed that the lipid extraction from *Botryococcus brannii* dry biomass with chloroform/methanol (2:1, v/v) was most effective compared to extraction with other combinations (e.g. hexane/isopropanol (1:1, v/v), dichloroethanol/ ethanol (1:1, v/v), dichloroethane/ethanol (1:1, v/v)). The results from these studies indicate clearly that the solvent combination of chloroform and methanol has a high potential for lipid extraction from dry microalgal biomass. The suitable ratio of chloroform and methanol was different depending on the microalgal species and the extraction conditions.

In view of the results of the present study, the single step extraction method was highly effective in recovering the total lipids from the biomass paste. A high total lipids recovery of 92% (45.68±0.60% w/w measured lipid in dry biomass) from *N. salina* biomass paste was obtained using the optimal solvent mixture of chloroform/methanol/water at 17mL/9mL/3mL (ratio of 5.7:3:1, v/v) and a solvent to biomass ratio of 29:1, v/w (mL/g). With this optimized composition of the solvent mixture the conditions for the extraction (i.e. the volume of the solvent mixture (mL) per 1 g equivalent of dry biomass, extraction temperature (°C) and extraction time (h)) were optimized.

4.5.4 Optimization of the conditions for the extraction of total lipids from the biomass paste

The optimal extraction solvent mixture identified from the previous experiments (Section 4.5.3) was used to optimize the conditions for extraction of the total lipids from *N. salina* biomass paste. A randomized, replicated 2^3 factorial experimental design with center points (Table 4.12) was used. A maximal yield of total lipids was the objective. The extraction conditions included the volume of the solvent mixture (25, 29, 33 mL), the extraction temperature (25, 35, 45 °C) and the extraction time (2, 4, 6 h). The amount of biomass paste extracted was fixed at 1 g dry equivalent of dry biomass as paste. The composition of the solvent mixture was fixed so that the ratio of chloroform, methanol and water was always 5.7:3:1 (v/v). The *N. salina* biomass paste used was the same as in the previous experiments (Section 4.5.3). The total lipids extraction procedure and the design of the experiment were fully explained in Chapter 3, Section 3.2.9.4.

The results in Table 4.12 show the effect of the extraction conditions on the total lipids extracted from the biomass paste. In Table 4.13 these data are summarized in terms of the average value of the total lipids extracted in replicate experiments. The data show that the total lipids extracted increased with increasing volume of the solvent. The highest measured total lipids content in the biomass was 47.7±0.4 % of dry biomass (96.3% recovery). The extraction conditions were 33 mL of solvent mixture, 25°C extraction temperature, and 2 h of extraction time. By contrast, the lowest total lipids extraction was 42.9±0.9 % of dry biomass (86.6% recovery), with 25 mL of solvent mixture, at 45°C of temperature, and 2 h of extraction time. The experimental results for total lipids extraction (Table 4.12) were also analysed using ANOVA.

Table 4.12 The measured and predicted response of the total lipids extracted (% of dry biomass) from *N. salina* biomass paste at different extraction conditions

Number	Run order	Volume of each solvent (mL)			Total volume of solvent mixtures (mL)	Temperature (°C)	Time (h)	Total lipids extracted (% of dry biomass)	
		Chloroform	Methanol	Water				Measured[a]	Predicted
1	10	19.4	10.2	3.4	33	25	2	48.0	47.7
2	2	19.4	10.2	3.4	33	25	2	47.5	47.7
3	14	19.4	10.2	3.4	33	25	6	47.2	47.4
4	6	19.4	10.2	3.4	33	25	6	47.6	47.4
5	4	19.4	10.2	3.4	33	45	2	47.5	47.1
6	12	19.4	10.2	3.4	33	45	2	46.8	47.1
7	16	19.4	10.2	3.4	33	45	6	47.4	47.2
8	8	19.4	10.2	3.4	33	45	6	47.0	47.2
9	18	17	9	3	29	35	4	45.1	45.2
10	17	17	9	3	29	35	4	45.3	45.2
11	1	14.7	7.8	2.5	25	25	2	44.4	44.3
12	9	14.7	7.8	2.5	25	25	2	44.3	44.3
13	5	14.7	7.8	2.5	25	25	6	44.6	44.0
14	13	14.7	7.8	2.5	25	25	6	43.4	44.0
15	3	14.7	7.8	2.5	25	45	2	42.2	42.9
16	11	14.7	7.8	2.5	25	45	2	43.6	42.9
17	15	14.7	7.8	2.5	25	45	6	44.1	43.5
18	7	14.7	7.8	2.5	25	45	6	43.0	43.5

[a] Extracted using the single step extraction method (Figure 3.22); the total lipids content in this biomass was 49.5±1.7% on dry basis (100% recovery) measured using the standard extraction method (Figure 3.11).

Table 4.13 Summary of the effect of the extraction conditions on the total lipids extracted (% dry biomass), the calculation based on experimental measured from Table 4.12

Extraction conditions			Total lipids extracted (% of dry biomass)*[a]
Volume of solvent (mL)	Temperature (°C)	Time (h)	
33	25	2	47.7±0.4
33	25	6	47.4±0.2
33	45	2	47.1±0.5
33	45	6	47.2±0.3
29	35	4	45.2±0.1
25	25	2	44.3±0.1
25	25	6	44.0±0.9
25	45	2	42.9±0.9
25	45	6	43.5±0.8

* Data are presented as mean ± standard deviation of the duplicate sets of data in Table 4.12.

[a] The average of the duplicate runs shown in Table 4.13.

The ANOVA results of the total lipids extraction are presented in Table 4.14. The ANOVA analysis, confirmed that the volume of solvent (mL) and extraction temperature (°C) had statistically significant effects on the total lipids extracted (p<0.05). In addition, the ANOVA results also indicated that there were no significant effects of interactions among the extraction conditions on the total lipids extracted (p<0.05). Figure 4.32, the Pareto chart, shows the magnitude and the importance of the effects of extraction conditions on the total lipids extracted. Based on Figure 4.32, the solvent volume had the most significant effect on

Table 4.14 Analysis of variance (ANOVA) for the total lipids extraction

Source	DF	Seq SS	Adj SS	Adj MS	*F* value	*P* value
Main effect	3	55.922	55.922	18.640	58.58	0.000
Volume of solvent (mL)	1	54.132	54.132	54.132	170.12	0.000
Temperature (°C)	1	1.788	1.788	1.788	5.62	0.042
Time (h)	1	0.000	0.000	0.000	0.000	0.976
2-Way interactions	3	0.857	0.857	0.285	0.90	0.479
Volume of solvent* Temperature	1	0.299	0.299	0.299	0.94	0.357
Volume of solvent*Time	1	0.716	0.071	0.071	0.22	0.647
Temperature*Time	1	0.486	0.486	0.486	1.53	0.248
3-Way interactions	1	0.088	0.088	0.088	0.28	0.611
Volume of solvent* Temperature*time	1	0.088	0.088	0.088	0.28	0.611
Curvature	1	0.182	0.182	0.182	0.57	0.468
Residual error	9	2.863	2.863	0.318		
Pure error	9	2.863	2.863	0.318		
Total	17	59.914				

R^2 = 95.22%, R^2(pred) = 80.57%, R^2(adj) = 90.97%

the total lipid extraction, compared to the effects of extraction temperature and the extraction time. Figure 4.32 shows no synergic interaction among the extraction conditions on the yield of total lipids extracted. The measured extraction results (Table 4.12) were evaluated and coefficients estimated for the total lipids extraction in terms of coded values in order to fit a third order interaction polynomial model. This polynomial model had a high coefficient of determination (R^2) of 0.95 and therefore it can be used to predict the total lipids extracted

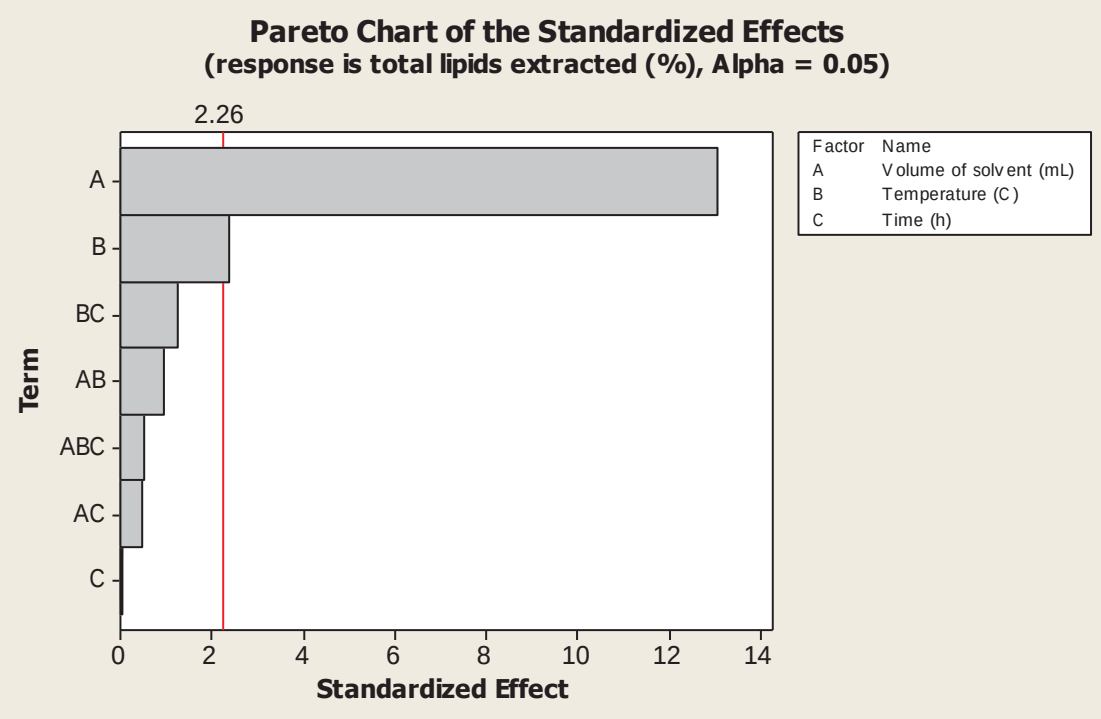


Figure 4.32. The Pareto chart for the effect of the extraction conditions on the total lipids extracted.

from *N. salina* biomass paste for any combination of the extraction conditions. This third order interaction polynomial model is as follows:

$$Y = 45.52+1.84X_1-0.33X_2+0.004X_3+0.14X_1X_2-0.07X_1X_3+0.17X_2X_3-0.07X_1X_2X_3-0.32 \quad (4.6)$$

In this equation, Y is the predicted response of the total lipids extracted (% by dry biomass); 45.52 is the overall mean value of the measured results; 1.84, –0.33 and 0.004 are the main effect coefficients estimated by the least-squares method; 0.14, –0.07 and 0.17 are the 2-way interaction coefficient estimated by the least-squares method; –0.07 is the 3-way interaction coefficients estimated by the least-squares method; –0.32 is the random error term; X_1, X_2, and X_3 are the coded variables that represent the three extraction conditions, i.e. the volume

of solvent (mL), the extraction temperature (°C) and extraction time (h), respectively. The predicted responses of the total lipids extracted (% of dry biomass), calculated using Equation 4.6, are shown in Table 4.12 as the predicted values.

The response surface plots of the polynomial model (Equation 4.6) for total lipids extracted are shown in Figure 4.33. These plots show a relatively strong influence of the volume of solvent (Figure 4.33 a and b) on the total lipid extracted, compared to the influences of the extraction temperature and the extraction time. Based on the response surface, the optimized extraction conditions were: 33 mL of solvent mixture for 1 g dry equivalent of paste biomass; 25°C extraction temperature; and a 2 h extraction time. For validation, an additional experiment using the optimal extraction conditions was carried out in duplicate. The total lipid extracted (% of dry biomass) obtained experimentally was 47.6±0.4% (96.1% recovery) compared to a value of 47.7% (96.4% recovery) predicted by the model. There was no significant difference between the predicted and the measured data.

The effect of the volume of solvent on the total lipids extracted from the algal biomass was consistent with some of the published findings. Suganya and Renganathan (2012) studied the effect of the ratio of solvent to biomass in the range 3:1 to 7:1 (v/w, mL/g) on oil extraction from dry biomass of *Ulva lactuca*. The measured oil content in the biomass increased from 9% to 10.9% of dry biomass when the solvent to biomass ratio increased from 3:1 to 6:1 (v/w, mL/g). A further raising of the solvent to biomass ratio to above 6:1 (v/w, mL/g) did not much improve the oil recovery (Suganya & Renganathan, 2012). Similar results were reported by Yang et al. (2014). The ratio of solvent to biomass was found to have stronger effect on the lipid recovery from *Picochlorum* sp. wet biomass when compared with the other extraction conditions (extraction temperature, extraction time) (Yang et al., 2014). The best ratio of solvent to wet biomass was 5:1 (v/w, mL/g) (Yang et al., 2014).

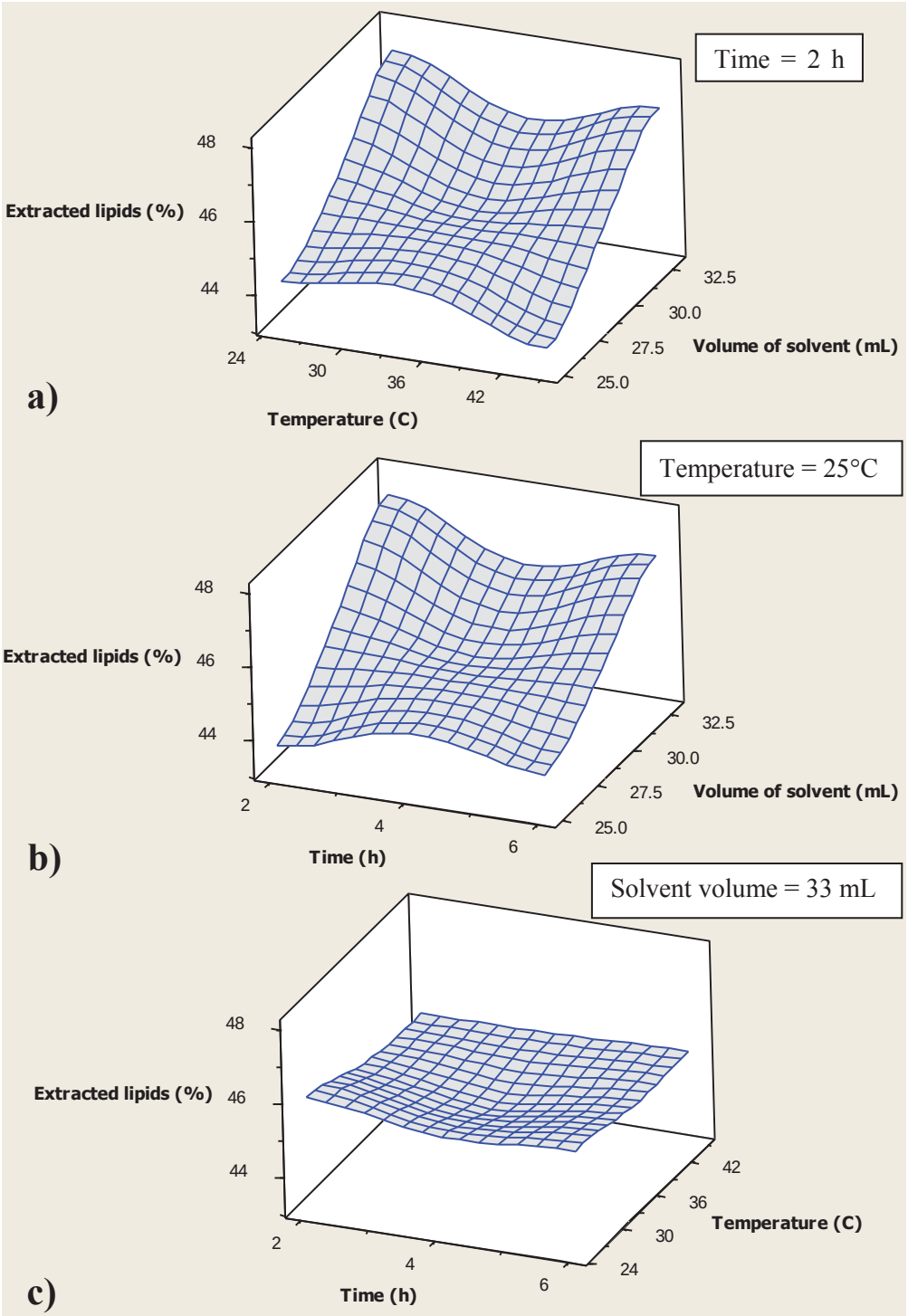


Figure 4.33. Response surface plots of the total lipids extracted (% of dry biomass): a) dependence of extracted lipids on the volume of the solvent (mL) and the extraction temperature (°C); b) dependence of extracted lipids on the volume of the solvent (mL) and the extraction time (h); c) dependence of extracted lipids on the extraction temperature (°C) and the extraction time (h).

In some of the other studies of solvent extraction of algal biomass, extraction temperature has been found to have a significant positive effect on the total lipids extracted (Yang et al., 2014). Cooney et al. (2009) explained that extraction at a high temperatures increases the solvation power of a solvent to dissolve the oils to shorten the extraction time. Suganya and Renganathan (2012) observed that an increase in extraction temperature in the range of 35-55°C enhanced the lipid recovery from *Ulva lactuca* dry biomass. Using hexane, the measured lipid content increased from 7% to 9.75% of dry biomass as the temperature increased from 35 to 55°C (Suganya & Renganathan, 2012). Other studies have found no major influence of the extraction temperature in concurrence with the present study. For example, Yang et al. (2014) found that the lipid recovery from *Picochlorum* sp. wet biomass was only slightly affected when extraction temperature was increased in the range of 20-50°C. The optimal extraction temperature was 26°C (Yang et al., 2014). Therefore, the use of a high temperature for lipids extraction as suggestion by Cooney et al. (2009) and Sheng et al. (2011) needs to be carefully considered as it affects the energy requirement of a production process without necessarily having any benefit.

In this study, the increase in extraction time from 2 h to 6 h had no significant effect. In contrast, Yang et al. (2014) reported that an increase in extraction time from 2.5 min to 47.5 min had a significant effect on the lipid recovery from *Picochlorum* sp. wet biomass. The optimal extraction time was 37 min. Suganya and Renganathan (2012) also observed the extraction time to be an important factor for lipid recovery from *Ulva lactuca* dry biomass. Lipid recovery increased nearly 2-fold by an increase in extraction time from 20 to 140 min (Suganya & Renganathan, 2012). Further increase in extraction time to 140 min did not increase recovery (Suganya & Renganathan, 2012). Similarly, Cooney et al., (2009) reported that increase in the extraction time from 2 h to 20 h had no effect on the amount of lipids extracted from *Nannochloropsis* dry biomass using the Bligh and Dyer (1959) method. From

these results, it may be inferred that the extraction time may be a significant factor for an extraction duration of < 2 h, but not for higher durations.

In the view of the results, the residual flocculant in the algal biomass and whether the biomass is dry or paste, do not influence the total lipids recovery when extracted using the standard extraction method (modified Bligh & Dyer, 1959). The optimal extraction solvent mixture for *N. salina* biomass paste is chloroform:methanol:water in the volume ratio of 5.7:3:1. Using this mixture a solvent volume of 33 mL for a paste biomass equivalent of 1 g dry biomass is optimal. The optimal extraction temperature and time are 25°C and 2 h, respectively. Under these optimal extraction conditions, more than 96% of the lipid in dry biomass can be recovered from the biomass paste. The optimal solvent composition identified here reduced the extraction time to 22% of the time needed in the modified Bligh and Dyer (1959) method. Furthermore, in modified Bligh and Dyer (1959) method the solvent composition for extracting 1 g of biomass was 25 mL chloroform and 20 mL methanol. In the method developed in this study the solvent used had only 77.6% of the chloroform used in the modified Bligh and Dyer (1959) method. Also, in the method developed here, the methanol required was only 51.0% of that of the modified Bligh and Dyer (1959) method.

CHAPTER 5

SUMMARY AND CONCLUSIONS

5.1 Summary

This work focused on flocculation–sedimentation as a method of recovering the biomass of microalgae from the aqueous broth. In addition, the possible effects of the presence of flocculants in the recovered biomass paste, on solvent extraction of the oils were examined.

A selection of microalgae having different cell size, morphology and ionic strength requirements for growth were examined for recovery by flocculation using inexpensive, readily available and safe inorganic flocculants (aluminum sulfate, ferric chloride). The algae were grown photoautotrophically in batches in freshwater and marine media to characterize biomass and oil productivities. Of the five microalgae examined, highest lipid productivities were observed for the marine alga *N. salina* and the freshwater alga *C. vulgaris* grown in full strength seawater.

Batch flocculation studies demonstrated that all algae could be effectively recovered by flocculation–sedimentation using either of the two flocculants. However, the minimum flocculant dosage required for 95% removal of the biomass from a broth in a standardized treatment depended on the following factors: the flocculant; the microalga, particularly its cell size; the ionic strength of the culture medium; and the initial concentration of the biomass in the broth. The microalgal species (its cell size/morphology, surface charge) was the strongest influence on the flocculation behaviour. The flocculant dose required for 95% removal of the biomass from the broth in a standardized treatment increased linearly with the

biomass concentration in the broth. In nearly all cases, aluminum sulfate was a more effective flocculant, requiring a lower dosage, on account of the relatively high surface charge density of the Al^{3+} ion compared to Fe^{3+}. Also, aluminum sulfate was less expensive overall than ferric chloride. Therefore, in subsequent work on a continuous flocculation–sedimentation, only aluminum sulfate was examined.

The optimal biomass recovery conditions identified in batch flocculation studies were used as a basis for designing a continuous flow flocculation–sedimentation process. This process was developed for the marine alga *N. salina* as it had the highest oil productivity in marine media. Any commercial process for producing fuel oils from microalgae will need to use marine algae as freshwater is in short supply globally (Becker, 1995; Chisti, 2007, 2012; Chen et al., 2013a; Chisti, 2013). Also, *N. salina* has a relatively small cell and any flocculation–sedimentation process capable of removing it from the broth could be likely adapted to microalgae having larger cells.

The continuous flocculation–sedimentation process proved quite effective: 86% of the algal biomass originally in the broth could be recovered within ~2.5 h of treatment. Notwithstanding this, the continuous flocculation–sedimentation required twice the optimal aluminum sulfate dosage that had been identified for the batch operation.

During flocculation, the cationic flocculants irreversibly attached to the negatively charged algal cells. Therefore, there was concern that adhering flocculant might interfere with the solvent extraction of the oil from the biomass. Studies revealed that a suitable mixture of Bligh and Dyer (1959) solvents (chloroform, methanol, water) could remove the oils from the flocculated biomass paste as effectively as from the same biomass paste obtained without the use of flocculants. Furthermore, an appropriately selected extraction solvent mixture was as effective in recovering the oils from a biomass paste as from freeze dried biomass. This

proved that a prior drying step was unnecessary for attaining a quantitative recovery of the oil from the biomass.

Statistically designed experiments were used to identify an optimal solvent composition of recovery the oil from harvested biomass paste of *N. salina*. Using the optimized solvent composition, the optimal conditions for extraction of the biomass paste were identified. The optimal solvent composition for extracting 1 g dry equivalent of the biomass paste of *N. salina* was 5.7:3:1 by volume mixture of chloroform, methanol and water. The optimal volume of the solvent mixture for extracting biomass paste equivalent to 1 g of dry biomass, was 33 mL. The extraction conditions were 25°C and 2 h.

5.2 Novelty and contributions of this work

The major novel contributions of this study are as follows:

1. It comparatively characterized batch flocculation–sedimentation of multiple microalgae including freshwater and marine species of different cell size/morphology, including a marine diatom. Using a microalga that could be grown both in seawater and freshwater (i.e. *C. vulgaris*), effects of ionic strength of the culture media on flocculation behaviour was characterized in the first ever study of its kind.

2. For the algae of interest, the minimum dosages required for 95% biomass removal by flocculation–sedimentation were established for two commercially viable and safe flocculants. The effects of algal biomass concentration in the broth on flocculant dosage required for 95% biomass recovery under standard treatment conditions were quantified.

3. Flocculants irreversibly adsorbed onto the biomass of microalgae were shown to not interfere with lipid recovery from the biomass paste of any microalga. For all algae, solvent extraction of oils from biomass paste (~86% moisture, w/w) was shown to be as effective as extraction from freeze-dried biomass.

4. Based on the above studies, a continuous flow flocculation–sedimentation process was designed and characterized for the one alga that demonstrated the highest oil productivities (i.e. *N. salina*). The optimal flocculant dosage of the one identified preferred flocculant was established for the target alga in continuous flocculation–sedimentation.

5. For *N. salina*, the one alga that demonstrated the highest lipid productivity, an optimal solvent composition was empirically established for a much simplified one-step extraction compared with the modified Bligh and Dyer (1959) extraction procedure that is widely used. Using the above mentioned solvent composition, the optimal extraction conditions were established for the biomass paste, using statistically designed experiments.

5.3 Conclusions

Based on the results of this work, the following are the main conclusions:

1. *N. salina* and *C. vulgaris* grown in full strength seawater media proved to have the highest lipid productivity of 31.4±0.6 and 23.9±0.6 mg L^{-1} d^{-1}, respectively.

2. A lipid productivity of 23.9±0.6 mg L^{-1} d^{-1} for *C. vulgaris* grown in full strength seawater media was comparable to a lipid productivity of 22.4±1.2 mg L^{-1} d^{-1} for the same alga grown in freshwater media.

3. All microalgae could be effectively harvested by flocculation–sedimentation using either aluminum sulfate or ferric chloride.

4. In nearly all cases, aluminum sulfate was a somewhat superior flocculant than ferric chloride.

5. The optimal flocculant dosage (mg L^{-1}) for 95% recovery of the biomass following a standardized treatment increased linearly with increasing concentration of the microalgal biomass in the broth. In other words, the flocculant dosage for a given level of biomass recovery under standardized processing conditions increased with an increase in the cell specific surface area in the range of 26–450 μm^2 $cell^{-1}$. Therefore, the main mechanism of the flocculation appeared to be cell surface charge neutralization.

6. The efficiency of microalgal biomass recovery by flocculation depended on the following factors: the type of flocculant; the flocculant dose; the biomass concentration in the broth; the microalgal species; and the ionic strength of the culture broth. The microalgal species appeared to be the most important variable.

7. In a continuous flocculation–sedimentation process designed for *N. salina*, a maximum recovery efficiency of 86% was attained using aluminum sulfate (229 mg L^{-1}) and a residence time of about 148 min. Potentially this flocculation efficiency may be enhanced by a redesign of the sedimentation tank, a subject of possible study in future work outside this thesis.

8. The flocculant adhering to the algal biomass paste did not interfere with extraction of the oils via a modified Bligh and Dyer (1959) method.

9. The optimal extraction conditions for total recovery the lipids from 1 g (dry basis) of *N. salina* biomass paste were as follows: solvent volume of 33 mL; a solvent composition of 5.7:3:1 by volume of chloroform, methanol and water; a temperature of 25°C; and an extraction time of 2 h. These extraction conditions could recover 96.1% of the oil present in the biomass. Compared to the standard extraction method (modified Bligh and Dyer (1959) method), the single step optimized extraction method developed in this work reduced the solvent mixture volume to ~52% and the extraction time to ~22%.

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APPENDIX

EXPERIMENTAL DATA

Data for the spectrophotometric calibration curves for Figure 3.3

Figure 3.3 A

Absorbance 680 nm	Dried biomass concentration (g L^{-1})
0.0	0.0±0.0
0.062	0.012±0.001
0.120	0.023±0.001
0.129	0.025±0.003
0.150	0.029±0.001
0.170	0.032±0.005
0.187	0.035±0.002
0.217	0.039±0.002
0.236	0.044±0.001
0.257	0.050±0.000
0.303	0.059±0.000
0.365	0.070±0.003
0.448	0.088±0.002

Data are presented as mean ± standard deviation of triplicate runs

Data for the spectrophotometric calibration curves for Figure 3.3 (Cont.)

Figure 3.3 B

Absorbance 680 nm	Dried biomass concentration (g L^{-1})
0.0	0.0±0.0
0.101	0.013±0.001
0.163	0.022±0.001
0.196	0.026±0.001
0.229	0.030±0.003
0.274	0.035±0.002
0.298	0.039±0.000
0.381	0.051±0.001
0.492	0.065±0.001
0.585	0.077±0.001

Data are presented as mean ± standard deviation of triplicate runs.

Figure 3.3 C

Absorbance 680 nm	Dried biomass concentration (g L^{-1})
0.0	0.0±0.0
0.084	0.021±0.003
0.140	0.040±0.001
0.177	0.052±0.000
0.210	0.059±0.001
0.224	0.067±0.002
0.293	0.089±0.001
0.321	0.097±0.000
0.391	0.116±0.002
0.470	0.146±0.002
0.584	0.175±0.000

Data are presented as mean ± standard deviation of triplicate runs.

Data for the spectrophotometric calibration curves for Figure 3.4

Figure 3.4 A

Absorbance 680 nm	Dried biomass concentration (g L^{-1})
0.0	0.0±0.0
0.08	0.040±0.000
0.093	0.045±0.001
0.105	0.052±0.001
0.129	0.060±0.001
0.148	0.072±0.002
0.194	0.090±0.004
0.253	0.121±0.002
0.395	0.181±0.003

Data are presented as mean ± standard deviation of triplicate runs.

Figure 3.4 B

Absorbance 680 nm	Dried biomass concentration (g L^{-1})
0.0	0.0±0.0
0.164	0.032±0.003
0.168	0.035±0.001
0.187	0.037±0.001
0.206	0.040±0.000
0.227	0.044±0.002
0.246	0.049±0.001
0.268	0.054±0.000
0.299	0.061±0.001
0.332	0.069±0.003
0.381	0.081±0.002
0.459	0.0969±0.002

Data are presented as mean ± standard deviation of triplicate runs.

Data for the spectrophotometric calibration curves for Figure 3.4 (Cont.)

Figure 3.4 C

Absorbance 680 nm	Dried biomass concentration (g L^{-1})
0.0	0.0±0.0
0.08	0.039±0.001
0.086	0.045±0.001
0.115	0.054±0.000
0.131	0.068±0.001
0.185	0.090±0.001
0.264	0.135±0.002
0.387	0.204±0.001
0.507	0.270±0.000

Data are presented as mean ± standard deviation of triplicate runs.

Data for the growth curves of microalgae for Figure 4.1

Data for Figure 4.1 *C. minor*

Time (d)	Biomass concentration ($g\ L^{-1}$)	Time (d)	Biomass concentration ($g\ L^{-1}$)	Time (d)	Biomass concentration ($g\ L^{-1}$)
0	0.444±0.012	19	1.855±0.052	38	2.435±0.016
1	0.601±0.019	20	1.991±0.056	39	2.454±0.021
2	0.738±0.030	21	2.073±0.590	40	2.452±0.024
3	0.840±0.020	22	2.091±0.048	41	2.463±0.021
4	0.943±0.028	23	2.144±0.064	42	2.489±0.016
5	1.032±0.032	24	2.193±0.034	43	2.507±0.028
6	1.081±0.042	25	2.228±0.051	44	2.547±0.077
7	1.206±0.040	26	2.248±0.057	45	2.567±0.136
8	1.289±0.220	27	2.271±0.063	46	2.570±0.044
9	1.402±0.023	28	2.297±0.054	47	2.598±0.043
10	1.484±0.030	29	2.297±0.051	48	2.591±0.050
11	1.550±0.072	30	2.285±0.029	49	2.595±0.027
12	1.593±0.058	31	2.339±0.029	50	2.581±0.042
13	1.637±0.062	32	2.361±0.016	51	2.583±0.020
14	1.675±0.010	33	2.368±0.024	52	2.583±0.029
15	1.699±0.000	34	2.380±0.037	-	-
16	1.774±0.063	35	2.383±0.020	-	-
17	1.811±0.050	36	2.385±0.032	-	-
18	1.834±0.051	37	2.408±0.020	-	-

Data are presented as mean ± standard deviation of triplicate runs.

Data for the growth curves of microalgae for Figure 4.1 (Cont.)

Data for Figure 4.1 *Neochloris* sp.

Time (d)	Biomass concentration ($g\ L^{-1}$)	Time (d)	Biomass concentration ($g\ L^{-1}$)	Time (d)	Biomass concentration ($g\ L^{-1}$)
0	0.441±0.000	19	2.502±0.082	38	3.752±0.039
1	0.561±0.018	20	2.622±0.108	39	3.767±0.029
2	0.709±0.013	21	2.631±0.095	40	3.851±0.041
3	0.922±0.027	22	2.791±0.065	41	3.989±0.070
4	0.997±0.027	23	2.854±0.068	42	3.926±0.067
5	1.099±0.018	24	2.914±0.061	43	4.001±0.081
6	1.240±0.046	25	2.989±0.072	44	4.091±0.041
7	1.285±0.058	26	3.037±0.044	45	4.079±0.023
8	1.430±0.075	27	3.073±0.099	46	4.085±0.018
9	1.532±0.101	28	3.214±0.039	47	4.107±0.085
10	1.637±0.102	29	3.277±0.061	-	-
11	1.736±0.093	30	3.307±0.052	-	-
12	1.907±0.093	31	3.379±0.080	-	-
13	2.027±0.103	32	3.428±0.050	-	-
14	2.136±0.082	33	3.536±0.051	-	-
15	2.211±0.066	34	3.635±0.103	-	-
16	2.301±0.095	35	3.629±0.031	-	-
17	2.361±0.064	36	3.689±0.029	-	-
18	2.451±0.118	37	3.722±0.022	-	-

Data are presented as mean ± standard deviation of triplicate runs.

Data for the growth curves of microalgae for Figure 4.1 (Cont.)

Data for Figure 4.1 *C. vulgaris* (freshwater)

Time (d)	Biomass concentration ($g\ L^{-1}$)	Time (d)	Biomass concentration ($g\ L^{-1}$)	Time (d)	Biomass concentration ($g\ L^{-1}$)
0	0.471±0.000	19	2.958±0.064	38	3.689±0.144
1	0.641±0.000	20	3.022±0.076	39	3.545±0.253
2	0.962±0.034	21	3.081±0.088	40	3.619±0.171
3	1.238±0.034	22	3.086±0.074	41	3.715±0.127
4	1.466±0.024	23	3.146±0.091	42	3.813±0.201
5	1.729±0.054	24	3.184±0.124	43	3.825±0.212
6	1.780±0.063	25	3.212±0.087	44	3.839±0.241
7	1.831±0.070	26	3.254±0.076	45	3.914±0.239
8	1.976±0.083	27	3.295±0.070	46	3.953±0.224
9	2.111±0.029	28	3.241±0.173	47	3.952±0.122
10	2.236±0.086	29	3.296±0.129	48	3.951±0.222
11	2.342±0.121	30	3.327±0.078	49	4.033±0.192
12	2.415±0.101	31	3.458±0.139	50	4.053±0.178
13	2.505±0.096	32	3.486±0.144	51	4.041±0.191
14	2.601±0.117	33	3.528±0.146	52	4.068±0.189
15	2.685±0.088	34	3.556±0.143	53	4.057±0.171
16	2.759±0.058	35	3.603±0.158	-	-
17	2.816±0.062	36	3.594±0.112	-	-
18	2.881±0.064	37	3.673±0.125	-	-

Data are presented as mean ± standard deviation of triplicate runs.

Data for the growth curves of microalgae for Figure 4.1 (Cont.)

Data for Figure 4.1 *C. vulgaris* (seawater)

Time (d)	Biomass concentration ($g\ L^{-1}$)	Time (d)	Biomass concentration ($g\ L^{-1}$)	Time (d)	Biomass concentration ($g\ L^{-1}$)
0	0.411±0.010	15	1.972±0.138	30	2.735±0.203
1	0.475±0.010	16	2.042±0.156	31	2.819±0.142
2	0.680±0.042	17	2.084±0.151	32	2.850±0.172
3	0.788±0.032	18	2.159±0.128	33	2.908±0.182
4	0.856±0.050	19	2.215±0.112	34	2.969±0.204
5	1.050±0.070	20	2.269±0.102	35	3.009±0.229
6	1.221±0.055	21	2.377±0.122	36	2.995±0.170
7	1.322±0.062	22	2.414±0.151	37	3.051±0.151
8	1.408±0.089	23	2.510±0.151	38	3.063±0.163
9	1.523±0.109	24	2.550±0.173	39	3.119±0.154
10	1.593±0.114	25	2.552±0.114	40	3.222±0.180
11	1.736±0.072	26	2.562±0.121	41	3.250±0.177
12	1.766±0.121	27	2.590±0.126	42	3.268±0.169
13	1.813±0.114	28	2.611±0.130	43	3.266±0.161
14	1.942±0.130	29	2.707±0.179	-	-

Data are presented as mean ± standard deviation of triplicate runs.

Data for the growth curves of microalgae for Figure 4.1 (Cont.)

Data for Figure 4.1 *N. salina*

Time (d)	Biomass concentration ($g\ L^{-1}$)	Time (d)	Biomass concentration ($g\ L^{-1}$)	Time (d)	Biomass concentration ($g\ L^{-1}$)
0	0.598±0.005	19	2.474±0.061	38	3.364±0.015
1	0.660±0.027	20	2.562±0.011	39	3.373±0.017
2	0.845±0.024	21	2.624±0.018	40	3.390±0.019
3	0.968±0.044	22	2.639±0.022	41	3.440±0.026
4	1.118±0.012	23	2.650±0.053	42	3.522±0.026
5	1.294±0.009	24	2.730±0.016	43	3.540±0.011
6	1.391±0.038	25	2.791±0.012	44	3.593±0.026
7	1.488±0.016	26	2.871±0.010	45	3.637±0.010
8	1.550±0.058	27	2.894±0.022	46	3.654±0.030
9	1.708±0.030	28	2.932±0.023	47	3.728±0.028
10	1.761±0.016	29	2.968±0.029	48	3.813±0.009
11	1.875±0.047	30	3.064±0.020	49	3.927±0.037
12	1.919±0.032	31	3.135±0.018	50	3.945±0.012
13	2.025±0.049	32	3.155±0.033	51	3.892±0.054
14	2.131±0.014	33	3.202±0.022	52	3.898±0.053
15	2.192±0.020	34	3.214±0.012	53	3.919±0.040
16	2.272±0.019	35	3.241±0.010	54	3.936±0.063
17	2.333±0.013	36	3.276±0.021	-	-
18	2.395±0.030	37	3.320±0.028	-	-

Data are presented as mean ± standard deviation of triplicate runs.

Data for the growth curves of microalgae for Figure 4.1 (Cont.)

Data for Figure 4.1 *C. fusiformis*

Time (d)	Biomass concentration (g L^{-1})	Time (d)	Biomass concentration (g L^{-1})	Time (d)	Biomass concentration (g L^{-1})
0	0.422±0.007	11	0.910±0.032	22	1.860±0.281
1	0.461±0.020	12	1.019±0.013	23	1.897±0.246
2	0.492±0.020	13	1.132±0.020	24	1.933±0.241
3	0.509±0.013	14	1.263±0.027	25	1.996±0.216
4	0.531±0.015	15	1.354±0.020	26	1.933±0.241
5	0.592±0.007	16	1.406±0.037	27	1.918±0.255
6	0.679±0.013	17	1.485±0.027	28	1.933±0.246
7	0.718±0.026	18	1.602±0.130	29	1.949±0.243
8	0.770±0.034	19	1.707±0.175	30	1.959±0.258
9	0.801±0.032	20	1.798±0.197	-	-
10	0.853±0.072	21	1.850±0.176	-	-

Data are presented as mean ± standard deviation of triplicate runs.

Data for the flocculation of *C. minor* for Figure 4.4

Biomass concentration (C_b) at 0.1 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	100±0.0	100±0.0
5	93.1±6.0	98.9±2.0
10	90.8±4.0	93.1±6.9
15	8.6±1.0	36.2±12.0
20	5.6±0.5	4.1±0.2
25	11.8±1.1	3.6±0.6

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. minor* for Figure 4.4 (Cont.)

Biomass concentration (C_b) at 0.5 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	97.9±1.8	98.2±1.9
50	95.9±0.9	60.8±5.5
100	26.7±7.3	1.4±0.1
150	2.3±0.7	0.3±0.2
200	0.9±0.2	5.6±1.6
250	0.8±0.3	3.8±2.3

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 1.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	98.5±1.3	98.3±1.2
50	96.8±0.7	81.5±4.4
100	92.8±3.8	66.9±2.2
150	64.3±3.7	43.8±2.8
200	30.4±2.7	21.9±1.7
250	8.6±4.4	0.7±0.2
300	1.5±0.6	0.4±0.1

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. minor* for Figure 4.4 (Cont.)

Biomass concentration (C_b) at 2.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	99.9±0.1	98.5±1.4
100	99.0±0.2	83.3±4.2
200	94.2±1.8	66.5±5.1
300	45.8±2.3	48.7±6.1
400	20.2±4.0	29.6±4.9
500	9.4±2.4	13.3±0.9
600	4.7±0.2	0.3±0.1

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 3.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	97.2±1.1	97.9±1.3
200	93.5±1.7	73.2±1.3
400	43.8±0.8	42.6±0.9
600	24.7±0.7	18.8±3.8
800	10.9±2.7	0.9±0.1
1000	4.4±0.4	5.1±0.7

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *Neochloris* sp. for Figure 4.5

Biomass concentration (C_b) at 0.1 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	66.8±1.3	69.3±1.2
2	15.4±1.3	20.1±1.3
4	12.8±1.0	9.0±0.7
6	11.8±0.3	4.5±0.2
8	10.3±1.8	3.4±1.3
10	5.3±0.6	3.7±0.9

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 0.5 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	61.3±5.2	63.1±7.4
5	15.4±1.3	8.1±0.5
10	6.7±1.8	1.9±0.2
15	4.4±0.9	1.0±0.4
20	2.7±0.6	0.5±0.1
25	1.7±0.4	0.5±0.1

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *Neochloris* sp. for Figure 4.5 (Cont.)

Biomass concentration (C_b) at 1.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	55.7±4.2	54.2±3.7
10	8.4±1.7	5.2±0.9
20	4.1±0.7	3.1±0.9
30	2.2±0.5	2.3±0.4
40	1.1±0.2	2.1±0.3
50	2.2±2.4	1.8±0.1

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 2.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	33.3±2.9	30.8±4.0
20	7.4±0.4	9.3±3.3
40	2.4±0.3	6.8±1.4
60	0.9±0.1	4.8±1.2
80	0.5±0.1	1.7±0.3
100	0.4±0.0	2.1±0.2

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *Neochloris* sp. for Figure 4.5 (Cont.)

Biomass concentration (C_b) at 3.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	25.1±2.7	23.1±2.7
20	14.8±0.3	-
40	2.4±0.2	-
60	1.2±0.3	-
80	0.6±0.2	-
100	0.5±0.3	5.0±2.1
200	-	1.1±0.4
300	-	0.4±0.1
400	-	0.2±0.1
500	-	0.2±0.1

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. vulgaris* (freshwater) for Figure 4.6

Biomass concentration (C_b) at 0.1 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	95.9±1.4	92.7±1.0
5	94.2±0.8	91.3±1.3
10	95.7±1.5	91.5±1.4
15	92.2±2.4	90.9±1.2
20	53.0±9.3	89.0±2.5
25	8.5±2.0	3.7±1.2
30	8.7±1.1	4.5±1.2
40	7.3±0.4	-
50	5.1±0.3	-

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. vulgaris* (freshwater) for Figure 4.6 (Cont.)

Biomass concentration (C_b) at 0.5 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	97.9±0.3	99.7±0.3
50	96.0±0.4	97.5±0.6
100	82.0±9.1	23.9±2.9
125	7.4±1.6	0.2±0.1
150	2.8±0.8	0.1±0.1
175	0.6±0.1	11.9±1.3
200	0.7±0.2	51.3±2.6
250	1.4±0.4	59.4±1.4

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 1.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	93.3±0.5	96.2±1.4
100	91.7±1.0	75.9±0.4
200	91.0±2.3	37.0±4.1
250	53.2±4.1	0.7±0.1
300	4.9±0.7	0.1±0.0
350	0.5±0.1	76.4±1.1
400	1.1±0.2	81.6±0.7
500	2.9±0.7	86.5±0.9

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. vulgaris* (freshwater) for Figure 4.6 (Cont.)

Biomass concentration (C_b) at 2.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	97.7±0.7	97.4±0.8
200	96.1±0.3	77.4±5.0
400	87.1±1.5	38.2±3.8
500	-	8.0±0.4
600	30.2±1.3	0.1±0.0
700	-	23.7±3.9
800	14.2±1.6	32.3±1.7
1000	5.2±1.2	43.2±1.1

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 3.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	97.2±0.9	96.4±1.0
200	95.4±1.0	81.9±1.9
400	93.7±1.2	58.2±0.3
600	89.1±1.5	33.2±1.1
700	-	15.6±1.1
800	44.5±3.6	1.0±0.7
900	-	0.1±0.0
1000	12.1±2.4	12.2±2.3
1100	10.9±0.7	-
1200	10.8±0.6	-
1300	8.9±2.3	-
1400	8.8±0.7	-
1600	5.51±1.05	-

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. vulgaris* (seawater) for Figure 4.7

Biomass concentration (C_b) at 0.1 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	96.1±0.5	93.2±3.6
5	88.3±0.5	90.2±2.3
10	34.2±3.6	62.8±6.7
15	15.5±0.3	11.4±0.9
20	14.7±1.9	4.1±0.9
25	27.2±2.9	2.9±1.1

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 0.5 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	96.5±3.8	93.5±3.8
20	36.2±2.9	60.2±2.3
40	4.4±1.3	14.3±2.2
60	4.5±0.6	1.8±0.5
80	6.0±1.1	1.2±0.3
100	13.6±1.4	2.5±0.2

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. vulgaris* (seawater) for Figure 4.7 (Cont.)

Biomass concentration (C_b) at 1.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	94.2±1.0	98.7±0.7
50	8.0±0.1	9.8±1.3
100	2.7±0.2	1.0±0.3
150	3.6±0.4	1.8±0.1
200	5.6±0.1	3.2±0.3
250	16.2±1.3	5.1±0.8

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 2.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	96.4±0.7	94.2±2.1
50	51.6±3.3	51.3±2.8
100	4.4±0.4	10.5±3.3
150	1.0±0.1	1.4±0.4
200	1.1±0.3	0.9±0.3
250	2.8±0.11	1.4±0.2

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. vulgaris* (seawater) for Figure 4.7 (Cont.)

Biomass concentration (C_b) at 3.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	91.7±2.2	94.3±2.2
100	28.9±5.4	35.3±3.2
200	1.6±0.2	2.5±0.3
300	1.3±0.2	0.6±0.0
400	2.1±0.8	18.4±2.4
500	7.6±0.5	5.5±1.3

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *N. salina* for Figure 4.8

Biomass concentration (C_b) at 0.1 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	98.9±0.5	94.9±2.4
5	94.8±0.9	85.2±2.9
10	49.6±0.6	72.3±1.6
15	10.5±0.3	49.2±0.1
20	10.7±0.3	25.3±0.1
40	7.9±0.1	1.7±0.0

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *N. salina* for Figure 4.8 (Cont.)

Biomass concentration (C_b) at 0.5 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	92.6±3.7	97.9±2.4
50	49.4±1.4	60.7±2.9
100	21.3±1.0	26.0±1.6
150	0.5±0.0	1.4±0.1
200	0.3±0.0	1.1±0.1
250	0.3±0.0	0.4±0.0

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 1.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	91.5±1.6	94.6±4.2
50	57.6±2.6	64.8±2.8
100	42.9±1.6	52.5±2.4
150	26.4±1.4	35.5±2.1
200	7.6±1.2	17.0±1.3
250	1.4±0.1	1.2±0.2

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *N. salina* for Figure 4.8 (Cont.)

Biomass concentration (C_b) at 2.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	95.9±3.0	93.5±5.2
100	56.0±2.1	68.3±3.6
200	41.3±3.3	55.2±2.4
300	27.2±1.7	32.2±1.6
400	7.6±0.3	19.3±2.6
500	2.0±0.5	4.3±0.2

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 3.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	98.9±0.2	97.1±1.1
200	64.4±3.5	74.0±0.5
400	22.8±1.1	53.3±2.5
600	7.9±0.4	35.0±2.9
800	1.7±0.1	18.8±1.1
1000	0.2±0.0	0.4±0.0

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. fusiformis* for Figure 4.9

Biomass concentration (C_b) at 0.1 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	71.5±1.3	76.1±0.8
5	6.7±0.5	36.1±3.7
10	1.4±0.3	11.5±2.6
15	1.2±0.6	2.9±0.5
20	0.9±0.3	1.2±0.3
25	0.9±0.3	0.7±0.3

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 0.5 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	61.1±1.7	63.0±0.9
10	36.4±1.8	-
20	21.0±3.3	-
30	3.1±0.4	-
40	0.8±0.1	-
50	0.5±0.1	6.1±0.4
60	-	3.5±0.4
70	-	2.1±0.4
80	-	1.0±0.2
90		0.6±0.1
100	0.4±0.0	0.6±0.2
150	0.3±0.1	0.6±0.1
200	0.3±0.0	0.5±0.1
250	0.3±0.0	0.6±0.1

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. fusiformis* for Figure 4.9 (Cont.)

Biomass concentration (C_b) at 1.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	59.2±0.5	61.9±0.8
50	49.7±4.8	29.5±1.2
100	42.7±2.8	19.3±0.3
150	1.7±0.1	2.5±0.1
200	1.3±0.1	0.3±0.0
250	1.1±0.1	0.3±0.1

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 1.5 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	61.7±3.7	67.6±1.7
50	56.3±2.0	58.3±2.6
100	45.7±2.2	37.0±1.6
150	31.6±1.4	23.6±3.0
175	25.8±2.3	15.5±2.6
200	19.9±2.1	5.6±1.2
225	5.3±1.0	4.2±0.2
250	4.6±1.0	5.1±0.4

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. fusiformis* for Figure 4.9 (Cont.)

Biomass concentration (C_b) at 2.0 g L^{-1}

Flocculant concentration (mg L^{-1})	Biomass in broth (%)	
	Flocculation with alum	Flocculation with ferric chloride
0	68.8±2.6	73.2±2.5
100	62.6±2.3	62.7±2.0
150	41.0±0.9	35.3±2.9
200	31.4±2.7	15.6±2.2
250	14.1±1.6	5.1±0.8
300	4.8±0.7	4.6±1.0
350	5.7±1.1	5.0±0.7
400	8.1±0.4	7.2±0.9
500	9.8±1.6	7.4±1.4

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. minor* for Figure 4.10

Biomass concentration (C_b) at 0.1 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	100±0.0	0	100±0.0
1.46×10^{-5}	93.1±6.0	3.08×10^{-5}	98.9±2.0
2.92×10^{-5}	90.8±4.0	6.17×10^{-5}	93.1±6.9
4.38×10^{-5}	8.6±1.0	9.25×10^{-5}	36.2±12.0
5.85×10^{-5}	5.6±0.5	1.23×10^{-4}	4.1±0.2
7.31×10^{-5}	11.8±1.2	1.54×10^{-4}	3.6±0.6

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. minor* for Figure 4.10 (Cont.)

Biomass concentration (C_b) at 0.5 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	97.9±1.8	0	98.2±1.9
1.46×10^{-4}	95.9±0.9	3.08×10^{-4}	60.8±5.5
2.92×10^{-4}	26.7±7.3	6.17×10^{-4}	1.4±0.1
4.38×10^{-4}	2.3±0.7	9.25×10^{-4}	0.3±0.2
5.85×10^{-4}	0.9±0.2	1.23×10^{-3}	5.6±1.6
7.31×10^{-4}	0.8±0.3	1.54×10^{-3}	3.8±2.3

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 1.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	98.5±1.3	0	98.3±1.2
1.46×10^{-4}	96.8±0.7	3.08×10^{-4}	81.5±4.4
2.92×10^{-4}	92.8±3.8	6.17×10^{-4}	66.9±2.2
4.38×10^{-4}	64.3±3.7	9.25×10^{-4}	43.8±2.8
5.85×10^{-4}	30.4±2.7	1.23×10^{-3}	21.9±1.7
7.31×10^{-4}	8.6±4.4	1.54×10^{-3}	0.7±0.2
8.77×10^{-4}	1.5±0.6	1.85×10^{-3}	0.4±0.1

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. minor* for Figure 4.10 (Cont.)

Biomass concentration (C_b) at 2.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	99.9±0.1	0	98.5±1.4
2.92×10^{-4}	99.0±0.2	6.17×10^{-4}	83.3±4.2
5.85×10^{-4}	94.2±1.8	1.23×10^{-3}	66.5±5.1
8.77×10^{-4}	45.8±2.3	1.85×10^{-3}	48.7±6.1
1.17×10^{-3}	20.2±4.0	2.47×10^{-3}	29.6±4.9
1.46×10^{-3}	9.4±2.4	3.08×10^{-3}	13.3±1.0
1.75×10^{-3}	4.7±0.2	3.70×10^{-3}	0.3±0.1

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 3.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	97.2±1.1	0	97.9±1.3
5.85×10^{-4}	93.5±1.7	1.23×10^{-3}	73.2±1.3
1.17×10^{-3}	43.8±0.8	2.47×10^{-3}	42.6±0.9
1.75×10^{-3}	24.7±0.7	3.70×10^{-3}	18.8±3.8
2.34×10^{-3}	10.9±2.7	4.93×10^{-3}	0.9±0.1
2.92×10^{-3}	4.4±0.4	1.00×10^{-2}	5.1±0.7

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *Neochloris* sp. for Figure 4.11

Biomass concentration (C_b) at 0.1 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	66.8±1.3	0	69.3±1.2
5.85×10^{-6}	15.4±1.3	1.23×10^{-5}	20.1±1.3
1.17×10^{-5}	12.8±1.0	2.47×10^{-5}	9.0±0.7
1.75×10^{-5}	11.8±0.3	3.70×10^{-5}	4.5±0.2
2.34×10^{-5}	10.3±1.8	4.93×10^{-5}	3.4±1.3
2.92×10^{-5}	5.3±0.6	6.17×10^{-5}	3.7±0.9

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 0.5 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	61.3±5.2	0	63.1±7.4
1.46×10^{-5}	15.4±1.3	3.08×10^{-5}	8.1±0.5
2.92×10^{-5}	6.7±1.8	6.17×10^{-5}	1.9±0.2
4.38×10^{-5}	4.4±0.9	9.25×10^{-5}	1.0±0.4
5.85×10^{-5}	2.7±0.6	1.23×10^{-4}	0.5±0.1
7.31×10^{-5}	1.7±0.4	1.54×10^{-5}	0.5±0.1

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *Neochloris* sp. for Figure 4.11 (Cont.)

Biomass concentration (C_b) at 1.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	55.7±4.2	0	54.2±3.7
2.92×10^{-5}	8.4±1.7	6.17×10^{-5}	5.2±0.9
5.85×10^{-5}	4.1±0.7	1.23×10^{-4}	3.1±0.9
8.77×10^{-5}	2.2±0.5	1.85×10^{-4}	2.3±0.4
1.17×10^{-4}	1.1±0.2	2.47×10^{-4}	2.1±0.3
1.46×10^{-4}	2.2±2.4	3.08×10^{-4}	1.8±0.1

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 2.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	33.3±2.9	0.00	30.8±4.0
2.92×10^{-5}	7.4±0.4	1.23×10^{-4}	9.3±3.3
5.85×10^{-5}	2.4±0.3	2.47×10^{-4}	6.8±1.4
8.77×10^{-5}	0.9±0.1	3.70×10^{-4}	4.8±1.2
1.17×10^{-4}	0.5±0.1	4.93×10^{-4}	1.7±0.3
1.46×10^{-4}	0.4±0.0	6.17×10^{-4}	2.1±2.2

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *Neochloris* sp. for Figure 4.11 (Cont.)

Biomass concentration (C_b) at 3.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	25.1±2.7	0	23.1±2.7
5.85×10^{-5}	14.8±0.3	6.17×10^{-4}	5.0±2.1
1.17×10^{-4}	2.4±0.2	1.23×10^{-3}	1.1±0.4
1.75×10^{-4}	1.2±0.3	1.85×10^{-3}	0.4±0.1
2.34×10^{-4}	0.6±0.2	2.47×10^{-3}	0.2±0.1
2.92×10^{-4}	0.5±0.3	3.08×10^{-3}	0.2±0.2

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. vulgaris* (freshwater) for Figure 4.12

Biomass concentration (C_b) at 0.1 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	95.9±1.3	0	92.7±1.0
1.46×10^{-5}	94.2±0.8	3.08×10^{-5}	91.3±1.3
2.92×10^{-5}	95.7±1.5	6.17×10^{-5}	91.5±1.4
4.38×10^{-5}	92.2±2.4	9.25×10^{-5}	90.9±1.2
5.85×10^{-5}	53.0±9.3	1.23×10^{-4}	89.0±2.5
7.31×10^{-5}	8.5±2.0	1.54×10^{-4}	3.7±1.2
8.77×10^{-5}	8.7±1.1	1.85×10^{-4}	4.5±1.2
1.17×10^{-4}	7.3±0.4	-	-
1.46×10^{-4}	5.1±0.3	-	-

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. vulgaris* (freshwater) for Figure 4.12 (Cont.)

Biomass concentration (C_b) at 0.5 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	97.9±0.3	0	99.7±0.3
1.46×10^{-4}	96.0±0.4	3.08×10^{-4}	97.5±0.6
2.92×10^{-4}	82.0±9.1	6.17×10^{-4}	23.9±2.9
3.65×10^{-4}	7.4±1.6	7.71×10^{-4}	0.2±0.1
4.38×10^{-4}	2.8±0.8	9.25×10^{-4}	0.1±0.1
5.11×10^{-4}	0.6±0.1	1.08×10^{-3}	11.9±1.3
5.85×10^{-4}	0.7±0.2	1.23×10^{-3}	51.3±2.6
7.31×10^{-4}	1.4±0.4	1.54×10^{-3}	59.4±1.4

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 1.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	93.3±0.5	0	96.2±1.4
2.92×10^{-4}	91.7±1.0	6.17×10^{-4}	75.9±0.4
5.85×10^{-4}	91.0±2.3	1.23×10^{-3}	37.0±4.1
7.31×10^{-4}	53.2±4.1	1.54×10^{-3}	0.7±0.1
8.77×10^{-4}	4.9±0.7	1.85×10^{-3}	0.1±0.0
1.02×10^{-3}	0.5±0.1	2.16×10^{-3}	76.4±1.1
1.17×10^{-3}	1.1±0.2	2.47×10^{-3}	81.6±0.7
1.46×10^{-3}	2.9±0.7	3.08×10^{-3}	86.5±0.9

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. vulgaris* (freshwater) for Figure 4.12 (Cont.)

Biomass concentration (C_b) at 2.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	97.7±0.7	0	97.4±0.8
5.85×10^{-4}	96.1±0.3	1.23×10^{-3}	77.4±5.0
1.17×10^{-3}	87.1±1.5	2.47×10^{-3}	38.2±3.8
1.75×10^{-3}	30.2±1.3	3.08×10^{-3}	8.0±0.4
2.34×10^{-3}	14.1±1.6	3.70×10^{-3}	0.1±0.0
2.92×10^{-3}	5.2±1.2	4.32×10^{-3}	23.7±3.9
-	-	4.93×10^{-3}	32.3±1.7
-	-	1.00×10^{-2}	43.2±1.1

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 3.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	97.2±0.9	0	96.4±1.0
5.85×10^{-4}	95.4±1.0	1.23×10^{-3}	81.9±1.9
1.17×10^{-3}	93.7±1.2	2.47×10^{-3}	58.2±0.3
1.75×10^{-3}	89.1±1.5	3.70×10^{-3}	33.2±1.1
2.34×10^{-3}	44.5±3.6	4.32×10^{-3}	15.6±1.1
2.92×10^{-3}	12.1±2.4	4.93×10^{-3}	1.0±0.7
3.21×10^{-3}	10.9±0.7	1.00×10^{-2}	0.1±0.0
3.51×10^{-3}	10.8±0.6	1.00×10^{-2}	12.2±2.3
3.80×10^{-3}	8.9±2.3	-	-
4.09×10^{-3}	8.8±0.7	-	-
4.68×10^{-3}	5.5±1.1	-	-

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. vulgaris* (seawater) for Figure 4.13

Biomass concentration (C_b) at 0.1 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	96.1±0.5	0	93.2±3.6
1.46×10^{-5}	88.3±0.4	3.08×10^{-5}	90.2±2.3
2.92×10^{-5}	34.2±3.6	6.17×10^{-5}	62.8±6.7
4.38×10^{-5}	15.5±0.3	9.25×10^{-5}	11.4±0.9
5.85×10^{-5}	14.7±1.9	1.23×10^{-4}	4.1±0.9
7.31×10^{-5}	27.2±2.9	1.54×10^{-4}	2.9±1.1

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 0.5 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	96.5±3.8	0	93.5±3.8
5.85×10^{-5}	36.2±2.9	1.23×10^{-4}	60.2±2.3
1.17×10^{-4}	4.4±1.3	2.47×10^{-4}	14.3±2.2
1.75×10^{-4}	4.5±0.6	3.70×10^{-4}	1.8±0.5
2.34×10^{-4}	6.0±1.1	4.93×10^{-4}	1.2±0.3
2.92×10^{-4}	13.6±1.3	6.17×10^{-4}	2.5±0.2

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. vulgaris* (seawater) for Figure 4.13 (Cont.)

Biomass concentration (C_b) at 1.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	94.2±1.0	0	98.7±0.7
1.46×10^{-4}	8.0±0.1	3.08×10^{-4}	9.8±1.3
2.92×10^{-4}	2.7±0.2	6.17×10^{-4}	1.0±0.3
4.38×10^{-4}	3.6±0.4	9.25×10^{-4}	1.8±0.1
5.85×10^{-4}	5.6±0.1	1.23×10^{-3}	3.2±0.3
7.31×10^{-4}	16.2±1.3	1.54×10^{-3}	5.1±0.8

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 2.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	96.4±0.7	0	94.2±2.1
1.46×10^{-4}	51.6±3.3	3.08×10^{-4}	51.3±2.8
2.92×10^{-4}	4.4±0.4	6.17×10^{-4}	10.5±3.3
4.38×10^{-4}	1.0±0.1	9.25×10^{-4}	1.4±0.4
5.85×10^{-4}	1.1±0.3	1.23×10^{-3}	0.9±0.3
7.31×10^{-4}	2.8±0.1	1.54×10^{-3}	1.4±0.2

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. vulgaris* (seawater) for Figure 4.13 (Cont.)

Biomass concentration (C_b) at 3.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	91.7±2.2	0	94.3±2.2
2.92×10^{-4}	28.9±5.4	6.17×10^{-4}	35.3±3.2
5.85×10^{-4}	1.6±0.2	1.23×10^{-3}	2.5±0.3
8.77×10^{-4}	1.3±0.2	1.85×10^{-3}	0.6±0.0
1.17×10^{-3}	2.1±0.8	2.47×10^{-3}	18.4±2.4
1.46×10^{-3}	7.6±0.5	3.08×10^{-3}	5.5±1.3

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *N. salina* for Figure 4.14

Biomass concentration (C_b) at 0.1 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	98.9±0.5	0	94.9±2.4
1.46×10^{-5}	94.8±0.9	3.08×10^{-5}	85.2±2.9
2.92×10^{-5}	49.6±0.6	6.17×10^{-5}	72.3±1.6
4.38×10^{-5}	10.5±0.3	9.25×10^{-5}	49.2±0.1
5.85×10^{-5}	10.7±0.3	1.23×10^{-4}	25.3±0.1
1.17×10^{-4}	7.9±0.1	2.47×10^{-4}	1.7±0.0

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *N. salina* for Figure 4.14 (Cont.)

Biomass concentration (C_b) at 0.5 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	92.6±3.7	0	97.9±2.4
1.46×10^{-4}	49.4±1.4	3.08×10^{-4}	60.7±2.9
2.92×10^{-4}	21.3±1.0	6.17×10^{-4}	26.0±1.6
4.38×10^{-4}	0.5±0.0	9.25×10^{-4}	1.4±0.1
5.85×10^{-4}	0.3±0.0	1.23×10^{-3}	1.1±0.1
7.31×10^{-4}	0.3±0.0	1.54×10^{-3}	0.4±0.0

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 1.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	91.5±1.6	0	94.6±4.2
1.46×10^{-4}	57.6±2.6	3.08×10^{-4}	64.8±2.8
2.92×10^{-4}	42.9±1.6	6.17×10^{-4}	52.5±2.4
4.38×10^{-4}	26.4±1.4	9.25×10^{-4}	35.5±2.1
5.85×10^{-4}	7.6±1.2	1.23×10^{-3}	17.0±1.3
7.31×10^{-4}	1.4±0.1	1.54×10^{-3}	1.2±0.2

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *N. salina* for Figure 4.14 (Cont.)

Biomass concentration (C_b) at 2.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	95.9±3.0	0	93.5±5.2
2.92×10^{-4}	56.0±2.1	6.17×10^{-4}	68.3±3.6
5.85×10^{-4}	41.3±3.3	1.23×10^{-3}	55.2±2.4
8.77×10^{-4}	27.2±1.7	1.85×10^{-3}	32.2±1.6
1.17×10^{-3}	7.6±0.3	2.47×10^{-3}	19.3±2.6
1.46×10^{-3}	2.0±0.5	3.08×10^{-3}	4.3±0.2

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 3.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	98.9±0.2	0	97.1±1.1
5.85×10^{-4}	64.4±3.5	1.23×10^{-3}	74.0±0.5
1.17×10^{-3}	22.8±1.1	2.47×10^{-3}	53.3±2.5
1.75×10^{-3}	7.9±0.4	3.70×10^{-3}	35.0±2.9
2.34×10^{-3}	1.7±0.1	4.93×10^{-3}	18.8±1.1
2.92×10^{-3}	0.2±0.0	1.00×10^{-2}	0.4±0.0

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. fusiformis* for Figure 4.15

Biomass concentration (C_b) at 0.1 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	71.5±1.3	0	76.1±0.8
1.46×10^{-5}	6.7±0.5	3.08×10^{-5}	36.1±3.7
2.92×10^{-5}	1.4±0.3	6.17×10^{-5}	11.5±2.6
4.38×10^{-5}	1.2±0.6	9.25×10^{-5}	2.9±0.5
5.85×10^{-5}	0.9±0.3	1.23×10^{-4}	1.2±0.3
7.31×10^{-5}	0.9±0.3	1.54×10^{-4}	0.7±0.3

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 0.5 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	61.1±1.7	0	63.0±0.9
2.92×10^{-5}	36.4±1.8	3.08×10^{-4}	6.1±0.4
5.85×10^{-5}	21.0±3.3	3.70×10^{-4}	3.5±0.4
8.77×10^{-5}	3.1±0.4	4.32×10^{-4}	2.1±0.4
1.17×10^{-4}	0.8±0.1	4.93×10^{-4}	1.0±0.2
1.46×10^{-4}	0.5±0.1	5.55×10^{-4}	0.6±0.1
2.92×10^{-4}	0.4±0.0	6.17×10^{-4}	0.6±0.2
4.38×10^{-4}	0.3±0.1	9.25×10^{-4}	0.6±0.1
5.85×10^{-4}	0.3±0.0	1.23×10^{-3}	0.5±0.1
7.31×10^{-4}	0.3±0.0	1.54×10^{-4}	0.6±0.1

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. fusiformis* for Figure 4.15 (Cont.)

Biomass concentration (C_b) at 1.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	59.2±0.5	0	61.9±0.9
1.46×10^{-4}	49.8±4.8	3.08×10^{-4}	29.5±1.2
2.92×10^{-4}	42.7±2.8	6.17×10^{-4}	19.3±0.3
4.38×10^{-4}	1.7±0.1	9.25×10^{-4}	2.5±0.1
5.85×10^{-4}	1.3±0.1	1.23×10^{-3}	0.3±0.0
7.31×10^{-4}	1.1±0.1	1.54×10^{-3}	0.3±0.1

Data are mean values ± SD of triplicate experiments.

Biomass concentration (C_b) at 1.5 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	61.7±3.7	0	67.6±1.7
1.46×10^{-4}	56.3±2.0	3.08×10^{-4}	58.3±2.6
2.92×10^{-4}	45.7±2.2	6.17×10^{-4}	37.0±1.6
4.38×10^{-4}	31.6±1.4	9.25×10^{-4}	23.6±3.0
5.11×10^{-4}	25.8±2.2	1.08×10^{-4}	15.5±2.6
5.85×10^{-4}	19.9±2.1	1.23×10^{-4}	5.6±1.2
6.58×10^{-4}	5.3±1.0	1.39×10^{-4}	4.2±0.2
7.31×10^{-4}	4.6±1.0	1.54×10^{-4}	5.1±0.4

Data are mean values ± SD of triplicate experiments.

Data for the flocculation of *C. fusiformis* for Figure 4.15 (Cont.)

Biomass concentration (C_b) at 2.0 g L^{-1}

Aluminum sulfate concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)	Ferric chloride concentration (mole of M^{+3} L^{-1})	Biomass in broth (%)
0	68.8±2.6	0.00	73.2±2.5
2.92×10^{-4}	62.6±2.3	6.17×10^{-4}	62.7±2.0
4.38×10^{-4}	41.0±0.9	9.25×10^{-4}	35.3±2.9
5.85×10^{-4}	31.4±2.7	1.23×10^{-3}	15.6±2.2
7.31×10^{-4}	14.1±1.6	1.54×10^{-3}	5.1±0.8
8.77×10^{-4}	4.8±0.7	1.85×10^{-3}	4.6±1.0
1.02×10^{-3}	5.7±1.1	2.16×10^{-3}	5.0±0.7
1.17×10^{-3}	8.1±0.4	2.47×10^{-3}	7.2±0.9
1.46×10^{-3}	9.8±1.6	3.08×10^{-3}	7.4±1.4

Data are mean values ± SD of triplicate experiments.

Data for the relationship between microalgal biomass concentration and the optimal flocculant dose of each microalgal species for Figure 4.16

C. minor

Biomass concentration (g L^{-1})	Optimal flocculant dosage (mg L^{-1})	
	Aluminum sulfate	Ferric chloride
0.1	20.1	20.1
0.5	144.0	96.9
1.0	274.6	239.6
2.0	600.0	563.6
3.0	1000.0	753.1

Data for the relationship between microalgal biomass concentration and the optimal flocculant dose of each microalgal species for Figure 4.16 (Cont.)

***Neochloris* sp.**

Biomass concentration (g L^{-1})	Optimal flocculant dosage (mg L^{-1})	
	Aluminum sulfate	Ferric chloride
0.1	10.1	6.0
0.5	13.4	7.4
1.0	17.5	10.1
2.0	25.7	55.2
3.0	35.6	98.6

***C. vulgaris* (freshwater)**

Biomass concentration (g L^{-1})	Optimal flocculant dosage (mg L^{-1})	
	Aluminum sulfate	Ferric chloride
0.1	50.0	25.0
0.5	140.0	120.0
1.0	300.0	220.0
2.0	1000.0	700.0
3.0	1600.0	700.0

***C. vulgaris* (seawater)**

Biomass concentration (g L^{-1})	Optimal flocculant dosage (mg L^{-1})	
	Aluminum sulfate	Ferric chloride
0.1	20.0	20.0
0.5	40.0	58.0
1.0	75.0	75.0
2.0	100.0	130.0
3.0	200.0	200.0

Data for the relationship between microalgal biomass concentration and the optimal flocculant dose of each microalgal species for Figure 4.16 (Cont.)

N salina

Biomass concentration (g L^{-1})	Optimal flocculant dosage (mg L^{-1})	
	Aluminum sulfate	Ferric chloride
0.1	37.5	37.5
0.5	140.0	145.0
1.0	220.0	240.0
2.0	440.0	500.0
3.0	700.0	940.0

C. fusiformis

Biomass concentration (g L^{-1})	Optimal flocculant dosage (mg L^{-1})	
	Aluminum sulfate	Ferric chloride
0.1	10.0	15.0
0.5	30.0	60.0
1.0	150.0	150.0
1.5	250.0	210.0
2.0	310.0	250.0

Data for the parity plot of the measured flocculant dosage and the dosage calculated using Equation 4.2 for Figure 4.17

C. minor

Biomass concentration ($g\ L^{-1}$)	Optimal flocculant dosage ($mg\ L^{-1}$)			
	Measured aluminum sulfate	Calculated aluminum sulfate	Measured ferric chloride	Calculated ferric chloride
0.1	20.1	31.90	20.1	25.78
0.5	144.0	159.49	96.9	128.91
1.0	274.6	318.98	239.6	257.82
2.0	600.0	637.96	563.6	515.64
3.0	1000.0	956.94	753.1	773.46

***Neochloris* sp.**

Biomass concentration ($g\ L^{-1}$)	Optimal flocculant dosage ($mg\ L^{-1}$)			
	Measured aluminum sulfate	Calculated aluminum sulfate	Measured ferric chloride	Calculated ferric chloride
0.1	10.1	1.18	6.0	3.22
0.5	13.4	5.90	7.4	16.12
1.0	17.5	11.80	10.1	32.23
2.0	25.7	23.59	55.2	64.46
3.0	35.6	35.39	98.6	96.69

***C. vulgaris* (freshwater)**

Biomass concentration ($g\ L^{-1}$)	Optimal flocculant dosage ($mg\ L^{-1}$)			
	Measured aluminum sulfate	Calculated aluminum sulfate	Measured ferric chloride	Calculated ferric chloride
0.1	50.0	50.32	25.0	26.53
0.5	140.0	251.58	120.0	132.63
1.0	300.0	503.16	220.0	265.25
2.0	1000.0	1006.32	700.0	530.50
3.0	1600.0	1509.48	700.0	795.75

Data for the parity plot of the measured flocculant dosage and the dosage calculated using Equation 4.2 for Figure 4.17 (Cont.)

***C. vulgaris* (seawater)**

Biomass concentration ($g\ L^{-1}$)	Optimal flocculant dosage ($mg\ L^{-1}$)			
	Measured aluminum sulfate	Calculated aluminum sulfate	Measured ferric chloride	Calculated ferric chloride
0.1	20.0	6.29	20.0	6.77
0.5	40.0	31.45	58.0	33.87
1.0	75.0	62.90	75.0	67.74
2.0	100.0	125.80	130.0	135.48
3.0	200.0	188.70	200.0	203.22

N salina

Biomass concentration ($g\ L^{-1}$)	Optimal flocculant dosage ($mg\ L^{-1}$)			
	Measured aluminum sulfate	Calculated aluminum sulfate	Measured ferric chloride	Calculated ferric chloride
0.1	37.5	22.96	37.5	29.01
0.5	140.0	114.79	145.0	145.03
1.0	220.0	229.58	240.0	290.06
2.0	440.0	459.16	500.0	580.12
3.0	700.0	688.74	940.0	870.18

C. fusiformis

Biomass concentration ($g\ L^{-1}$)	Optimal flocculant dosage ($mg\ L^{-1}$)			
	Measured aluminum sulfate	Calculated aluminum sulfate	Measured ferric chloride	Calculated ferric chloride
0.1	10.0	15.46	15.0	13.27
0.5	30.0	77.30	60.0	66.35
1.0	150.0	154.59	150.0	132.69
1.5	250.0	231.89	210.0	199.04
2.0	310.0	309.18	250.0	265.38

Data for flocculant demand (ng) per unit cell surface area (μm^2) at various initial biomass concentrations for Figure 4.18

C. minor

Biomass concentration (g L^{-1})	Flocculant demand (ng) per unit cell surface area (μm^2)	
	Aluminum sulfate	Ferric chloride
0.1	1.98×10^{-5}	1.98×10^{-5}
0.5	2.78×10^{-5}	1.87×10^{-5}
1.0	2.68×10^{-5}	2.35×10^{-5}
2.0	3.16×10^{-5}	2.97×10^{-5}
3.0	2.86×10^{-5}	2.16×10^{-5}

***Neochloris* sp.**

Biomass concentration (g L^{-1})	Flocculant demand (ng) per unit cell surface area (μm^2)	
	Aluminum sulfate	Ferric chloride
0.1	2.47×10^{-5}	1.48×10^{-5}
0.5	7.41×10^{-6}	4.36×10^{-6}
1.0	5.87×10^{-6}	1.29×10^{-5}
2.0	4.38×10^{-6}	1.23×10^{-5}
3.0	2.88×10^{-6}	9.26×10^{-6}

***C. vulgaris* (freshwater)**

Biomass concentration (g L^{-1})	Flocculant demand (ng) per unit cell surface area (μm^2)	
	Aluminum sulfate	Ferric chloride
0.1	7.85×10^{-5}	3.92×10^{-5}
0.5	5.62×10^{-5}	4.81×10^{-5}
1.0	4.92×10^{-5}	3.61×10^{-5}
2.0	8.57×10^{-5}	6.00×10^{-5}
3.0	9.47×10^{-5}	4.14×10^{-5}

Data for Flocculant demand (ng) per unit cell surface area (μm^2) at various initial biomass concentrations for Figure 4.18 (Cont.)

***C. vulgaris* (seawater)**

Biomass concentration ($g\ L^{-1}$)	Flocculant demand (ng) per unit cell surface area (μm^2)	
	Aluminum sulfate	Ferric chloride
0.1	6.61×10^{-5}	6.61×10^{-5}
0.5	2.78×10^{-5}	4.03×10^{-5}
1.0	2.36×10^{-5}	2.36×10^{-5}
2.0	1.63×10^{-5}	2.11×10^{-5}
3.0	2.36×10^{-5}	2.36×10^{-5}

N salina

Biomass concentration ($g\ L^{-1}$)	Flocculant demand (ng) per unit cell surface area (μm^2)	
	Aluminum sulfate	Ferric chloride
0.1	5.54×10^{-5}	5.54×10^{-5}
0.5	4.36×10^{-5}	4.52×10^{-5}
1.0	3.36×10^{-5}	3.66×10^{-5}
2.0	3.50×10^{-5}	3.97×10^{-5}
3.0	3.73×10^{-5}	5.01×10^{-5}

C. fusiformis

Biomass concentration ($g\ L^{-1}$)	Flocculant demand (ng) per unit cell surface area (μm^2)	
	Aluminum sulfate	Ferric chloride
0.1	2.69×10^{-5}	4.03×10^{-5}
0.5	2.02×10^{-5}	4.03×10^{-5}
1.0	5.91×10^{-5}	5.91×10^{-5}
1.5	6.31×10^{-5}	5.30×10^{-5}
2.0	6.50×10^{-5}	5.24×10^{-5}

Data for the flocculation efficiency of *N. salina* biomass in the continuous flocculation-sedimentation process at various dosages of aluminum sulfate for Figure 4.21

Sedimentation time (h)	Percentage removal of biomass from broth at different dosage of aluminum sulfate			
	Dosage 114.5 mg L^{-1} (control)	Dosage 171.7 mg L^{-1}	Dosage 229.0 mg L^{-1}	Dosage 343.5 mg L^{-1}
0.0	40.2±0.5	69.6±0.3	75.3±0.3	68.6±0.3
0.5	42.4±0.7	74.7±0.7	79.7±0.1	76.3±0.1
1.0	39.6±0.5	72.1±0.6	78.3±0.3	77.5±0.9
1.5	38.7±0.7	71.8±0.6	76.3±0.2	75.2±0.6
2.0	35.7±2.5	70.4±0.8	77.8±0.2	75.9±0.8
2.5	40.6±2.1	69.8±0.4	77.9±0.1	76.3±1.7
3.0	41.9±0.8	70.4±0.5	75.9±0.2	74.9±0.3
3.5	46.0±0.9	71.1±0.7	75.3±0.0	75.1±0.3
4.0	48.0±1.0	70.2±0.3	74.8±0.1	75.4±1.3

Data are mean values ± SD of duplicate experiments.

Data for the flocculation efficiency of *N. salina* biomass in the continuous flocculation-sedimentation process at various total flow rates for Figure 4.24

Sedimentation time (h)	Percentage removal of biomass from broth at different total flow rates				
	At 56.5 mL min^{-1} (control)	At 67.8 mL min^{-1}	At 45.2 mL min^{-1}	At 33.9 mL min^{-1}	At 22.6 mL min^{-1}
0.0	75.3±0.3	72.6±0.7	75.8±1.2	74.8±0.3	85.6±0.3
0.5	79.7±0.1	75.2±0.1	75.4±0.4	79.4±0.9	86.2±0.1
1.0	78.3±0.3	73.1±1.1	78.5±0.7	81.4±0.9	84.8±1.6
1.5	76.3±0.2	71.8±0.3	78.1±0.4	83.8±0.7	85.2±0.7
2.0	77.8±0.2	72.5±0.9	79.8±0.6	78.4±1.8	85.0±0.4
2.5	77.9±0.1	75.9±0.1	76.9±0.1	82.0±0.9	86.6±0.2
3.0	75.9±0.2	75.5±1.0	79.9±0.7	83.5±1.2	87.9±0.3
3.5	75.23±0.0	75.2±0.3	79.4±0.3	82.5±0.8	87.2±0.8
4.0	74.8±0.1	-	77.6±0.1	83.6±0.1	85.4±0.1
4.5	-	-	77.3±0.2	83.0±0.7	85.4±0.9
5.0	-	-	80.0±0.4	81.7±0.1	85.7±0.5

Data are mean values ± SD of duplicate experiments.

Data for the flocculation efficiency of *N. salina* biomass in the continuous flocculation-sedimentation process at various total flow rates for Figure 4.24 (Cont.)

Sedimentation time (h)	Percentage removal of biomass from broth at different total flow rates				
	At 56.5 mL min^{-1} (control)	At 67.8 mL min^{-1}	At 45.2 mL min^{-1}	At 33.9 mL min^{-1}	At 22.6 mL min^{-1}
5.5	-	-	-	84.5±0.3	85.7±0.4
6.0	-	-	-	83.8±0.3	86.5±0.1
6.5	-	-	-	83.1±0.2	87.2±0.5
7.0	-	-	-	-	84.7±0.4
7.5	-	-	-	-	86.4±0.6
8.0	-	-	-	-	86.0±0.2
8.5	-	-	-	-	84.8±0.4
9.0	-	-	-	-	84.4±0.7
9.5	-	-	-	-	87.0±0.23
10.0	-	-	-	-	86.1±0.1

Data are mean values ± SD of duplicate experiments.

Data for Figure 4.28

Alga	Percentage of the flocculant dose remaining in the microalgal biomass after washing and drying	
	Aluminum sulfate	Ferric chloride
C. minor	68.3±5.9	76.3± 3.4
Neochloris sp.	49.4±4.2	21.4±6.5
C. vulgaris (freshwater)	65.4±3.9	75.1±4.9
C. vulgaris (seawater)	31.6±5.6	27.5±4.5
N. salina	81.8±4.0	48.9±4.9
C. fusiformis	48.2±3.6	49.4±3.5

Data are mean values ± SD of triplicate experiments.

Data for Figure 4.29

Alga	Percentage of moisture in biomass paste	
	Oven dried	Freeze dried
C. minor	68.7± 1.5	67.3 ±0.2
Neochloris sp.	72.6±0.2	74.1±1.0
C. vulgaris (freshwater)	75.4±0.0	75.1±0.1
C. vulgaris (seawater)	77.1±0.2	76.4±0.4
N. salina	79.9±2.6	77.8±0.2
C. fusiformis	87.8±0.0	86.5±0.0

Data are mean values ± SD of triplicate experiments.

Data for Figure 4.30

Alga	Percentage of the total lipids in biomass paste	
	Dry biomass	Wet biomass
C. minor	28.7±0.4	27.2±2.9
Neochloris sp.	16.6±0.3	17.0±0.9
C. vulgaris (freshwater)	30.8±0.7	30.1±1.1
C. vulgaris (seawater)	33.2±2.6	31.9±3.5
N. salina	53.7±0.1	50.9±0.7
C. fusiformis	22.8±1.0	20.7±1.7

Data are mean values ± SD of triplicate experiments.