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# Algea Suspension Dewatering with an Inclined Gravity Settler

Jing Hou  
*Cleveland State University*

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ALGAE SUSPENSION DEWATERING WITH AN INCLINED  
GRAVITY SETTLER

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Bachelor of Engineering in Biochemical Engineering  
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Submitted in partial fulfillment of requirements for the degree  
MASTER OF SCIENCE IN CHEMICAL ENGINEERING  
at the  
CLEVELAND STATE UNIVERSITY  
December 2011

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This thesis has been approved

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**To my advisor and my friends**

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# ALGAE SUSPENSION DEWATERING WITH AN INCLINED GRAVITY SETTLER

JING HOU

## **ABSTRACT**

Biofuel has been studied for several decades as a feasible substitute for petroleum, which may solve the energy crisis. Having a high oil content and growth rate, algae biofuels have received lots of attention recently, and have been considered as one of the most promising technologies to reduce the dependence of fossil fuels. In the process of producing algae biofuel, the dewatering process constitutes a significant part of the cost of the algae biofuel, impeding progress towards large-scale commercial use. An inclined settler utilizes gravity to separate algae cells from the fluid, with little energy consumption. The separation ability of a rectangular downward inclined gravity settler was evaluated with two strains of algae, *Scenedesmus dimorphus* and *Chlorella vulgaris*, which exhibit different oil content levels. The functional parameters of recovery rate and concentration ratio were used to evaluate the gravity settler's separation ability with these two cell lines. Experiments were operated to verify that the gravity settler system kept a steady and constant performance for larger scales. From the current experimental data, this gravity settler showed a stable ability in dewatering two strains of algae cells, at both small and large scale. These performances insured the possibility of a two stage gravity settler, which can significantly increase the biomass concentration, compared to that of the regular one stage.



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# **CHAPTER I**

## **INTRODUCTION**

### **1.1 Biodiesel**

Oil is the main source of energy and is used in industrial settings and for transportation, worldwide. The United States consumes 25% of the world's petroleum, 43% of the gasoline, and 25% of the natural gas; despite the fact the country contains only 5% of the world's human population. According to Oil and Gas Journal (O&GJ) estimates, worldwide reserves at the beginning of 2004 were 1.27 trillion barrels of oil and 6,100 trillion cubic feet of natural gas (Palligarnai et al., 2008). Currently, the consumption rate of world energy is roughly 85 million barrels per day of oil and 260 billion cubic feet per day of natural gas. At this rate, the proven oil reserves would last 40 years, at most, and the natural gas reserves only 64 years. Since diesel is not a renewable energy, the increasing demand of energy always brought a surge of oil price. Based on oil prices from West

Texas Intermediate, reported by Dow Jones & company in Wall Street Journal, a sharp increase of oil price appeared in the past ten years (Fig1.1), which indicated a dramatically raised demand of energy. This widespread use of petroleum-based fuels also causes serious environmental problems, most notably environmental pollution and global warming (Meher et al., 2006; Gerpen., 2005). Therefore, an urgent need exists to develop new energy resources.

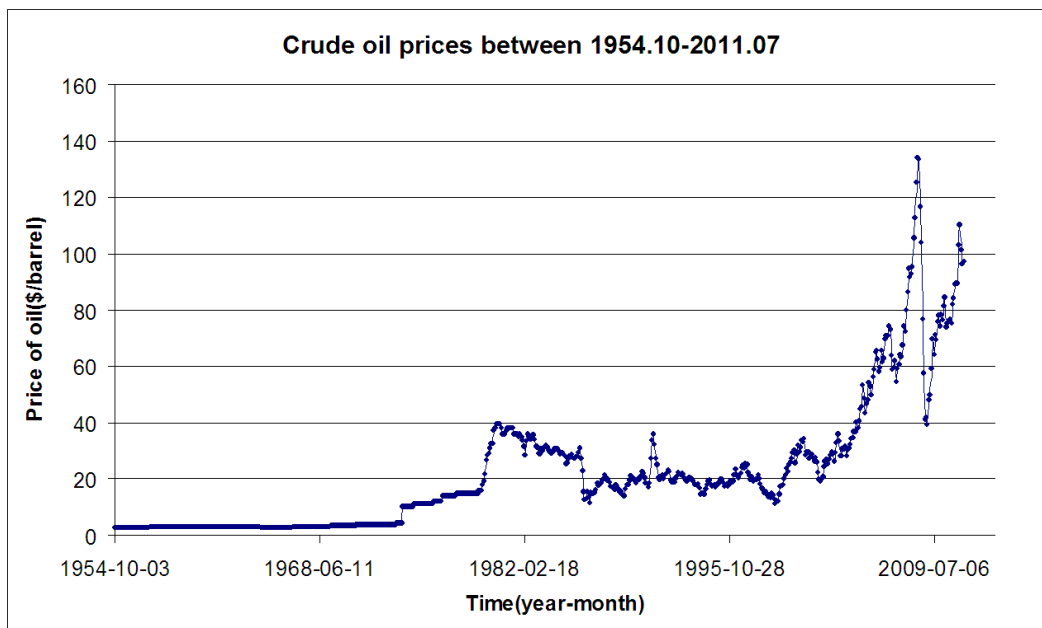


Fig1.1 Oil price change from 1954 to 2011  
(<http://research.stlouisfed.org/fred2/series/OILPRICE?cid=98>)

Biodiesel has the potential to substitute for petroleum and solve energy crisis problems. It is a clean-burning and renewable fuel source produced from grease, animal fat and vegetable oil. It is non-toxic and bio-degradable and burns with low-emissions. Many studies have already

been done with various types of plants, in search of the one that has the highest productivity of biodiesel.

## **1.2 Algae biodiesel**

Biofuel has been utilized as a viable fuel for more than 50 years (Barnwal and Sharma, 2005; Demirbas, 2005; Felizardo et al., 2006; Fukuda et al., 2001; Knothe et al., 1997; Kulkarni and Dalai, 2006; Meher et al., 2006; Metting, 1996; Van Gerpen, 2005). Terrestrial crops traditionally considered the best sources of biodiesel include soybeans, corn, canola, jatropha, coconut, and palm oil. 30 years ago, algae were considered as a competitive biodiesel source due to a short growth cycle and high lipid content. It neither affects the environment in a negative manner nor does it compete for food resources (Chisti, 1980). Microalgae have been suggested to be the only efficient and productive source of oil for biodiesel to fulfill the requirements for large scale biodiesel production.

### **1.2.1 Algae have shorter growth cycle**

The growth cycle for most terrestrial crops, like soybeans, corn and canola lasts four to six months. In comparison, the growth cycle for most microalgae lasts two to four weeks. Microalgae commonly double their

biomass within 24 hours. During exponential growth phase, the doubling times can be as short as 3.5 hours (Chisti, 2007).

### **1.2.2 Algae have high lipid content**

The oil content in terrestrial crops is commonly lower than 5% of the weight of the dry biomass. Microalgae have much higher oil contents, ranging from 20% to 50% of the weight of the dry biomass (Table 1.1) (Chisti, 2007). Some microalgal species even contain lipid levels as high as 80% of the dry biomass weight (Spolaore et al., 2006).

Various oil seed crops have been cultivated in experiments to evaluate their annual biodiesel yields. The results indicated microalgae biodiesel production exceeded the other yields, from 5.87L/m<sup>2</sup> to 13.69L/m<sup>2</sup> annually. These numbers indicated algae are able to produce 10-30 times the amount of oil per year, compared to other high oil producing plants. Based on the numbers above, it was estimated that if 1.1-2.5% of the total land area of the US were used for algae cultivation, enough fuel will be produced to meet the requirement of half of the total US transportation fuel consumption (Table 1.2) (Chisti, 2007; Gong et al., 2011).

Table1.1: Oil content of microalgae (Chisti, 2007)

Microalga	Oil content (% dry weight)
<sup>a</sup> Botryococcus braunii	25-75
Chlorella sp.	28-32
Cryptocodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella primolecta	23
Isochrysis sp.	25-33
Monallanthus salina	>20
Nannochloris sp.	20-35
Nannochloropsis sp.	31-68
Neochloris oleoabundans	35-54
Nitzschia sp.	45-47
Phaeodactylum tricornutum	20-30
<sup>a</sup> Schizochytrium sp.	50-77
Tetraselmis sueica	15-23

<sup>a</sup> Algae strains which have very high lipid content.

Table1.2: Comparison of some sources of biodiesel(Gong et al., 2011)

Crop	Oil yield (L/m <sup>2</sup> ·year)	Land area needed for Cultivation (*10 <sup>10</sup> m <sup>2</sup> ) <sup>a</sup>	% of existing Cropland area <sup>a</sup>
Corn	1.72*10 <sup>-2</sup>	1540	846
Soybean	4.46*10 <sup>-2</sup>	594	326
Canola	1.19*10 <sup>-1</sup>	223	122
Jatropha	1.892*10 <sup>-1</sup>	140	77
Coconut	2.689*10 <sup>-1</sup>	99	54
Oil palm	5.950*10 <sup>-1</sup>	45	24
<sup>b</sup> Micro-algae	13.69	2	1.1
<sup>c</sup> Micro-algae	5.87	4.5	2.5

<sup>a</sup> For meeting 50% of all transport fuel needs of the US.

<sup>b</sup> 70% oil (by wt) in biomass.

<sup>c</sup> 30% oil (by wt) in biomass.



### **1.2.3 Algae do not compete for food resources**

Unlike many typical oilseed crops requiring large inputs of water, ammonia, and physical spaces, algae are maintained in liquid culture, demanding no farmland. It does not compete with any agricultural or food resource. Some algae species can even live in an extreme environment, like very high salinity, for example, or in brackish or waste water; low temperatures, and varying luminosities. Thus, minerals from industrial and living waste water could be utilized to feed into algae pond as nutrition; excess CO<sub>2</sub> emissions may be used as well, since algae grow faster at high CO<sub>2</sub> concentrations and produce more biomass for energy production (Chisti, 2007).

### **1.2.4 Algae biodiesel is a green energy**

Carbon dioxide (CO<sub>2</sub>) is converted into biomass during the photosynthesis process by algae, and then released when the biofuel is burned to produce energy. Other than the common greenhouse gas, CO<sub>2</sub>, algae biodiesel does not produce any environmentally damaging gas. The amount of CO<sub>2</sub> produced in energy release is equal to the amount consumed during algal growth. It is believed that energy is produced in a CO<sub>2</sub>-neutral manner, contrary to conventional energy carriers.

### **1.2.5 Limits of economic viability of algae biodiesel**

There are three theoretical limits restraining the economic viability of algae biodiesel.

First, compared to the price of diesel, the capital costs of the algae cultivation are high regardless of whether the culture is maintained in an open raceway pond or in photobioreactors. The cost in cell culturing takes at least 40% of the bio-diesel production cost (Chisti, 2007).

Second, the maximum algae biomass concentration is low. Dense algae solution blocks light from reaching algae cells and eventually limits cell growth. At this point, even optimal conditions are provided to the algae culture, the maximum biomass concentration achieved never exceeds 10g/L due to the light limitation. The algae biomass concentration commonly obtained in raceway pond is about 0.5g/L (Morweiser et al., 2010; Chisti, 2007). The low biomass concentration results in a large amount of water that must be removed prior to oil extraction. This process of removing the water and concentrating the cells, called the “dewatering” process, is both energy and equipment intensive and accounts for at least 15% of the total biodiesel production cost.

Third, oil extraction process commonly accounts for 16% of the total algae biodiesel cost (Gong et al., 2011).

### 1.3 General procedures of algae biodiesel production

The process to produce biodiesel from algae could be roughly stated as three steps: algae cultivation, biomass harvest and oil extraction.

(Fig 1.1)

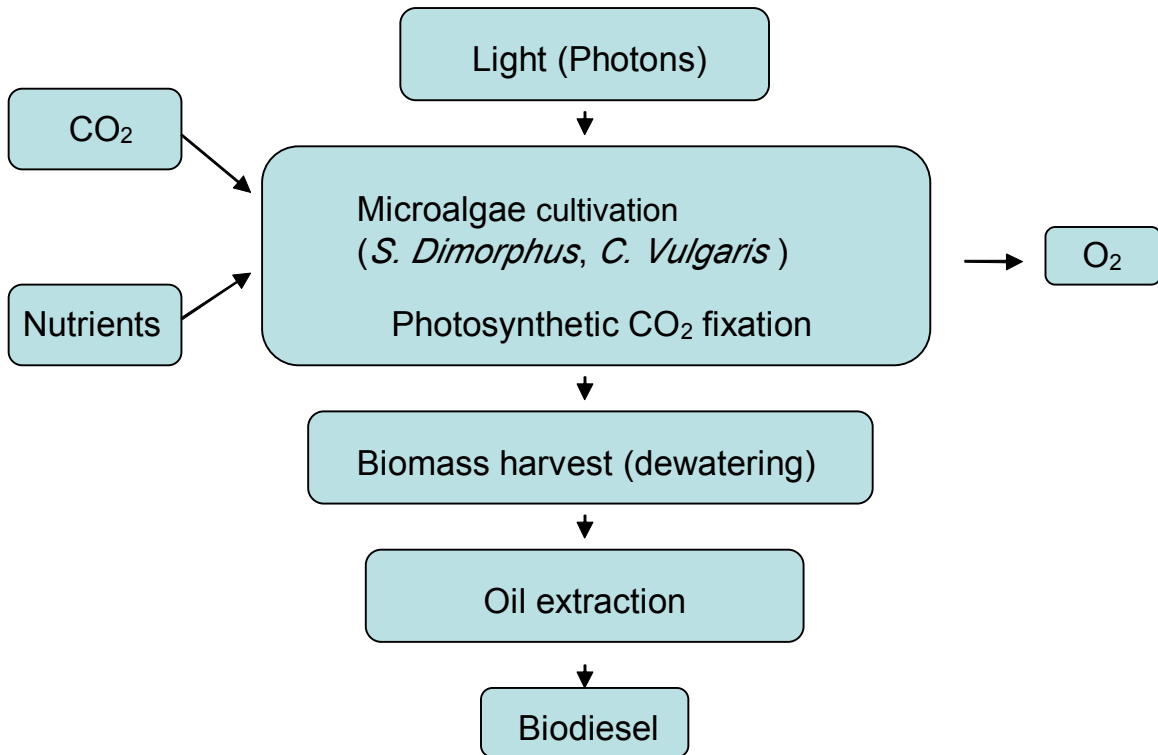


Fig.1.2 Biodiesel production in microalgae

#### 1.3.1 Algae cultivation

Given water, CO<sub>2</sub>, minerals, appropriate temperature and light, algae could be raised in open raceways, a photobioreactor, a marine environment, or even waste water (Fig 1.1) (Chisti, 2007).

Open raceways easily provide abundant sunlight, nutrients, and carbon dioxide needed for algae growth. These open raceways were

originally considered the best algae cultivation method for large scale production. The disadvantages to raceways are their vulnerability to contamination due to the openness of the raceway area, and also the difficulty of harvesting due to the constant water flow.

Photo-bioreactors are another common algae culture method.

Photo-bioreactors usually consist of multiple transparent tanks or tubes, constantly provided with optimum CO<sub>2</sub> inflow, nutrients, temperature and light. Multiple bioreactor tanks are connected, but the whole system is closed to avoid contamination. Since slight changes in pH and nutrient level can be detrimental to algae growth and productivity, a complex control system is required to maintain the optimum growth conditions. The high cost of this system is the limitation of photo-bioreactor in algae cultivation.

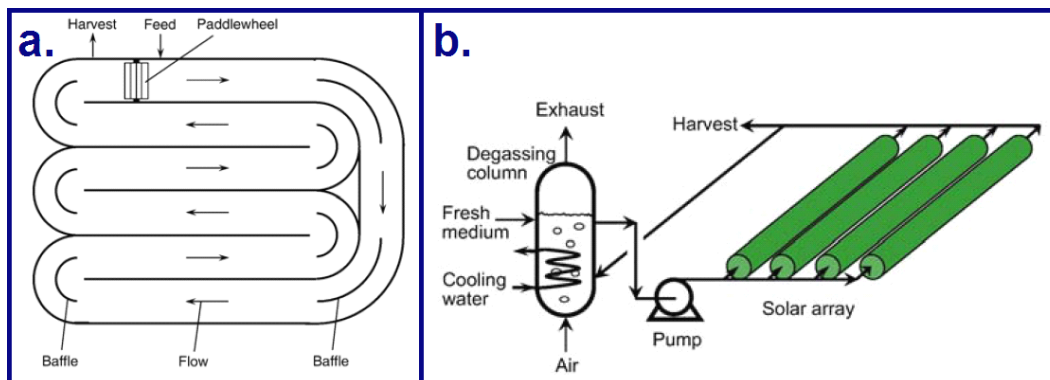


Fig 1.3 View of raceway pond and photobioreactor.  
a.Raceway pond. b. Photo-bioreactor

### **1.3.2 Biomass harvest (Dewatering)**

After algae cultivation, the process of separating the algae culture from the growing media takes place. This is called biomass harvesting or dewatering.

### **1.3.3 Oil extraction**

The final process of algae biodiesel production is oil extraction from the biomass gathered during harvesting. Extraction methods of lipids from the algae biomass are divided into two categories, the mechanical method and the chemical method. Mechanical crushing is the simplest way to extract lipids and it is often used in conjunction with chemicals.

Ultrasonic-assisted extraction uses ultrasonic waves to create bubbles around the algae cells. When these bubbles break near the cells, the shock wave assists in cell wall breakage, thus releasing the cell's contents (Mercer et al., 2011). Microwave-assisted extraction is another method currently in use. Since microwave heating releases very little heat to the environment, this method was applied to algae cells for oil extraction. When using microwaves, moisture inside cells evaporates, generating a significant amount of pressure that stresses the algae cell wall. Lipids inside the cells are released when the cell walls rupture under high pressures (Sundar et al., 2011; Mandel et al., 2007).

Many chemical methods have been developed for lipid extraction because of the low expense and high efficiency of these processes. Some chemical agents, like benzene, ether and hexane have been widely used in the food industry, for example, for oil extraction and have also been used as solvents for algae biodiesel extraction.

#### **1.4 Current dewatering technologies**

Many methods have been developed to dewater algae cultures. The following four are, the most commonly used and frequently discussed: flocculation, flotation, filtration and centrifugation.

##### **1.4.1 Flocculation**

Flocculation is a harvesting method utilizing chemical flocculants, such as alums and ferric chloride, to separate the algae from mediums such as colloids. "Chitosan" is the agent most commonly used for water purification and for algae harvesting (Ravi et al., 2002). The efficiency of this method is highly dependent on the pH value of the medium. Plus, the cost to remove the flocculants from the algae after separation is expensive. Overall, it is a possible, but limited, method for large-scale biofuel production.

### **1.4.2 Flotation**

Flotation is another method for harvesting algae, and it is usually combined with the flocculation method. Algae are flocculated with Cationic *N*-Cetyl-*N*-*N*-*N*-trimethylammonium bromide (CTAB), anionic sodium dodecylsulfate (SDS) and the nonionic Triton X-100, and simultaneously bubbled with air. These flocculants caused algae aggregation and froth accumulation on the top of the medium. Then algae is then easily separated and removed. SDS has been shown to remove 90% algae, achieving maximum efficiency for this method (Chen et al., 1998). The combination of flotation and flocculation improves the efficiency of algae separation, but it is still far too expensive for commercial use on a large scale.

### **1.4.3 Filtration**

Filtering is a third harvesting method, in use since the early seventies (Golueke et al., 1965; Sim et al., 1988). Benemann et al., 1980 reported their filtration system using the cloth pore size at 12  $\mu\text{m}$  achieved a recovery rate surpassing 80%. However, the blinding of the filters proved problematic. Blinding of the filter refers to deposited algae biomass clogging the pores in the cloth, significantly reducing the rate of filtration across the filter. The filtration method is commonly considered to have high

recovery rate and low cost, but lacks the ability to run continuously, a necessary factor in long-term commercial production.

#### **1.4.4 Centrifugation**

The use of centrifugation is not a new concept and the process is widely used in the food industry and for algal harvesting (Golueke et al., 1965). It is fast and effective in harvesting algae with recovery rate in excess of 90%. However, the cost of centrifugation and subsequent water removal process is high. Based on experiments, it is estimated the energy demand can reach 1.3 kWh/m<sup>3</sup> (kilowatt hour of energy per cubic meter of pond water) on average (Sim et al., 1988). If the cost of electricity is estimated at 9.8 cents/kWh (Average retail price of electricity in the United States in 2010) and the biomass is estimated to reach 2.5 g/L, the calculation of the cost of centrifugation would be \$12.74 per ton of algae biomass, which contributes \$0.8 per gallon to the cost of biodiesel and makes algae biodiesel less competitive than diesel fuel in price.

#### **1.5 An inclined gravity settler**

In 1998, an inclined rectangular particle settler separating particles from bulk liquid was patented (Thompson et al., 1998). This device has inclined layers which were connected to a vertical tank containing fluid and

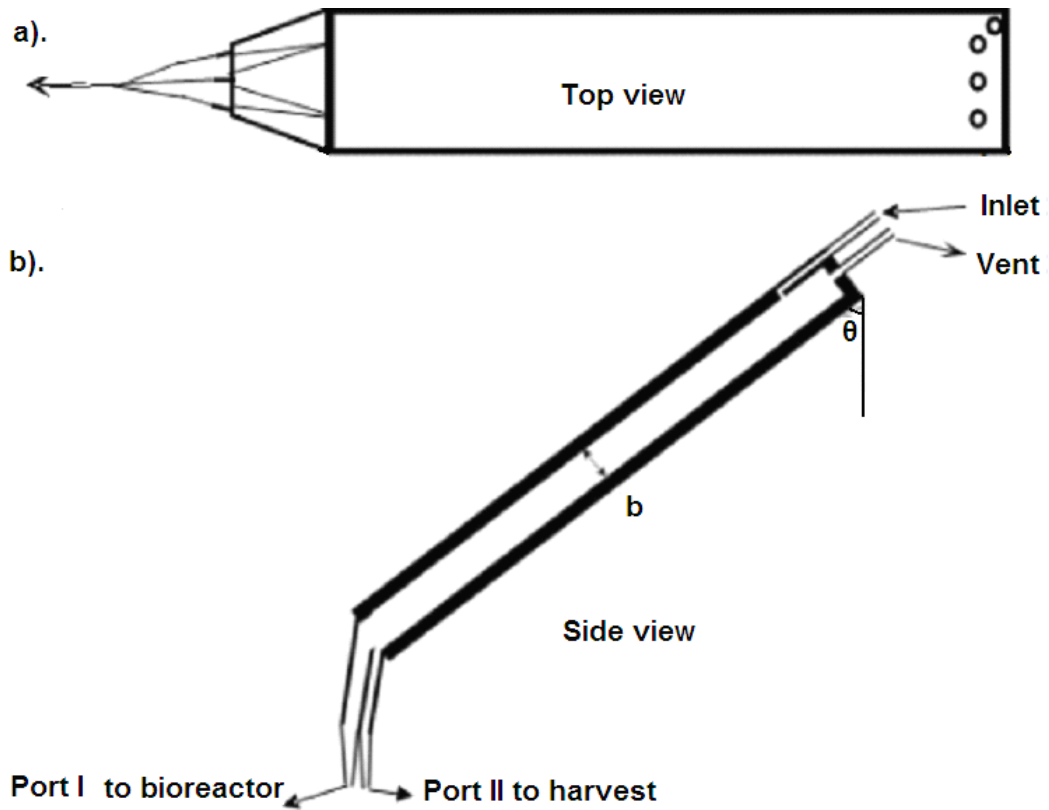


particles. This liquid flows upward from the tank to the settler over the surfaces of the layers and then settles on these surfaces. Separation is achieved after the particles settle and slide down the incline where they are collected.

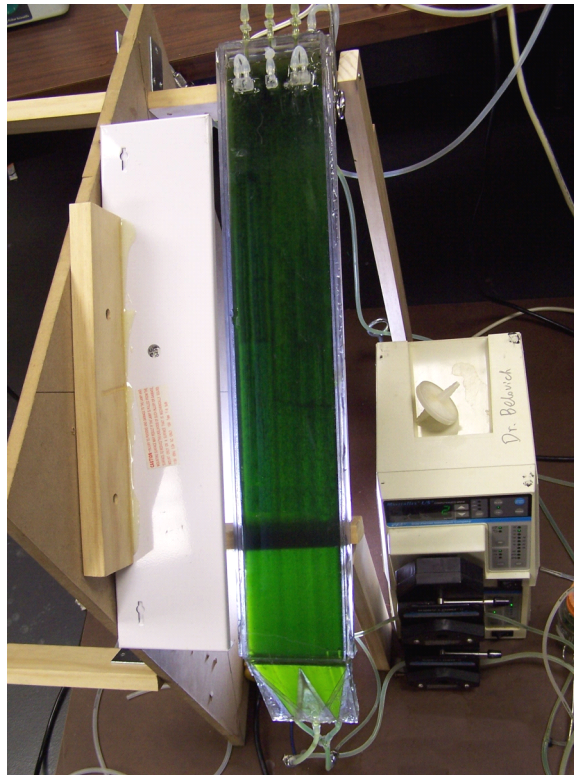
There are two outlets at the bottom of the settler. The settler is designed to have the port "I" export the concentrated stream, while the port "II" export the diluted stream. For industrial use, the concentrated stream is further processed for concentration. The diluted stream should be recycled to the cell culture process to reuse the water and diluted unconsumed nutrition (Batt et al., 1990).

The outlet of the concentrated stream and the inlet stream of an upward flow settler are located very close, both are at the bottom of the settler. A potential risk of clogging and vortex flow exists in this design. The downward flow settler avoided this problem by changing the inlet flow from the bottom to the top. The gravity settler investigated in this thesis is a downward flow inclined rectangular gravity settler, similar in appearance with the upward flow settler (Wang and Belovich, 2009). The device is connected to a bioreactor. The schematic of the top view and side view of this device is shown in Fig 1.4 (Wang, 2009). A photo of the system in operation is shown in Fig 1.5.

The output from the large gravity settler was found approximately 1.6L/day (Wang, 2009). An algae biodiesel company (Solix BioSystems, Inc., Fort Collins, Colorado) reported in 2009 to have an annual production of 3000 gallons of algal biofuels per acre per year. Therefore, the requirements for dewatering at industrial production levels can be estimated as 8296.8 L/ day·acre, assuming the biomass concentration is 5g/L and the density of biofuel is 0.8g/cm<sup>3</sup>. A settler should be able to be scaled up to this level if it is to be aimed for a commercial application. From previous research, it was reported that mammalian cell suspension could be concentrated by settler at least by 4-fold (Wang, 2009), which meant 75% of the water in the cell suspension was removed. It can be anticipated that an even higher water removing rate may be achieved from the outlet if a two-stage settler configuration was used.



**Fig1.4** Schematic of the gravity settler. a). Top view. b).Side view.(Wang, 2009)



**Fig1.5** View of a gravity settler used in lab.

## 1.6 Cells lines

### 1.6.1 *Scenedesmus dimorphus*

*Scenedesmus dimorphus* is a unicellular alga, elliptical in shaped, long axis 10-20  $\mu\text{m}$  in size. It belongs to the Scenedesmaceae family of chlorophyceae class (Fig 1.6, Fig 1.7) (Ying et al., 2009; Trainor et al., 1965).

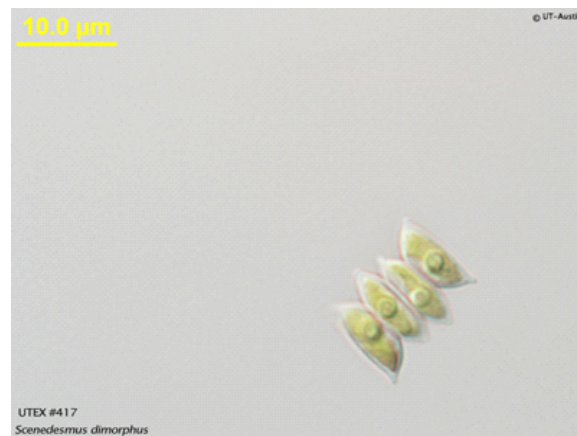


Fig 1.6 *Scenedesmus dimorphus*  
(<http://www.sbs.utexas.edu/utex/algaeDetail.aspx?algaeID=2937>)

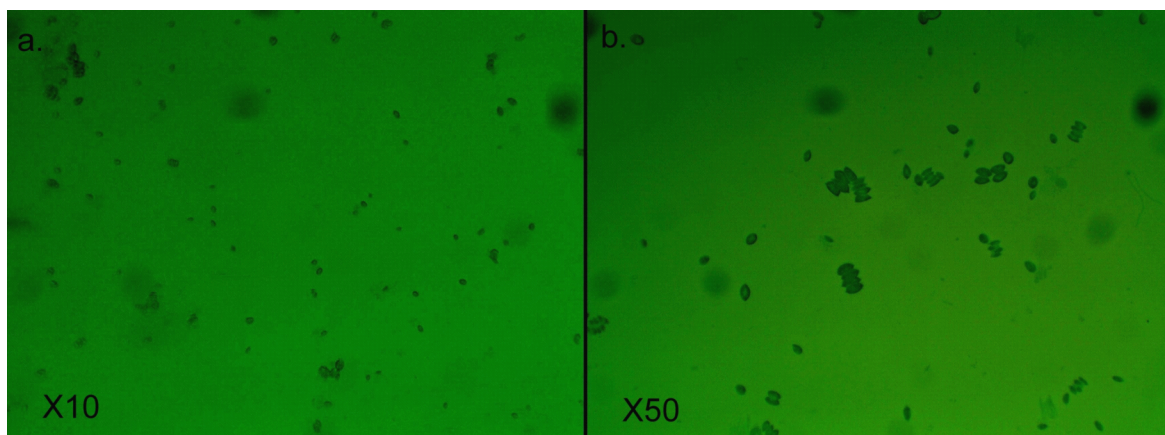


Fig 1.7 Microscope images of *Scenedesmus dimorphus* cells captured in Dr. Belovich's lab under different magnitudes. a. magnified by 10 times b. magnified by 50 times.

The cells usually grow as 2, 4, or 8 grouped together, and they grow both easily and rapidly in a wide range of nutritional conditions. They have

a high lipid content ranging from 16% to 40% by dry biomass weight, and lipid productivity between 41-54 mg·L<sup>-1</sup>day<sup>-1</sup> (Gong et al., 2011). The optimal growth temperature falls between 30-35 degrees Celsius. Cell cultures should be continuously agitated to avoid sedimentation of cells, which hinders cell growth (Li et al., 2010a; 2010b; 2010c; 2010d).

### **1.6.2 *Chlorella vulgaris***

*Chlorella vulgaris* is a type of single-celled green algae found in most bodies of fresh water, belonging to *chlorellaceae* family of phylum *Chlorophyta* division. Cells are spherical in shape and are approximately 2 to 10 µm in diameter (Fig 1.8, Fig 1.9). The optimal growth temperature falls between 20-30 degrees Celsius. The lipid content of *Chlorella vulgaris* is under the average level of most microalgae, which is 14%–30% by weight of dry biomass (Feng et al., 2011; Liu et al., 2008). However, this algae strain has great potential to be a resource for biodiesel production due to its fast growth and easy cultivation. Plus, researchers found its lipid content increased up to 40% by biomass dry weight if nitrogen concentration were reduced in the medium (Illman et al., 2000); the number could even go higher to 56.6% if Fe<sup>3+</sup> is provide at 1.2×10<sup>-5</sup> mol/L in media (Liu et al., 2008). *Chlorella Vulgaris* also had a high lipid

productivity of 147mg/L d<sup>-1</sup> if cells were maintained with a semi-continuous culture mode (Feng et al., 2011).

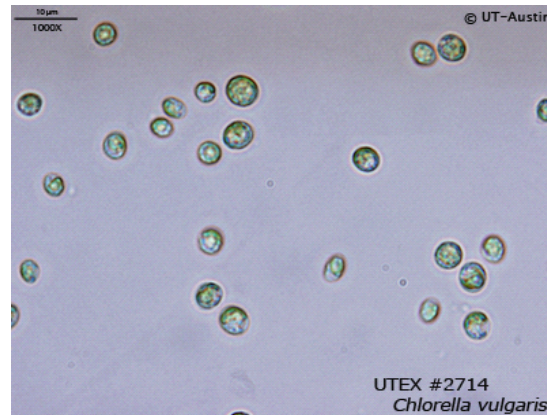


Fig 1.8 Chlorella Vulgaris  
(<http://www.sbs.utexas.edu/utex/algaeDetail.aspx?algaeID=5235>)

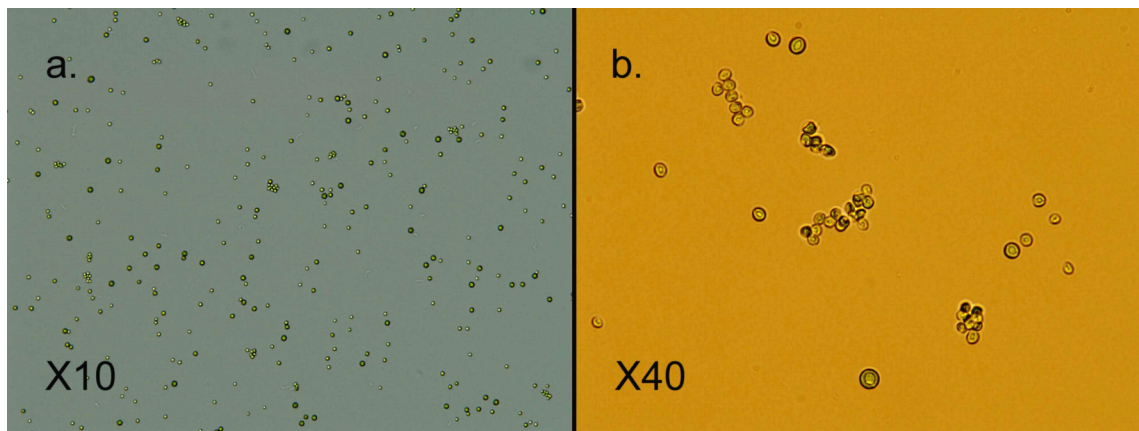


Fig 1.9 Microscope images captured at different magnitudes for Chlorella vulgaris cells in Dr. Belovich's lab. a. magnified by 10 times b. magnified by 40 times.

Both *Scenedesmus* and *Chlorella* have an exponential growth rate constant  $\mu = 0.03 \text{ h}^{-1}$ , which remains stable in the cell's optimal growth temperature range (Westerhoff et al., 2010). Even they were not the strains having very high oil content, they were often considered as a suitable source to produce biodiesel because they grow fast and can survive with a wide tolerance to different environments.

## 1.7 Objectives

The gravity settler was first designed to separate mammalian cells from media in order to recycle and reuse nutrients in the media. Since the mammalian cells tested have comparable cell size to algae (10  $\mu\text{m}$ ) cells, the settler was considered to be also useful for algae dewatering.

### 1.7.1 Aim1

The first aim of this study is to examine if that the gravity settler can effectively dewater algae cells.

From previous work, the settler was tested by using three types of mammalian cells: HB58, 9E10, R73. It was reported the settler could concentrate solutions at least by 4-fold (Wang, 2009), which would imply that 75% of the water in the cell suspension could be removed. Since these mammalian cells and algae cell all have similar size at 10 $\mu\text{m}$  in diameter and density close to water, the settler was expected to have similar performance in dewatering algae culture.

Due to the different lipid contents in algae cells, *Scenedesmus dimorphus* and *Chlorella vulgaris*, the densities of these two strains in culture may be slightly different. So, this study also aims to determine how the settler performance depends on the lipid content. In this study, we will also explore how the operating parameters, such as outlet flow rate ratio

and total inlet flow rate, affect the performance of the gravity settler in terms of algae dewatering.

### **1.7.2 Aim 2**

When the gravity settler was applied to dewater mammalian cells, 75% of water was removed from the cell suspension. For algae biodiesel application, most dewatering methods like filtration and centrifugation achieved dewatering rates surpassing 80%. It is expected that the settler could reach a higher dewatering rate by using a two-stage system configuration. The feasibility and efficiency of the two-stage settler for algae dewatering will be demonstrated in this thesis.

### **1.7.3 Aim 3**

The final aim of this study is to test the potential use of this gravity settler for large scale commercial projects. The theoretical equations representing settler design and scaling would then be verified experimentally as potential design criteria for scale-up for industrial applications.



## CHAPTER II

# METHODS AND MATERIALS

### 2.1 Cell lines

*Scenedesmus dimorphus* and *Chlorella vulgaris* were selected as the cell strains to conduct the settling experiment.

*Scenedesmus dimorphus* (UTEX number: 417) and *Chlorella vulgaris* (UTEX number: 2714) were initially obtained from the UTEX culture collection, stored in agar tubes. Cells were then stored as seed culture in liquid nitrogen (in 5% DMSO) and maintained in 1.5% agar prepared with modified Bold's 3N medium (3N-BBM). A Couple of weeks prior to the beginning, seed cells were thawed and inoculated into 3N-BBM media to grow until they reached the stationary growth phase. Cells were then transferred into a bioreactor and prepared for further experimentation.

## 2.2 3N-BBM media

For algae cultivation, *Scenedesmus Dimorphus* and *Chlorella Vulgaris* were cultured separately, both maintained in modified Bold-Basal Medium with 3-fold Nitrogen and Vitamins (3N-BBM+V).

The 3N-BBM+V media recipe is shown in the following three tables (table 2.1-2.3). Six types of stock solutions and one trace element solution were prepared before media preparation. Stock solutions required to make 1L media were measured by serological pipette (Cole-Parmer, 10mL) as follows: 30mL of NaNO<sub>3</sub>; 10 mL each of MgSO<sub>4</sub>, NaCl, KH<sub>2</sub>PO<sub>4</sub>, K<sub>2</sub>HPO<sub>4</sub>, CaCl<sub>4</sub>; 6mL of trace metal solution and 1 mL of each vitamin solution.

The measured stock solutions, minus vitamins, were mixed and diluted with distilled water in glass bottles or Erlenmeyer flasks until the total volume reached 1L. The glass bottles or Erlenmeyer flasks were sealed with a clean foam stopper or rubber stopper and then loosely covered with aluminum foil. Media was sterilized in autoclave at 121°C, 0.1MPa, for 30 minutes. After the media was cooled down in the autoclave, the aluminum foil needed tightening up around the neck of glass bottles or flasks in order to prevent bacterial contamination from air during the transportation from autoclave room to the laminar flow hood. Since vitamins easily decompose under high pressure and temperature, they were not autoclaved. Instead they were sterilized by 0.2 µm, sterile,

polypropylene (PP) syringe filters (Cole-Parmer, 25mm diameter), and added to the cooled, sterilized media in the laminar flow hood.

Table 2.1 Stock solutions for 3N-BBM

#	Component	Amount	Stock Solution	Final
1	NaNO <sub>3</sub> (Fisher BP360-500)	30 mL/L	10 g/ 400mL dH <sub>2</sub> O	2.94 mM
2	CaCl <sub>2</sub> ·2H <sub>2</sub> O (Sigma C-3881)	10 mL/L	1 g/ 400mL dH <sub>2</sub> O	0.17 mM
3	MgSO <sub>4</sub> ·7H <sub>2</sub> O (Sigma 230391)	10 mL/L	3 g/ 400mL dH <sub>2</sub> O	0.3 mM
4	K <sub>2</sub> HPO <sub>4</sub> (Sigma P 3786)	10 mL/L	3 g/ 400mL dH <sub>2</sub> O	0.43 mM
5	KH <sub>2</sub> PO <sub>4</sub> (Sigma P 0662)	10 mL/L	7 g/ 400mL dH <sub>2</sub> O	1.29 mM
6	NaCl (Fisher S271-500)	10 mL/L	1 g/ 400mL dH <sub>2</sub> O	0.43 mM
7	Trace elements solution (Prepared as below)	6mL/L		

(<http://www.sbs.utexas.edu/utex/mediaDetail.aspx?mediaID=29>)

Table2.2 Trace elements solution

#	Component	Concentration
1	Na <sub>2</sub> EDTA·7H <sub>2</sub> O	0.415g/500mL DI H <sub>2</sub> O
2	FeCl <sub>3</sub> ·6H <sub>2</sub> O	0.0485g/500mL DI H <sub>2</sub> O
3	MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.0205g/500mL DI H <sub>2</sub> O
4	ZnCl <sub>2</sub>	0.0025g/500mL DI H <sub>2</sub> O
5	CoCl <sub>2</sub>	0.001g/500mL DI H <sub>2</sub> O
6	NaMoO <sub>4</sub>	0.002g/500mL DI H <sub>2</sub> O

Table2.3 Vitamin solutions

#	Component	Amount	Concentration
1	H (biotin)	1 mL/L	0.0016g/
2	B1 (thiamine)	1 mL/L	0.0442g/
3	B12 (cyanocobalamin)	1 mL/L	40mL 50mM HEPES solution 0.0063g

### 2.3 Seed growth in sterile hood

The cultures were started by inoculating the cells from agar tubes into a 250 mL Erlenmeyer flask with sterilized 3N-BBM medium, sparged with 5% CO<sub>2</sub> in air at a flow rate of 0.1 LPM, and agitated on a stir plate at room temperature. Light was provided by 14 watt, 48" Accupro fluorescent bulbs on a 12 hours on/off cycle, with a measured intensity of 150-200 ft-candles at the liquid surface maintained in a sterile hood (Fig 2.1).

The growth of the cells was tracked by measuring absorbance of the cell suspension at wavelength of 600nm (Milton Roy, Spectronic Genesys 5). Previous work has shown that algae biomass concentration is linearly related to A<sub>600</sub>, up to A<sub>600</sub>=1.0, with slope of 0.62 gdw/L A<sub>600</sub><sup>-1</sup> (Kanani., 2012). Samples with higher biomass concentrations were diluted with 3N-BBM media to ensure A<sub>600</sub><1.0.

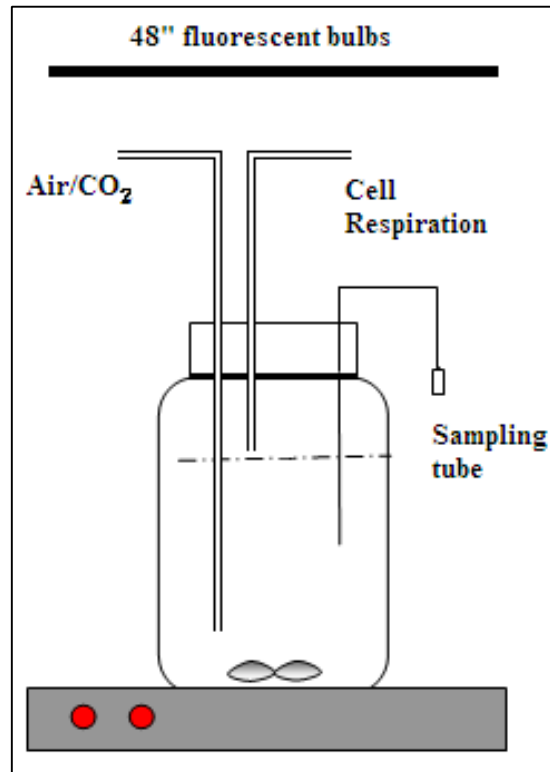


Fig 2.1 Cell culture maintained in the sterile hood

## 2.4 Algae growth phases

A typical batch algae growth included the following five phases (Fig 2.2): lag phase, exponential phase, phase of declining relative growth, stationary phase and death phase. The lag phase was a period of adaption of algae to the new media and environment, which occurred right after inoculation. The exponential phase was also called the logarithmic growth phase. Algae biomass and cell number density were increasing exponentially with time in this phase. Afterwards, the phase of declining relative growth started, which is also called deceleration growth phase. In this phase, algae cell growth decelerated due to the complete consumption

of one or more essential nutrients such as  $\text{NaNO}_3$ . The stationary phase started at the end of the deceleration phase. Algae cells were still metabolically active, but the growth rate was equal to the death rate. During this phase, the net growth rate was zero; the total cell number was almost constant. The dry weight of algae biomass density at this stage was about 2g/L, the absorbance was 3.23 at light wavelength of 600nm. Once the algae growth reached this stationary phase (Dry weight 2g/L,  $A_{600}=3.23$ ), cells needed to be inoculated from the seed flask into 2L glass bottles containing sterilized 3N-BBM medium. If algae cells were not transferred in time, they would go in to the death phase, and the cell number would sharply decline in a short time.

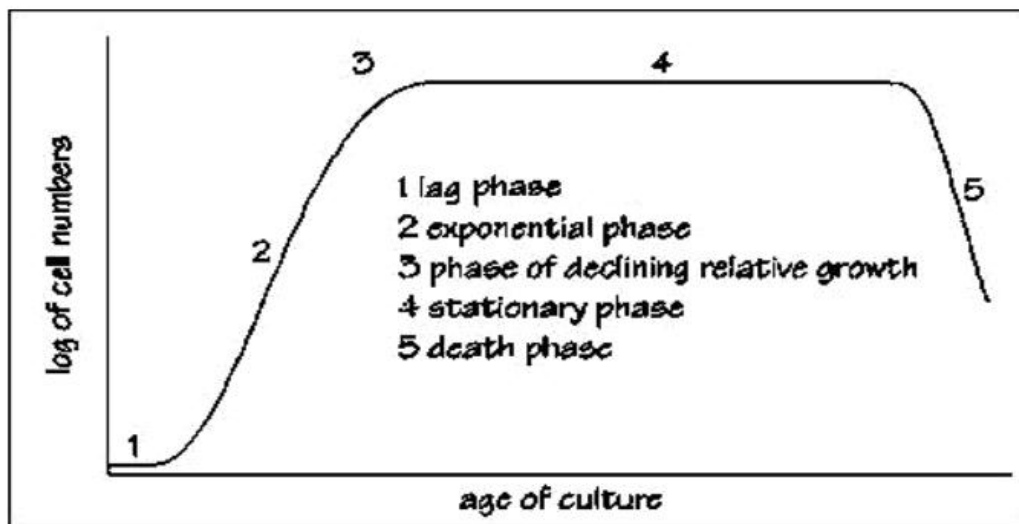


Fig 2.2 Five growth phases of algae culture(Lavens et al., 1996)

## 2.5 Amplified culture in shaker bath

Once cells were inoculated into 2L glass bottles with sterile 3N-BBM+V medium, they were maintained in a water shaker bath with temperature at 30°C (30°C for *Scenedesmus dimorphus*, 27°C for *Chlorella vulgaris*) shaking at the speed about 150 rpm (Fig2.3). Each culture was sparged with 5% CO<sub>2</sub> in air at a total gas flow rate of 0.1 LPM. Illumination was provided by seven 14-watt, 24" fluorescent tubes (four Coralite Aquapro T-5/10,000K and three Accupro AFL/F14T5/14W/830) on a 12 hours on/12 hours off cycle, with a measured intensity of 515-550 ft-candles at the liquid surface.

The cell suspensions were maintained in a shaker bath, and not transferred to the bioreactor until the concentration of cell suspension reached a specific requirement of each experiment. Experiments for comparison purposes were required to have the same starting cell concentration levels in the bioreactor, so that the effects from other parameters could later be compared. Concentrated and sterilized NaNO<sub>3</sub> solution was added cell culture if growth was stopped before cell culture reached the required concentration. The optimal concentration for cells to retrieve growth was the one in fresh 3N-BBM. Exorbitant concentration of NaNO<sub>3</sub> was fatal to algae cells, caused a high osmotic pressure and terminated the culture. Adding NaNO<sub>3</sub> solution was precisely controlled

and monitored by a nitrate ion-selective electrode probe (Vernier, Inc., Beaverton, Oregon). A two-stage settler is an approach to dewater the cell suspension twice to improve the dewatering efficiency. To mimic operation of a second stage, a cell concentration in the second stage was approximately five times of that in the normal biomass concentration was used. In order to get the cell suspension with a high concentration for the second stage, the bottles were removed out the shaker bath and settled overnight. Supernatant was then removed from the bottles until the absorbance of the cell suspension was five times of the absorbance of the cell suspension in the first stage.



Fig2.3 Shaker water bath and lighting configuration.



## 2.6 Tracking the concentration of the essential nutrient: $\text{NaNO}_3$

As a key nutrient ingredient in 3N-BBM+V media,  $\text{NO}_3^-$  has very important effects on algae growth (Li et al., 2010d). Tracking and monitoring the concentration of  $\text{NaNO}_3$  in 3N-BBM+V media is necessary, especially when there was specific requirement for density of the cell culture. A nitrate Ion-selective Electrode (ISE) Probe (Vernier, Inc., Beaverton, Oregon) (Fig 2.4) was used to monitor the nitrate ion concentration in the media.



Fig 2.4 Vernier Nitrate Ion-selective Electrode Probe  
([http://www.vernier.com/images/cache/product.no3-bta.\\_waterquality.\\_hero.001.1280.721.jpg](http://www.vernier.com/images/cache/product.no3-bta._waterquality._hero.001.1280.721.jpg))

The nitrate concentration that could be detected by the probe ranged from 0.1 to 14000mg/L ( $7 \times 10^6$  M to 1M). A software "Go!Link" was provided with the probe for calibration, reading, and recording the ion concentrations. Probe was soaked in high standard  $\text{NaNO}_3$  solution at 100mg/L for half an hour, and then calibrated from 1mg/L to 100mg/L. Any measurement with a concentration higher than 100mg/L should be diluted

with distilled water to ensure the solution is within a concentration lower than 100 mg/L. The reading displayed on the software "Go!Link" indicated the concentration of  $\text{NO}_3^-$  in solution. In order to determine the concentration of  $\text{NaNO}_3$  in media, an equation used for calculation is shown as below (Fig 2.5).

Example :  
 Reading on "Go!Link" was 100mg/L, that means the concentration of  $\text{NaNO}_3$  is:

$$\frac{100 \text{ mg N}}{1 \text{ L}} \times \frac{1 \text{ g N}}{1000 \text{ mg N}} \times \frac{85.0 \text{ g NaNO}_3}{14.0 \text{ g N}} = 0.607 \text{ g NaNO}_3 / \text{L solution}$$

Fig 2.5 Calculation for concentration of  $\text{NaNO}_3$  based on ISE probe reading (<http://www.vernier.com/files/manuals/ise-bta.pdf>)

## 2.7 Cell count

Hemocytometer (Cole parmer, Product ID: EW-79001-00) (Fig 2.6) was used for cell counting. The cell density was accurately determined by counting cell numbers in a unit volume on the hemocytometer.

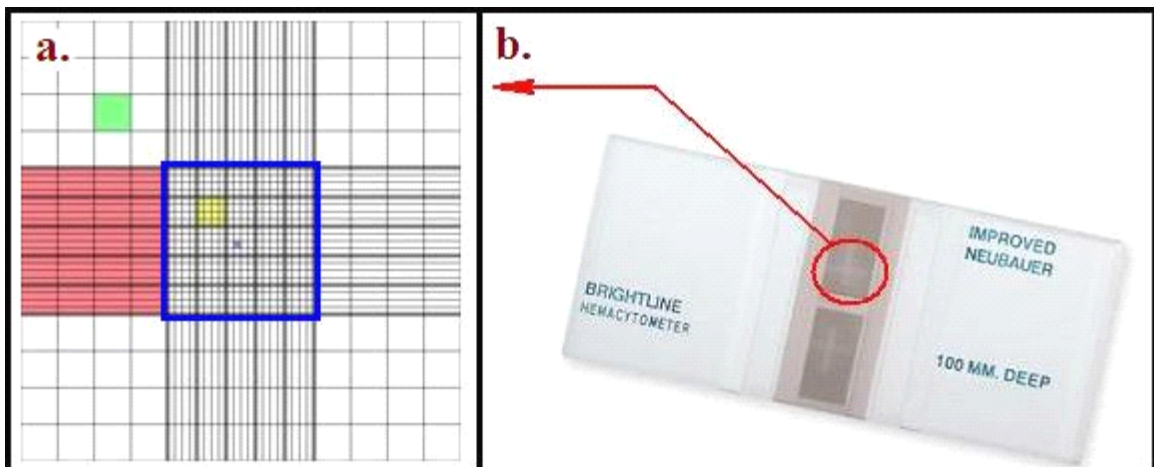
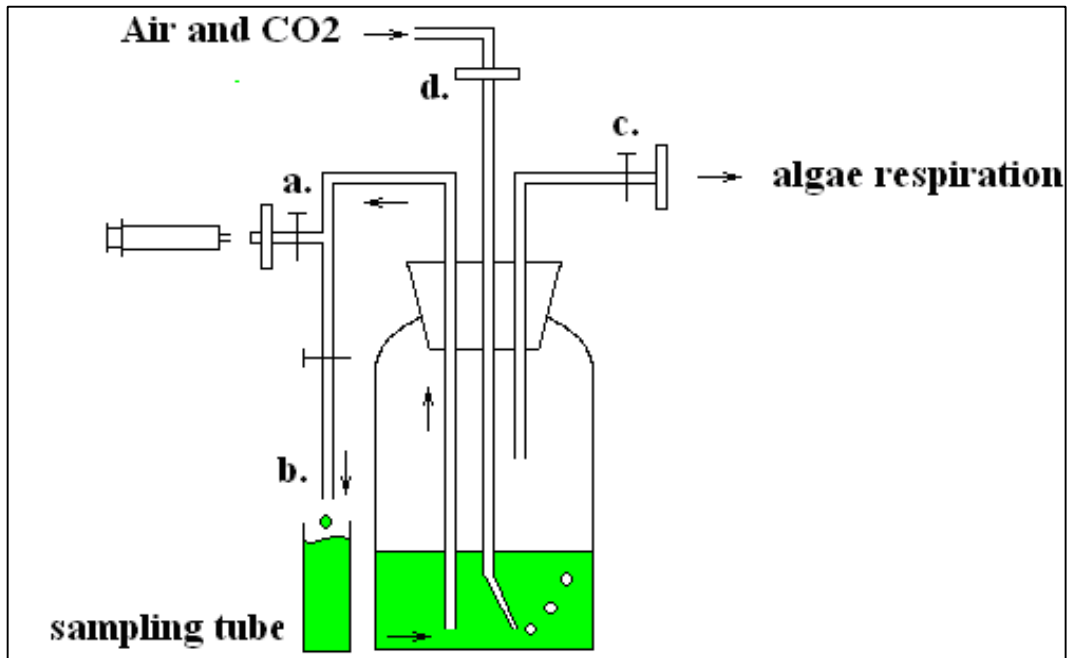


Fig 2.6 a. Enlarged hemocytometer grid b. Hemocytometer ([http://www.coleparmer.com/catalog/large\\_image.asp?sku=7900100&img=7900100.jpg](http://www.coleparmer.com/catalog/large_image.asp?sku=7900100&img=7900100.jpg))

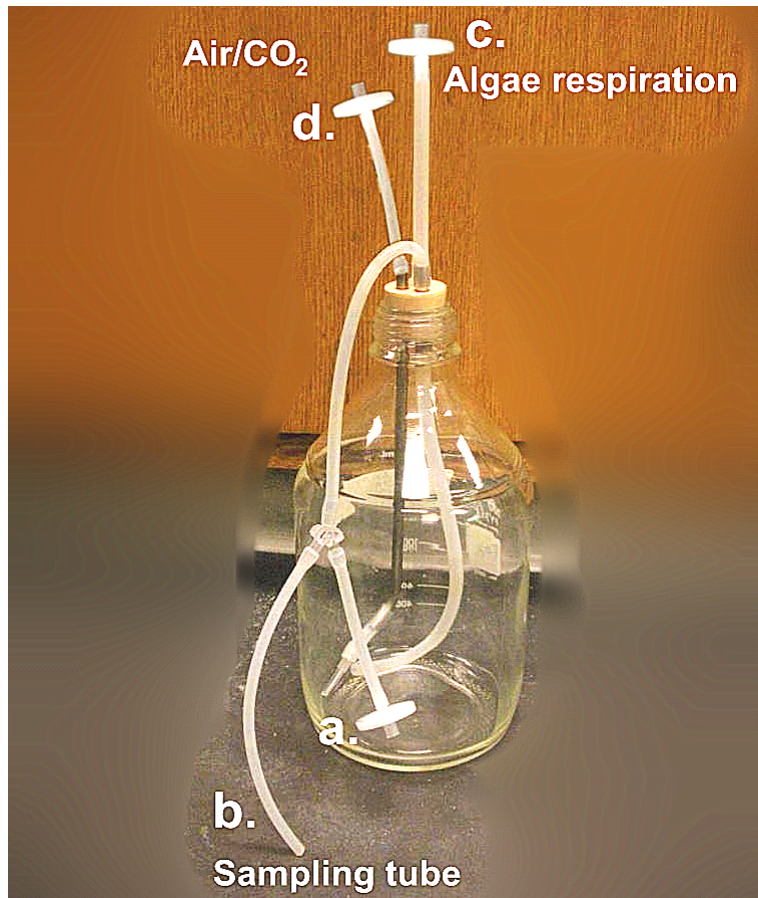
One drop of cell suspension was put on the grid of the hemacytometer, covered with a thin glass cover. The suspension volume above the central square, under the glass cover was 0.1 $\mu$ L. Cell numbers inside the central square could be precisely counted under a microscope. The cell density could then be calculated by cells per liter.

## **2.8 Sampling system on bioreactor**

Algae culture has to be maintained in a sterile environment, since bacteria and other organisms compete with algae for nutrition in the media. Plus, the activities of bacteria may affect the algae sedimentation process in the settler. The inoculation process from the algae tube to the seed jar was strictly controlled in an aseptic environment, and then the algae culture was maintained in a sterile closed system. In order to track the growth of algae, the culture was sampled from the closed system once per day. During the sampling process, a high risk was induced to the algae cultures in contamination, since the closed system was opened and connected to the outside non-sterile environment to take a sample. A sampling system to prevent contamination was operated. The schematic and depiction of the system are shown in Fig 2.7 and Fig 2.8 as follows.



**Fig 2.7** Schematic of the sampling system that prevents the culture from being contaminated during the sampling process



**Fig 2.8** Actual looking of the sampling system

The algae culture was sparged with 5% CO<sub>2</sub> in air at a total flow rate of 0.1 LPM through a 0.2 µm sterile syringe filter placed on port d. Respiratory gas from algae metabolism and unused CO<sub>2</sub> and air mixture were released through a same size filter on port c. Most bacteria and other contaminants larger than 0.2 µm in diameter were blocked outside of the system by these filters while the necessary gas transportation was guaranteed.

Before sampling, ports a and c were sealed to accumulate enough air pressure in order to press algae suspension out through sampling port b. After adequate algae suspension was collected in a sampling tube, port b could be closed right away to stop the suspension flow and port c was quickly opened to release the high air pressure inside the jar. For the fluid retained in the sampling port, a syringe was applied and connected to port a to return the top half of the fluid back to the jar, and the bottom half to the sampling tube. It is necessary to accomplish this step, since the fluid retained in the line connected to port b, connected to the algae suspension is non-sterile. At this point, the sampling process was accomplished and the algae culture was isolated from the outside non-sterile environment by filtered air.

## 2.9 Gravity Settler

The settler was configured as a downward flow inclined rectangular panel used to remove water and concentrate the algae culture (Fig1.6).

Two settlers different in size, but identical were manufactured and operated to compare the output capacity. Both have the inclination angle  $\theta$  of 55 degrees. The dimensions for the large settler were 60 cm in length, 9.5 cm in width, 1 cm in depth, while the smaller one was 59 cm in length, 4.5 cm in width and 1cm in depth. The larger settler had a surface area of 570 cm<sup>2</sup>, while the smaller one had surface area of 265 cm<sup>2</sup>. Both were manufactured of polycarbonate (Size: 3/8 inches, 1/4 inches), a material which was tough (almost unbreakable), autoclavable, transparent as glass, and much lighter when compared to glass and metal. The adhesive used during manufacturing was Max bond epoxy, the low viscosity type (Polymer Composites, Inc., Los Angeles, California).

Three inlets at the top of the settler were used to feed cell suspensions. A vent located close to these inlets was used to adjust the filling liquid level. Two outlets were located at the bottom of the settler to converge the concentrated and diluted flows, respectively.

The gravity settler was autoclaved before each experiment, in order to provide a sterile environment during the dewatering process.

For each experiment, the same filling level to the very top of the settler was maintained, since it determined the settling surface.

The gravity settler was set up in a system as shown in Fig 2.9. The bioreactor was fed with a mix of 5% CO<sub>2</sub> and 95% of air at a total flowrate of 0.1LPM for the respiration and the carbon intake of algae. Fresh 3N-BBM media was added daily into the bioreactor, necessary to compensate for evaporation.

The upper inlets of the gravity settler were connected to the bioreactor. One lower outlet for concentrated flow and the other outlet for diluted flow were both filled back to the bioreactor. Two pumps separately controlled flow rates of two outlets. There were a total of three sampling ports in this system. One port was connected to the bioreactor in order to monitor the inlet flow concentration. The other two ports were connected to two outlets.

Both the system and modified testing system were closed and sterile. 5% CO<sub>2</sub> in air fed into the bioreactor was filtered by 0.2 µm filter (Cole Parmer, syringe filter, 0.2µm). The sampling method was the same as used for cell culture, which effectively excluded contamination from air during the sampling process.

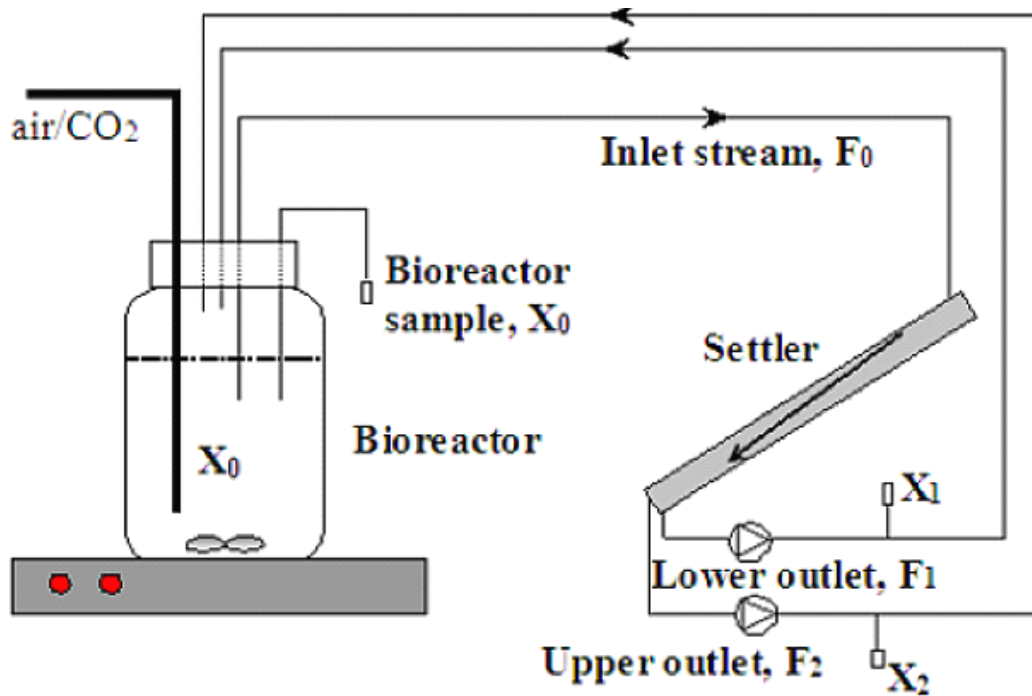


Fig 2.9 System schematic for laboratory tests

There were two experiments operated with *C. Vulgaris* cells, both were run in the 2.5L bioreactor which connected to the small gravity settler. Each experiment included three trials, which were conducted with one parameter changed at three levels. In the first experiment, the flowrate ratio between the concentrated outlet and the diluted stream of the gravity settler was maintained constantly at 1:9, while the total inlet flowrate varied at 2.22mL/min, 7.78mL/min and 13.33mL/min. In the second experiment, the total inlet flowrate of the gravity settler were 7.78mL/min, 7.81mL/min and 7.82mL/min for three trials. It was still considered as a constant, since the difference was induced by the error of the equipment. The flowrate ratio between the concentrated stream and the diluted stream was changed in three trials, which were 1:4, 1:9, and 1:18.



Four experiments were operated with *S. Dimorphus* cells. The first and second were run in the 2.5L bioreactor and the small gravity settler. The third and the fourth were run in the 5L bioreactor and the big gravity settler. Each experiment had three trials setup strictly at the same condition, with only one parameter changed. In the first experiment, the outlet flowrate ratio was 1:9, and the inlet flowrate changed in three different trials were 2.28mL/min, 7.78mL/min and 13.33mL/min. The second experiment was set with the same equipment parameters as the first one, the only difference was the higher biomass concentration of the cell suspension. Usually the biomass concentration of the cell suspension used in the experiment was the maximum what cells can reach at their growth, the one used in the second experiment was condensed as five times as the regular, in order to mimic the condition of the second stage of a two-stage settler. The third experiment was set of the outlet flowrate ratio at 1:9, and the changed inlet flowrate at 4.47mL/min, 16.44mL/min and 28.66mL/min for three trials. The fourth experiment had inlet flowrate fixed at 20mL/min, and the changed outlet flowrate ratio at 2:20, 3:20 and 4:20.

For each trial, when the gravity settler was originally set up, samples were taken every 6 hours during operation. The mass balance of the settler was monitored by calculating the cell density of the inlet flow and the two outlet flows. Once mass balance in the gravity settler was verified for three

consecutive samples, it was considered that the system had reached the steady state. Samples were taken once per day; data collection then lasted three to five days, depending on the trial. The concentration ratio and recovery rate were calculated from the measured absorbance from each sampling port.

## **2.10 Bioreactor**

A 2.5L (B. Braun biotech, Allentown, PA) bioreactor with 1L working volume and a 7.5L (New Brunswick Scientific, Edison, NJ) bioreactor with 5L working volume were used for the small and large scale tests. Both bioreactors were autoclaved before each operation. The 3N-BBM media was autoclaved in bottles and then transferred into the bioreactors by filtered sterile air. Cell culture in the bioreactor was agitated at speed of 130 rpm. Illumination was provided by six 14-watt, 24" fluorescent tubes (four Coralite Aquapro T-5/10,000K and three Accupro AFL/F14T5/14W/830) on a 12 hours on/12 hours off cycle, with a measured intensity of 515-550 ft-candles at the liquid surface. The 2.5L small bioreactor was used with the small scale gravity settler for both *S. Dimorphus* and *C. Vulgaris* cells; the 5L bioreactor was only operated with *S. Dimorphus*.

## 2.11 Lipid content estimation

After the completion of each dewatering experiment, samples were taken from the bioreactor for lipid content estimation. Samples were dried in an oven overnight at 50°C.

The sample weight was tracked until the it was constant, which indicated the sample was fully dried. Each sample was then placed in glass tubes for further extraction. An empty glass tube was weighed, and the weight was recorded as W1. Then the dried algae samples were ground with mortar and pestle, placed into the same glass tube, weighed as W2. Furthermore, 10mL of hexane and isopropanol solvents (volume ratio at 3:2) was added into the glass tube to extract oil from the dried algae flakes. The glass tube was placed on a shaker and left running at the speed of 200 RPM overnight (around 18~24 hours) to have the algae lipid fully dissolved in the solvent. The algae flakes settled down after the shaker was stopped. The top supernatant was removed into another empty glass tube called lipid tube (weight W3) and then dried for two to five days until the tube weight did not change anymore. The final weight of lipid tube and lipids inside of the tube was weighed as W4. The lipid content level was calculated as below:

$$\text{Weight of Biomass} = W2 - W1 \quad 2.1$$

$$\text{Weight of lipid} = W4 - W3 \quad 2.2$$

$$\text{Lipid content level} = [(W4-W3) / (W2-W1)] * 100\%$$

2.3

## **2.12 Theory**

### **2.12.1 Principles of Settler Design and Operation**

The cell separation was realized by having the algae cells settled on the lower surface of the settler, where algae cells formed a thin sediment layer that slides down to be collected at the bottom of the settler. The kinematic theory for inclined sedimentation was developed about eighty years ago (Ponder., 1925; Nakamura., 1937).

In Batt's research in 1990, it was indicated the inlet flowrate could be represented by equation 2.4, when the settler was operated in an ideal condition and cells were completely separated from media. It showed the inlet flowrate  $S$  is equal to the vertical settling velocity of the particles multiplied by the horizontal projected area of the channel surface available for sedimentation. (Batt. et al., 1990; Davis. et al., 1991):

$$S = vw(L\sin\theta - b\cos\theta)$$

2.4

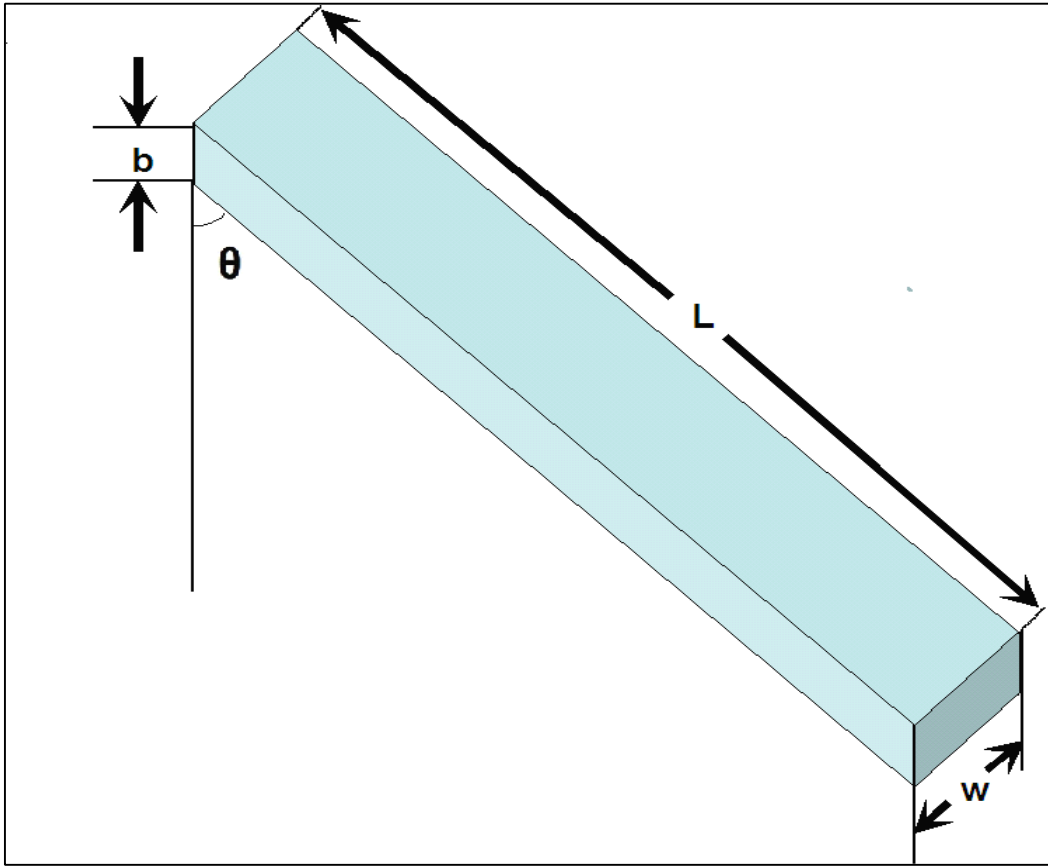


Fig 2.10 3D view of the gravity settler

Where  $S$  is the inlet flowrate of the suspension need to be dewatered by the inclined settler,  $v$  is the cell settling velocity,  $w, L, b$  are the width, length and depth of the settler respectively.  $\theta$  is the angle between the longitudinal axis of the gravity settler and the vertical(Fig 2.10). The quantity  $w(L \sin \theta - b \cos \theta)$  is the projected area of the inclined gravity settler.

Considering the settler used in this thesis, which had  $L=59$  cm,  $d=1$ cm, it can be seen that the  $L \gg b$ . The equation 2.4 could be simplified to equation 2.5(Batt et al., 1990; Davis et al., 1991; Searles et al., 1994):

$$S = v \cdot wL \sin \theta \quad 2.5$$

The cell settling velocity,  $v$ , can be theoretically calculated by Stoke's

law when Reynold's number is less than 0.2 as follows:

$$v = \frac{gd_p^2(\rho_p - \rho)}{18\mu} \times 100\% \quad 2.6$$

Where  $d_p$  =particle diameter,  $\mu$ =fluid dynamic viscosity,  $\rho_p$ = density of the solid particle,  $\rho$ = density of the carrying fluid, and  $g$  is the gravity acceleration constant. In other words, the cell settling velocity "v" (Equation 2.6) is determined by inherent parameters of both the algae-cell-particle and media carrier. In an actual lab experiment, as long as the algae species and media were not changed, the cell settling velocity "v" was a constant according to equation 2.6.

In that case, the volumetric production rate of the settler is linearly related to the projected area of the gravity settler with a slope of  $v$  (Equation 2.5). A larger projected area can generate a higher volumetric production rate  $S$ , since  $v$  is a constant.

During the settling, algae cells need to slide down to the outlet of gravity settler, instead of attaching to the lower surface and accumulating. In order to maintain the basic function of the settler, the inclination angle  $\theta$ , therefore, has an upper limit. From previously published work, the settlers were tested with inclination angles of 25 or 30° by Batt et al. (1990) and Davis et al. (1991). As well as, the accumulation of mammalian cells was observed to be virtually eliminated at the angle of 55°. Therefore, the

inclination angle of 55° was used in this thesis.

### 2.12.2 Calculations and functional parameters.

Mainly, there are two parameters used to evaluate the performance of the gravity settler: concentration rate and recovery rate.

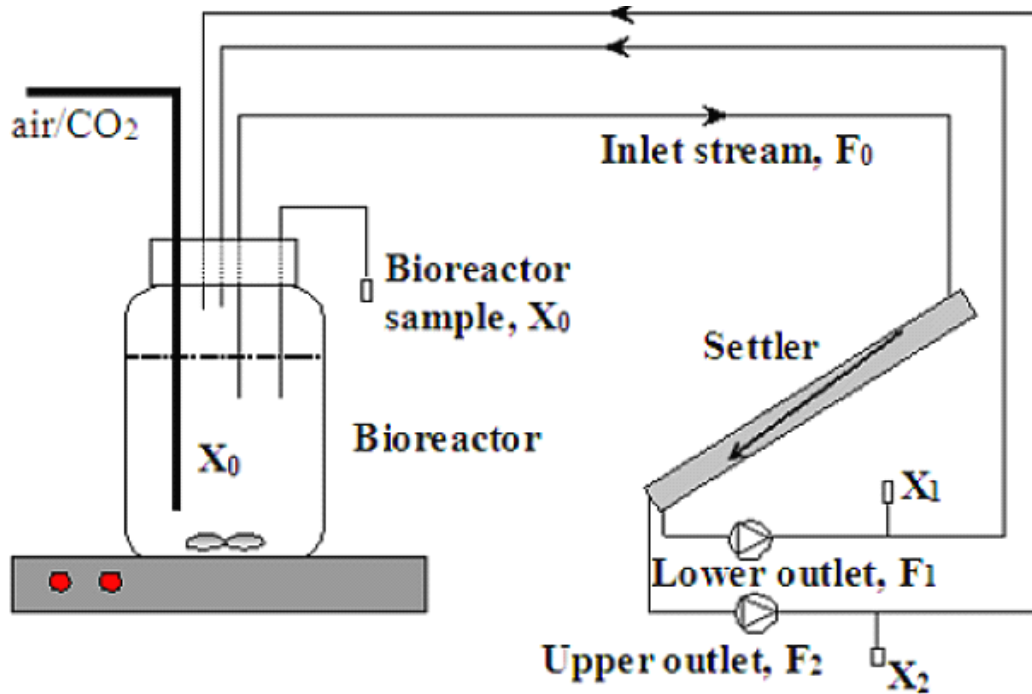


Fig 2.11 System schematic for lab tests

#### 2.12.2.1 Concentration ratio

The concentration ratio,  $C$ , is defined as (Fig 2.11):

$$C = \frac{X_1}{X_0} = \frac{0.62 \times A_1}{0.62 \times A_0} = \frac{A_1}{A_0} \quad 2.7$$

where  $X_1$  is the concentration of the biomass from the concentrated stream, while  $X_0$  is the concentration of the biomass in the bioreactor. Algae

biomass concentration is linearly proportional to  $A_{600}$ , with slope of 0.62 gdw/L  $A_{600}^{-1}$  (Kanani., 2012). Equation 2.7 can be simplified by using  $A_1$  and  $A_0$ , where  $A_1$  is the absorbance of biomass concentration from the concentrated stream; and  $A_0$  is corresponding to the biomass concentration in the bioreactor.

This parameter  $C$  is used to quantitatively show how dense the algae suspension is after dewatering, compared to the algae suspension that was in the bioreactor.

#### 2.12.2.2 Flowrate ratio

The flowrate ratio  $F$ , is defined as:

$$F = \frac{F_1}{F_0} \tag{2.8}$$

where  $F_1$  is the flow rate of the concentrated stream;  $F_0$  is the inlet flowrate to the settler.

#### 2.12.2.3 Recovery rate

The biomass recovery rate,  $R$ , is defined as:

$$R = C \times F = \frac{A_1 \times F_1}{A_0 \times F_0} \tag{2.9}$$

where  $R$  indicates how much algae cells could be recovered compared to the total amount of the input algae cells.



In industrial application, recovery rate is also an important parameter used to direct the design of the settler. Based on requirements of how concentrated the cell suspension should be after dewatering and how much algae suspension should be dewatered per unit time,  $R$  could be used to determine the inlet flowrate should be applied to the settler and the dimensional parameters of the settler, such as the width, length and depth.

In the ideal condition, if the cells were all separated from media and recovered at the bottom of the settler, the  $R_{\text{ideal}} = 1$ . However, due to other uncontrollable factors during actual experimentations, this is not usually the case.

The theoretical inlet flowrate  $S$  indicates the volumetric rate goes into the system when algae were ideally 100% dewatered.

In other words, if the  $F_0$  in equation 2.9 was substituted by  $S$ , the equation equals to 1:

$$R = C \times F = C \times \frac{F_1}{F_0} = C \times \frac{F_1}{S} = 1 \quad 2.10$$

$$\Rightarrow F_1 = \frac{S}{C} \quad 2.11$$

However, the actual inlet flowrate  $F_0$  is always greater than  $S$ , which made the  $R$  never as high as 100%. If the  $F_0$  in equation 2.9 is substituted by equation 2.11, a relation between the recovery  $R$ , ideal inlet flowrate  $S$  and actual inlet flowrate  $F_0$  reveals as follows:

$$R = C \times \frac{F_1}{F_0} = C \times \frac{1}{F_0} \times \frac{S}{C} = \frac{S}{F_0}$$

2.12

## CHAPTER III

### RESULTS AND DISCUSSION

#### 3.1 Linear relationship between the cell count and absorbance $A_{600}$

The data set in table 3.1 included the cell density ( $10^6$  cells/mL) and the absorbance  $A_{600}$ . The cell density was acquired by counting cells on a hemacytometer. A Pearson correlation was computed by SPSS (Version.PASW statistic 18.0) between these two variable, a statistically significant result showed there was a strong positive relationship between  $A_{600}$  and cell density ( $R=0.993$ ,  $P<0.01$ ), with the coefficient at  $19.195 \cdot 10^5$  cells/mL  $\cdot A_{600}$ .

Based on this relationship, the cell suspension density could be estimated via  $A_{600}$ .

Table 3.1 Absorbance  $A_{600}$  Versus Cell density

$A_{600}$	1.326	0.442	0.164	0.633	0.336	0.179	0.101
$10^6$ cells/mL	24.75	10.55	2.95	12.6	5.8	3.4	1.85

Table 3.2 Correlation of Absorbance A<sub>600</sub> and Cell density

		Absorbance A <sub>600</sub>	Cell density
Absorbance A <sub>600</sub>	Pearson Correlation	1	.993**
	Sig. (2-tailed)		.000
	N	7	7
Cell density	Pearson Correlation	.993**	1
	Sig. (2-tailed)	.000	
	N	7	7

\*\* . Correlation is significant at the 0.01 level (2-tailed).

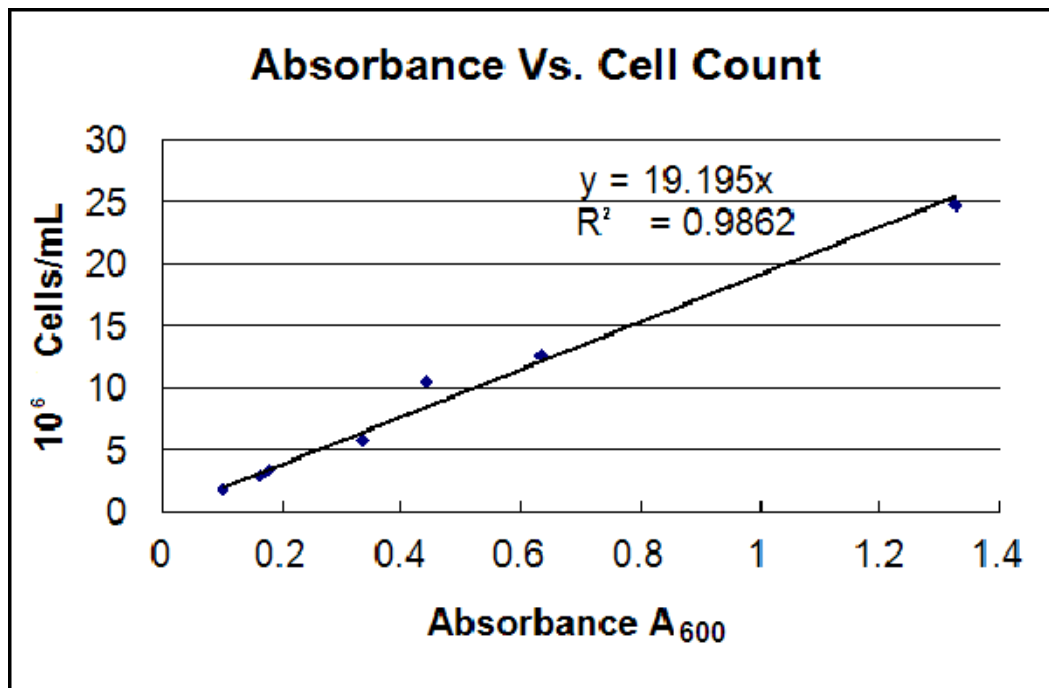


Fig 3.1 Absorbance A<sub>600</sub> vs. Cell density

### 3.2 Lipid content comparison between *S. Dimorphus* cells and *C.*

#### *Vulgaris* cells

Oil extraction was performed on three different batches of algae culture, *Scenedesmus dimorphus* cells from a 2.5L bioreactor,

*Scenedesmus dimorphus* cells from a 5L bioreactor and *Chlorella vulgaris* cells from a 2.5L bioreactor. All cultures were sampled after they reached the stationary phase. The lipid extraction followed protocols described in section 2.11.

Table 3.3 below shows values from each lipid extraction step. Since the dried biomass was hand ground in a mortar, there was a biomass weight loss during the grinding and the transfer from the mortar to the lipid extraction tube. The recovery rate showed that less than 90% of the biomass went into the next extraction process. To reduce errors from this step, the calculation of the lipid content was based on the extracted oil weight and the weight of the ground biomass.

Table 3.3 Lipid content extraction for *Scenedesmus dimorphus* and *Chlorella vulgaris* cells

	Dried Biomass(g)	Ground Biomass(g)	Recovery Rate	Lipid (g)	Lipid content= (oil/Biomass)*100%
SD 2.5L <sup>a</sup>	0.104	0.091	87.2%	0.008	8.8%
SD 5L <sup>b</sup>	0.137	0.117	85.3%	0.010	8.6%
CV 2.5L <sup>c</sup>	0.155	0.134	86.5%	0.009	6.7%

a. *Scenedesmus dimorphus* cells from the 2.5L bioreactor

b. *Scenedesmus dimorphus* cells from the 5L bioreactor

c. *Chlorella vulgaris* cells from the 2.5L bioreactor

Hand grinding only roughly broke the dried biomass down to small pieces, which may have prevented the complete extraction from the biomass. The measured lipid content values (8% for *S. Dimorphus* cells, 6% for *C. vulgaris* cells) were much lower compared to those reported previously by (Gong et al., 2011; Feng et al., 2011; Liu et al., 2008). The

lipid content in *S. Dimorphus* cells was reported from 16% to 40%, and 14%-30% in *C. vulgaris* cells. Basically, the *S. Dimorphus* cells have 30% higher lipid content, compared with *C. vulgaris* cells. Though the grinding process was limited, the result revealed that the oil content in *S. Dimorphus* cells was 30% higher than that of *C. vulgaris* cells (Fig 3.2), which coincided with previous studies (Gong et al., 2011; Feng et al., 2011; Liu et al., 2008).

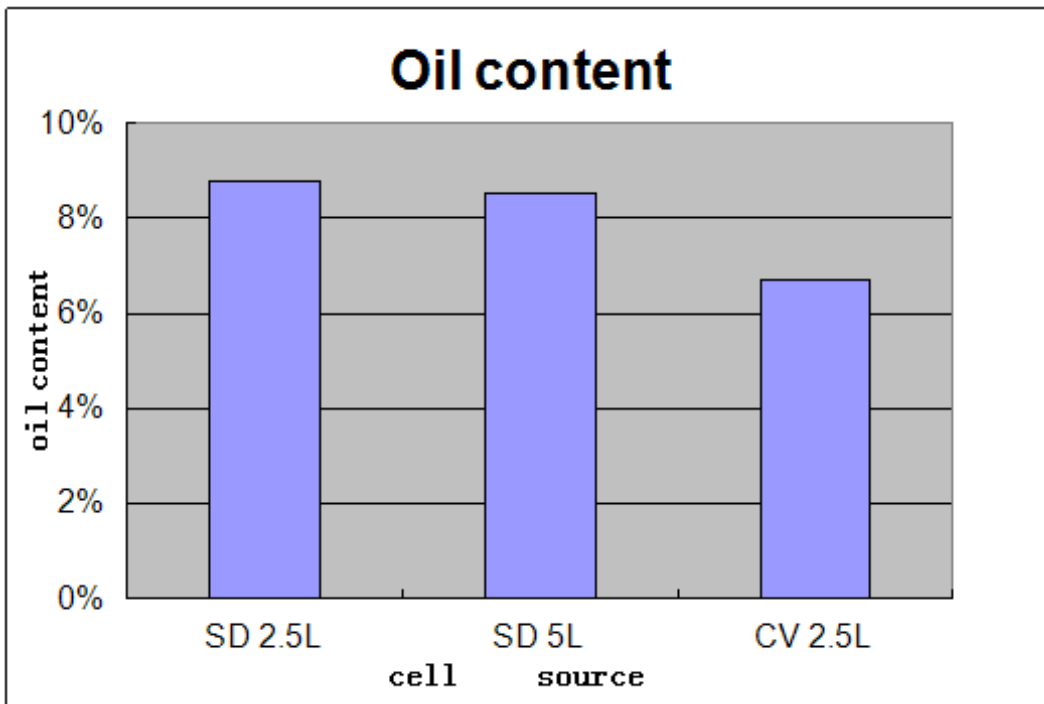


Fig 3.2 Oil content measurement for different samples

### 3.3 Dewatering performance comparison of two algae strains

The gravity settler dewatering performances for different algae cells were explored. These trials were carried with *Scenedesmus dimorphus* and *Chlorella vulgaris* cells in a 2.5 L bioreactor to research whether/how

the oil content, morphology, and size differences of the two species, would affect the settler's performance.

Fig 3.3 shows the recovery rate and concentration ratio with *Chlorella vulgaris* cells. Fig 3.4 displays the results for the same parameters but with *Scenedesmus dimorphus* cells. The data from Figs 3.3 and 3.4 are summarized in Figs 3.5 and 3.6, which the comparison of the settler performances with the two cell strains. P-values were calculated between the points that were at the same inlet flowrate.

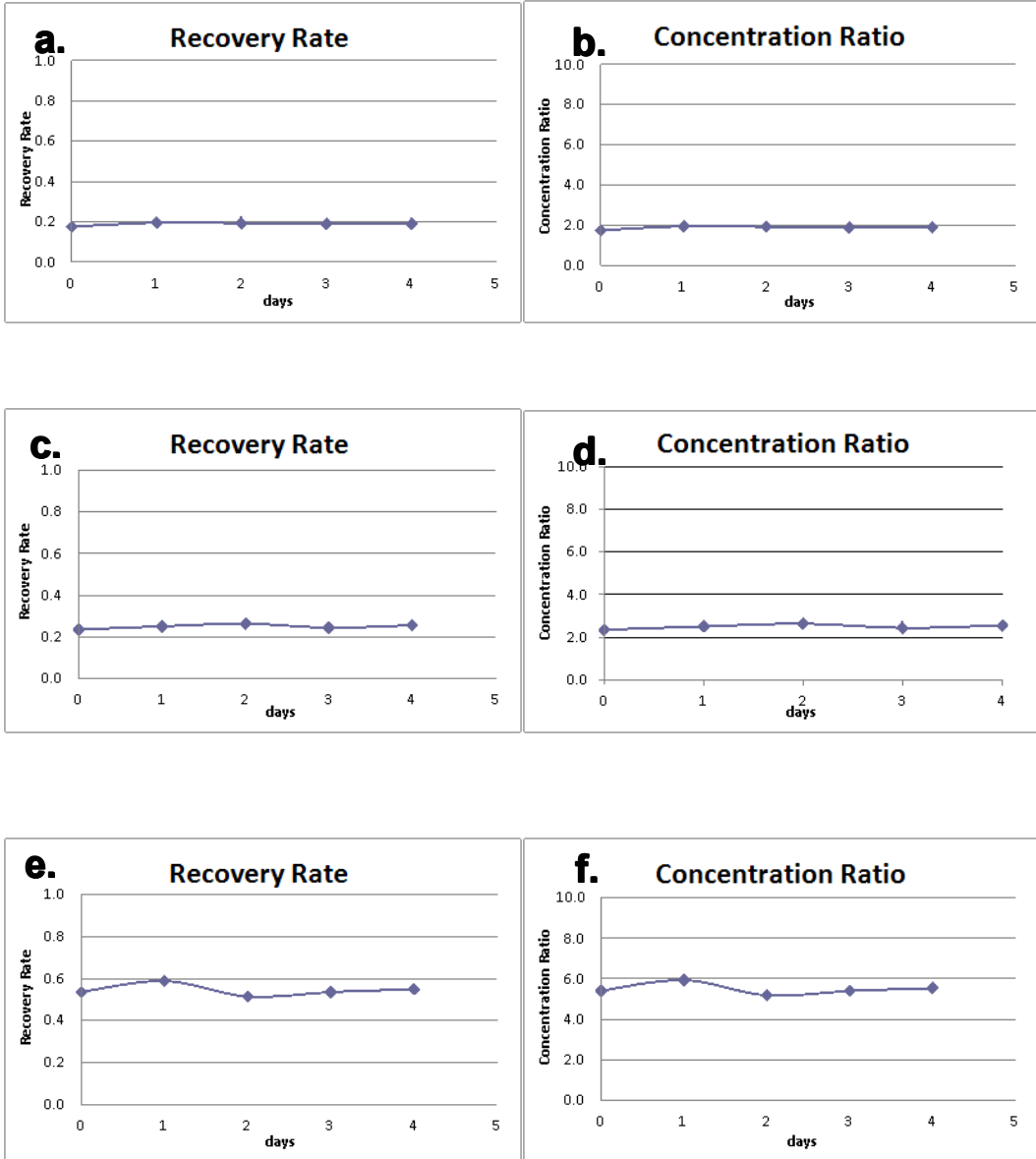


Fig 3.3 Recovery rate and concentration ratio reported from *Chlorella vulgaris* batch in 2.5L bioreactor with the small settler of a flowrate ratio  $F=1:9$ , time=0 is considered the start of steady state. a.b. Inlet flowrate=13.33 mL/min; c.d. Inlet flowrate=7.78mL/min; e.f. Inlet flowrate=2.22ml/L.



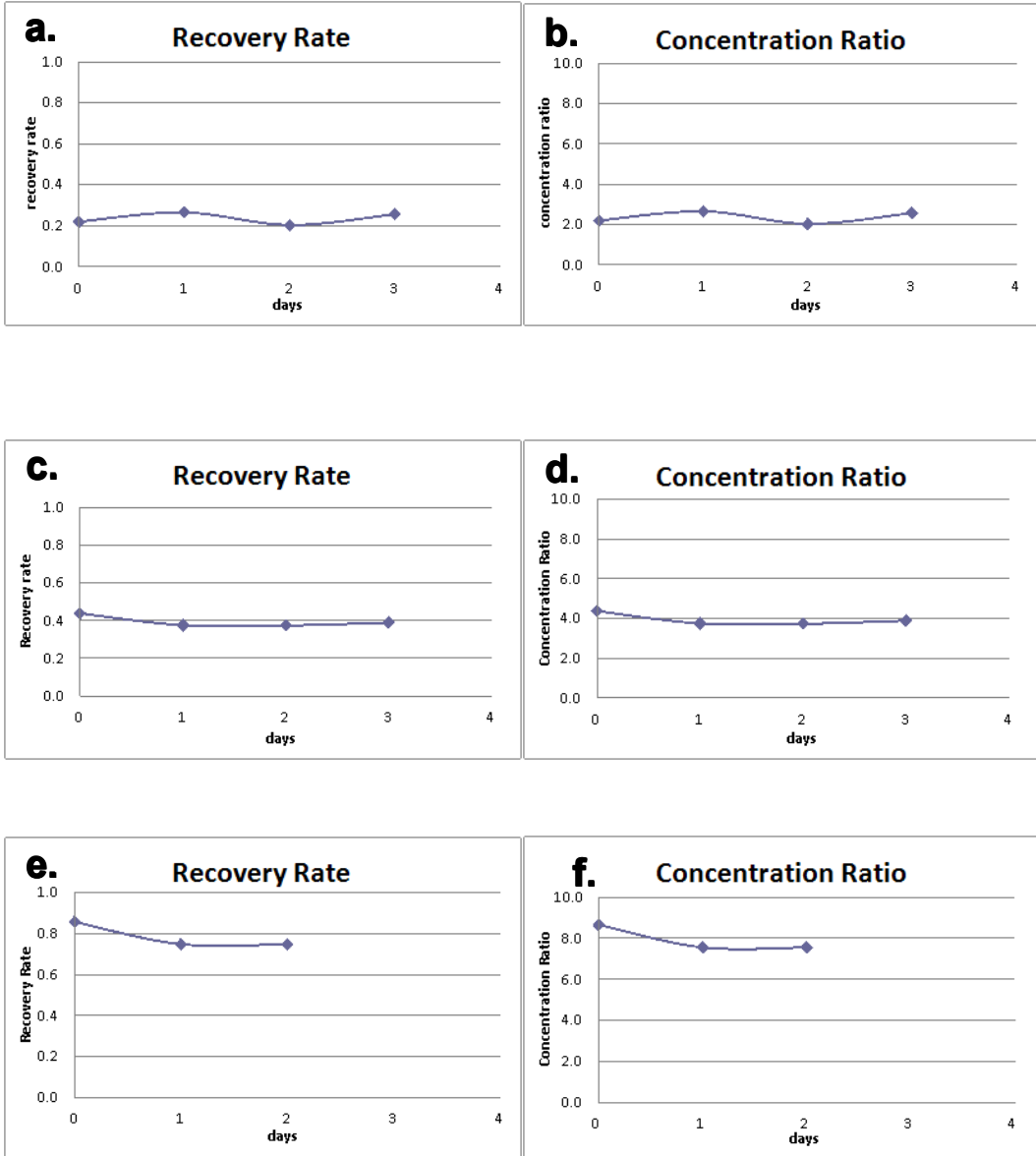


Fig 3.4 Recovery rate and concentration ratio reported from *Scenedesmus dimorphus* batch in 2.5L bioreactor with the small settler of a flowrate ratio  $F=1:9$ , time=0 is considered the start of steady state. a.b. Inlet flowrate=13.33 mL/min; c.d. Inlet flowrate=7.78mL/min; e.f. Inlet flowrate=2.22ml/L.

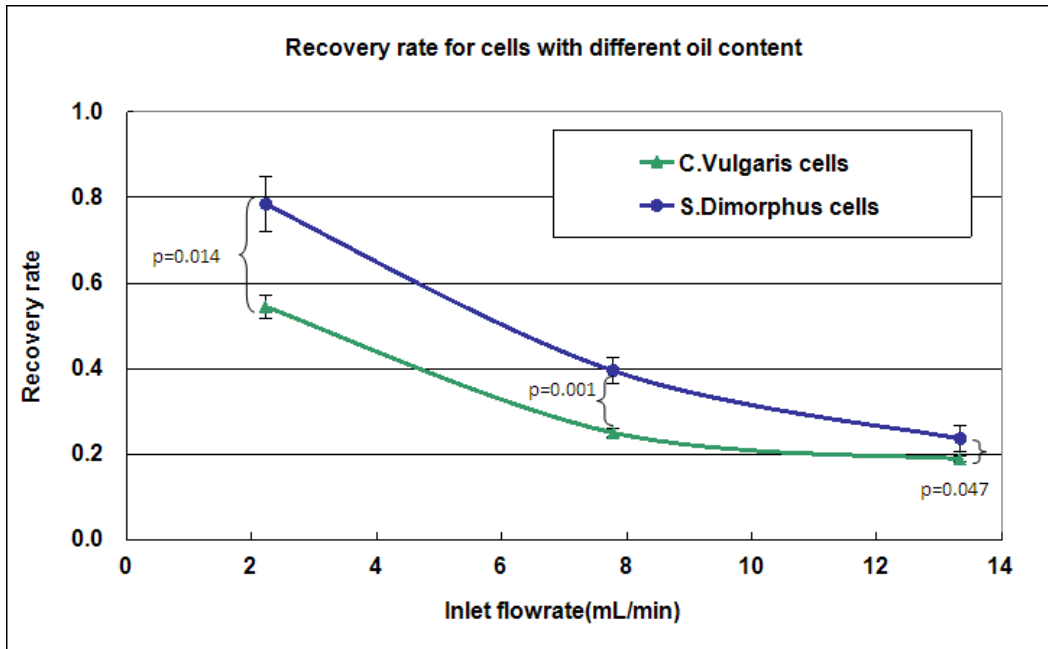


Fig 3.5 Recovery rate Vs. two different algae species. (Error bars are standard deviations)  $n=5$  for *C. Vulgaris*;  $n=3-4$  for *S. Dimorphus*. Systems were operated with a 2.5 L bioreactor, connected to the smaller settler, with the outlet flowrate ratio=1:9.

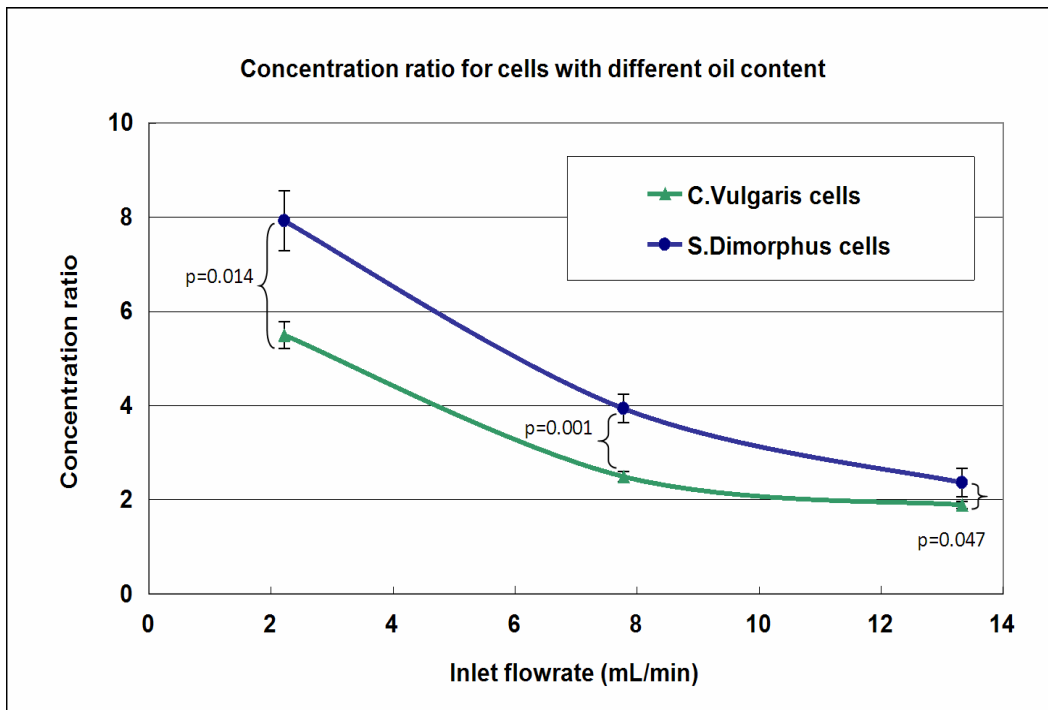


Fig 3.6 Concentration ratio Vs. two different algae species. (Error bars are standard deviations)  $n=5$  for *C. Vulgaris*;  $n=3-4$  for *S. Dimorphus*. Systems were operated with a 2.5 L bioreactor, connected to the smaller settler, with the outlet flowrate ratio=1:9.

These figures reveal that there is a significant difference in settler performance for *Scenedesmus Dimorphus* cells and *Chlorella Vulgaris* ( $P < 0.05$ ). The differences became smaller for higher inlet flowrates.

This trial was designed to test the settler's performance for cells with different morphology, size, and oil content.

Recalling equation 2.5, 2.6:

$$S = v \cdot wL \sin \theta \quad 2.5$$

$$v = \frac{gd_p^2(\rho_p - \rho)}{18\mu} \quad 2.6$$

If  $v$  from equation 2.5 is substituted by equation 2.6, it yields:

$$S = wL \sin \theta \cdot \frac{gd_p^2(\rho_p - \rho)}{18\mu} \quad 3.1$$

Recalling the equation 2.12 in chapter II:

$$R = \frac{S}{F_o} \quad 2.12$$

The two equations 3.1 and 2.10 could be rearranged as:

$$R = \frac{gd_p^2(\rho_p - \rho)wL \sin \theta}{18\mu \cdot F_o} \quad 3.2$$

For the two gravity tests with *Scenedesmus dimorphus* cells and *Chlorella vulgaris* cells, the only parameters changed were  $\rho_p$ , the density of the algae cell, and  $d_p$  the diameter of the algae cell. Here,  $\rho_p$  is the parameter related to oil content of cells. The cells that have higher oil content have lower density, since oil is lighter compared to water.

Therefore, if the two algae strains were similar in cell diameter, the strain with a lower density and higher oil content would have a lower Recovery rate. However, the result in this test that *Scenedesmus dimorphus* cells had a higher recovery rate with high oil content, which conflicted to the assumption above.

It was noticed that the *Scenedesmus dimorphus* cells are oblong shaped, the length of each *Scenedesmus dimorphus* cell was 10~20  $\mu\text{m}$ , while *Chlorella vulgaris* cells were round and 2~10  $\mu\text{m}$  in diameter. *Scenedesmus dimorphus* cells existed in clusters with two cells, four cells, or even eight cells, which increased the particle diameter by eight times, whereas the *Chlorella vulgaris* cells exist mostly individually. The impact from the diameter difference to the recovery rate became even more significant since it was the second power of the diameter represented in equation 3.2.

$$R = \frac{gd_p^2(\rho_p - \rho)L \sin \theta}{18\mu \cdot F_0} \quad 3.2$$

In summary, this test was designed to explore the influence from different cell oil content levels on the performance of a gravity settler. Whereas, it was found that the cell diameter had a stronger impact on the performance.

### 3.4 Effect of the inlet flowrate on the performance of a gravity settler

The data set used in fig3.3 and fig 3.4 could be used to analyze the effects of inlet flowrate on the performance of a gravity settler.

In the last section, data were compared vertically to see which group had higher recovery rate at each inlet flowrate point. In this section, data will be analyzed horizontally to see within each group, how the inlet flowrate affects the settler performance.

Recalling the equation 3.2, it was displayed that R would be linear to  $1/F_0$  within one group if the equation 2.12 was valid.

$$R = \frac{S}{F_0} \quad 2.12$$

Which yields

$$R = \frac{gd_p^2(\rho_p - \rho)wL \sin\theta}{18\mu \cdot F_0} \quad 3.2$$

eg. For the group of *S. Dimorphus* cells, the diameter of cells  $d_p$ , the fluid viscosity  $\mu$ , the density of the algae cell particle  $\rho_p$ , the density of the carrying fluid  $\rho$ , gravity acceleration constant  $g$ ,  $\theta$ ,  $w$  and  $L$  which were fixed values as long as the gravity settler was not changed. All these parameters did not change when the inlet flowrate was changed from 2.22mL/min to 13.33mL/min. According to equation 3.2, the recovery rate should correlate linearly with the reciprocal of the inlet flowrate.

A linear regression was calculated by SPSS (PASW statistic 18.0) between the recovery rate and reciprocal of the inlet flowrate (Table 3.4 and 3.5) for both *S. Dimorphus* cells and *C. Vulgaris* cells, the result showed a statistically significant, strong and positive linear relationship between the recovery rate and the reciprocal of the inlet flowrate ( $P < 0.01$ ).

The slope of the linear line generated from the *S. Dimorphus* cell data set was  $1.88 \pm 0.14$  mL/min, which was higher than  $1.30 \pm 0.08$  mL/min, from the *C. Vulgaris* cell data set. The slope differences were mainly caused by the cell particle diameter differences from the two algae strains, as discussed in last section.

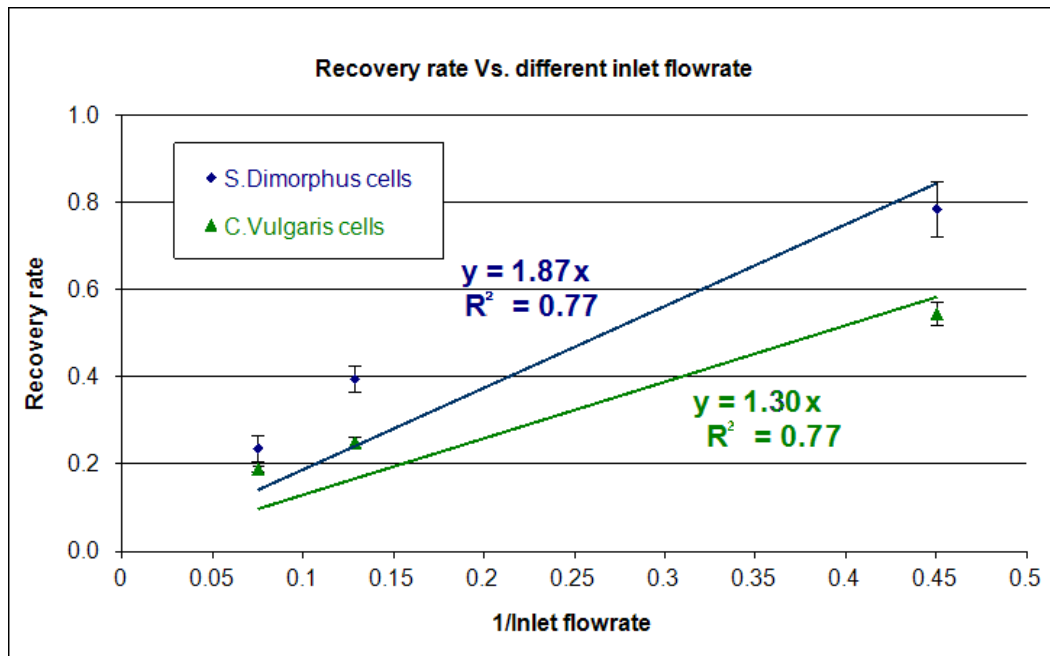


Fig 3.7 Recovery rate verse 1/inlet flowrate (Error bars are standard deviations). Each data point  $n=5$  for *C. Vulgaris*;  $n=3-4$  for *S. Dimorphus*. Systems were operated with a 2.5 L bioreactor, connected to the smaller settler, with the outlet flowrate ratio=1:9.

Table 3.4 Linear regression between R and 1/F<sub>0</sub> (SD cells) n=11

Coefficients <sup>a,b</sup>						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	SD cells	1.875	.143	.973	13.374	.000
a. Dependent Variable: Recovery rate of SD cells						
b. Linear Regression through the Origin						

Table 3.5 Linear regression between R and 1/F<sub>0</sub> (CV cells) n=15

Coefficients <sup>a,b</sup>						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	CV cells	1.296	.075	.978	17.353	.000
a. Dependent Variable: Recovery rate of CV cells						
b. Linear Regression through the Origin						

According to equation 2.8, 2.9, 2.12 and 3.2, there should be a relation between concentration ratio and recovery rates if the equation 2.12 was valid:

$$R = \frac{A_1 \cdot F_1}{A_0 \cdot F_0} = C \cdot F = C \cdot \frac{F_1}{F_0} = \frac{S}{F_0} \quad 3.3$$

$$C = \frac{S}{F_1} \quad 3.4$$

S was determined by inherent parameters of the algae cells (cell size and density of the cell particle), of the media (density of the carrying fluid) and the dimensions of the gravity settler (width, length and inclination angle), which were constants in one experiment within one cell strain. The S was

therefore a constant in this case, and the concentration ratio should be linear with  $1/F_1$  according to equation 3.4, if the equation 2.12 was valid.

A statistical regression analysis was conducted between the concentration ratio and the reciprocal of the lower outlet flowrate (Table 3.5 and 3.6). A linear relationship in between was found statistically significant ( $P < 0.01$ ) in both the *S. Dimorphus* cell dataset and the *C. Vulgaris* cell dataset (Fig 3.8).

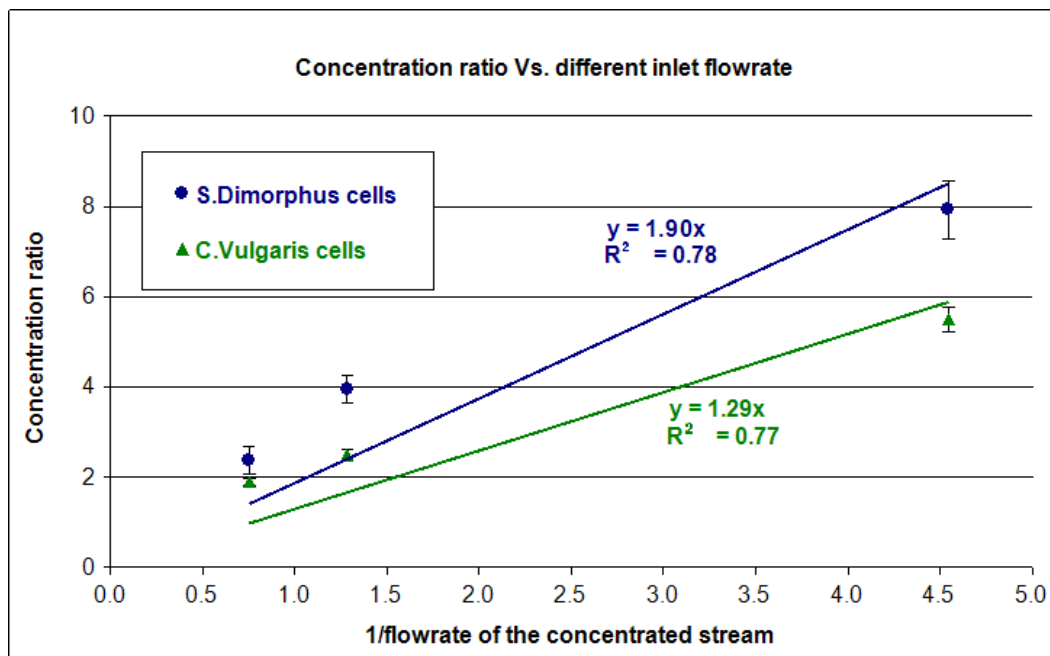


Fig 3.8 concentration ratio verse the  $1/\text{flowrate}$  of the concentrated stream (Error bars are standard deviations).  $n=5$  for *C. Vulgaris*;  $n=3-4$  for *S. Dimorphus*. Systems were operated with a 2.5 L bioreactor, connected to the smaller settler, with the outlet flowrate ratio=1:9.



Table 3.5 Linear regression between C and  $1/F_1$  (SD cells) n=11

<b>Coefficients<sup>a,b</sup></b>						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	SD cells	1.900	.147	.972	12.961	.000
a. Dependent Variable: Concentration ratio of SD cells						
b. Linear Regression through the Origin						

Table 3.6 Linear regression between c and  $1/F_1$  (CV cells) n=15

<b>Coefficients<sup>a,b</sup></b>						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	CV cells	1.294	.074	.978	17.453	.000
a. Dependent Variable: Concentration ratio of CV cells						
b. Linear Regression through the Origin						

As a summary, the recovery rate was found linearly related to the inverse of the inlet flowrate, and the concentration ratio was linearly related to the inverse of the flowrate of the concentrated stream, when the S was a constant, as predicted by equation 2.12. The recovery rate was an important parameter to evaluate the dewatering ability of the gravity settler. A high recovery rate represented a high percentage of the cells separated from the media.

For industrial applications of this system, a trade off should be made between the recovery rate and the inlet flowrate to achieve the optimal

productivity, considering the equation 2.12 stated the relation between recovery rate and inlet flowrate.

This equation 2.12 also helped to obtain the concentrated stream of a certain concentration, which can meet a specified requirement for industrial production. At first, the settler system should be recorded of different concentration ratios when the system was tested at different outlet flowrates. Second, the appropriate outlet flowrate could then be calculated and setup to produce the right concentrated flow, considering the linear relationship between concentration ratio and outlet flowrate.

### **3.5 Effects of the flowrate ratio on the performance of the gravity settler**

This part included two experiments, aiming to test how the flowrate ratio  $F (F=F_1/F_0)$  affects the performance of the gravity settler. One experiment was operated with *S. Dimorphus* cells in a 5L bioreactor (Fig 3.9). The total inlet flowrate was 20mL/min, the inlet and outlet flowrate were setup based on flowrate ratios ( $F=F_1/F_0$ ) at 0.1, 0.15 and 0.2. The other experiment (Fig 3.10) was run with the *C. Vulgaris* cells in a 2.5 L bioreactor. The inlet flowrate was 7.78mL/min, and the flowrate ratios were 0.1, 0.15 and 0.18.

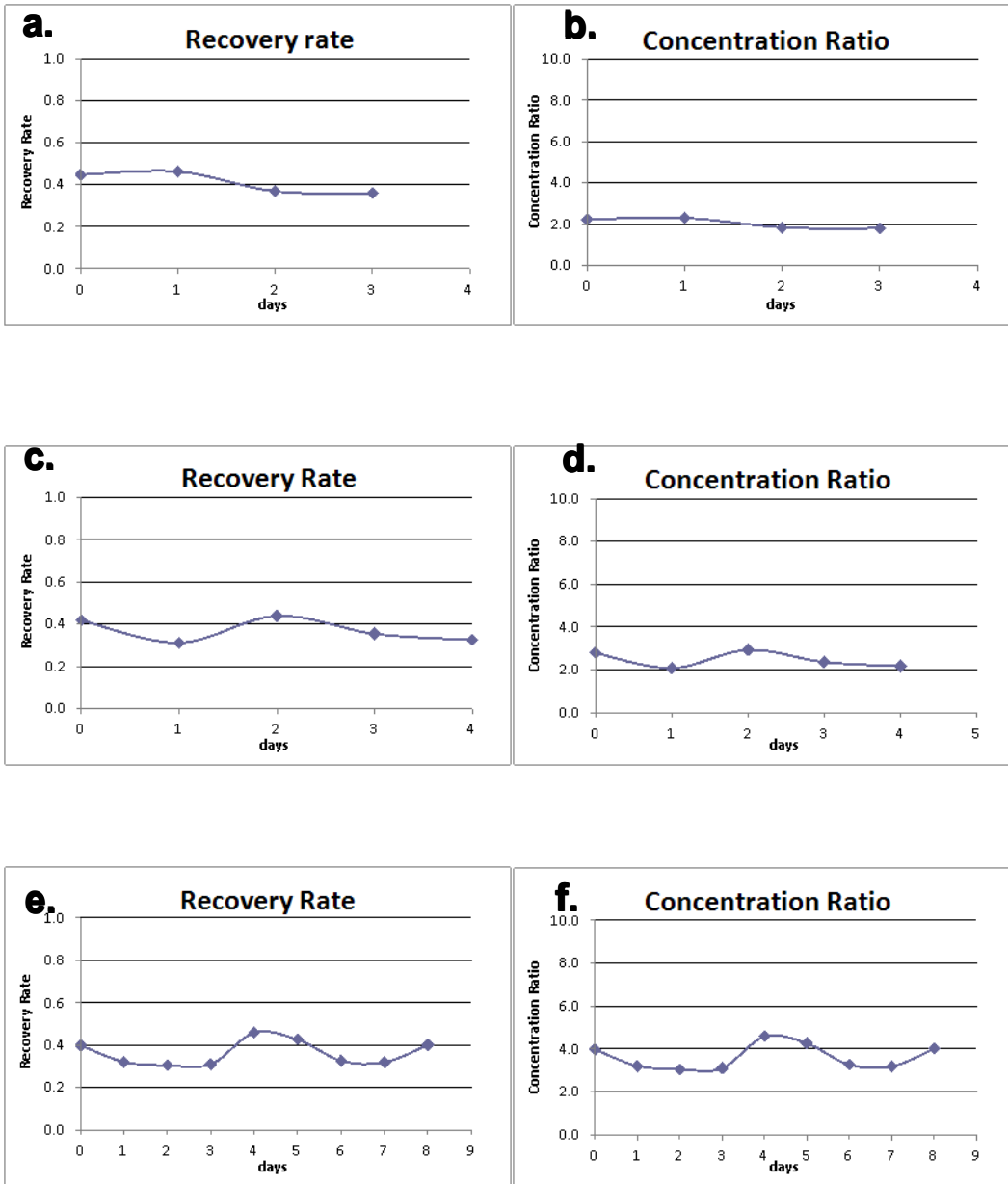


Fig 3.9 Recovery rate and concentration ratio reported from *Scenedesmus dimorphus* batch. Systems were operated with a 5 L bioreactor, connected to the large settler, with the inlet flowrate  $F_0=20\text{ml/min}$ , outlet flowrate ratio ( $F$ ) varies. a.b.  $F=4/20=0.20$ ; c.d.  $F=3/20=0.15$ ; e.f.  $F=2/20=0.1$

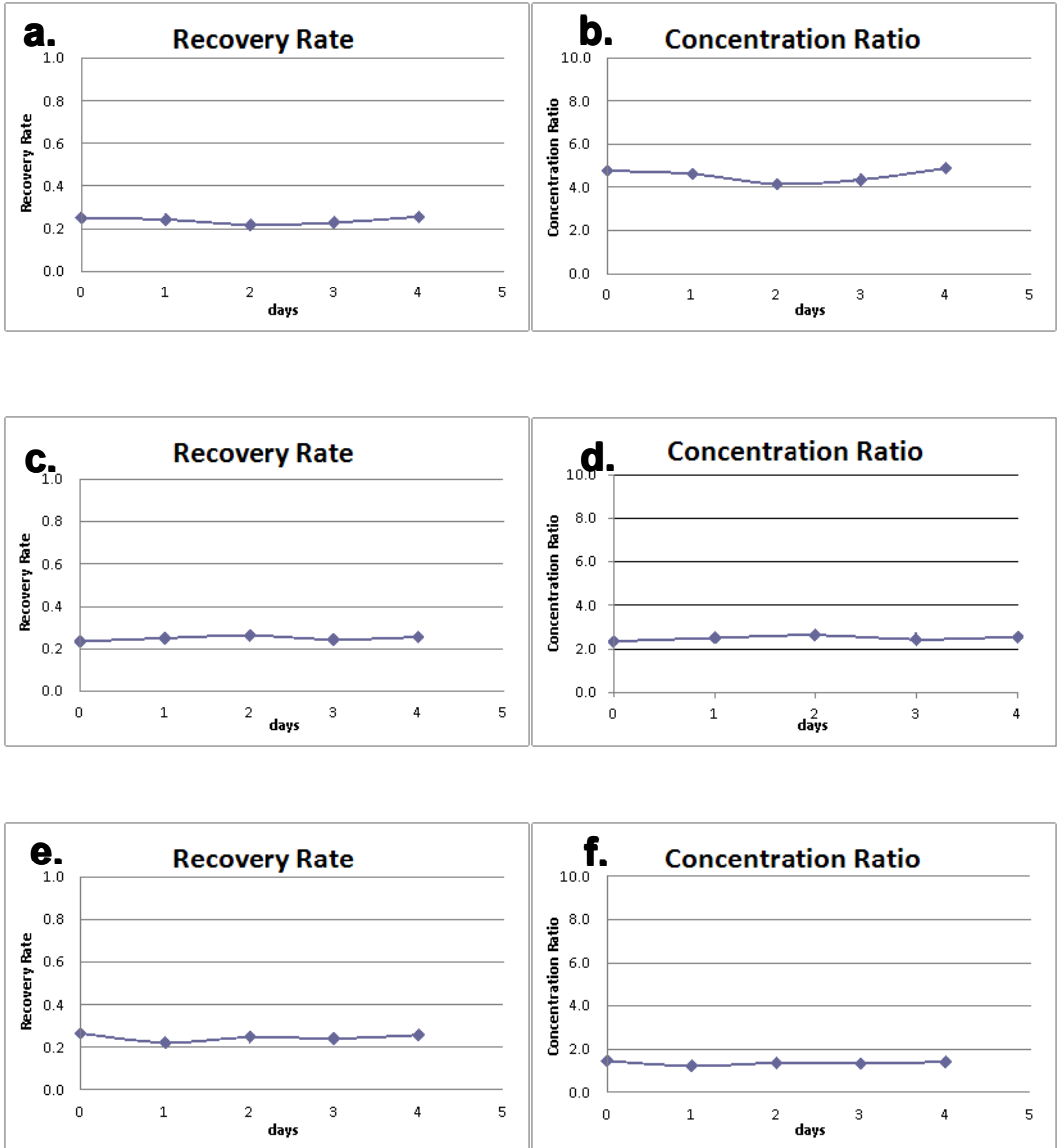


Fig 3.10 Recovery rate and concentration ratio reported from *Chlorella vulgaris* batch. Systems were operated with a 2.5 L bioreactor, connected to the small settler, with the inlet flowrate  $F_0=7.78\text{ml/min}$ , outlet flowrate ratio ( $F$ ) varies. a.b.  $F=0.41/7.81\approx 0.05$ ; c.d.  $F=0.78/7.78\approx 0.1$ ; e.f.  $F=1.42/7.82\approx 0.2$ .

Recalling equation 3.2,

$$R = \frac{gd_p^2(\rho_p - \rho)wL \sin\theta}{18\mu \cdot F_0} \quad 3.2$$

which is derived based the equation 2.12. Equation 3.2 stated that the recovery rate was determined by the size of the gravity settler, the diameter of the cells, the density of cell particles, and the inlet flowrate. Therefore, when the inlet flowrate were fixed, the recovery rate should not change, regardless of change on the outlet flowrate, if the equation 2.12 was valid.

Figure 3.11 shows the actual recovery rate for different flowrate ratios.

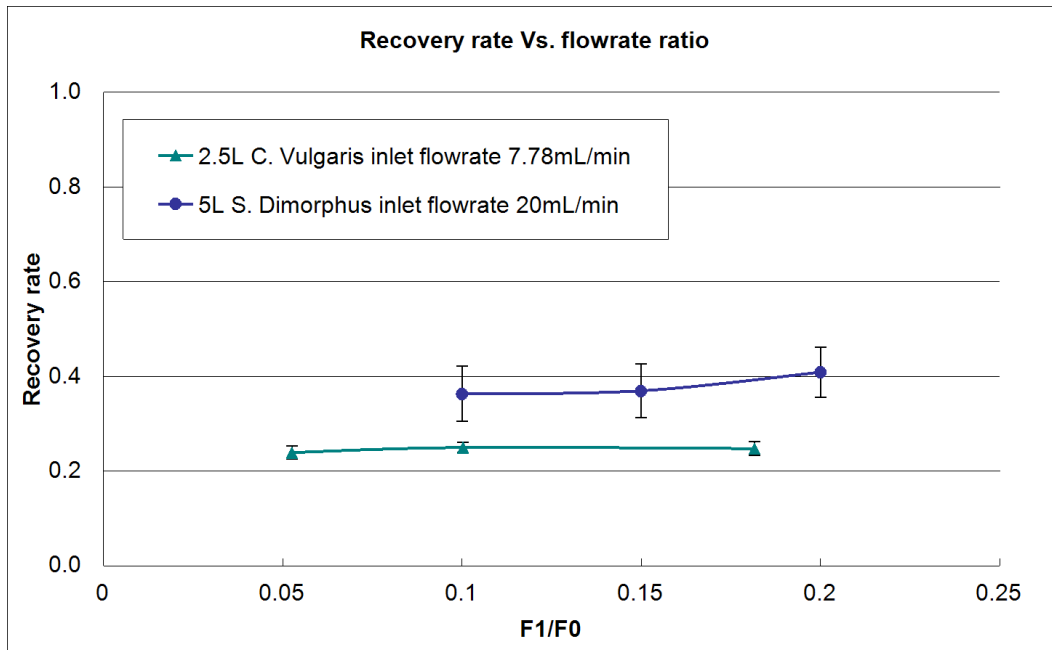


Fig 3.11 Recovery rate Vs. flowrate ratio ( $F_1/F_0$ ) with two algae strains(Error bars are standard deviations);  $n=5$  for *C. Vulgaris*;  $n=3-8$  for *S. Dimorphus*. Systems were operated with a 2.5 L bioreactor, the smaller settler for *C. Vulgaris* batch; a 5L bioreactor with a larger settler for *S. Dimorphus* batch. The outlet flowrate ratios are 0.1,0.15 and 0.25 for group and *S. Dimorphu* 0.05, 0.1, 0.2 for *C. Vulgaris* group.

In this graph, both trials for two cell strains show that the recovery rate fluctuates only in a minimal range when the outlet flowrate ratio was changed. A statistic test, non-parametric Kruskal Wallis Test, was conducted on the dataset above to see if there was a significant difference between recovery rates within one trial (Table 3.7 and 3.8).

The Kruskal-Wallis test is a statistical non-parametric method to compare the differences between two groups of data, while the source population of the tested data do not have to have a normal distribution, and the two compared groups of data do not have to have equal variance. Data points (Fig 3.10) discussed above should be similar at different outlet flowrate ratio for each cell line, if the equation 2.12 was valid. Therefore, it is suspicious the data were from a normal distribution, and Kruska-Wallis was the appropriate test to discuss the data difference.

The first column in table 3.7 and 3.8 shows different values of the outlet flowrate. The second to fourth column indicate the recovery rate and error measured from experiments for each cell line. The fifth column shows which two groups are compared, with the group name marked in the first column. The last two columns show the probability when the data difference appeared by chance.

Overall, there was not a statistically significant difference ( $P > 0.05$ )

between the recovery rates when they were acquired at different outlet flowrate ratio for each cell line, corroborating the validation of equation 3.2.

Table 3.7 Kruskal Wallis test result for recovery rate comparison for *S. Dimorphus* cells

outlet	Recovery rate			compared groups	Asymp. Sig.	
flowrate ratio( $F_1/F_0$ )	S. Dimorphus cells					
0.1 <sup>a</sup>	0.363	±	0.058	a,b	0.641	>0.0 5
0.15 <sup>b</sup>	0.370	±	0.056	b,c	0.142	
0.2 <sup>c</sup>	0.409	±	0.053	a,c	0.165	

Table 3.8 Kruskal Wallis test result for recovery rate comparison for *C. Vulgaris* cells

outlet	Recovery rate			compared groups	Asymp. Sig.	
flowrate ratio( $F_1/F_0$ )	C. Vulgaris cells					
0.05 <sup>a</sup>	0.247	±	0.015	a,b	0.917	>0.0 5
0.10 <sup>b</sup>	0.250	±	0.011	b,c	0.248	
0.18 <sup>c</sup>	0.239	±	0.014	a,c	0.465	

Based on the equation 2.12, the concentration ratio was expected to correlate linear with the reciprocal of the flowrate ratio  $F$  with a slope of the value of the recovery rate, when  $F_0$  was a constant set up in the experiment.

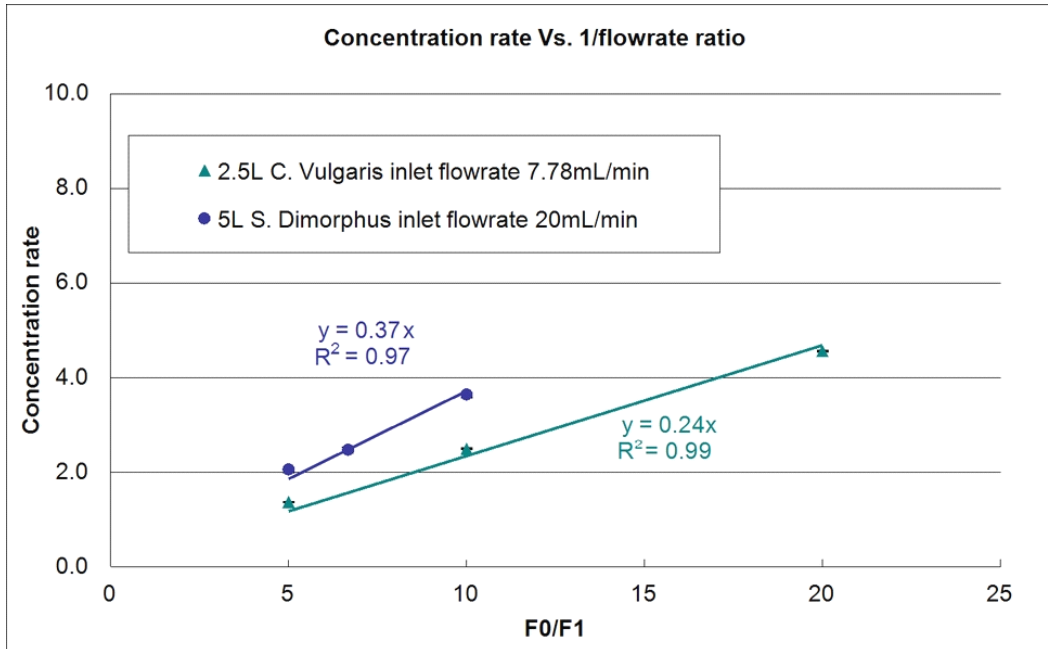


Fig 3.11 concentration ratio vs. outlet flowrate ratio ( $F_0/F_1$ ) with two algae strains (Error bars are standard deviations);  $n=5$  for *C. Vulgaris*;  $n=3-8$  for *S. Dimorphus*. Systems were operated with a 2.5 L bioreactor, the smaller settler for *C. Vulgaris* batch; a 5L bioreactor with a larger settler for *S. Dimorphus* batch. The outlet flowrate ratios are 0.1, 0.15 and 0.20 for group and *S. Dimorphus* 0.05, 0.1, 0.2 for *C. Vulgaris* group.



Table 3.9 Linear regression between C and  $F_0/F_1$  (CV cells) n=15

Coefficients <sup>a,b</sup>						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	$F_0/F_1$	.242	.004	.998	63.908	.000
a. Dependent Variable: Concentration ratio						
b. Linear Regression through the Origin						

Table 3.10 Linear regression between C and  $F_0/F_1$  (SD cells) n=17

Coefficients <sup>a,b</sup>						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	$F_0/F_1$	.371	.013	.989	27.660	.000
a. Dependent Variable: Concentration ratio						
b. Linear Regression through the Origin						

The experimental data is presented in Fig. 3.11, the data shows a strong positive linear relationship for SD cells at the significance of 0.000 ( $P < 0.01$ ), as well as a strong positive linear relationship for CV cells (Sig=0.000;  $P < 0.01$ ).

In summary, the statistical analysis showed that

a. the outlet flowrate ratio ( $F = F_1/F_0$ ) did not have any significant impact on the recovery rate  $R$ ;

b. the reciprocal of the outlet flowrate ratio ( $F_0/F_1$ ) was found linearly related to the concentration ratio  $C$ ,

when the inlet flowrate  $F_0$  was a constant and  $S$  did not change. These above two results are consistent with the idea of the hypothesis 2.12 and the equation 3.4 derived from the hypothesis.

$$R = \frac{S}{F_0} \quad 2.12$$

$$C = \frac{S}{F_1} \quad 3.4$$

### **3.6 The scale-up experiment**

As discussed above, the recovery rate of the gravity settler was determined by the size of the settler, the density and diameter of the cells, and the inlet flowrate. Since the ultimate usage of the gravity settler would be for industrial purposes, it is therefore necessary to confirm if the settler would be able to be scaled up without attenuating its dewatering ability, so that the settler could meet the demands of the large production requirement in industry.

Two settlers were hand manufactured with different dimensions. The larger one was 60cm in length, 9.5cm in width and 1cm in depth; the smaller one was 59cm in length, 4.5cm in width and 1cm in depth.

Recovery rate and concentration ratio are the two main parameters used to evaluate the dewatering ability of the settler. The scale-up

experiment is aimed to test if the two settlers have similar performances in these two parameters.

During the scale-up experiment, the cell strain, the type of media and the declination angle were fixed for both two settlers. The only different variables were the width  $w$  and length  $L$  and inlet flowrate  $F_0$ , but the ratio  $wL/F_0$  were set up same for both two settlers. According to the equation 2.12, the recovery rate  $R$  and concentration ratio  $C$  measured from experiments should be similar for both settlers, if the hypothesis was valid and the system was able to be scaled up.

The surface area  $wL$  is 570 cm<sup>2</sup> for the large settler, and 265.5 cm<sup>2</sup> for the small gravity settler. The inlet flowrate  $F_0$  was set up at different levels, but the ratio  $wL/F_0$  was always ensured to be same for both settlers. For example, the ratio was  $wL/F_0=119\text{min/cm}$  for both settlers using  $F_0=2.22\text{mL/min}$  for the smaller settler and  $F_0=4.47\text{mL/min}$  for the larger settler. In each trial, it was ensured the ratios from each settler were almost the same, even the inlet flowrates were set up as different.

The following two groups of figures (Fig 3.12 and Fig 3.13) show the change in recovery rate and concentration ratio for both settlers as a function of the operating time at different inlet flowrates. Fig 3.12 is from the data acquired from the large settler, Fig3.13 is from the small settler. Both settlers were operated with *S. Dimorphus* cells. If the hypothesis was

valid and the system was able to be scaled-up, the R and C at same position in each figure should be similar.

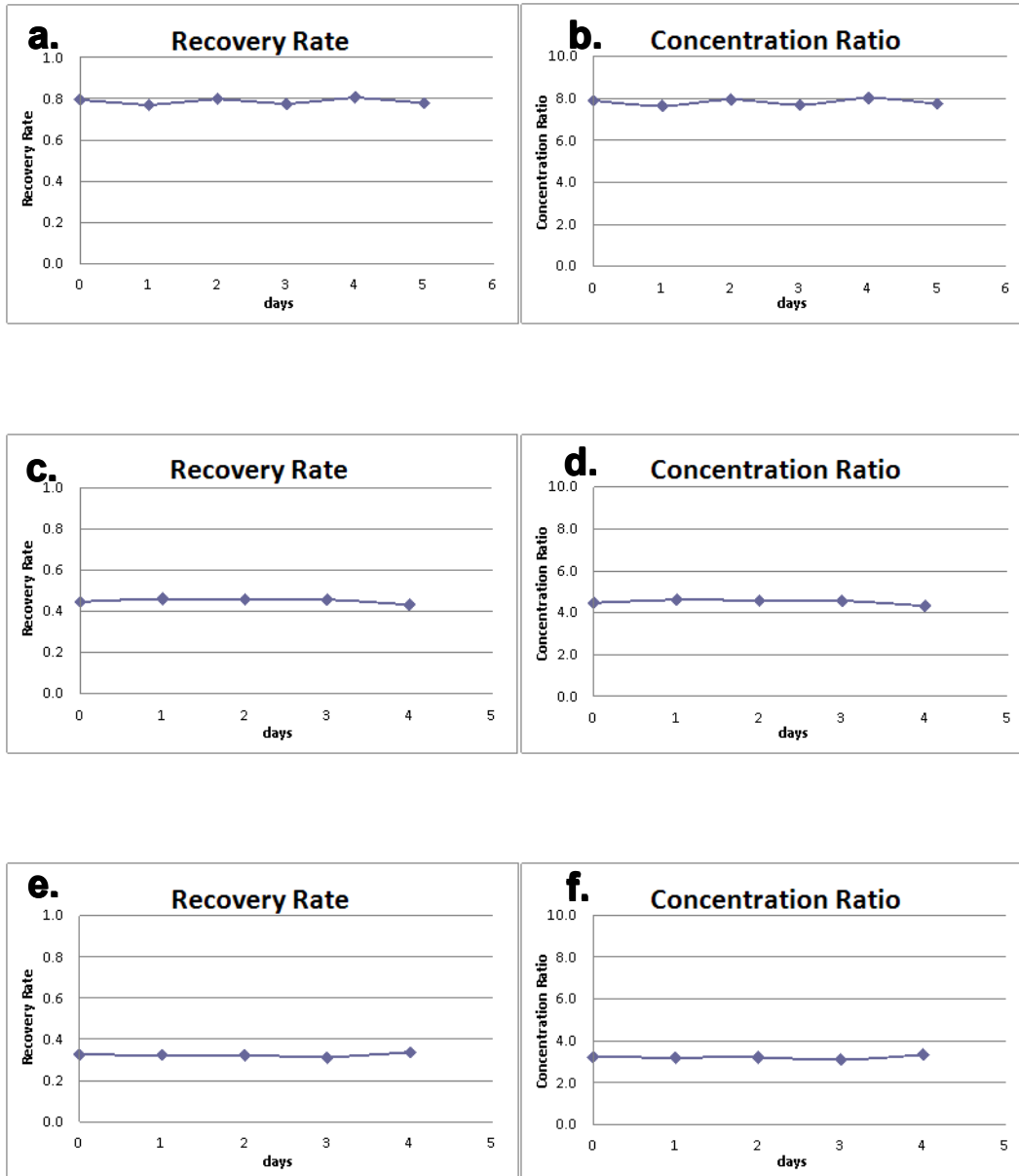


Fig 3.12 Recovery rate and concentration ratio with achieved by the large settler with a 5L bioreactor with *S. Dimorphus* cells. The flowrate ratio  $F=1:10$ , the inlet flowrate varies; a.b.  $F_{\sigma}$  of 4.77 mL/min; c.d.  $F_{\sigma}$ =16.44 mL/min; e.f.  $F_{\sigma}$ =28.66 mL/min.

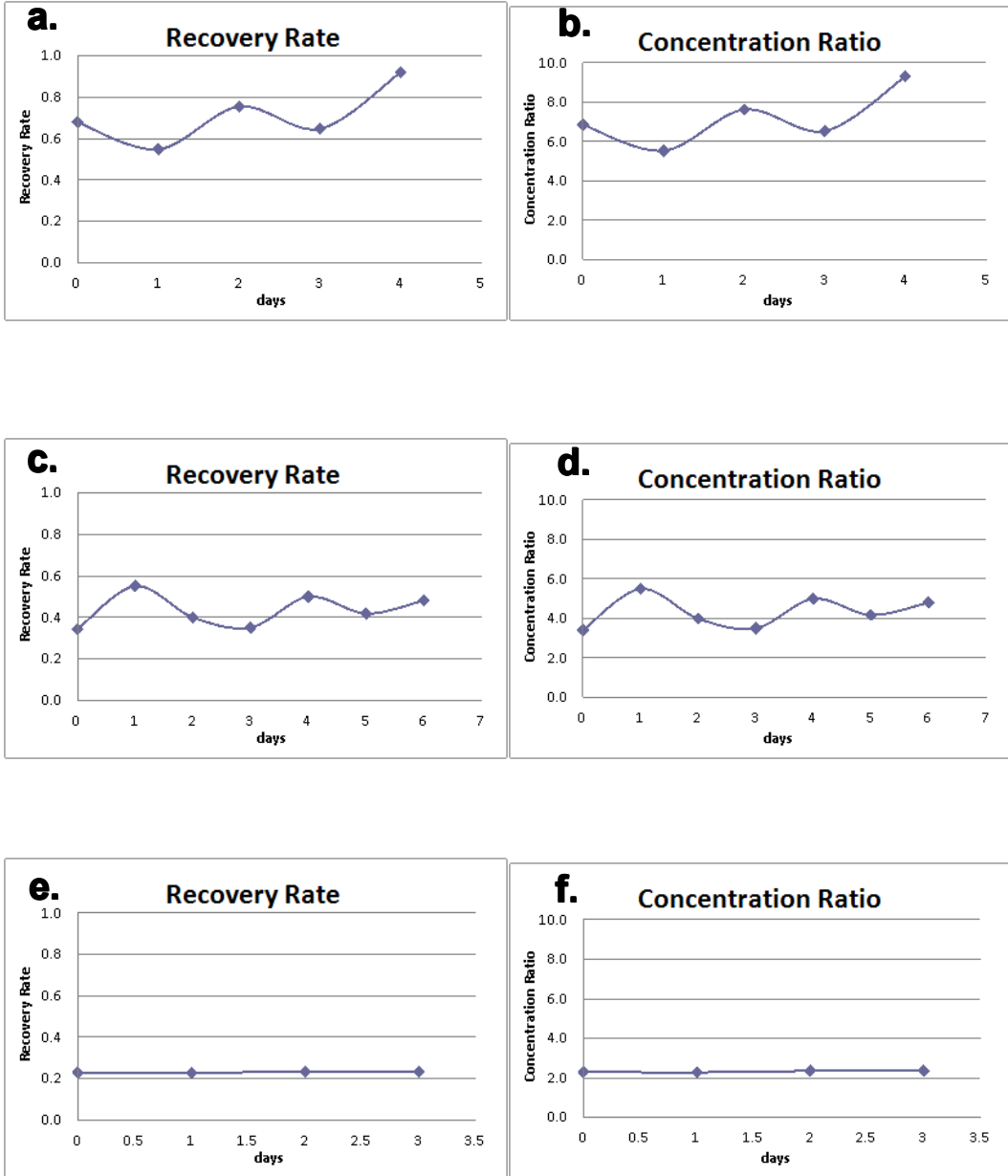


Fig 3.13 Recovery rate and concentration ratio with achieved by the small gravity settler with a 2.5L bioreactor with *S. Dimorphus* cells. The outlet flowrate ratios  $F=1:9$ , the inlet flowrate  $F_0$  varies; a.b.  $F_0$  of 2.22 mL/min; c.d.  $F_0=7.78$  mL/min; e.f.  $F_0=13.33$  mL/min.

Fig 3.14 and Fig 3.15 depict the recovery rate R and the concentration ratio C changes at different  $F_0/wL$  levels with the large and the small settlers. The two lines shown in each figure are intuitively observed to be close. A One-Way ANOVA statistical analysis was conducted with SPSS (Version.PASW statistic 18.0) to this dataset to further discuss if there was a difference between the two lines (Table 3.11). The data from the large settler was marked as group 1, while the data from the small settler was marked as group 2. The One-Way ANOVA test can tell if there are significant differences within comparisons of the two groups at different  $F_0/wL$  levels.

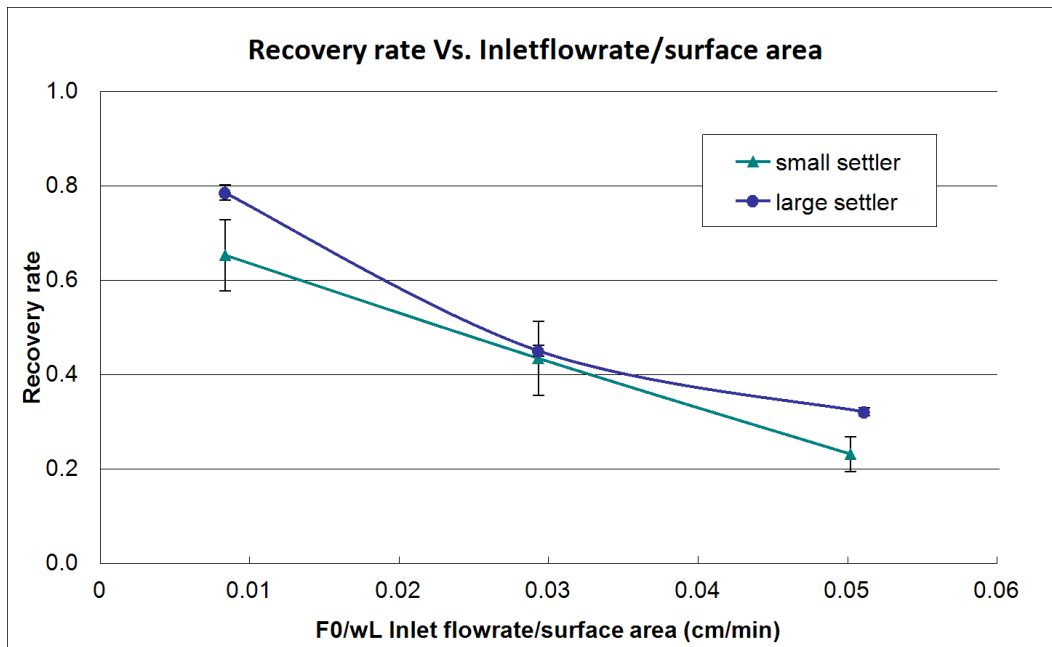


Fig 3.14 Recovery rate Vs. Inlet flowrate/surface area, operated by the large gravity settler with a 5L bioreactor with *S. Dimorphus* cells, also by the small gravity settler with a 2.5L bioreactor (Error bars are standard deviations). The outlet flowrate ratios  $F=1:9$ , the inlet flowrate varies;  $F_0$  of 4.77, 16.44 and 28.66 mL/min for the large settler;  $F_0$  of 2.22, 7.78, 13.33mL/min for the small settler.(n=5 or 6 for large settler; n=4-7 for small settler.)

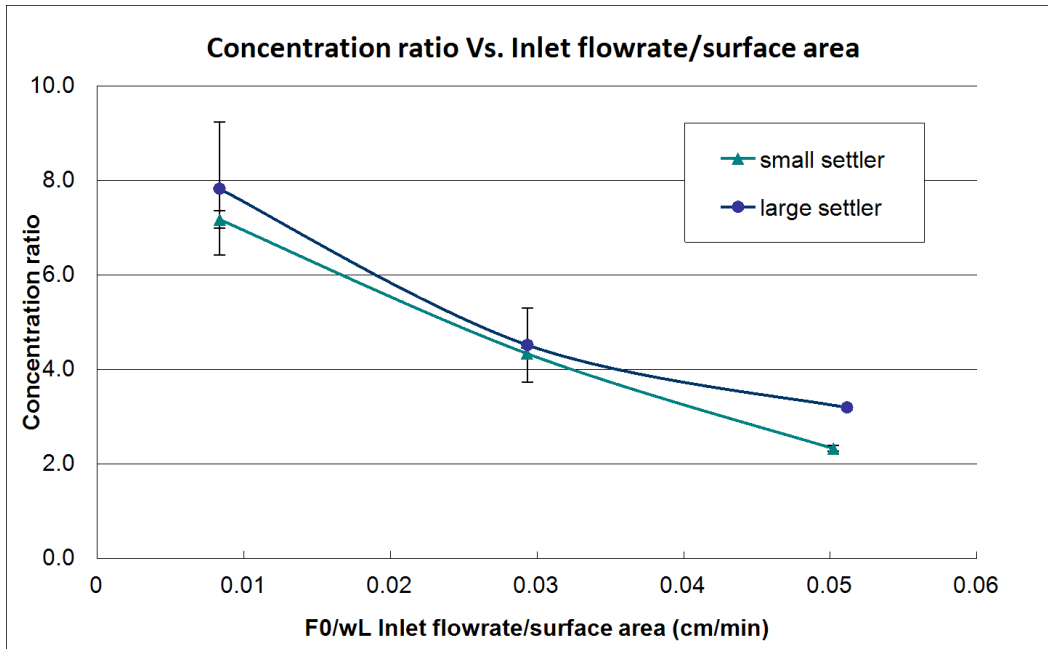


Fig 3.15 concentration ratio Vs. Inlet flowrate/surface area operated by the large gravity settler with a 5L bioreactor with *S. Dimorphus* cells, also by the small gravity settler with a 2.5L bioreactor (Error bars are standard deviations). The outlet flowrate ratios  $F=1:9$ , the inlet flowrate varies;  $F_0$  of 4.77, 16.44 and 28.66 mL/min for the large settler;  $F_0$  of 2.22, 7.78, 13.33 mL/min for the small settler. (n=5 or 6 for large settler; n=4-7 for small settler.)

Table 3.11 One-Way ANOVA test for recovery rate  
(Group1: large settler Group 2: small settler)

		Sum of Squares	df	Mean Square	F	Sig.
$F_0/wL=0.008$ cm/min	Between Groups	.019	1	.019	379.224	.000
	Within Groups	.000	7	.000		
	Total	.019	8			
$F_0/wL=0.029$ cm/min	Between Groups	.001	1	.001	.190	.672
	Within Groups	.038	10	.004		
	Total	.038	11			
$F_0/wL=0.050$ cm/min	Between Groups	.049	1	.049	18.716	.002
	Within Groups	.024	9	.003		
	Total	.072	10			

Table 3.12 One-Way ANOVA test for concentration ratio  
(Group1: large settler Group 2: small settler)

		Sum of Squares	df	Mean Square	F	Sig.
$F_0/wL=0.008$ cm/min	Between Groups	1.159	1	1.159	1.292	.285
	Within Groups	8.074	9	.897		
	Total	9.233	10			
$F_0/wL=0.029$ cm/min	Between Groups	.093	1	.093	.249	.629
	Within Groups	3.742	10	.374		
	Total	3.835	11			
$F_0/wL=0.050$ cm/min	Between Groups	1.788	1	1.788	361.413	.000
	Within Groups	.035	7	.005		
	Total	1.823	8			

The result of analysis for recovery rate shows only the data points from the medium  $F_0/wL$  level are similar ( $P>0.05$ ). The data points from the low and high  $F_0/wL$  levels are significantly different ( $P<0.05$ ). However, analysis for concentration ratio shows data are similar from both the low and the medium  $F_0/wL$  levels ( $P>0.05$ ), while the data from high  $F_0/wL$  level are significantly different ( $P<0.05$ ).

Fortunately, the recovery rate and concentration ratio are actually higher than expected using the larger settler.

This result is moderately different from the expectation to the scaled-up system. The difference may be induced by the error of equipment, since the pump used to control the outlet flowrate was not exactly accurate when it was run at the very low flowrate like 0.22ml/min (Inlet flowrate=2.22 ml/min, outlet flowrate ratio=1:9, outlet flowrate at the concentrated



stream=0.22ml/min). The difference may also come from too many times dilution during the absorbance measurement. When the outlet flowrate was low as 0.22ml/min, the concentration ratio approximately reached 8 (Fig 3.15), which implied a very condensed outlet stream. Samples had to be diluted for 25 times to ensure the absorbance measurement was conducted within the calibration range of the spectrometer.

In summary, the gravity settler was tested to have a stable performance when its surface area was scaled up from 265.5cm<sup>2</sup> to 570cm<sup>2</sup>, and only when the system was run at a medium inlet flowrate level at (7.78ml/min for the small settler, 16.44ml/min for the large settler). More experiments are required to prove the settler's stable performance at high inlet flowrate level and low inlet flowrate level in future research.

### **3.7 The two-stage experiment**

The one-stage gravity settler had been discussed above. It was found that the recovery rate and concentration ratio were related with flowrate as follows (Equation 3.4), if the equation 2.12 was valid.

$$R = \frac{S}{F_0} \tag{2.12}$$

$$C = \frac{S}{F_1} \tag{3.4}$$

S is determined by the cell type, the type of media and the size of the gravity settler. Therefore, with experimental subjects and equipment fixed, therefore S is a constant. At this point, in order to get higher recovery rate and concentration ratio, the inlet flowrate  $F_0$  and outlet flowrate  $F_1$  had to be decreased. This sometimes severely impairs the total productivity of the system.

A two-stage gravity settler was able to solving this problem since it is capable to achieve high concentration ratio without sacrifice flowrate. After one stage dewatering, the cell suspension is usually concentrated by five times, if the concentrated suspension could be then dewatered in a second stage settler. The cell suspension will hopefully be concentrated by 25 times.

Two bioreactors, one with low biomass concentration at 1.0 g/L, the other with high biomass concentration at 5.0 g/L (five times of the 1.0g/L), were used to imitate the two stages dewatering.

The following two figures (Fig 3.18; Fig 3.19) showed how the recovery rate R and concentration ratio C changed at different inlet flowrates with a lower and a high biomass concentration.

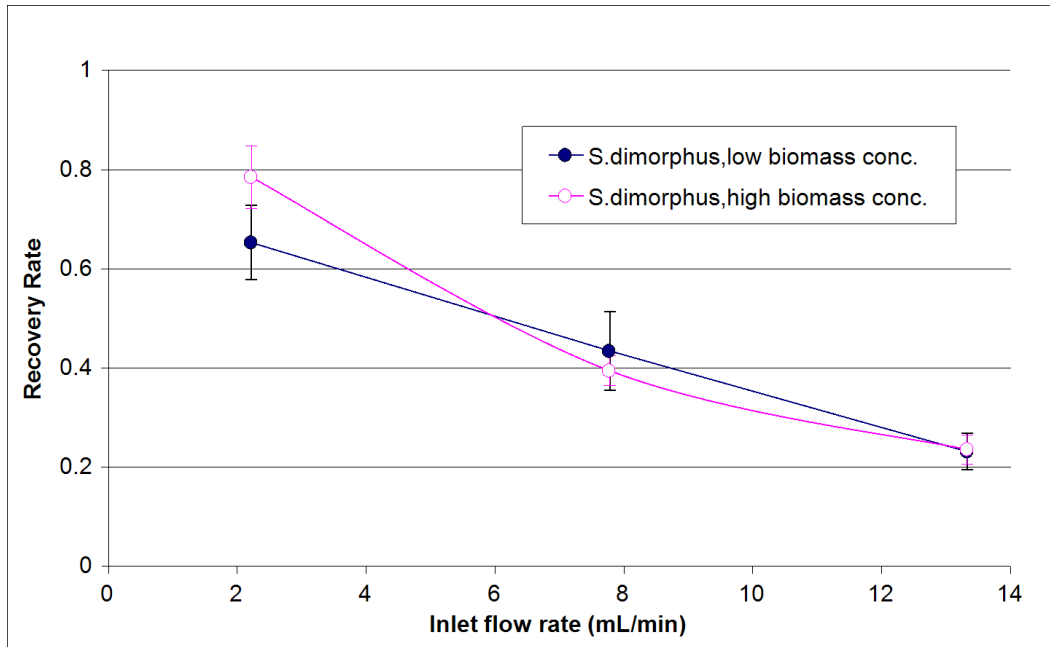


Fig 3.18 Recovery rate Vs. different biomass concentration; operated by the small gravity settler with *S. Dimorphus* cells in a 2.5L bioreactor (Error bars are standard deviations). The low biomass concentration was 1.0g/L; the high biomass concentration was 5.0 g/L. The flowrate ratio  $F=1:10$ , the inlet flowrate varies;  $F_{\sigma}$  of 2.22, 7.78 and 13.33 mL/. (n=3 or 4 for high biomass concentration; n=5-7 for the low biomass concentration.)

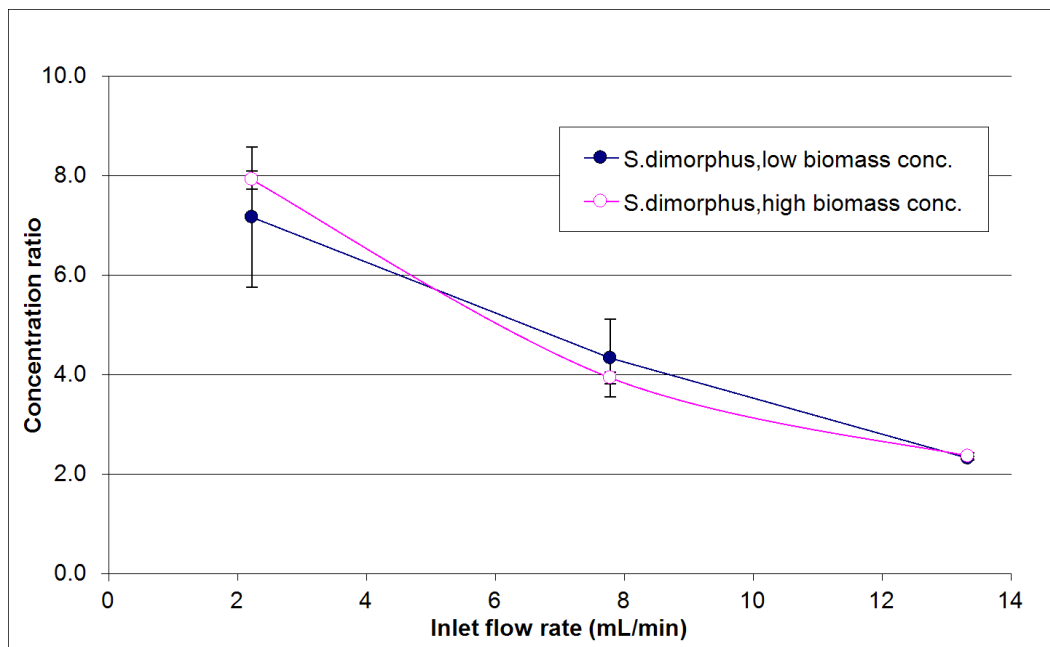


Fig 3.19 Concentration factor Vs. different biomass concentration; operated by the small gravity settler with *S. Dimorphus* cells in a 2.5L bioreactor (Error bars are standard deviations). The low biomass concentration was 1.0g/L; the high biomass concentration was 5.0 g/L. The flowrate ratio  $F=1:10$ , the inlet flowrate varies;  $F_{\sigma}$  of 2.22, 7.78 and 13.33 mL/. (n=3 or 4 for high biomass concentration; n=5-7 for the low biomass concentration.)

The figures above qualitatively shows that the settlers operated with different inlet biomass concentrations had similar dewatering ability.

The following figures 3.20 and 3.21 depict the relationship between  $R$  and  $1/F_0$ , as well as the relationship between  $C$  and  $1/F_1$ .

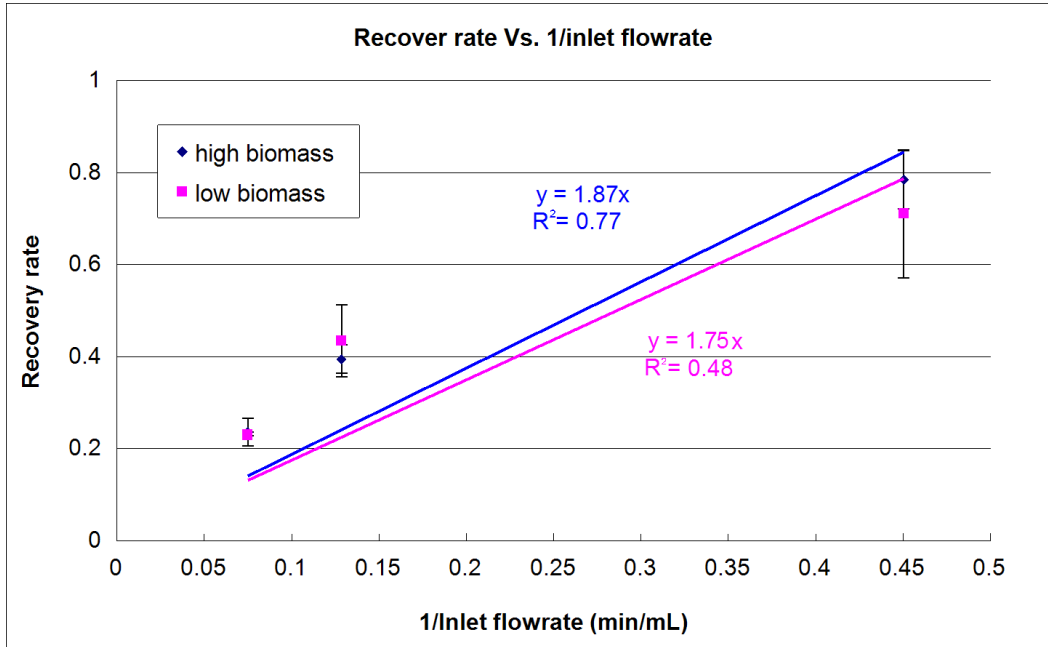


Fig 3.20 Recovery rate Vs.  $1/\text{inlet flowrate } F_0$ ; operated by the small gravity settler with *S. Dimorphus* cells in a 2.5L bioreactor (Error bars are standard deviations). The low biomass concentration was 1.0g/L; the high biomass concentration was 5.0 g/L. The outlet flowrate ratios  $F=1:9$ , the inlet flowrate varies;  $F_0$  of 2.22, 7.78 and 13.33 mL/. (n=3 or 4 for high biomass concentration; n=5-7 for the low biomass concentration.)

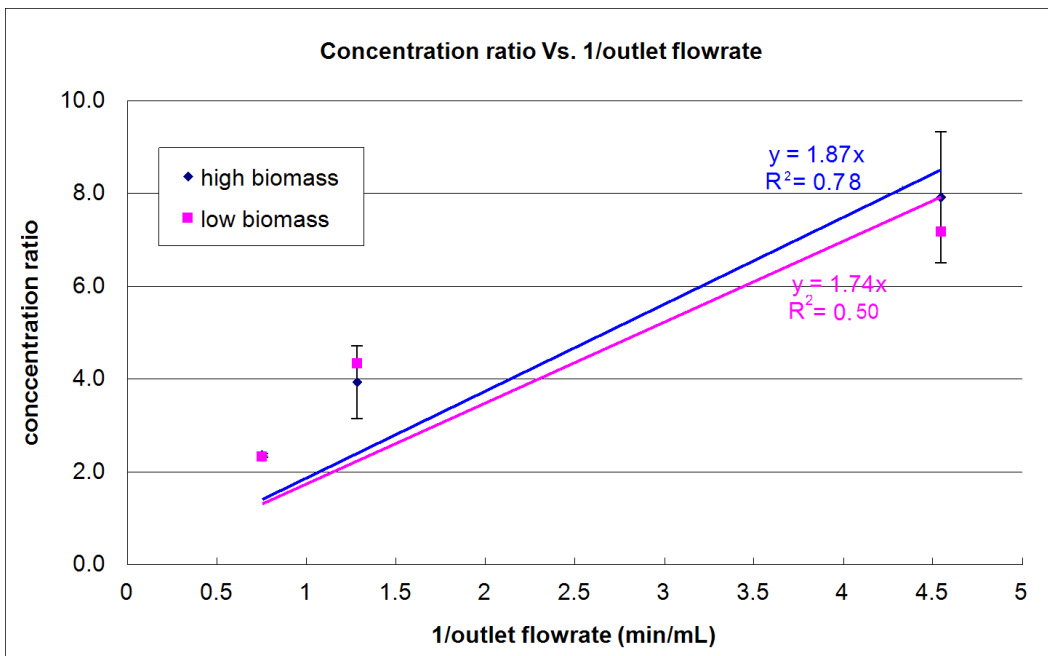


Fig 3.21 concentration ratio vs.  $1/\text{outlet flowrate } F_1$ ; operated by the small gravity settler with *S. Dimorphus* cells in a 2.5L bioreactor (Error bars are standard deviations). The low biomass concentration was 1.0g/L; the high biomass concentration was 5.0 g/L. The flowrate ratio  $F=1:10$ , the inlet flowrate varies;  $F_0$  of 2.22, 7.78 and 13.33 mL/. (n=3 or 4 for high biomass concentration; n=5-7 for the low biomass concentration.)

Table 3.13 Linear regression between R and  $1/F_0$  (High biomass)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	$1/F_0$	1.875	.148	.971	12.888	.000
a. Dependent Variable: Recovery rate						
b. Linear Regression through the Origin						

Table 3.14 Linear regression between R and  $1/F_0$  (Low biomass)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	$1/F_0$	1.747	.169	.932	9.927	.000
a. Dependent Variable: Recovery rate						
b. Linear Regression through the Origin						

Table 3.15 Linear regression between C and  $1/F_1$  (High biomass)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	$1/F_1$	1.872	.147	.972	12.961	.000
a. Dependent Variable: concentration ratio						
b. Linear Regression through the Origin						

Table 3.16 Linear regression between C and  $1/F_1$  (Low biomass)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	$1/F_1$	1.744	.167	.940	10.646	.000
a. Dependent Variable: concentration ratio						
b. Linear Regression through the Origin						

A statistical regression analysis was conducted on the data showed in fig 3.20 and 3.21. Tables 3.13 to 3.16 show the analytical results. The table 3.13 and 3.14 show there are statistical significant linear relationships between the recovery rate and the reciprocal of the inlet flowrate for both the high biomass group and low biomass group. Between the concentration ratio and the reciprocal of the outlet flowrate  $1/F_1$ , there are also statistically significant linear relationships shown for both groups.

This result is consistent with the equation 2.12. Also,  $S=1.9 \pm 0.15$ ,  $1.7 \pm 0.17$ , similar to that obtained before.

A non-parametric statistic test, Kruskal Wallis Test, was used to compare if there was a significant difference of settler performances between two groups by using software SPSS (Version. PASW statistic 18.0).

Table3.17 Kruskal Wallis Test Result for Recovery Rate Comparison

Inlet flowrate (ml/min)	Recovery rate		Asymp. Sig.
	Low biomass conc.	High biomass conc.	
2.22	0.710 ± 0.140	0.785 ± 0.063	0.456
7.78	0.435 ± 0.079	0.395 ± 0.030	0.450
13.33	0.232 ± 0.004	0.236 ± 0.030	1.000

Table 3.18 Kruskal Wallis Test Result for concentration ratio Comparison

Inlet flowrate (ml/min)	concentration ratio				Asymp. Sig.
	Low biomass conc.		High biomass conc.		
2.22	7.167	± 1.411	7.919	± 0.637	0.456
7.78	4.334	± 0.784	3.936	± 0.302	0.450
13.33	2.321	± 0.037	2.363	± 0.299	1.000

The first columns in table 3.17 and 3.18 indicate the inlet flowrate when data were collected. The second to fourth column show the recovery rate/concentration ratio and its standard deviation from the low biomass group, the fifth to seventh column show data from high biomass group. The recovery rates (or concentration ratio), as the parameter to estimate settler performance, were compared between the low biomass group and high biomass group. The last two columns show the probability of that the difference from comparison is presented by chance.

It is shown there is no significant difference between the high biomass and low biomass groups ( $P > 0.05$ ).

Thus, it could be concluded that the gravity settler performance is not affected by the biomass concentration; this suggests that the idea of a two-stage gravity settler could be applicable. A two-stage settler, with highly improved dewatering ability, makes the settler more economical and suitable for industrial production, compared to the single gravity settler configuration.



Since the system needs to be operated continuously, the outlet flowrate from the first stage will determine the inlet flowrate of the stage following. Too many stages of dewatering will lead to a low final outlet flowrate, which could impair the overall biomass recovery rate. To achieve the optimum amount of stages, a trade off must be made between a high production rate and a highly biomass concentration of the production. Usually, the enterprise scale and the productivity of a biodiesel factory are already fixed, therefore the number of stages required can be determined based on the factory's production capacity.

## **CHAPTER IV**

### **CONCLUSION AND RECOMMENDATIONS**

#### **4.1 Conclusions**

The performance of the gravity settler was tested in this thesis research. The dewatering ability of the gravity settler was quantitatively evaluated by two parameters, Recovery rate and concentration ratio.

Recovery rate refers to the amount of cells that can be recovered per unit time; concentration ratio refers to the cell density of the dewatered stream, compared to the cell density of the inlet stream from the bioreactor.

From the definition of recovery rate in equation 2.9, it was stated that the recovery rate is linearly related to the concentration ratio, with a coefficient of flowrate ratio ( $F = F_1/F_0$ ). If the gravity settler was set up with a known inlet flowrate, the recovery rate could be determined based on

equation 2.12 and the specific requirements of production. The concentration ratio was inversely related to the flowrate ratio, whereas the recovery rate was determined by inlet flowrate.

If this downward-flow gravity settler will be used in large system, it must be scalable. Two settlers that only differed in width were used in the experiment. The results showed that the settler performance obeyed the theory design equations 2.5 and 2.6, irrespective of the settler size. In industrial applications, a high inlet flowrate is usually required to provide a high production rate. Since the recovery rate was inversely related to inlet flowrate, a high inlet flowrate means a lower the recovery rate. Thus, the scalable is done by increasing the width of the settler.

The settler was also tested as a two-stage settler. It was shown that a two-stage system is feasible, with a concentration factor up to 35 can be achieved.

## **4.2 Recommendations**

1. The declining angle was set up at 55 degrees in this experiment.

As reported by other researchers, the angle was limited because cells

would block the outlet when the angle went higher. However, different cell strains have varied morphological characters and cell diameters, which may have different maximum declining angles for the gravity settler. It is necessary to explore the applicable declining angles of different algae strains, since higher recovery rate and concentration ratio could be achieved by increasing this angle.

2. Algae cells with different oil content are also different in cell density. The cell density was a parameter in theory that influenced the recovery rate. However, the two cell strains used for comparison had great differences in cell diameter; therefore the comparison result was more influenced by the cell diameter than the oil content. In the future study, the same cell strain could be cultured in different conditions to have cells accumulated different levels of oil content. The effects from different oil content on the performance of the gravity settler could be then investigated, excluding the influence from other parameters, such as the cell diameter. Alternatively, two strains of cells having comparable cell diameters could also be used.

3. There are two ways to scale up the gravity settler. One is to

enlarge the width of the settler, in order to increase its working capacity; the other one is to use multiple layers to realize the surface area enlargement. The first method was verified in this thesis research, which showed that the settler had a constant ability of dewatering when it was scaled up by width. A multiple-layers approach would consume less materials and have a lower capital cost compared to the 1<sup>st</sup> method. Therefore scaling-up using multiple layers should be also tested and verified in the future.

4. The multiple stages dewatering can obtain a higher concentrated outlet stream compared to the single stage settler, but outlet stream with high concentration has a risk to block the outlet. Currently, the dewatering ability of a two stage settler was tested and verified in this research. In the future, it is necessary to find the maximum number of stages could be implemented.

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