Culturing and Harvesting Marine Microalgae for the Large-scale Production of Biodiesel

This thesis is presented for the degree of Masters of Engineering Science in the school of Chemical Engineering

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Declaration

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Abstract

In the commercial production of biodiesel from marine microalgae, the cost and efficiency of harvesting technique affects the overall cost and production of biodiesel. The commercial harvesting techniques being used for harvesting microalgae include centrifugation and filtration preceded by flocculation. Centrifugation and filtration are very high cost processes and different flocculation techniques like chemical flocculation, auto-flocculation and bio-flocculation (microbial flocculation) are being developed to achieve more efficiency in flocculation of algal biomass at lower costs. In this project, 'Electroflocculation'- a common process for flocculating contaminants, organic matter and metal ions from waste water was applied to flocculate marine microalgae.

The studies presented in the thesis aim to

1. determine the effect of electroflocculation on the flocculation of marine microalgae at lab scale

2. investigate the factors affecting electroflocculation i.e. current density, time, material of electrodes, distance between electrodes, salinity of the cultures and pH

3. scale-up the lab scale electroflocculation process to pilot-scale and investigate the cost effectiveness of pilot-scale electroflocculation process

4. theoretically optimize, design and analyse the costs for electroflocculation process, based on experiments performed and data available in literature

5. culture marine microalgae species starting from lab-scale to outdoor raceway ponds and study the reliability and stability of the cultures over a long period

The lab-scale experiments on electroflocculation of marine microalgae showed that this technique successfully flocculated the microalgae from the culture solution and the floccs floated to the surface that can be easily scrapped off and used for further dewatering or extraction purposes. Investigation of factors affecting electroflocculation indicated that factors like current density, time, distance between electrodes and electrode material should be optimized for lowering the costs. The higher salinity of cultures and pH around 7 are favourable factors for harvesting marine microalgae using electroflocculation.

Following the success of lab-scale experiments a 100L pilot-scale setup was built to analyse the cost effectiveness of electroflocculation at this scale. Results showed that minimum power requirement of 0.168kWh/m^3 was noted with more than 95% removal efficiency and concentration factor of 25 times was achieved.

The study also introduced a several key factors in understanding the optimization, design, and cost analysis of the process and to overcome the process drawbacks of electroflocculation.

The results enhance the current understanding of the electroflocculation process and further studies required to apply electroflocculation as a harvesting technique at large scale in the process of production of biodiesel from microalgae in saline water.

Prior to harvesting the marine microalgae species were cultured in laboratory upto 20L and outdoor raceway ponds upto 400L. The growth rate and productivities of the microalgae cultures in outdoor raceway ponds were investigated regularly over a period of 9 months and productivities of 1-5$gm^{-2}day^{-1}$ were reported. The study of effect of changing environmental factors on the growth rate and productivities showed that the marine microalgae species are reliable and stable and suitable for large scale culturing in the production of biodiesel.

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Chapter 1. Introduction

1.1 Background

The large scale production of renewable energy resources like biodiesel is a research focus all over the world because of the insufficient fuel resources and a constantly increasing demand for petroleum products and green house gas emissions. Large scale production of biodiesel from marine microalgae could be one of the solutions. The production of biodiesel from marine microalgae compared to irrigated crops is more beneficial because of the availability of the sea water and high oil productivities of microalgae in less area with lower quality land, in contrast to the productivity of terrestrial energy crops in large areas with high quality farming land (Chisti, 2007). There are also concerns that energy crops for developed nations will compete with basic foodstuffs for developing nations which acerbate the income gap between rich and poor and lead to further poverty and starvation in developing countries. A variety of microalgae species can grow over a wide range of salinities ranging from freshwater to saturated brines (Borowitzka, 2008). It is very uneconomical to use fresh water algae for biodiesel production due to increasing demand of scarce fresh water for domestic usage, industrial and irrigation purposes. Therefore, marine microalgae promise to be the commercial option in the production of biodiesel.

The idea of using microalgae as a source of fuel is not new; the concept was first experimentally demonstrated by Golueke and Oswald (1957-1960) by growing microalgae on flue gases and converting the biomass to methane gas (Benemann & Oswald, 1996). After 1979, the Aquatic Species Program of DOE-SERI started focusing on renewable energy from algal lipids due to energy shortage (Benemann & Oswald, 1996). The Israeli Seambiotics Company founded in 2003 produces algae biomass with approximately 30% oil content using CO_2 from coal power plant flue gas. In 2007, Li et al studied the properties of biodiesel obtained from *Chlorella* and was comparable to conventional diesel fuel and complies with the US standard for Biodiesel (ASTM 6751), which can be expanded hetero-trophically for *Chlorella* fermentation for biodiesel production at large scale (Li *et al.*, 2007). The current study

is a part of AP6 funded project lead by Prof. Michael Borowitzka working on large scale production of biodiesel from marine microalgae.

Literature study (above) shows that considerable work has been done all around the world in the production of biodiesel from microalgae. Research done by scholars strengthens the hope of obtaining a renewable 'quality fuel' from microalgae. Major trouble lies in reducing the costs required for the processes involved in the production of fuel. These processes are culturing, harvesting and extracting.

Figure 1.1 shows the four important steps in the production of biodiesel from microalgae. Initially for the commercial production of biodiesel from microalgae, it requires large scale culturing of microalgae species. The commercial culture of microalgae is more than 30 years old and the main species are *Chlorella* and *Spirulina* for health food, *Dunaliella* salina for β-*carotene*, *Haematococcus pluvialis* for *astaxanthin* etc (Borowitzka, 1999). The microalgae species reported in literature for the production of biofuels are mainly *Chlorella*, *Botryococcus braunii*, *Scenedesmus* (Benemann & Oswald, 1996) and many other species of green algae and diatoms. A collection of over 300 species that can be used for biodiesel production are housed at the University of Hawaii and is available to researchers (Sheehan *et al.*, 1998).

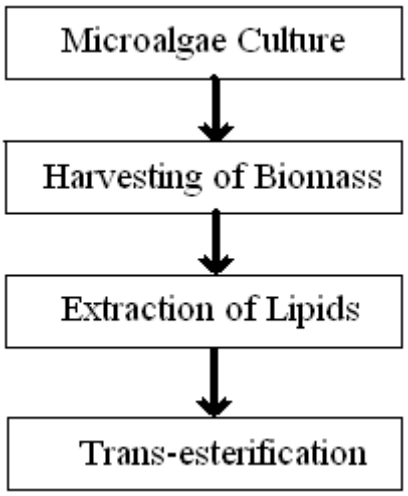

Figure 1.1 Steps involved in the process of production of biodiesel

It is really necessary that the microalgal cultures grow well outside the laboratory in order to be able to grow them in hectares of area for greater productivities of microalgal biomass. Therefore, the cultures for commercial production of microalgae

are grown in shallow open-air ponds with no artificial mixing, paddle wheel mixed ponds, circular ponds with a rotating mixing arm, with pond area ranging in hectares, also other commercial options include tubular photobioreactor systems, large plastic bags with upto 1000L volume, and carboys for small scale production (Borowitzka, 1999). It is essential to study the growth patterns of microalgae cultures in outdoor raceway ponds to analyse the productivities and their growth response to different atmospheric conditions. Culturing is an important step to demonstrate the viability of the cultures at large scale and also this stage provides the feed for the next stage i.e. 'harvesting', which is the main focus of this project.

After culturing the microalgae, the biomass needs to be separated from the water for further processing known as 'harvesting'. The most commonly used techniques for harvesting microalgae at large scale are centrifugation, filtration, flotation and flocculation. However, most of these techniques are very expensive, few are immature and some may not be efficient for large scale harvesting. Most of the times centrifugation and filtration are preceded by flocculation and coagulation to improve the harvesting efficiency and reduce costs (Shelef *et al.*, 1984; Grima *et al.*, 2003).

Followed by harvesting, the biomass is treated to extract the lipids (fats, oils, sugars and functional bioactive compounds) out of the cells; this process is known as 'extraction'. The microalgae species contain high levels of lipids/oils in the range of 1 – 40 % of dry weight, in certain species under specific conditions the lipid content could be as high as 85% (Becker, 1994). Lipids extracted from microalgae are then processed and trans-esterified to produce biodiesel.

In summary, in the process of commercial production of biodiesel, harvesting and extraction processes are not completely developed and are very costly and thus the cost of biodiesel produced is very high (Borowitzka & Borowitzka, 1988; Chisti, 2007). Compared to current fuel prices the cost of bio-diesel from microalgae has to be competitive and so the research focus should be on the development of low cost harvesting and extraction methods. This project will investigate a new technique of harvesting marine microalgae for the large scale biodiesel production and study the cost effectiveness of the method.

1.2 Thesis Organisation

Chapter 2 is a literature review on culturing and harvesting techniques for marine microalgae. It also highlights the parameters influencing the growth of microalgae and limitations of the present harvesting techniques. This chapter also identifies the knowledge gap and introduces a new technique of harvesting marine microalgae.

Chapters 3 and 4 describe the materials and methods and results for culturing marine microalgae species in outdoor raceway ponds and new harvesting technique implemented. The experimental design and procedure for pilot-scale harvesting setup is also explained in chapter 4.

Chapter 5 presents a several key factors in understanding the optimization, design, and cost analysis of the new harvesting technique and suggestions to overcome the process drawbacks.

Finally, conclusions of these studies are summarised in chapter 6, where future work will also be recommended.

Chapter 2. Literature Review

2.1 Culturing Microalgae

The commercial production of microalgae is more than 30 years old and the common microalgae species being grown are *Chlorella*, *Spirulina*, *Dunaliella salina* and *Haematococcus pluvialis*. All of these species grown commercially have great value in health food and pharmaceutical industry (Borowitzka, 1999) and currently as a renewable energy source. The photosynthetic growth of microalgae requires light, carbon dioxide, water and inorganic salts, and the energy is stored in the form of lipids in the cells (Chisti, 2007). A variety of microalgae species are grown in presence of the media which provides the microalgae with nitrates, phosphates, trace metals and vitamins, essential for growth. The recipes for different types of media necessary for the growth of particular species of microalgae are described in detail by Guillard and Ryther (Guillard & Ryther, 1962; Guillard, 1975).

Culturing microalgae is the first stage in the process of production of biodiesel form microalgae. Therefore, it is essential to study and understand the different types of culturing systems and the factors affecting the growth of microalgae.

2.1.1 Microalgae Culturing Systems

Microalgae can be cultured in laboratory starting from tissue culture flasks upto carboys, bags and tanks. Microalgae for aquaculture are generally cultured at small scale in 20-40L or in large plastic bags upto 1000L (Borowitzka, 1999). The commercial microalgae culturing systems include tanks, extensive open ponds, raceway ponds, fermenters and photobioreactors (Borowitzka, 1999). Most of the enclosed photobioreactors are tubular reactors, stirred tank reactor, air-lift reactor and bag cultures (Pohl *et al.*, 1988; Hu *et al.*, 1996; Pulz, 2001).

Table 2.1 shows the commercial microalgae culturing systems located in different countries, the species cultured and approximate volume. The maximum microalgae culture volume cultured reported is upto $1x10^9$L in extensive open ponds for the species *Dunaliella salina*. It is easy to culture marine microalgae in such large

extensive ponds because many marine microalgae are tolerant to the range of salinities and moreover high salt content is unfavourable for any bacteria and other harmful organisms. Therefore, this is one of the reasons for culturing marine microalgae at large scale, which provides large feedstock for the biodiesel production.

Table 2.1 Commercial microalgae culture systems currently in use different locations with approximate volume and the algal species cultured (Borowitzka, 1999)

Culture System	Algae	Approximate Volume (L)	Location
Tanks	Many species	1×10^4	World wide
Extensive open ponds	*Dunaliella salina*	1×10^9	Australia
Circular ponds with rotating arm	*Chlorella spp.*	1.5×10^4	Taiwan, Japan
Raceway ponds	*Chlorella spp., Spirulina spp., Dunaliella salina*	3×10^4	Japan, Taiwan, USA, Thailand, China, India, Vietnam, Chile, Israel
Cascade system with baffles	*Chlorella spp.*	3×10^4	Czech Republic, Bulgaria
Large Bags	Many species (used for aquaculture)	1×10^3	World wide
Fermenters (heterotrophic)	*Chlorella spp., Crypthecodinium cohnii*	$> 10^3$	Japan, Taiwan, Indonesia, USA

2.1.2 Factors Influencing the Growth of Microalgae

Light

Microalgae are capable of carrying out photosynthesis and cellular division in presence of light. The light intensity diminishes with the depth of the culture. Also if the biomass is highly concentrated, it would not allow light to pass through to the lower layers. Therefore effective use of light is most important concern while culturing microalgae (Richmond, 2004a). The light intensity of 2500-5000lux is optimal for the growth of microalgae (Hoff & Snell, 2001). Depending on the structure and species most algae respond to suitable wavelengths (Hoff & Snell, 2001). If the temperature and nutritional requirements are satisfied, light is the major limiting factor of productivity and growth (Richmond & Zou, 1999). However in outdoor raceway ponds, light is rarely the sole limiting factor for algal growth and production of biomass (Dodd, 1986).

Light-dark cycle

Photosynthesis is a process comprising two steps, light reactions that take place when the cells are illuminated, and carbon-fixation reactions. The carbon fixation reactions are also known as dark reactions that occur both in the presence and absence of light. In the light reactions microalgae absorb light energy and convert the light energy into chemical energy. This chemical energy is stored in the cells in the form of high energy compounds. The microalgae use this energy during the dark for carbon dioxide fixation reactions (Iverson, 2006).

Jacob-Lopes (2008) studied the effect of the photoperiod on the biomass production and carbon dioxide fixation rates using a photosynthetic culture of the cyanobacterium *Aphanothece microscopica Nägeli* in bubble column photobioreactors. The duration of the light regime was the important factor for the better performance of the photobioreactor. There was a linear reduction in the carbon dioxide fixation rate and biomass production with the reduction in light regime. However during the 12:12 (night: day) cycle the biomass production and carbon dioxide fixation was average, suggesting the pre-adaptation of the microalgae (Jacob-Lopes *et al.*, 2009).

Light-dark cycle is an uncontrollable factor for large scale open systems and therefore location and climate must be chosen, which provides maximum light regime to the microalgae.

Temperature

Microalgae have an optimal temperature for growth. Below the optimal temperature, plant growth rates increase with increase in temperature. Above the optimal temperature the growth rate decreases with increase in temperature because of the inactivation of the proteins and the cell components (Moisan *et al.*, 2002). Microalgae that grow better at higher temperatures have higher optimum growth temperature (Suzuki & Takahashi, 1995). Microalgae are capable of tolerating a range of temperatures and depending on the response to temperature variations, the nutritional requirements, cell composition and rates and nature of metabolism will vary (Richmond & Zou, 1999). Most algae can easily tolerate temperatures upto 15°C lower than their optimal; however rise in temperature by 2 to 4°C may damage the culture (Richmond & Zou, 1999).

Maintaining an optimum temperature for culturing the selected microalgae species at large scale is an essential requirement for maximum growth rate and lipid productivities in the process of biodiesel production.

Nutrients

The growth of microalgae is dependent on a proper balance of nutrients in the culture. The microalgae utilises light energy to obtain most of the nutritional needs from inorganic compounds. Usually microalgae species require phosphates, nitrates, and carbon for proper growth (Richmond, 1988). Similar to optimum temperature each microalgae have its own optimum nutrient concentration (Cronar & Fallowfield, 1997).

Kjell Reitan and Jose Rainuzzo (1994) studied the effect of nutrient limitation on fatty acid and lipid content in marine microalgae and found that increasing the extent of phosphorus limitation resulted in increased lipid content in the *Bacillariophyceae* and *Prymnesiophyceae* and decreased lipid content in the green flagellates *N. atomus* and *Tetraselmis* sp (Reitan *et al.*, 1994). Nutrient limitation is one of the most critical factors affecting the algal lipid metabolism, and most microalgae found respond to N-limitation by increasing their lipid content (Borowitzka, 1988b).

In the large scale culturing of microalgae for biodiesel production cost of nutrients contributes to the total costs, however, providing an optimum nutrient concentration is necessary for maximum lipid productivities and better growth rates.

Mixing

In the field of microalgal research, some excellent research have reported that the turbulence, mixing rate or stirring speed have very decisive affects on the growth of microalgae and high productivities (Borowitzka, 1996; Changhai *et al.*, 2005). However the study of fluid dynamics in microbial cultures is not given much importance. Although for efficient trapping of light by each cell mixing plays an important role. The more efficiency we achieve in this task the more will be the production of the biomass. The situation becomes more complex with the higher density of the cells and longer light path. Therefore, proper mixing is required for equal distribution of nutrients and light in the microalgal culture. To reduce the

complexity of the system Changahi and Yingying (2005) developed a relationship between the circulation velocity, cell density and the growth rate of *Parietochloris incisa* in 10 cm light-path flat plate photobioreactors (Changhai *et al.*, 2005). The results obtained clearly indicated the relationship between the liquid dynamic equations and the output. Thus, developing a mathematical equation could help in finding the optimal velocity for microalgal cultures in different systems.

Salinity

Every microalgae species have a optimum salinity range (Brand, 1984). The salinity will increase during hot conditions due to evaporation and decrease during rainfall. Although microalgae have been found to tolerate wide range of salinities, change in salinities due to evaporation and rainfall found to be the major factors affecting the growth of marine microalgae (Borowitzka & Borowitzka, 1990; Fabregas *et al.*, 2000).

Effect of pH

To explain the effect of change in pH, Goldman et al. (1982) studied two freshwater and two marine algal species grown in cultures with pH ranges from 7.6 to 10.6. The fresh water species grew well upto pH 10.6 and one of them was adversely affected by increased pH. The marine species however failed at pH 10.6. Studies show that marine species cannot tolerate pH values above 9.5, with an exception of few species like *Phaeodactylum tricornutum* (Goldman *et al.*, 1982). Microalgae are sensitive to pH changes and therefore pH control is essential for keeping the growth rates higher (Rocha *et al.*, 2003).

2.1.3 Summary

Outdoor raceway ponds and tanks are being widely used all over the world for culturing microalgae. These open ponds are more favourable to marine microalgae species growing at higher salinities because these species could, upto most extent, protect themselves from any bacteria or other harmful organisms, allowing large scale production of biomass.

It is essential to study the factors affecting the growth of microalgae cultures to analyse the productivities and their growth response to these factors. Also, study of factors like light, temperature, pH, nutrients, mixing and salinity in outdoor systems like raceway ponds is an important step in culturing microalgae to demonstrate the viability of the cultures in different conditions. Literature study shows that microalgae species have their own optimum range for these parameters and the species selected for culturing should be maintained at these optimum values for higher productivities and growth rates.

2.2 Harvesting Microalgae

Harvesting microalgae is the process of removal of algal biomass from the culture medium.

The harvesting of microalgae is one of the most difficult and thus far least satisfactorily resolved problems in algal mass culture (Benemann & Oswald, 1996). The algal mass (dry weight) in the microalgae cultures is very low and thus it becomes hard to remove lower mass contents from large amount of medium. There are many techniques cited in literature for harvesting marine microalgae but none of them are universally accepted as economic and efficient. However, sufficient information is available to develop promising techniques for low cost harvesting of microalgae (Benemann & Oswald, 1996).

The economic recovery of the microalgae biomass is an essential component for the production of biodiesel from microalgae feedstocks. A range of harvesting techniques is currently available and the aim of this review is to compare existing and future technologies and to report on the technical and economic considerations for each option. The commercially available harvesting techniques include centrifugation, filtration, sedimentation, flotation and flocculation.

2.2.1 Centrifugation

Centrifugal processes rely on the generation of a centrifugal force which accelerates the movement and separation of particles based on a density difference between the

particle and the surrounding medium. Centrifugation processes may be batch, semi batch or continuous. Equipment available for centrifugation is divided into fixed wall devices (hydroclone) and rotating wall devices (sedimenting centrifuges) (Shelef *et al.*, 1984). Among the various types of centrifuges, the major types of centrifuges used for harvesting microalgae include multi-chamber centrifuges, disc-stack centrifuges, nozzle centrifuges and decanters.

A multi-chamber centrifuge consists of vertical concentric cylinders. The feed is passed through these cylinders and is subjected to progressively higher accelerations. This results in deposition of larger particles at the inner chamber and finer particles at the outer chamber (Mohn, 1980b). A disc stack centrifuge is assembled with disc stack plates where the solids are removed continuously under gravitation and the clear liquid is collected from the top (Shelef *et al.*, 1984; Oswald, 1988b). A decanter centrifuge is very commonly used equipment for solid liquid separations. Decanter centrifuge consists of a solid cylindrical bowl rotating at high speed inside which a screw conveyor is fixed rotating at slower speed, the solids are removed by the conveying motion and the clear liquid leaves the bowl at the other end (Records & Sutherland, 2001). Table 2.2 gives a summary of energy requirements for different centrifugation processes.

Table 2.2 Energy requirements for different centrifugation equipments

Device	Energy requirements	Source
Multi-chamber centrifuges	15 kWh/m³	Borowitzka (1988)
Disc-stacked and nozzle	0.7-1.3 kWh/m³	Mohn (1980) and Oswald (1988)
Decanter	8 kWh/m³	Mohn (1980)
Hydrocyclones	0.3 kWh/m³	Mohn (1980)

In summary, the centrifugation process is most commonly used process for microalgae harvesting and have been well developed for the separation of microalgae. The high operational costs as well as high maintenance and capital costs has restricted its usage at large scale. The major costs for centrifugation are depreciation and maintenance costs.

2.2.2 Filtration

There are different types of filtration processes available however each of these processes is restricted with some design conditions or the size of microalgae or process requirements and must be developed further to increase the efficiency of filtration and reduce the cost. Filtration is suitable for small scale harvesting of microalgae because at large scale many equipment handling problems occur.

Operating filter presses for the recovery of microalgae are suitable only for large size of microalgae like *Coelastrum proboscideum* and *Spirulina platensis* but fail to recover organisms approaching bacterial dimensions e.g., *Scenedesmus*, *Dunaliella*, *Chlorella* (Mohn, 1978). Using the diaphragm filter small size microalgae like Scenedesmus can be harvested with upto 100% filtration efficiency (Borowitzka & Borowitzka, 1988) .

Vacuum band filters are suitable for microalgae with more coarse particles like *Spirulina*. The cost required for operating these filters is very high and therefore these are not suitable for large scale filtration (Borowitzka & Borowitzka, 1988).

The cylindrical sieving machines and vibrating sieves could not be operated because of the cost. Sand filters operate discontinuously because they have to be rinsed off after certain residence time. Newly modified sand filters can be cleaned continuously using mammoth pump, however fluidization is the problem (Borowitzka & Borowitzka, 1988).

Microfiltration and ultrafiltration are promising processes in the biotechnology industry for microalgae separation. Using ultrafiltration and microfiltration techniques for large-scale harvesting of microalgae is limited by membrane-fouling phenomena. (Rossignol *et al.*, 1999).

Rossignol (1999) also studied cross-flow microfiltration and ultrafiltration for the harvesting of two marine microalgae, *Haslea ostrearia* and *Skeletonema costatum*. In cross flow filtration the culture of solid liquid suspension runs parallel to the membrane and perpendicular to the permeation flux. From his study it was evident that the ultrafiltration proves to be more efficient at low pressures and low tangential

velocities for long term run than microfiltration. The cross flow filtration has many advantages over the conventional filtration, centrifugation, flocculation and flotation. Energy requirements, while dependent on operating pressures and feed characteristics have been estimate to be 3-10 $kWh.m^{-3}$ (Rossignol *et al.*, 1999). However more recently, energy costs of 0.38-0.51 $kWh.m^{-3}$ with permeate fluxes of ~$20L.m^{-2}.h^{-1}$ and concentration factors of 20-46 were reported (Danquah *et al.*, 2009).

For cross-flow filtration the major costs are membrane replacement and pumping. Ultrafiltration and microfiltration can be more cost-effective than centrifugation if only small volumes (e.g., <2 m3 day−1) are to be filtered. However for large scale production of biomass (e.g., >20 m3 day−1), centrifugation could be a more economic method of recovering the biomass. (Grima et al., 2002)

A summary of energy requirements of filtration techniques mentioned above is shown in Table 2.3.

Table 2.3 Energy requirements for different filtration equipments

Device	Energy requirements	Source
Filter presses	3-5 kWh/m³	Borowitzka (1988)
Vacuum band filter	34 kWh/m³	Borowitzka (1988)
Cylindrical sieve and vibrating sreens	3 kWh/m³	Borowitzka (1988)
Cross flow microfiltration	0.38-0.51kWh/m³	Danquah (2009)

Large scale harvesting of microalgae using filtration mainly depends on the filtration media and the properties of the feed. The filtration media and the properties of the feed also affect the capacity of the equipment and the cost. Membrane fouling and higher energy requirements are the greatest problems in filtration of microalgae as discussed earlier. Filtration processes like cross flow microfiltration could be one of the options for large scale harvesting, but further development and modifications are required to lower the equipment handling cost and maintenance cost.

2.2.3 Sedimentation

Sedimentation is a simple process of solid-liquid separation under gravity. Sedimentation processes are primarily divided into two types- clarification and thickening. In clarification the clarity of the overflow is of major importance and the

feed suspension is usually dilute. In thickening the thickness of underflow is of more importance and the feed slurry is usually more concentrated (Svarovsky, 1979; Svarovsky, 1985).

The major advantages of sedimentation are low power requirement, low design cost and low manpower requirement. The sedimentation rates depend on cell density, cell size, cell motility and type of water flow (turbulent/laminar). In sedimentation the recoveries are too low for large scale harvesting however sedimentation can be used as one of the primary harvesting process to concentrate the microalgae.

2.2.4 Flotation

Flotation is a gravity separation process based on the attachment of air or gas bubbles to solid particles, which then are carried to the liquid surface and accumulate as float which can be skimmed off. The success of flotation depends on the instability of the suspended particles. The lower the instability the higher is the air particle contacts. The attachment of an air bubble to a particle depends on air, solid and aqueous phases contact angle. Smaller air bubbles are more efficient because of larger surface area per unit volume and lower buoyancy (Koren & Syversen, 1995).

Froth flotation

In the process of froth flotation air is continuously pumped into a flotation cell. The bubble size in froth flotation is large and results in lower flotation efficiencies. The pH of the culture can also govern the stability of the air-microalgae mixture and while concentration factors of between 50 and 200 have been reported (Levin *et al.*, 1962a). Not much literature is available on usage of froth flotation for large scale harvesting of microalgae, this process may not be suitable for harvesting microalgae due to lower flotation efficiencies. In today's currency a cost estimate from information provided by Levin *et al*. (1962) is \$AU 0.46 kg^{-1} of dry algae.

Dissolved air flotation

Dissolved air flotation is a separation process for the removal of solids by introducing a stream of fine air bubbles. Air supersaturated water is injected under pressure into a flotation cell. Higher floatation efficiencies are obtained due to micro-fine bubbles. Favourable flotation conditions for bubble attachment or adhesion to particles requires

a reduction in the charge of particles (Edzwald, 1995), therefore, usually DAF is operated with chemical flocculants. Operating costs of DAF systems are usually higher than costs of sedimentation units due to higher cost requirements for producing micro-fine air bubbles and use of chemical flocculants.

Bio-flotation

Bio-flotation of microalgae by photosynthetically produced dissolved oxygen can be a rapid and effective harvesting technique. It can be used in conjunction with chemical flocculation and removal of 80–90% of algal cells can be achieved in high rate raceway ponds (Koopman & Lincoln, 1983). The minimum dissolved oxygen concentration reportedly required is 14 mg/L (Chillie *et al.*, 1966). This value approximates twice the concentration of dissolved oxygen in seawater at 20°C. During the night time the amount of dissolved oxygen is minimal due to restricted photosynthesis. Therefore continuous harvesting is not a feasible option using this process.

2.2.5 Flocculation

One of the most promising harvesting techniques of the microalgae produced appears to be flocculation. Microalgal cells carry a negative charge that prevents aggregation of cells in suspension. The surface charge can be neutralized or reduced by adding flocculants such as multivalent cations and cationic polymers to the broth (Grima *et al.*, 2003). Ideally, the flocculants used should be inexpensive, non-toxic, and effective in low concentration. In addition, the flocculant should be selected so that further downstream processing is not adversely affected by its use.

Auto flocculation

Photosynthesis in microalgae increases the pH of the water. Increased pH can result in the precipitation of the Mg, Ca and phosphate ions (Becker, 1994). These precipitates form a chemical flocculating agent as these salts have a positive surface charge and may interact with the negative charge on the microalgae causing flocculation of the algal species. For this process the pH needs to be maintained above 10 which is not suitable for cultures maintained at lower pH.

Chemical flocculation

In the past few decades, a lot of research is done on flocculating microalgae using different chemicals (Shelef *et al.*, 1984; Zhu *et al.*, 2005) in waste water treatment and production of microalgal feeds. Study shows that the most economic option is addition of alum(Becker, 1994). Use of chemical flocculation in harvesting microalgae from waste water, fresh water and brackish water has been phenomenal. However, at high ionic strengths or high salinities the flocculation process is inhibited by reduction in chemical activity of the flocculants, masking of the active sites and shrinkage of polymer molecules to its smallest dimensions (Bilanovic *et al.*, 1988; Sukenik *et al.*, 1988; Becker, 1994). Thus, it is evident that very high chemical dosage is required to flocculate marine microalgae with high salinities, experimental results show that for *Isochrysis galbana* and *Chlorella stigmatophora*, an alum or ferric chloride dosage of 5 to 10 times higher was required for flocculation compared to fresh water cultures (Sukenik *et al.*, 1988). Additions of high concentration of chemicals to the microalgae feed may result in high contamination in the supernatant or recycle.

Usually after the addition of flocculants like alum to the microalgae feed a constant mixing is provided for upto 3 minutes for the formation of sufficiently large flocs and then tiny air bubbles are diffused to assist the flocs to float to the surface and then the flocs are scrapped off. This process of flocculation and flotation can be achieved in a dissolved air flotation unit (Becker, 1994).

Chemical flocculants could be organic or inorganic, an example of organic chemical flocculant is chitosan, a chemical agent obtained by de-acetylation of Beta-N-acetyl-D-glucosamine, when added to few species of microalgae without adjusting the pH, 100 % flocculation efficiency was obtained at Chitosan concentrations above 40mg/l (Morales *et al.*, 1985).

The major concerns with the use of chemical flocculation are high operating costs, cost of chemicals and residual metal salts dissolved in the growth media and metal salts incorporated in recovered biomass. In1988 the Aquatic Species Program concluded that chemical flocculation was too expensive for the production of biofuels from microalgae (Sheehan *et al.*, 1998).

Bio-flocculation

Bio-flocculation is spontaneous flocculation of the algal cells due to secretion of extra cellular polymeric substances (EPS) under stress conditions (Shipin *et al.*, 1999). Mishra and Jha (2009) found that under salt stress microalgae like Dunaliella salina excrete EPSs, and the concentration increases with increase in concentration of salt (Mishra & Jha, 2009). Many EPS/EBF (extracellular biopolymeric flocculants) producing microorganisms include microalgae, bacteria, fungi and yeast (Salehizadeh & Shojaosadati, 2001), therefore bio-flocculation is also known as microbial flocculation. Lee A. K. et al studied microbial flocculation as a separation technique for harvesting marine microalgae by using organic carbon as a substrate for the growth of flocculating microbes, recovery efficiencies of over 90% and concentration factor of 226 were achieved (Lee *et al.*, 2008).

In microbial flocculation micro-organisms like bacteria are added to the culture medium to form bulk mass with the algal species. Because of the harmless and biodegradable intermediates, recently the use of microbial flocculants has been promoted as a solution to environmental problems (Yim *et al.*, 2007).

Further research is to be done to find suitable micro-organisms that are efficient in producing EPSs at high salinities and other growth conditions, for this process to be used at large scale as a primary harvesting method.

2.2.6 Electroflocculation

Electroflocculation is a common process for flocculating contaminants, organic matters and metal ions from waste water (El-Naas *et al.*, 2009). Most of the literature available on electroflocculation technique is in the waste water treatment (Mollah *et al.*, 2004; Escobar *et al.*, 2006) as well as hard water treatment (Malakootian & Yousefi, 2009).

Electroflocculation is the process involving electrolytic addition of coagulating metal ions directly from sacrificial electrodes. The metal ions then coagulate/adsorb the contaminants, ultrafine particles, oil drops and organic matters like algae, these coagulated agglomerates/precipitates then attach to the gas bubbles or are captured by

the gas bubbles released during the process of electrolysis and floated to the top (Koren & Syversen, 1995; Poelman *et al.*, 1997; Robinson, 2000).

Microalgae carry a negative charge that prevents aggregation of cells in suspension (Grima *et al.*, 2002). P. Sridhar and his colleagues (1987) studied electroflocculation for removal of algal cells, using a bipolar cell with aluminium electrodes, in reservoir water. Results showed that electroflocculation effectively removed the algal population from the reservoir water with a carry-over of Al^{3+} ions (Sridhar *et al.*, 1988). In the method of electroflocculation, electrode dissolution and deposition takes place in the electrolyte/microalgae culture solution. The oxidation and reduction reaction take place at the surface of the electrodes. The anode plays a sacrificial electrode that donates positive metal ions into the solution. The positive ions then combine with the negatively charged microalgae to form flocs and these flocs eventually float to the top with the gas bubbles released at the cathodes and form a thin green mat that can be easily scrapped off. When the gas bubbles in the green mat escape the flocs settle to the bottom due to higher density.

To understand the process of electroflocculation it is really important to study the factors affecting the process of electroflocculation. The major factors controlling the process of electroflocculation are current, voltage, time, current density, electrode material, surface area of electrodes, pH and salinity (conductivity) of the solution.

Current, Voltage and Time

Current, voltage and time are three important factors that determine the power or cost required for electroflocculation. The power consumed by the electroflocculation unit is the product of voltage and current (Koren & Syversen, 1995). Amount of current passed through the culture solution will determine the ion concentration of the metal ions in the culture required to flocculate a certain volume of culture. At a fixed value of current the voltage will vary depending on the resistance offered by the culture solution. Lower the resistance offered by the culture solution less will be the voltage required.

Time plays an important role in determining the power required in kWh for complete flocculation of the microalgae without damaging the microalgae cells by charge flow. The power required can be calculated using equation 2.1,

$$P = \frac{V \times I \times t}{1000} (2.1)$$

Where,

P= Power consumption (kWh)

V= Voltage (V)

I= Current (A)

t= time (h)

Electrode material

The common material used in most electroflocculation processes is aluminium, and better removal efficiencies were achieved using aluminium (Novikova & Shkorbatova, 1982; Vik *et al.*, 1984; Sridhar *et al.*, 1988; Poelman *et al.*, 1997; Robinson, 2000; Mameri *et al.*, 2001; Schulz *et al.*, 2009) however, not all of the references listed tried all the possible metal combinations. Use of iron(Fe) rather than aluminium is also recommended as one of the better electrode materials due to fouling of aluminium and slower removal efficiencies (Kobya *et al.*, 2003; Schulz *et al.*, 2009; Zodi *et al.*, 2009), however these results were based on short term experiments. Balkan and Kolesnikova studied Al and Fe as anodic material for the removal of organic material from water and found that Fe electrodes gave higher efficiency compared to Al electrodes, the reason stated is the larger size of initial Fe^{3+} ions (10-30μm) compared to Al^{3+} ions (0.05-1μm)(Balkan & Kolesnikova, 1996), this conclusion was based on a single experiment using chemical adsorption of oxygen as the only measure of separation of organic matter. Balmer and Foulds studied different electrode materials such as alloys of aluminium, iron, steel, copper, brass, bronze and phosphor bronze to determine the effect of different metal ions on the floc formation and separation, and found that all materials produced enough floccs and gave a high

degree of separation. However, they recommended that the cheapest and easily accessible electrode materials should be used (Balmer & Foulds, 1986).

In the large scale harvesting of marine microalgae for biodiesel production, the electrode material plays an important role in the removal efficiency, cost, downstream treatment of the floccs, reuse of the supernatant and disposal of the final residual biomass. Therefore considering these parameters into account the material chosen should be- highly efficient in removal of microalgae, easily available and cheap, should not interfere with the extraction process, safe for the growth of the microalgae when the supernatant is reused and non-toxic to the environment when the residual biomass is disposed or able to be used as animal fodder.

Surface area of electrodes

The surface area of electrodes will determine the size of electroflocculation setup. More the surface area of electrodes, higher will be the volume between the electrodes if the distance is kept constant and large volume of microalgae culture will be flocculated, although it will affect the current density and surface to volume ratio. According to Holt et al. 1999, the surface area to volume ratio (S/V) is an important scale-up parameter, and the optimal values reported are between 15-45 (m^2/m^3) (Holt *et al.*, 1999). As the S/V ratio increases the optimal current density decreases(Mameri & Yeddou, 1998).

Thus for large scale electroflocculation setup the surface area of electrodes could be determined by estimating the optimal S/V ratio and an array of bipolar electrodes is recommended for the flocculation of maximum volume of microalgae culture.

Current density

Current density is the ratio of current delivered to the electrode to the active surface area of the electrode (A/m^2). Current density determines the amount of metal ions released at the anode, the electrolytic bubble density production (Holt *et al.*, 1999) and time required for flocculation of the microalgae cells. In most literature optimal current densities ranging from 10-50A/m^2 have been reported (Holt *et al.*, 1999; Kobya *et al.*, 2003; Xu & Zhu, 2004), although Holt et al reported that higher current

densities upto 150A/m^2 may be required for processes involving flotation cells or large settling tanks.

Current density can be easily maintained by varying the current, however for a particular process an optimal current density should be estimated by experimentation. The current density could vary depending on the charge on the microalgae (zeta potential), the bubble density required for the proper flotation of the floccs and allowed concentration of metal ions in the supernatant and in the biomass flocculated. Current density also determines the power required therefore cost and time are important factors to be considered before finalising the optimal current density.

Distance between electrodes

Distance is directly proportional to voltage, so less the distance between the electrodes lower will be the required voltage to maintain the required amount of current flow/current density in the culture solution, however less volume of culture will be flocculated between the two electrodes. The optimal inter-electrode distance for electroflocculation processes reported is 0.5-3.0cm (Mameri & Yeddou, 1998; Daneshvar *et al.*, 2004) in few cases the optimal inter-electrode distance was gauged by optimizing the current density and time for maximum removal efficiency (Daneshvar *et al.*, 2004; Xu & Zhu, 2004).

Series of experiments needs to be performed to optimise the distance between electrodes for maximum culture flocculation at minimum power requirement. Shorter inter-electrode distances can cause short circuit due to high current density, therefore a distance of 1cm should be preferred as the shortest distance.

pH

The culture pH is an important factor in determining the efficiency and performance of the electroflocculation process. From literature it is evident that pH ranges from 6 to 8 are effective for electroflocculation(Holt *et al.*, 1999; Xu & Zhu, 2004). Few research studies show that a pH of 7 is optimal for electroflocculation processes (Escobar *et al.*, 2006; Irdemez *et al.*, 2006; Ghernaout *et al.*, 2008).

The culture pH determines the nature of reactions of the metal ion complexes in the solution and solubility of the product formed. The pH of a culture can be easily maintained by adding acid or base. The microalgae cultures used in this project are already maintained around pH 7.

Salinity (Conductivity)

Salinity is a measure of conductivity offered to the amount of current flow. Therefore, at higher salinities less power is consumed to maintain the required amount of current flow through the microalgae cultures (Kashefialasl *et al.*, 2006). However, not all marine microalgae can be grown at higher salinities, so the cost of electroflocculation will be higher for low salinity cultures of marine microalgae.

2.2.7 Summary

Literature review shows that there is no commercial cost effective harvesting technique available for harvesting marine microalgae at large scale in the process of production of biodiesel.

After considering all the harvesting options it was concluded that 'electroflocculation' was not used as a harvesting technique in the process of biodiesel production. Most of the work on electroflocculation has been done in waste water treatment. The method of electroflocculation as a harvesting technique for marine microalgae requires study and experimentation of the process and all the affecting factors.

This thesis will discuss electroflocculation as an effective way of harvesting marine microalgae in the process of large scale production of biodiesel from marine microalgae.

2.3 Objectives of the Research

The main objective of this research is to study electroflocculation as a cost effective technique for harvesting marine microalgae at large scale. The process of electroflocculation will be applied to two marine microalgae species MUR230 and MUR232 cultured in outdoor raceway ponds. The process parameters like current, voltage, time, material of electrodes, distance between electrodes and surface area of electrodes will be studied at lab-scale. Pilot-scale experiments will be performed to demonstrate the viability and cost effectiveness of electroflocculation process.

The secondary objective of the research is to successfully culture the species of MUR230 and MUR232 in out door raceway ponds upto 400L volume. As mentioned earlier it is really important to study the productivities and behavioural patterns of microalgae species outside the laboratory in order to be able to modelled at large scale. A study of change in the productivity and growth rate of marine microalgae species MUR230 and MUR232 with change in atmospheric parameters will be undertaken for a long term period of more than 10 months. The parameters like pH, D.O, temperature, rainfall, evaporation, and photosynthetically active radiation will be measured to compare the productivities and growth rates with changes in these parameters and study the behavioural patterns.

Chapter 3 will discuss the whole process of culturing the species MUR230 and MUR232 from lab-scale to out door raceway ponds and the results on productivities, growth rates and study of different parameters in out door raceway ponds.

Chapter 3. Culturing marine microalgae species MUR230 and MUR232

3.1 Introduction

Reliability and stability of microalgae cultures is most important requirement for commercial large-scale culturing for microalgal biomass to be used as a feedstock for the production of biodiesel from microalgae. This chapter will discuss the reliability and stability of two marine microalgae cultures over a period of one year.

5ml samples of the marine microalgae species MUR230 and MUR232 were obtained from Murdoch University, Perth, WA. These species were cultured at the University of Adelaide starting in laboratory with 1ml inoculum in the tissue culture flasks and cultured upto 400L in outdoor raceway ponds. The lab-scale culturing was commenced for a period of three months starting in October 2008 upto December 2009. The outdoor raceway ponds were managed for a period of nine months starting in January 2009 upto September 2009. The procedure for culturing these species from 1ml to 400L, preparation of media, managing and monitoring the outdoor raceway ponds and results for productivity and ash free dry weights (AFDW) are included in this chapter.

3.2 Materials and Methods

3.2.1 Procedure for Culturing MUR230 & MUR232 upto 1L

Initially 10 ml cultures of both species, MUR230 & MUR232, were inoculated. These cultures were inoculated in 50ml tissue culture flasks to maintain the shallowness of the media, by adding 1ml of microalgae samples to 9 ml F-media (For recipe please see section 3.2.3).

The culture tubes were kept on the shaker tables under light to enhance the growth of the microalgae. According to information obtained from Murdoch University, Perth the conditions required for growth of MUR230 & MUR232 are specified as follows:

circulation pattern: 90 oscillations per minute, light intensity: 2500-5000 lux, temperature: 16-27 °C, salinity: 6-7% wt/vol.

The growth of the microalgae was calculated by calculating the cell density using the Neubauer Improved Brand GMBH+ haemocytometer. (Procedure for counting cells using haemocytometer explained below, section 3.3) When the cell density in the culture tubes was in the exponential phase the inoculants were transferred into five 50ml culture tubes (2 ml each) again to prepare 10ml inoculants as the backup of the species and kept under required growth conditions on the shaker table. The ten 10ml inoculants in 50ml flasks of both species were then transferred into 250ml flasks and cultured to 100ml (10 ml inoculant + 90 ml media) and again grown under required growth conditions. Out of the 100ml inoculums 10ml was removed to prepare 100ml cultures (for both species as backup) and remaining 90ml inoculants were then used to prepare 1000ml culture in 2L aerated flasks. Out of 1000ml inoculants 100ml was removed to prepare backup of 1000 ml culture and remaining 900 ml was used to inoculate 15L carboys. These carboys were then used to culture microalgae in 400L raceway ponds.

Figure 3.1Cultures of MUR230 and MUR232 inoculated in the laboratory from 1ml to 1L.

3.2.2 Procedure and Setup for Culturing MUR230 and MUR232 in 15L Carboys

30 litres of seawater was treated with charcoal, 5g of charcoal per litre, in two 20 litre plastic carboys. The seawater and charcoal were mixed for one hour using an electric stirrer. Addition of charcoal to the seawater removes any organic matter from the seawater. The charcoal treated sea water was then filtered through 2 layers of Whatman No.1 filter paper using the vacuum pump setup. This removes large particles of charcoal from the seawater. The seawater was further filtered through .45 micron cellulose acetate filter to remove minute particles of charcoal, unwanted

material and bacteria from the seawater. Now 30g salt per litre of seawater was added to adjust the salinity to approximately 7% for the growth of MUR strains.

To prepare F-media 2ml of nitrates and trace metal solutions were added per litre of seawater. This mixture was autoclaved for 20 min at 120°C. After autoclaving 2ml of phosphates was added per litre of seawater (phosphates were added after autoclaving to prevent precipitation). This F-media was now ready for inoculation. Now the 20L carboys were washed with detergent and autoclaved. The tubing, air stone, plastic joints and 500ml conical flask were also autoclaved to avoid any harmful bacteria from entering into the carboys.

Setup for the photobioreactors: A 0.25µm air filter was attached to the air pump. The filtered dry air was passed through a tube to a 500ml conical flask containing R.O water with a stopper having inlet and outlet tubes. A valve was attached to the tube at the entrance of the conical flask to control the air flow. The input to the conical flask was filtered dry air and output was filtered humid air. The humid air then passed through the tubing connected with a T- joint into two carboys. Two air stones were attached to the end of the two tubes in the carboys for proper dispersion of air. The tubing running into the carboys was hold by the cotton plugs at the entrance of the carboys. This whole setup was placed in a light cabinet with light intensity of more than 2500lux.

The carboys were inoculated to 15L in three steps. Initially 900 ml of inoculum of both species was added to the designated carboys which were then inoculated to 5 litres by adding four litres of media to it. After few days (one week) when the cell count was in the range of 10^6, 5L of media was again added to take the inoculum upto 10L. Similarly after one more week the volume was increased to 15L.

Figure 3.2 15L Cultures of MUR230 and MUR232 cultured from 1L flasks

These 15 litres of inoculum in the carboys were now ready to inoculate the outdoor raceway ponds.

3.2.3 Culturing MUR230 and MUR232 in Outdoor Raceway Ponds

The research done by Terry and Raymond (1985) shows that high productivity of microalgal biomass can be obtained in outdoor raceway ponds. Outdoor raceway ponds are open ponds with a mixing system and a chamber to direct the culture. The culture mixing serves a variety of purposes, including prevention of cell settling, elimination of thermal stratification, distribution of nutrients and carbon dioxide, removal of photosynthetically produced oxygen and enhancement of light utilization efficiency (Terry & Raymond, 1985).

The Figure 3.3 below shows the Outdoor raceway ponds located on the roof of Earth & Environmental Sciences department at the University of Adelaide, Australia. Dimensions of the pond shown above are 0.4x1.0x2.0m (height x width x length). The capacity of these ponds is 800L however these ponds were cultured to the volume of

400L to maintain the shallowness of the culture and prevent spillage during the mixing and high rainfall.

Figure 3.3 Outdoor raceway ponds located on the roof, at University of Adelaide

Initially the microalgae species MUR230 and MUR232 were cultured in laboratory in the carboys upto 15L with cell count of $5x10^5$cells/ml as described in section 3.1.2. These 15L cultures of both species were used as inoculum for the out door raceway ponds. Natural filtered seawater (30µm) was collected from SARDI located on west coast of Adelaide in a 1000L tanker. The seawater was then pumped into the 200L black drums using a submersible pump. These drums were placed on the roof near the ponds. No further filtration of seawater was done at this scale.

Now the F-media for culturing microalgae was prepared (see section 3.2.5). The salinity of seawater was adjusted to 7.0% wt/vol by adding pool salt. Initially the ponds were inoculated by adding 15L inoculum of each species to approximately 200L of media in each pond. Approximately after a week when the cell concentration reached upto $6x10^5$ cells/ml the ponds were inoculated to 400L. This stage onwards both the ponds were maintained at 400L as a final culture volume. For the proper mixing of the cultures the speed of paddle wheels for both the ponds is maintained at 40rpm.

The growth of the cultures was recorded by counting the number of cells everyday in the morning. The cells were counted using the haemocytometer under the microscope (see section 3.2.6). Simultaneously, the salinity of the cultures was measured everyday and for the amount of evaporation rate, the ponds were topped up by fresh water to maintain the salinity at 7% wt/vol. The salinity was measured using the aqua depot salinity refractometer. Data logger (Unidata Pro Logger with Magpie data logging system) was installed on the roof near the ponds to log the temperature (Probes- Starlog 6536B), D.O (Probes- OxyGuard 420), pH (Probes- Greenspan PH100), electrical conductivity/salinity (Probes- Starlog 6536B) and PAR (photosynthetic active radiation). The data loggers sensed these parameters every 5 seconds and averaged it over 10 minute time interval and the values were logged every 10 minutes. The readings were downloaded weekly. Carbon dioxide was supplied to the ponds on the roof to increase the productivity of the microalgae cultures. The CO_2 cylinder was placed on the ground locked in a cage open to the atmosphere. The CO_2 was supplied to the roof through copper tubing running from the cylinders.

Depending on the growth rate of the microalgae the amount of culture to be removed and timeline of harvesting was decided. Upon harvesting of the particular amount of culture the ponds were refilled back to 400 L and cell density of 60x10^4 were maintained as constant every time after harvesting.

Figure 3.4 Cultures of MUR230 and MUR232 inoculated in the outdoor raceway ponds upto 400L from 15L inoculum

3.2.4 Procedure for Calculating the Harvest Volume

The daily cell count of both the species MUR230 and MUR232 gave the concentration of the species each day. Strategically for maintenance purposes both the species were harvested on a weekly basis. The harvest volume was decided on the basis of current cell concentration (cell count at the time of harvesting) of the species and the base concentration of $60x10^4$cells/ml. Following is the formula for the calculation of the harvest volume.

$$V = \frac{(x-60)\times 10^4\,(cells/ml)\times 400L}{x\times 10^4\,(cells/ml)} \quad (3.1)$$

Where,

V= Volume to be harvested (L)

x = Cell concentration at the time of harvesting (cells/ml)

(The base cell count of $60x10^4$ can be changed as per harvesting strategy/growth phase)

3.2.5 Preparation of Media

The microalgae species MUR230 and MUR232 selected for this project require F-media for their growth. Preparation of F-media includes treatment of seawater, methodology for the preparation of F-media for MUR232 and MUR232, recipe for the F/2 medium.

Seawater treatment

Natural filtered seawater (30 micron) was acquired from SARDI located on west coast of Adelaide. The natural seawater was then stored in the dark at low temperature (~ 10 ^{0}C) to inhibit microbial and microalgal growth. For microalgae culturing, the required amount of seawater was tapped and then treated as follows:

[Procedure for treatment of seawater is obtained from Murdoch University, No treatment was required for culturing of the outdoor raceway ponds]

5g of activated charcoal was added to every 10L of natural seawater and left to mix for 1hour. The charcoal was then left to settle overnight. The charcoal-treated seawater was then filtered through 3 layers of Whatman No.1 filter paper to remove large particles and the bulk of the charcoal. The filtered seawater was then filtered through 1 layer of cellulose ester filter paper (0.45 μm pore size) to remove residual charcoal and smaller particles/ organisms. The double-filtered seawater was then transferred into black carboys until further use. Seawater sterilisation was achieved by autoclaving the double-filtered seawater at 121°C for either 20min (for small volumes) or 1hour for 10L of liquid. After autoclaving, the sterile seawater was allowed to cool to room temperature and then stored in the dark in a cold room. The sterile seawater was once again brought to room temperature before adding nutrients for algal culturing.

Methodology for preparation of f-medium for MUR230 & MUR232

Initially the salinity of treated seawater was adjusted to 7%wt/vol. The f-medium nutrients were prepared in separate bottles as per the information obtained from University of Murdoch (Recipe for preparation of nutrients, see section 3.2.3). The

species MUR230 and MUR232 do not require silicate and vitamins for growth; therefore these components were omitted in the media preparation.

Each nutrient stock solution was autoclaved separately and allowed to cool to room temperature. For the trace metal stock solution, all the individual components were mixed in the appropriate ratios beforehand and autoclaved. The individual salt solutions do not need to be autoclaved.

For f-medium preparation, 2 ml of every sterile nutrient stock solution was added to every 1L of sterile treated seawater. One nutrient was added at a time and stirred well before addition of the next nutrient.

The medium was ready for algal culturing and any left-over medium was stored in the dark in a refrigerator.

Recipe for f/2 medium

f/2 Medium (Guillard & Ryther, 1962; Guillard, 1975)

This medium is commonly and widely used for marine microalgae especially diatoms, and added to seawater. The concentrations in the Table 3.1 below are half the concentrations required to prepare f-medium. Therefore, for the species MUR 230 and MUR 232 double the volume of f/2 medium was used to obtain the final required concentration of f-medium. MUR230 and MUR232 do not require vitamins and silicates for their growth therefore these nutrients are omitted from the Table 3.1.

For the growth of MUR230 and MUR232 the nutrients required are nitrates, phosphates and trace metals. These nutrients were prepared in R.O water upto 5L and preserved in cold and dark and removed whenever required. The concentration of each nutrient is given in Table 3.1 and the components of trace metal solution along with the required concentrations are given in Table 3.2. 2ml of each nutrient was added per liter of seawater to obtain the concentration of f-medium.

Table 3.1 Recipe for f/2-medium

Component	Stock Solution	Quantity	Molar Concentration in final medium
$NaNO_3$	75g/L	1ml	8.82×10^{-4} M
$NaH_2PO_4\ H_2O$	5g/L	1ml	3.62×10^{-5}
Trace metals	(See recipe below)	1ml	---

f/2 Trace Metal Solution

100ml stock of each of the metals required for the trace metal solution was prepared except $FeCl_3.6H_2O$ and $Na_2EDTA2.2H_2O$; these two metals were directly added to the trace metal stock solution of 5L.

Table 3.2 Recipe for Trace metal solution

Component	Stock Solution	Quantity	Molar Concentration in final medium
$FeCl_3$ $6H_2O$	---	3.15g	$8.82x10^{-4}M$
Na_2EDTA $2H_2O$	---	4.36g	$3.62x10^{-5}M$
$CuSO_4$ $5H_2O$	9.8g/L dH_2O	1ml	$3.93x10^{-8}M$
Na_2MoO_4 $2H_2O$	6.3g/L dH_2O	1ml	$2.60x10^{-8}M$
ZnSO4 $7H_2O$	22.0g/L dH_2O	1ml	$7.65x10^{-8}M$
$CoCl_2$ $6H_2O$	10.0g/L dH_2O	1ml	$4.20x10^{-8}M$
$MnCl_2$ $4H_2O$	180.0g/L dH_2O	1ml	$9.10x10^{-8}M$

3.2.6 Counting Cells Using a Hemacytometer under Light Microscope

There are different counting devices mentioned in the literature for counting cells like the Sedgwick-Rafter, Palmer-Maloney, Hemacytometer (0.2 and 0.1mm deep) used for counting cells(Andersen, 2005). For the species MUR230 and MUR232 a hemacytometer (Neubauer Improved Brand GMBH+) was used for counting the cells with the aid of a light microscope (Olympus IX50). The hemacytometer is a specialized microscope slide used to count cells, bacteria, organelles, etc. It has a thick base with grids on the surface; a coverslip is used to cover the grids. The coverslip is thick enough to stay flat under the pull of surface tension from the solution in the counting chambers. The hemacytometer has two chambers with nine 1mm squares in each chamber and several subdivisions of 250µm squares, 250x200 µm rectangles, 200 µm squares and 50 µm squares, this is a Neubauer ruling as shown in the figure below.

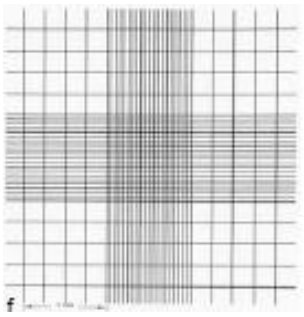

Figure 3.5 Neubauer ruling (Andersen, 2005)

To load the hemacytometer it is assembled by putting the coverslip in position. Now the microalgae samples are injected in both the chambers using a pipette. The centre square of the chamber is $1mm^2$, the cell counts for MUR230 and MUR232 were done by counting four of these squares. The cells counted in this square can be scaled upto cells per mL of sample by multiplying by a factor of 10^4.

3.2.7 Determination of Ash Free Dry Weight (AFDW)

The raceway ponds were maintained at a base cell concentration of $60x10^4$cells/ml. When the cell concentration increased to approximately $120x10^4$cells/ml, the required volume of culture to be harvested was calculated by using equation 3.1, and AFDW studies were undertaken on the harvested culture volume. Fresh media was added to the ponds to maintain the cell concentration at $60x10^4$cells/ml. Approximately 1L samples were taken from each pond after addition of the fresh media and AFDW studies were undertaken at this cell concentration.

200mL culture samples of both MUR230 and MUR232 were centrifuged in 250 mL conical tubes at 3750rpm for 10 minutes in a Beckman-Coulter centrifuge installed with a SX4750 swing bucket rotor. After centrifugation pellet was transferred into a pre-weighed glass vial. The biomass in the glass vial was dried overnight (or until constant weight) in a 70°C oven. The glass vial was re-weighed and the dry weight was recorded. The dry weight includes the weight of organic and inorganic material. Following the dry weight assay, the glass vial was placed in the furnace at 450°C for 8 to 10hrs. The glass vial was re-weighed and the ash content recorded. The AFDW was determined by the difference between the dry weight and ash. The above procedure was carried out for four 200mL samples of each species and the mean value was calculated and reported.

3.3 Results

3.3.1 Culturing MUR230 and MUR232 in Outdoor Raceway Ponds

The graphs in Figure 3.6 and Figure 3.7 represent the data for MUR230 and MUR232 cultures grown in outdoor raceway ponds over a period of 9 months. The data logger logged the parameters like temperature, DO, PAR, pH and conductivity (salinity).

From top to bottom in Figure 3.6 and Figure 3.7-
Graph 1 shows the daily PAR (solar irradiance) and temperature variance. The grey dots in the graph represent the PAR values. The average value of maximum irradiance was not noted as the data logger was not installed during summer. An average irradiance of 350µmols.s^{-1}.m^{-2} was observed during autumn whereas in winter the cultures received minimum average irradiance of 170µmols.s^{-1}.m^{-2}. The blue curve in the graph represents daily maximum temperature and pink curve indicates daily minimum temperature downloaded from the bureau of meteorology. Between the blue and pink line the blue data points represent the daily temperature data logged by the data logger. Minimum temperature of 5°C and maximum temperature of 45°C was noted.

Graph 2 indicates the daily change in pond height due to evaporation, rainfall and culture withdrawn for experimental purposes. An average evaporation of 20L/day from the $2m^2$ ponds with 400L volume was observed during summer, autumn and on windy days in winter. Lower evaporation rate was noted during winter and the culture volume increased during rainy days decreasing the salinity of the cultures.

Graph 3 is a plot of dissolved oxygen and growth curve. The black curve represents the growth curve. The growth curve was plotted using the daily cell count data. An average doubling time of approximately one week and two weeks was observed during summer and winter respectively, while maintaining the minimum culture concentration at $60x10^4$cells/ml. The average dissolved oxygen value represented by red curve was approximately 12mg/L during the day and 8mg/L at night. The fall in dissolved oxygen values was due to the restricted rate of photosynthesis during night.

Salinity and pH readings are plotted in graph 4. Initially when the data logger was not installed the salinity readings were calculated by measuring the refractive index of sea water using a refractometer represented by the dots once every week. Later on data logger logged the daily salinity readings with the help of conductivity probe installed in the ponds. The continuous black line represents the salinity data, logged by the data logger. The salinity of both the cultures MUR230 and MUR232 was maintained approximately around 7%wt/vol. However, decrease in salinity during heavy rainfall diluted the cultures and a minimum salinity of 5%wt/vol was noted. Similarly due to

evaporation when the ponds were not topped up with fresh water the salinity increased to 8%wt/vol. The cultures showed a stable growth at these minimum and maximum salinities. The red curve represents pH values logged by the data logger using the pH probes installed in the raceway ponds. The average pH values noted before CO_2 was supplied was approximately around 8.5, when the CO_2 supply was started for the proper growth of the species the pH was maintained at 7 using a solenoid valve to control the flow of CO_2.

Graph 5 presents the specific growth rate plotted using the daily values of cell counts for both the cultures. Each point represents the exponential growth of the microalgae during approximately 24 hour period.

Graph 6 is a plot of productivity reported in $gm^{-2}day^{-1}$. The productivity values indicate the amount of AFDW produced by the microalgae between two harvest times. The blank spaces represent too low productivities or negative productivities due to rainfall and removal of culture volume for experimental purposes. Higher productivities of approximately $5gm^{-2}day^{-1}$ were observed during summer and autumn and lowest productivities approximately $1gm^{-2}day^{-1}$ was reported during winter.

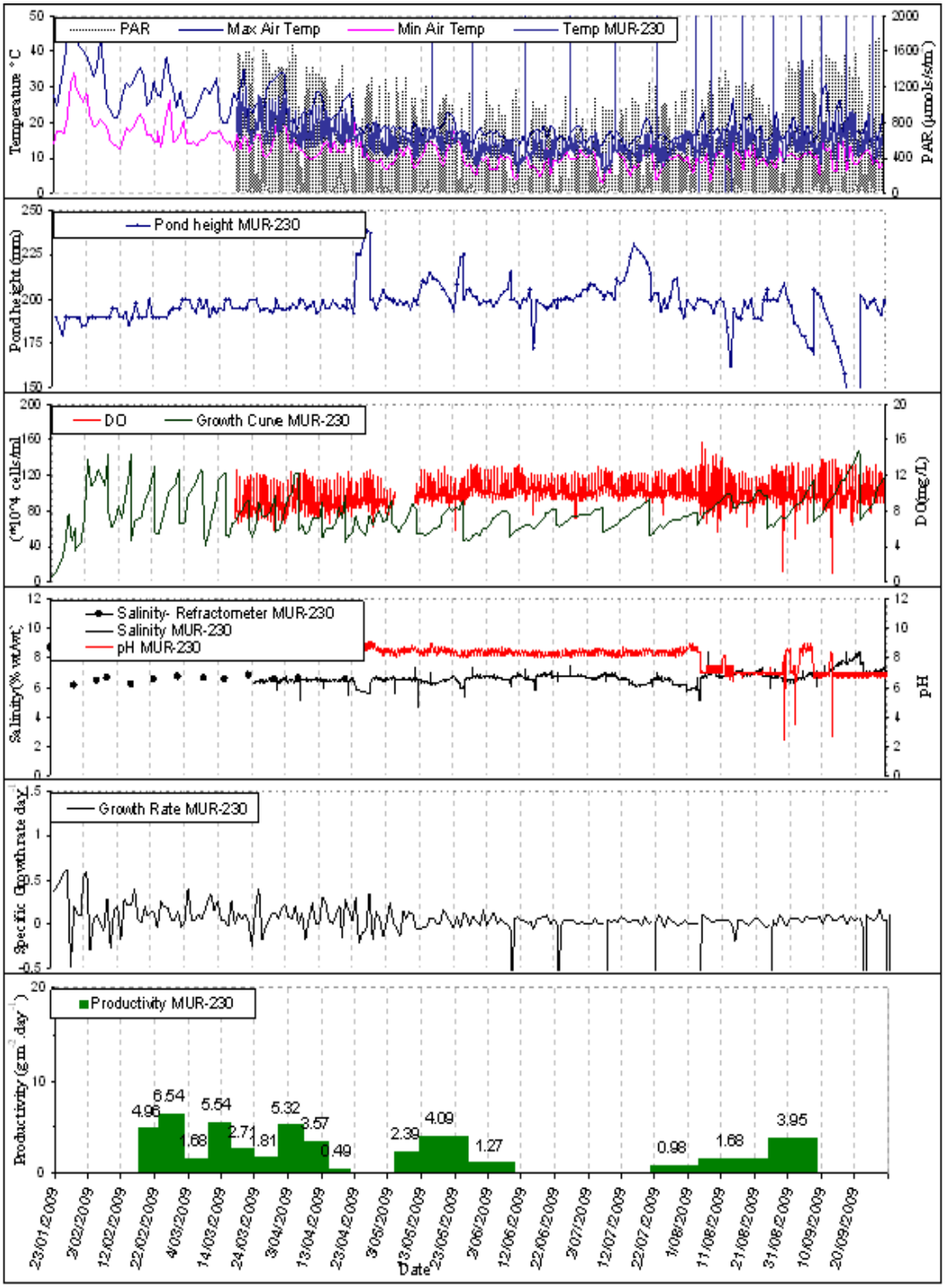

Figure 3.6 Pond data for MUR230

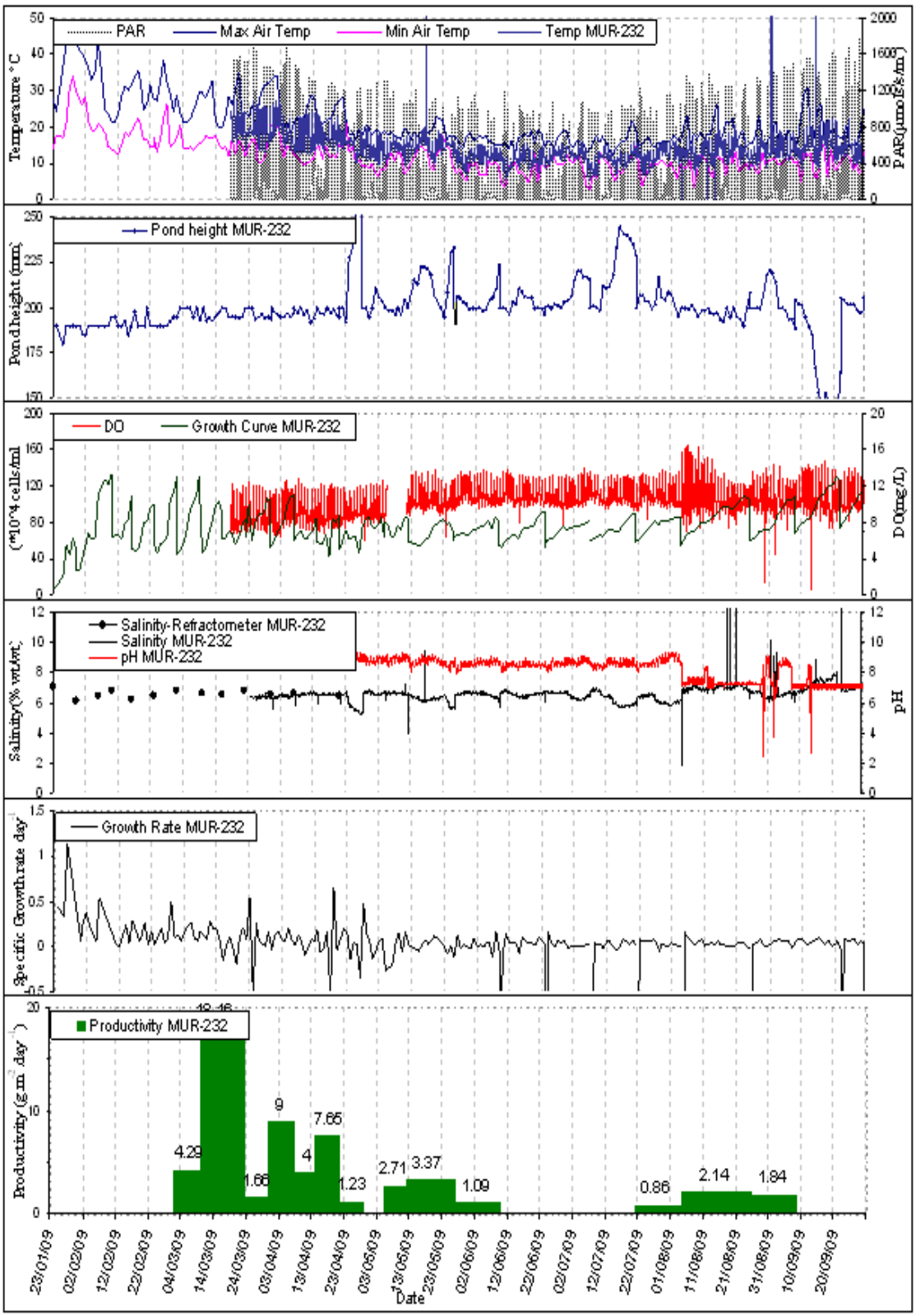

Figure 3.7 Pond data for MUR232

3.3.2 Ash Free Dry Weight (AFDW)

A summary of the AFDW results achieved for MUR230 and MUR232 grown in the outdoor raceway ponds, are reported in Table 3.3 and Table 3.4 respectively. Initially when the ponds were inoculated during summer, the AFDW studies were done on a weekly basis, when the growth rate was higher. During winter the growth rate decreased and the AFDW studies were done every fortnight. At harvest the AFDW determined was at a cell concentration of approximately $120x10^4$cells/ml. The AFDW determined after addition of media was at a cell concentration of approximately $60x10^4$cells/ml. Initially when only two replicates were studied (n=2) the standard deviation was higher (showing higher error in AFDW measurements), and was controlled by increasing the number of replicates to n=4. Average AFDW values of 300-500mg/L were measured for microalgae species MUR230 and higher AFDW values of 400-600mg/L were measured for MUR232.

Table 3.3 AFDW results for MUR230

Date	At harvest AFDW (mg/L)			After addition of media AFDW(mg/L)		
	(n=2)			(n=2)		
16/02/2009	412.5	±	70.7	160.0	±	106.1
23/02/2009	333.7	±	40.7	101.3	±	1.8
2/03/2009	330.0	±	14.1	258.8	±	19.4
9/03/2009	317.5	±	24.7	195.0	±	7.1
16/03/2009	361.2	±	19.4	240.0	±	0.0
23/03/2009	335.0	±	35.4	242.5	±	24.7
31/03/2009	315.0	±	3.54	233.75	±	26.52
7/04/2009	420.00	±	21.21	278.75	±	12.37
14/04/2009	403.75	±	40.66	370.00	±	21.21
21/04/2009	387.00	±	59.40	356.75	±	34.29
28/04/2009	325	±	10.61			
05/05/2009	326.25	±	12.37	261.25	±	5.30
12/05/2009	344.50	±	17.68	261.25	±	0.00
	(n=4)			(n=4)		
26/05/2009	547.50	±	54.85	472.50	±	25.33
9/06/2009	561.25	±	19.74	463.75	±	10.31
23/06/2009	465.00	±	12.25	405.00	±	8.16
21/07/2009	483.75	±	18.87	401.25	±	13.77
4/08/2009	470.00	±	96.87	352.50	±	13.23
25/08/2009	528.75	±	43.28	376.25	±	20.16
8/09/2009	652.50	±	31.22	406.25	±	48.37

Table 3.4 AFDW results for MUR232

Date	At harvest AFDW (mg/L)			After addition of media AFDW(mg/L)		
	(n=2)			(n=2)		
16/02/2009	348.8	±	72.5	163.8	±	8.8
23/02/2009	510.0	±	342.9	156.2	±	26.5
2/03/2009	1230.0	±	201.5	266.3	±	12.4
9/03/2009	416.3	±	12.4	223.8	±	8.8
16/03/2009	768.7	±	496.7	307.5	±	24.7
23/03/2009	945.0	±	756.6	408.8	±	5.3
31/03/2009	475.00	±	17.68	378.75	±	65.41
7/04/2009	693.75	±	295.22	405.00	±	0.00
14/04/2009	545.00	±	127.28	323.75	±	1.77
21/04/2009	591.63	±	36.24	453.12	±	9.37
28/04/2009	496.25	±	37.12			
05/05/2009	391.25	±	8.84	411.25	±	5.30
12/05/2009	506.00	±	7.78	357.88	±	10.08
		(n=4)			(n=4)	
26/05/2009	593.75	±	44.79	542.50	±	37.97
9/06/2009	618.75		41.51	515.00		12.25
21/07/2009	497.50		5.00	430.00		15.81
4/08/2009	490.00		1732	416.25		39.02
25/08/2009	641.25		29.55	471.25		40.29
8/09/2009	600.00		38.94	445.00		27.39

3.4 Conclusion

For the commercial large-scale culturing of microalgae it is essential to check the reliability and stability of the microalgae over a long term period. The marine microalgae species were cultured and monitored for a period of 3 months in the laboratory and 9 months in the outdoor raceway ponds.

Laboratory culturing was successfully undertaken starting with 5ml cultures of marine microalgae species MUR230 and MUR232 upto 20L carboys. The laboratory conditions like temperature, light and mixing speed were controlled and the species responded well under these conditions.

The major success was maintaining healthy cultures of MUR230 and MUR232 in outdoor raceway ponds under daily changing climatic conditions. Results show that the marine microalgae species of MUR230 and MUR232 were reliable and stable under these conditions.

- The microalgae species MUR230 and MUR232 can tolerate temperatures from 5°C in winter upto 45°C in summer. The growth rate and productivities decreased considerably during low temperature regime, which shows that these species grow better at higher temperatures between 30-40°C.
- The maximum solar irradiance required for the photosynthesis and growth of microalgae was available during summer and autumn.
- From the daily evaporative losses noted for outdoor raceway ponds we can conclude that at large scale this can make considerable difference for fresh water demand which will add to the total costs.
- An average doubling time of approximately one week and two weeks was observed during summer and winter respectively.
- The fall in dissolved oxygen values from 12mg/L in day to 8mg/L at night was due to the restricted rate of photosynthesis at night, which concludes longer light regimes is essential for higher productivities.
- The marine microalgae species MUR230 and MUR232 tolerated the salinities upto 8% and 5%wt/vol. Further studies for salinity tolerance have to be done to find the maximum and minimum tolerance limit of the cultures without crashing.
- It was difficult to study the effect of CO_2 on the productivity due to irregular harvesting schedule because of rainfall and culture removal for experimental purposes; however supply of CO_2 showed a positive impact on the growth of both the species.
- Average productivities of 1-5gm^2day^{-1} was recorded with average minimum productivities of 1gm^2day^{-1} during winter and average maximum productivities of 5gm^2day^{-1} during summer.
- An average AFDW of 0.3-0.6g/L was recorded for both the species.

Chapter 4 will discuss in detail the process of electroflocculation implemented for harvesting of the marine microalgae species MUR230 and MUR232 cultured in outdoor raceway ponds.

Chapter 4. Electroflocculation- A Potential Method of Harvesting Marine Microalgae

4.1 Introduction

Literature study shows that microalgae carry a negative charge (Becker, 1994; Grima *et al.*, 2002). The negatively charged microalgae are subjected to electric current flowing between to electrodes with a sacrificial metal anode. The positive metal ions from the sacrificial anode interact with or attract the microalgae, forming flocs or clumps. The flocs attach to the gas bubbles released during electrolysis of the culture solution and float to the surface, leaving behind a clear supernatant.

Experiments were performed at lab-scale and pilot-scale to apply the principle of electroflocculation to the microalgae species MUR230 and MUR232.

4.1.1 Factors Affecting Electroflocculation

Current, Voltage and Time

Current, voltage and time are three important factors that determine the power or cost required for electroflocculation. Amount of current passed through the culture solution will determine the ion concentration of the metal ions in the culture required to flocculate a certain volume of culture. The current tolerance limit for the microalgae was determined by measuring the maximum value of current that microalgae can tolerate during flocculation without getting damaged. At a fixed value of current the voltage will vary depending on the resistance offered by the culture solution. Lower the resistance offered by the culture solution less will be the voltage required. Time plays an important role in determining the power required in kWh for complete flocculation of the microalgae without damaging the microalgae cells by charge flow.

Current density

Current density is an important factor that determines the amount of metal ions released into the culture solution, density of bubbles released and the time required for

flocculation of the microalgae. In this project for lab-scale experiments current density was not given much importance because the initial aim was to justify the concept of electroflocculation for the species MUR230 and MUR232.

Electrode Material

The common material of electrode for electroflocculation experiments currently being used is Aluminium. Study is done with different electrode materials that include- aluminium, mild steel, SS-304, SS-316, copper and bronze to determine the effect of different metal ions on the floc formation and composition of supernatant.

Surface area of electrodes

The surface area of electrodes will determine the size of electroflocculation setup. More the surface area of electrodes, higher will be the volume between the electrodes and large volume of microalgae culture will be flocculated. The change in surface area of electrodes will affect the voltage. So, the surface area of electrodes can be varied depending on the load of the feed.

Distance between electrodes

Distance is directly proportional to voltage, so less the distance between the electrodes lower will be the required voltage to maintain the required amount of current flow through the culture solution, however less volume of culture will be flocculated. Series of experiments is performed to optimise the distance, for maximum culture flocculation at minimum power requirement.

pH

The optimal pH required for the electroflocculation process mentioned in literature is 7 (see section 2.3.6). The MUR230 and MUR232 cultures were maintained at pH 7 and therefore there was no need to adjust the pH before electroflocculation.

Salinity

Salinity is a measure of resistance offered to the amount of current flow. Therefore, at higher salinities less power is consumed to maintain the required amount of current flow through the microalgae cultures. However, not all marine microalgae can be

grown at higher salinities, so the cost of electroflocculation will definitely be higher for low salinity cultures of marine microalgae.

4.2 Materials and methods

4.2.1 Laboratory Study of Factors Affecting Electroflocculation

A series of experiments were undertaken to study the affects of current, voltage, time; electrode material, electrode surface area and distance between electrodes on electroflocculation performance. The experiments were performed in 1L cylindrical flask, Figure 4.1 shows the picture of lab-scale experimental setup and circuit diagram for the setup.

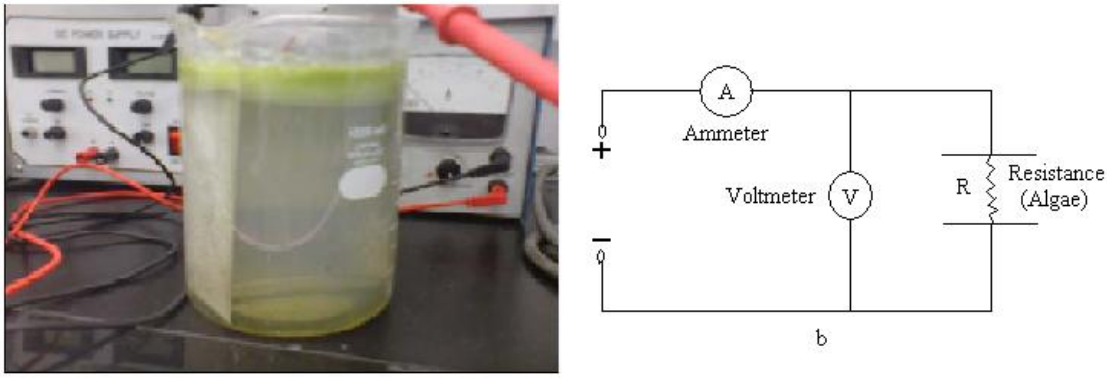

Figure 4.1 a. Lab-scale 1L electroflocculation setup b. Circuit diagram for the setup

Current, voltage and time

The electroflocculation experiments were performed in a 1L cylindrical flask with aluminium electrodes with fixed working electrode dimensions of 12.5cm x 7.5cm and 0.5 mm thickness. Initially a culture sample of MUR-230 was subjected to low current (0.1-1.0A) to study the minimum current required for electroflocculation. The current was then increased from 5A to 50A, in steps of 5A, to investigate the current tolerance limit of the microalgae and the time required for complete flocculation.

A heavy duty D.C supply with a current limit of 50A and voltage limit of 150V was used. Current and voltage values were noted from the ammeter and voltmeter, assembled in the D.C supply (Hewlett Packard6477C).

Current density

The current densities for lab-scale experiments were fixed at a higher value of more than 1000A/m^2, knowing that these values are practically invalid at large scale. The sole purpose of the lab-scale experiments was to study the electroflocculation process at lab-scale and evaluate the process. Therefore, the results presented below are reported for the current and voltage values.

Electrode Material

Electroflocculation experiments were performed for different materials of electrodes viz. SS316, SS304, aluminium, graphite, mild steel, galvanized steel, copper & bronze. The experiments were performed in a 1L cylindrical flask with all electrodes having a fixed working electrode dimension of 12.5cm x 7.5cm and 0.5mm thickness.

A D.C supply with current limit of 10A and voltage limit of 25V was used to perform the experiments. Current and voltage values were noted from the ammeter and voltmeter, assembled in the D.C supply.

Surface area of electrodes

Two experiments were performed with Aluminium electrodes of different surface areas. The thickness of electrodes in both scenarios was kept constant as 0.5 mm.

First experiment: Electrode surface areas of 87.5cm2.

Second experiment: Electrode surface areas of 43.75cm2.

A D.C supply with current limit of 10A and voltage limit of 25V was used to perform the experiments. Current and voltage values were noted from the ammeter and voltmeter, assembled in the D.C supply.

Distance between electrodes

A rectangular leak proof plastic box embedded with movable aluminium electrodes of surface area 30cm^2 was used to perform the experiments. The setup shown in figure 4.2 was prepared by the workshop at the School of Chemical Engineering workshop, University of Adelaide. The voltage required and current was recorded when the

position of the electrodes were moved. Due to the design of the box the time required for complete flocculation could not be determined.

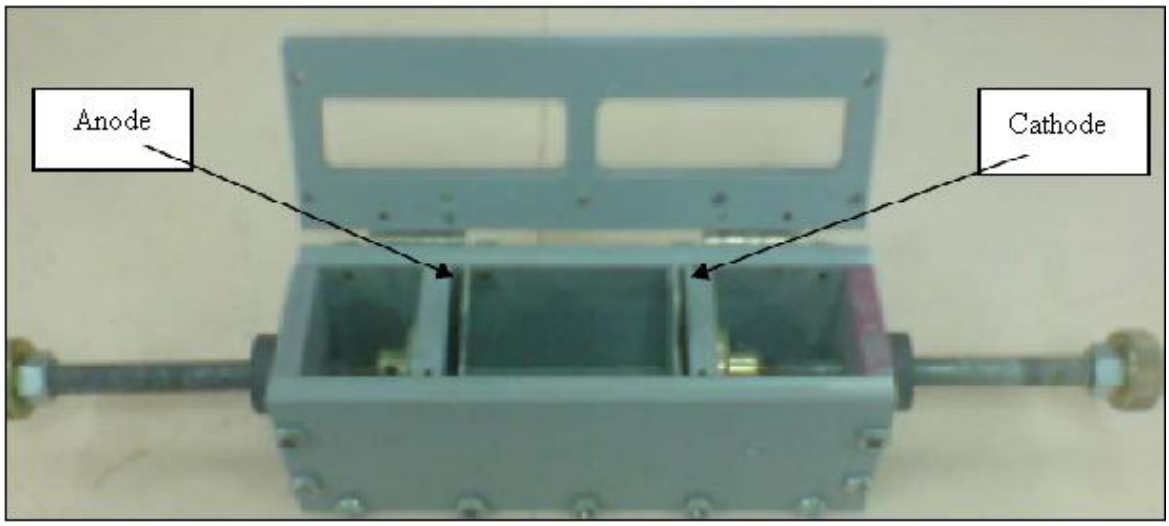

Figure 4.2 Equipment for the study of distance between electrodes

A D.C supply with current limit of 10A and voltage limit of 25V was used to perform the experiments. Current and voltage values were noted from the ammeter and voltmeter, assembled in the D.C supply.

Salinity

Two experiments were performed with salinity of the cultures maintained at 7% and 3.5 %. The experiments were performed in a 1L cylindrical flask with two aluminium electrodes having a fixed working surface area of 12.5cm x 7.5cm and 0.5mm thickness.

A D.C supply with current limit of 10A and voltage limit of 25V was used to perform the experiments. Current and voltage values were noted from the ammeter and voltmeter, assembled in the D.C supply.

4.2.2 Pilot-scale Study of Electroflocculation

A 100L electroflocculation module was constructed by the School of Chemical Engineering Workshop, at the University of Adelaide. The 100L module is shown in Figure 4.33, and was a replica of the design reported by E. Poelman et al (Poelman *et al.*, 1997). A series of experiments were undertaken to study the power requirement

using this setup for harvesting the species MUR 230 and MUR 232. The aim of the experiments was to compare the minimum power requirement of 0.3 kWh/m^3 claimed by E. Poelman et al, for removal efficiencies of more than 95%.

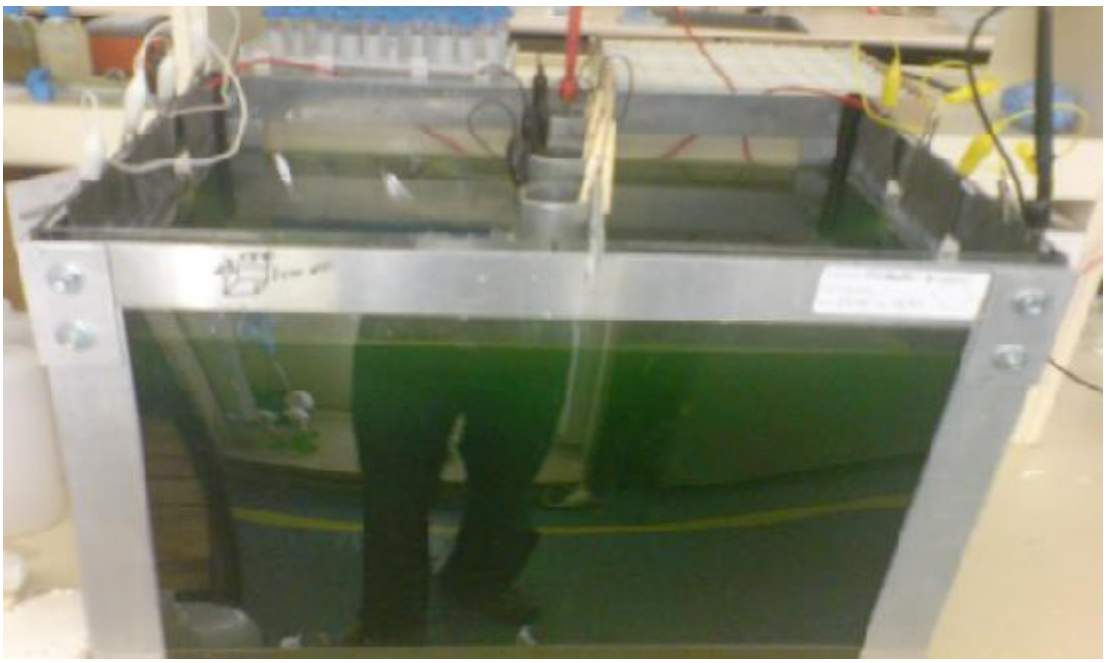

Figure 4.3 Electroflocculation setup at University of Adelaide with six lead cathodes and three aluminium anode tubes

The experiments were carried out in a 100 L vessel (LxWxH=59x42x50cm) made of transparent plastic material and reinforced with aluminium at the corners on the outer surface of the plastic to provide maximum strength. The electrodes were placed vertically and fixed to the walls with the help of wooden clips. Initially the cathodes used were lead sheets (50x10x0.1cm) and were placed along two opposite walls with three cathodes on each side of the anode. The anodes were hollow aluminium tubes (R x H = 2.5x50cm) and were placed in the centre of the vessel and held in position with the help of a sliding plastic sheet. The length of the electrodes submerged in the culture solution was 41cm.

Further experiments were carried out by using a combination of different types of electrodes as shown in Table 4.1. Table 4.1 gives the characteristics of different experiments with combination of electrodes, number of electrodes, distances between cathodes and anodes, voltage and current intensities applied. A D.C supply with current limit of 10A and voltage limit of 25V was used to perform the experiments.

Current and voltage values were noted from the ammeter and voltmeter, assembled in the D.C supply.

Table 4.1 Characteristics of different electroflocculation experiments

Experiment Name	Cathode Material	Cathode *T.A.S.A (cm^2)	Anode Material	Anode *T.A.S.A (cm^2)	Culture	Salinity (% wt/vol)
E.1	6 Pb sheets	2460	3 Al tubes	1931.1	MUR 230	6.94
E.2	6 Pb sheets	2460	3 Al tubes	1931.1	MUR 230	7.04
E.3	6 Pb sheets	2460	3 Al tubes	1931.1	MUR 232	7.02
E.4	2 Al plates	3362	1 Al plate	3206	MUR 230	7.04
E.5	6 Al plates	2460	3 Al tubes	1931.1	MUR 232	7.02

* T.A.S.A – Total active surface area of electrodes
Note 1- The distance between anodes and cathodes was kept constant at 29.5cm
Note 2- For experiment E.4 the anode was a Aluminium plate with equally spaced 4 slots of 1cm to allow free passage to the microalgae during electroflocculation

The cell concentration for the experiments for both the cultures was approximately 110x10^4cells/ml. The removal efficiency was measured by calculating the cells in the culture solution before flocculation and after flocculation. After electroflocculation 10 samples were taken with the help of a pipette near the bottom of the tank after 25 min and 30 min from the start of the experiments. The cell count was done using a haemocytometer by manually counting the cells under the microscope.

4.2.3 Recovery Efficiency and Concentration Factor

Recovery efficiency

Recovery efficiency is the ratio of amount of biomass removal after electroflocculation to the initial biomass concentration in the culture solution. The efficiency of electroflocculation process was determined by counting the number of cells in the culture solution before and after flocculation.

$$RE = \frac{V_0 C_0 - V_f C_f}{V_0 C_0} \times 100\% (4.1)$$

Where,

RE = Recovery efficiency

V_0 = Volume of culture before electroflocculation

C_0 = Concentration of cells in the culture before electroflocculation

V_f = Volume of supernatant

C_f = Concentration of cells in the supernatant

Concentration factor

Concentration factor is the ratio of initial volume of the culture to the volume of the floccs after electroflocculation and determines the amount of water removed from the biomass. Concentration factor was determined by measuring the bed volume of the flocculated microalgae.

$$CF = \frac{V_0}{V_b} (4.2)$$

CF = Concentration factor

V_0= Volume of culture before electroflocculation

V_b= Bed volume of the floccs after electroflocculation

4.3 Results and discussion

4.3.1 Laboratory Study of Factors Affecting Electroflocculation

The initial biomass concentration used in the following investigations was approximately 60 x 10^4cells/mL.

Current, Voltage and Time

A current of 0.1A was observed to initiate electroflocculation, although the electroflocculation rate was very slow the time taken for flocculation was several hours, which is very inefficient (exact hours not noted because the experiment was stopped after 1hr, where only partial flocculation was achieved). Increasing the current increased the voltage and reduced flocculation time, which indicates that more current allows more ions to pass through the culture solution and time required for forming floccs, is reduced. A voltage of 5.5V was required to achieve a current of 5A, and under this condition complete flocculation (complete flocculation in the thesis refers to removal efficiencies of 90-100%) of a fresh culture was achieved in 4mins.

Fresh cultures were then subjected to increasing currents of 5A, 10A, 15A, 20A, 25A, 30A, 35A, 40A and 50A. As shown in Figure 4.4, the time required for flocculating the microalgae decreased linearly with increase in current up to 25A. However, when the set current was 25A, and above, the time required for complete flocculation remained constant at 30seconds. Figure 4.4 shows linear increase in voltage with increase in current upto 35A. At currents above 35A the required voltage began to increase gradually.

Figure 4.5 shows the power consumption for the increasing values of current density for complete flocculation of 1L culture of MUR230. Increasing the current density upto 1000Am^{-2} decreased the power required for complete flocculation. Increase in current densities after this point increased the power requirements because higher voltage was required to maintain higher current densities and time required for complete flocculation was independent after current densities of 2500Am^{-2}.

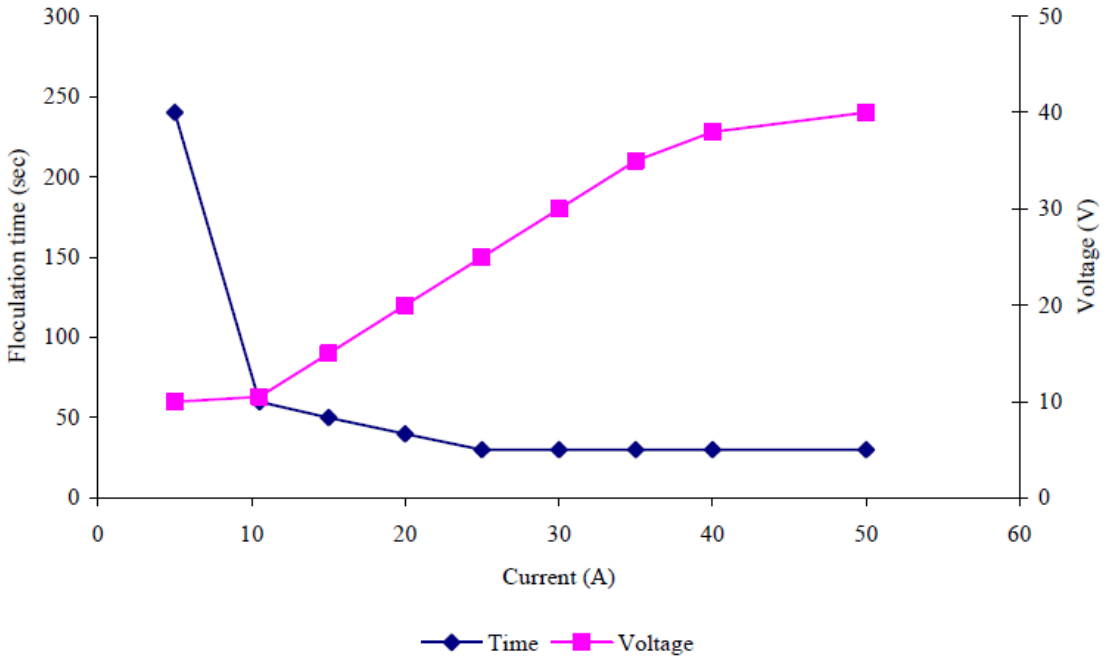

Figure 4.4 Effect of current on time and voltage required for complete flocculation of 1L culture of MUR230; Electrode material: Aluminium; Electrode surface area 93.75cm^2 ; Distance between electrodes:7cm; DC power supply 50A/150V

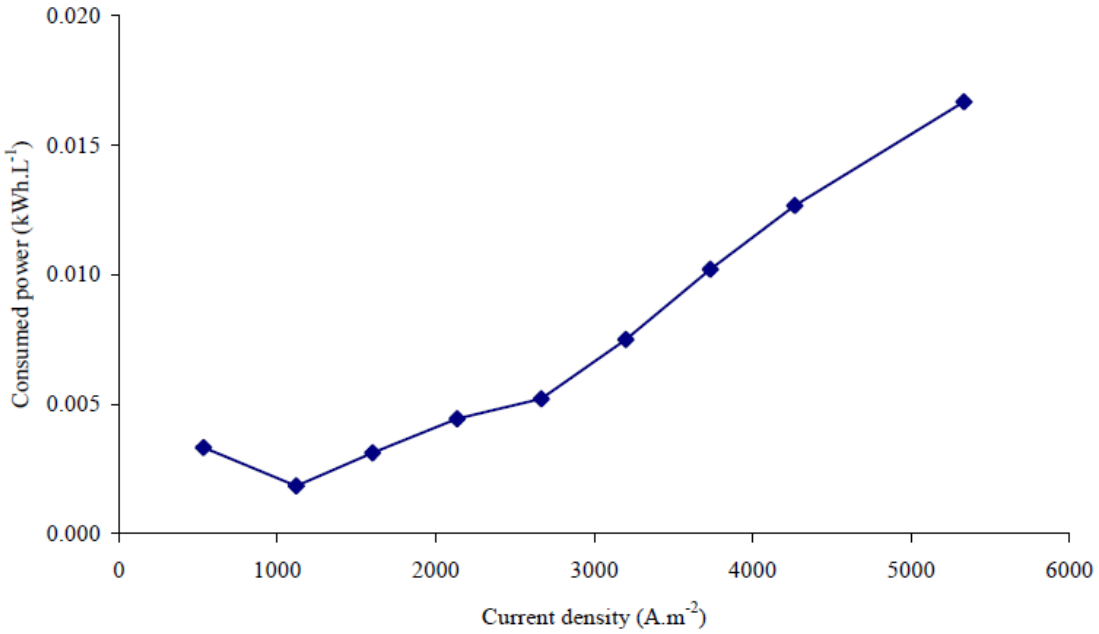

Figure 4.5 Power required for electroflocculation of MUR230 for increasing current density (calculation based on data from a 1L culture); Electrode material: Aluminium; Electrode surface area: 93.75cm^2; Distance between electrodes:7cm; DC power supply 50A/150V

The minimum power required for flocculation of 1L culture of MUR230 was 0.00183kWh/L, at current of 10.5A, voltage of 10.5V and time required for complete flocculation was 60 seconds. However, the volume between electrodes was only 656mL, yet 1,000mL of culture was flocculated. Therefore the water convections surrounding the electrode are important. Therefore, normalising the time to treat the 656mL (volume between electrodes), yield 39.36seconds.

Electrode Material

Table 4.2 shows the effect of different materials on the flocculation of MUR232. Identical results were obtained for MUR230 (not reported). With aluminium and galvanized steel the flocculation process was faster and clear supernatant was derived, also the process of formation of floccs was much better than other electrodes and the floccs floated faster to the surface compared to other electrode materials when the power was switched off. Figures 4.6-4.12 show flocculation results with different material of electrodes on MUR230 cultures.

Table 4.2 Electroflocculation experiments performed with different electrode materials

Electrode Material	Voltage (V)	Current (A)	Time (min)	Distance between electrodes(cm)	Flocculation Results
SS-304	12.8	10.54	1	7	Light green supernatant, good flocculation
Graphite*	27.6	8.86	1	8	No flocculation, culture burnt
SS-316	11.8	10.5	1	7	Light brown supernatant, good flocculation
Mild Steel	10.2	10.53	1	7	Dark green supernatant, good flocculation
Aluminium	9.2	10.5	1	7	Clear supernatant, very good flocculation
Galvanized Steel	9.5	10.52	1	7	Clear supernatant, very good flocculation
Copper	12.9	10.5	1	7	Dark green supernatant, average flocculation
Bronze*	17-28	10.3-8	1	7	Dark green supernatant, average flocculation

* indicated that the DC supply switched from constant current (10.5A) to constant voltage

Figure 4.6 Electroflocculation with SS-304 electrodes

Figure 4.7 Electroflocculation with SS-316 electrodes

Figure 4.8 Electroflocculation with Mild Steel electrodes

Figure 4.9 Electroflocculation with Aluminium electrodes

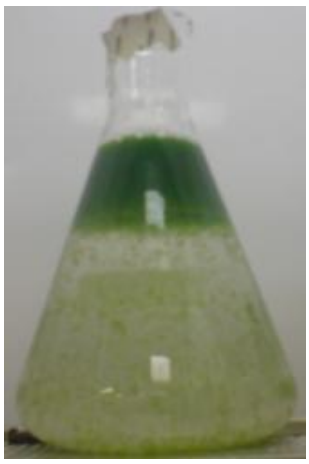

Figure 4.10 Electroflocculation with Galvanized steel electrodes

Figure 4.11 Electroflocculation with Copper electrodes

Figure 4.12 Electroflocculation with Bronze electrodes

Surface area of electrodes

The results from the two experiments are reported in Table 4.3. Reducing the surface area of the electrodes increased voltage and time required for complete electroflocculation. The output from the DC power supply was current controlled and thus remained constant. The two data points indicate that, the power required increased by approximately twice the value when the surface area of electrodes was cut down to half of the original surface area.

Table 4.3 Effect of surface area of electrodes on the power requirement

Electrode surface area(cm^2)	Culture Volume (L)	Distance (cm)	Voltage (V)	Current (A)	Time (min)	Power (kWh)
87.5	1	7	10.5	10.5	1	0.00183
43.75	1	7	11.9	10.5	1.5	0.00312

Distance between electrodes

Figure 4.13 shows the effect of changing distance between electrodes on the current and voltage in the electroflocculation system. From the graph it is seen that the voltage reached its maximum value at a distance of 8cm i.e after 8cm the D.C supply was running under constant voltage mode. Upto 8cm with increasing distance the current decreases gradually however, there is a gradual rise in the voltage due to an increase in resistance between the paths. The time required for complete flocculation of the cultures was not noted because of the design issues with the experimental setup, therefore the power requirement was not calculated. However, minimum distance provides minimum power requirement if the time is kept constant.

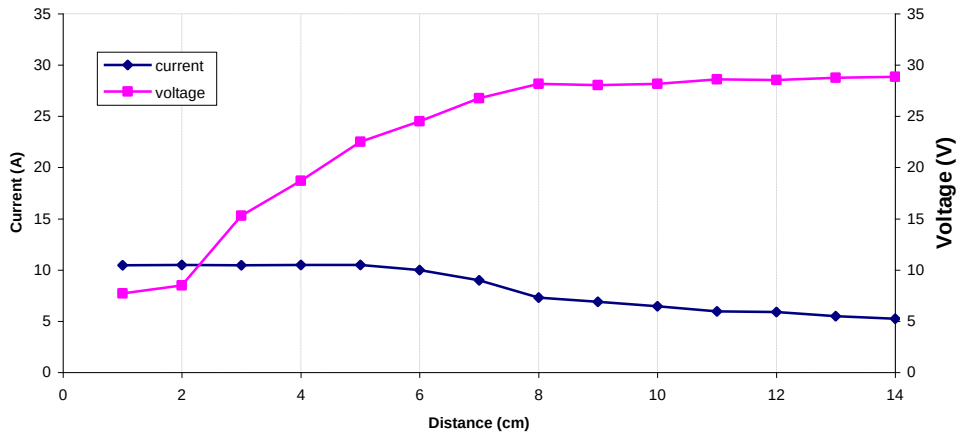

Figure 4.13 Effect of change in distance between electrodes on current and voltage

Salinity

Table 4.4 below shows the results obtained from electroflocculation of 1L samples of MUR230 at salinities 3.5% and 7%.

Table 4.4 Effect of salinity on the power requirement for electroflocculation

Salinity(%)	Current(A)	Voltage(V)	Time(min)	Power(kWh)
3.5	10.5	16.5	1	0.00288
7	10.5	11	1	0.00192

It was observed that the voltage decreased when the salinity was doubled; therefore the power requirement was higher at lower salinities. Results show that increase in salinity offers lower resistance to the current flow and thus results in low power usage.

4.3.2 Pilot-scale Study of Electroflocculation

The results of experiments described in Table 4.1 are summarized in Table 4.5. First experiment was performed to maintain a current flow 1A through the culture which required a voltage of only 1.5V in contrast to 26.5V reported by E.Poelman et al (Poelman *et al.*, 1997) the cultures did not flocculate at this current intensity even after 75min of electroflocculation. For later experiments the voltage was tuned to its highest limit on the D.C power supply to obtain maximum current output represented by values in Table 4.5 for experiments E.2 to E.5. The minimum power requirement of 0.168kWh/m^3 was noted for experiment E.4 with more than 95% removal

efficiency and concentration factor of 25 times was measured after 30 minutes. This power required was half the minimum power required by E.Poelman et al of 0.33kWh/m^3 for his experiment for the same removal efficiencies.

Table 4.5 Results for pilot-scale electroflocculation experiments

Experiment Name	Voltage (V)	Current (A)	Time (min)	Power (kWh/m^3)	Removal Efficiency (%)	Concentration Factor
E.1	1.5	1	75	0.018	0-10	-
E.2	4.3	9.6	30	0.206	> 95	>25
E.3	4.6	9.6	30	0.220	> 95	>25
E.4	3.5	9.6	30	0.168	> 95	>25
E.5	3.9	9.5	30	0.185	> 95	>25

4.3.3 Recovery Efficiency and Concentration Factor

The recovery efficiency and concentration factor was calculated from equation 4.1 and equation 4.2 respectively. The initial concentration of cells for the species MUR-230 was approximately 60x10^4cells/ml.

Table 4.6 Results for recovery efficiency and concentration factor

Microalgae Strain	Volume	Recovery efficiency	Concentration factor
MUR230	1L	90-99%(1-15min)	20
MUR232	1L	90-99%(1-15min)	20

The recovery efficiency varies with time. The recovery efficiency of 90% was noted at 1 min after flocculation and 99% at 15 min after flocculation. The more time (up to 15 min) given for the flocs to float or settle the better is the recovery of cells in the flocs. The concentration factor varies depending on whether the flocs are floated or settled. High concentration factor of 20 was measured for floated flocs compared to concentration factor of 10-15 when the flocs were settled.

4.4 Conclusions

4.4.1 Laboratory Study of Factors Affecting Electroflocculation

Current, voltage and time

From the experiments it was inferred that a minimal current of 0.1 A is enough to flocculate the microalgae however the time taken would be very inefficient. The current tolerated by the microalgae was 50A; however, the maximum current tolerance limit was not determined due to the limit of the D.C power supply. At and after 25A the 1L microalgae culture only took 30secs for complete flocculation, which indicates that there is a process of physical attachment of available charges on the microalgae and amount of Al^{3+} ions dissolved, that take specific amount of time to form flocs. For 1L culture of MUR230 the optimal values of current and voltage were 10.5A and 10.5V for a flocculation time of 1min. The current density based on the optimal value of current and electrode surface was 666A/m^2, which is not appropriate at large scale. Based on the optimal values of current, voltage and time the power required was 0.00183kWh/L.

Electrode material

Results from Table 4.2 and Figure 4.6-4.10 show the effect of different electrode materials on the floccs and supernatant. The change in colour of supernatant with change in electrode material could be due to the different ions of different metals dissolved in the solution during flocculation. Aluminium and galvanized steel gave the best results of flocculation with a clear supernatant and good floccs. However, the life of galvanized steel electrodes is finished as soon as the zinc layer is deteriorated. Therefore, Aluminium is the best option for electroflocculation at large scale.

Surface area of electrodes

Surface area plays an important role in determining the amount of ions dissolved into the culture solution, thus for more surface area more ions will be available for the microalgae to stick to and this will reduce the time required for electroflocculation, thus reducing the power requirement. Also from Table 4.3 it is evident that increase in

surface area reduces the voltage required to maintain the same amount of current flow through the cultures. Thus surface area of electrodes is an important factor to be considered in designing the electroflocculation process at large scale.

Distance between electrodes

Distance governs the voltage value because it represents the resistance between the electrodes. From Figure 4.13 we can conclude that minimum distance of 1cm to 3cm will provide an optimal power requirement. Similar to the surface area of electrodes distance plays an important role in determining the power, capacity and cost required for the electroflocculation process.

Salinity

Salinity is a measure of conductivity. Table 4.4 shows the importance of the conductivity of the culture solution. Higher salinity of the microalgae cultures provides less resistance to the current path and thus decreases the voltage requirement. Therefore, high salinity microalgae cultures require less power compared fresh water cultures.

Laboratory results conclude that the power required for harvesting marine microalgae using electroflocculation could be as low as 0.00183kWh/L or 1.83kWh/m^3.

4.4.2 Pilot-scale Study of Electroflocculation

The 100L electroflocculation setup constructed at University of Adelaide, with specially designed aluminium electrodes and high salinity cultures of MUR230 and MUR232 required a power input 0.168kWh/m^3 compared to 0.33kWh/m^3 reported by E.Poelman (Poelman *et al.*, 1997). Results show that changing the parameters like electrode materials, surface area and electrode design can significantly affect the power requirement for electroflocculation.

Optimisation of the process parameters is necessary to further reduce the costs at large scale.

4.4.3 Recovery Efficiency and Concentration Factor

Results for lab-scale and pilot scale studies show that recovery efficiencies of more than 90% and concentration factor of 20-25 times can be achieved using the electroflocculation as a harvesting technique.

4.4.4 Summary

In summary the study of electroflocculation as a harvesting technique was successfully commenced on the microalgae species MUR230 and MUR232. Although tremendous amount of research and experiments are required to explore all the possible combination of electrodes, optimize the distance between electrodes and surface area, design of cathodes and anodes, current density and time requirements to successfully use electroflocculation for large scale harvesting of microalgae.

Chapter 5 is a discussion on the theoretical method of optimization and design of the electroflocculation process. No experiments were undertaken for optimization and design of the electroflocculation process due to time limitation; however chapter 5 will discuss the essential steps to be followed for optimization and design of the electroflocculation process in future. The cost analysis and process drawbacks are also discussed in chapter 5.

Chapter 5. Electroflocculation – Optimization, Design, Cost Analysis and Process Drawbacks

5.1 Introduction

As described in previous chapters results conclude that electroflocculation is a promising technique for harvesting marine microalgae, although optimization of the process parameters is required for minimum cost, maximum removal efficiencies and high concentration factors at large scale. The optimisation of these parameters has to be undertaken in future. This chapter will also discuss the optimization and design theory, cost analysis and drawbacks of the electroflocculation process for harvesting marine microalgae.

5.2 Optimization of Electroflocculation Process

5.2.1 Optimization of Current Density

Current density can be easily maintained by varying the current, however for a particular process an optimal current density should be estimated by experimentation. Optimal current density depends on the charge on the microalgae, salinity of the culture (conductivity) and bubble density required for the flotation of floccs. For a particular volume of microalgae to be flocculated the current density can be optimised by measuring the percentage removal efficiency of the microalgal biomass. The increase in current density will increase the power requirement, although there will be an optimal current density for the lowest total cost of investment and operation for that particular equipment/volume.

Figure 5.1 shows a typical graph for the determination of optimal current density. At lower current densities, rate of biomass removal is lower due to fewer metal ions reacting with the microalgae and high power is required for good removal efficiencies due to longer time periods. Similarly at higher current densities power required is much higher due to high current and voltage required, although the time required for maximum removal efficiencies will decrease. There will be an optimal value in

between, which will give us the minimal power requirement with maximum removal efficiencies; this value will represent the optimal current density.

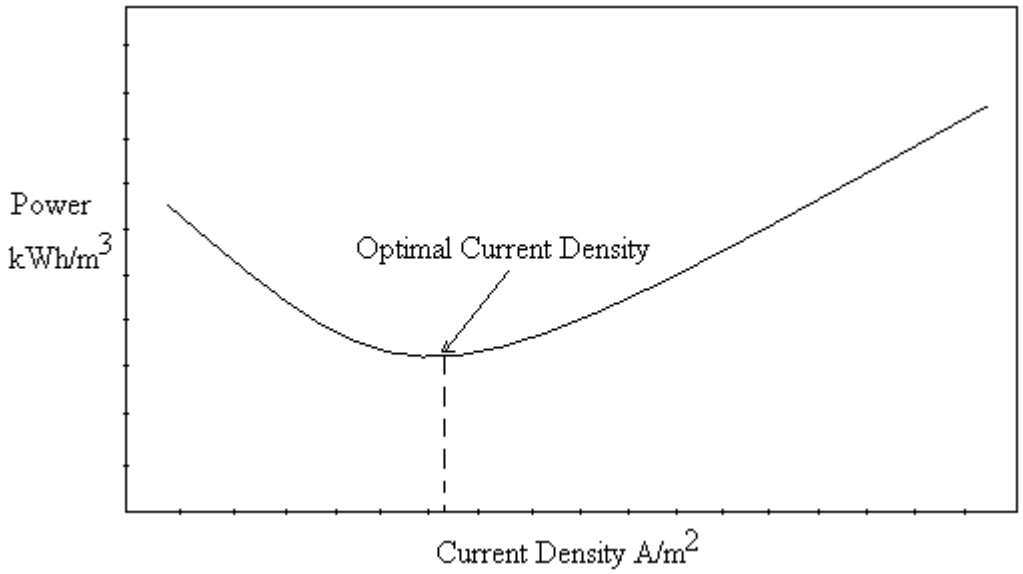

Figure 5.1 Graph representing the standard curve for estimating the optimal current density value at maximum removal efficiency and minimum power requirement for harvesting microalgae using electroflocculation; time required will be the time for complete flocculation of microalgae cultures at each value of current density

5.2.2 Optimization of Time

For an optimized current density, time can be optimized based on the removal efficiency. Thus, keeping the current density constant and measuring the removal of biomass from the culture solution will give us a threshold time limit at which we get maximum removal efficiency. After the threshold time there can be no more removal of biomass. Depending on the operating cost we can choose optimised time frame as time required for removal efficiencies between 80-99%.

Figure 5.2 shows a typical graph for the increase in time required for maximum removal efficiencies at constant current density. After the threshold limit the increase in time can not increase further removal of biomass and will only increase the power required. Therefore an optimal time range should be considered for exact optimal time depending on the cost and removal efficiency expected.

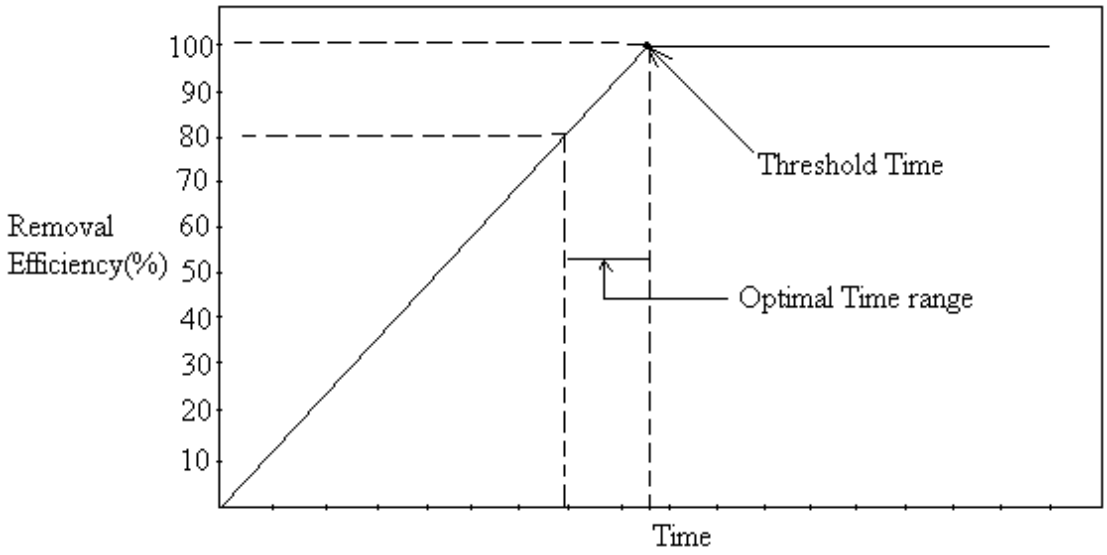

Figure 5.2 Figure representing optimal time range and threshold time at constant current density for microalgal biomass removal using electroflocculation

5.2.3 Optimization of Distance between Electrodes

The optimal inter-electrode distance for electroflocculation processes reported is 0.5-3.0cm (Mameri & Yeddou, 1998; Daneshvar *et al.*, 2004) in few cases the optimal inter-electrode distance was gauged by optimizing the current density and time for maximum removal efficiency (Daneshvar *et al.*, 2004; Xu & Zhu, 2004). For a particular volume of microalgae culture to be electroflocculated the optimal distance between electrodes could be determined by performing series of experiments with varying distance between electrodes for maximum removal efficiencies at constant current density and time.

5.2.4 Electrode Material

After experimenting with different electrode materials studied in Chapter 4, it was found that the Aluminium electrodes gave good recovery efficiency and less time was required for the flocs to float. However, laboratory experiments show that a combination of Al with SS-316, using Al as anode, resulted in low power consumption as shown in Table 5.1 below. The use of SS-316 in conjunction with Al, using SS-316 as Anode is not recommended because of the lower recovery efficiency and possibility of Chromium ions getting dissolved into the culture solution. Although

the resistivity of SS-316 is greater than aluminium, the reason for the drop in voltage has to be determined.

Table 5.1 Results for electroflocculation experiments with Al and combination of Al-SS-316 electrodes; Electrode surface area: 93.75cm^2; Distance between electrodes: 7cm

Electrodes	Current(A)	Voltage(V)	Time(min)
Anode- Al Cathode- Al	10.5	11	1
Anode- Al Cathode- SS-316	10.5	8.6	1

From the results in Table 5.1, the two data points indicate that changing the combination of electrode material resulted in low power consumption. Thus, for selecting the best material of electrodes for anodes and cathodes requires more experimentation to be undertaken and study of electrical potentials of metals has to be done.

5.2.5 Salinity and pH

From the results in Chapter 4 (section 4.3.1) it is evident that higher salinity decreases the power consumption for the flocculation of microalgae. However, salinity will be dependant on the type of marine microalgae cultured.

The culture pH determines the nature of reactions of the metal ion complexes in the solution and solubility of the product formed. From literature review (section 2.3.6) the optimal pH values reported for electroflocculation experiments is 7.

5.3 Design of Electroflocculation Unit

The design of electroflocculation unit depends on the design factors described in Figure 5.3.

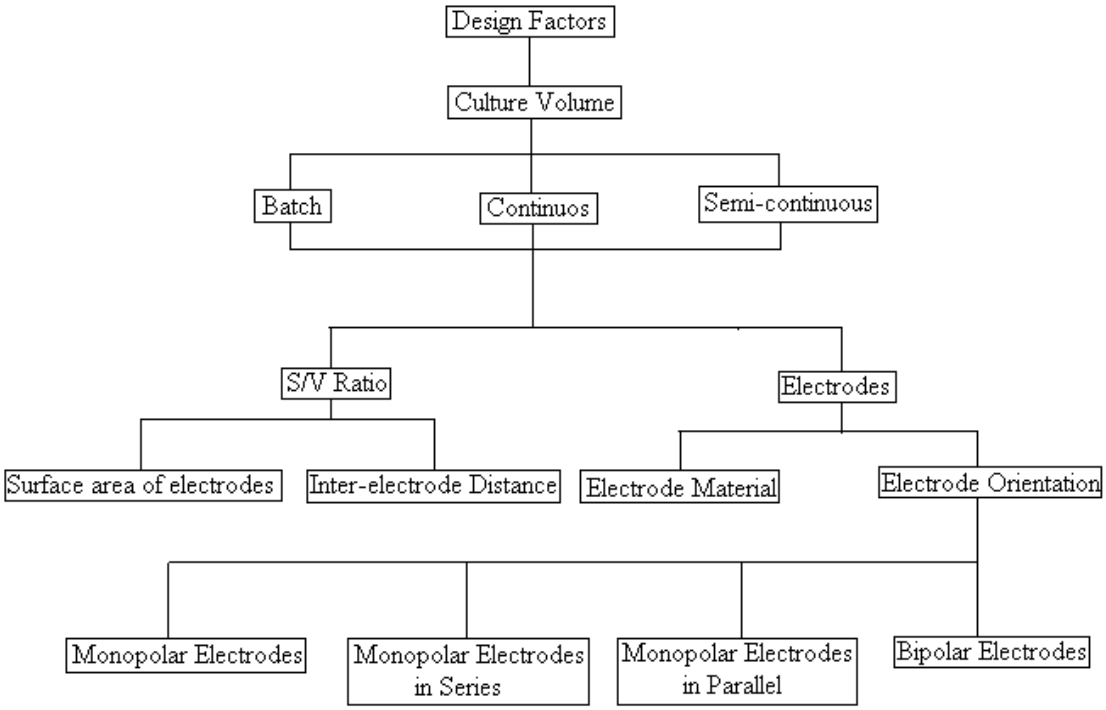

Figure 5.3 Design factors for electroflocculation unit

5.3.1 Design Factors

The major design factors are volume of the microalgae culture to be harvested, S/V ratio and electrode orientation.

Culture volume

The volume of microalgae culture to be harvested will decide whether harvesting has to be continuous, batch or semi-continuous. For lab scale and small scale harvesting the operation could be batch or semi continuous. Large scale harvesting should be undertaken in continuous mode for more productivity.

S/V ratio

According to Holt et al the surface area (of anode) to volume ratio (S/V) is an important scale-up parameter, and the optimal values reported are between 15-45 (m^2/m^3) (Holt *et al.*, 1999). The literature data available on S/V ratios is not enough and reliable for electroflocculation of marine microalgae at large-scale and therefore more experimentation needs to be done for a particular microalgae species to set an optimal value of S/V ratio.

The importance of S/V ratio is that if an optimum value for S/V ratio is known, this value will directly give the value of surface area of electrodes and help to optimize the distance between electrodes.

Electrode material and distance between electrodes

Optimization and determination of these parameters is briefly discussed in Section 5.2 of this chapter and same values can be used for design of the electroflocculation equipment. The optimised values of distance between electrodes may change with the volume to be harvested and S/V ratio.

Material of construction

The recommended material for the construction for the electroflocculation equipment is reinforced fibre glass. No metal is recommended for the construction of equipment to avoid current flow through the equipment and also to avoid any corrosion when flocculating the marine microalgae.

Electrode orientation

Depending on the type of electrode orientation the electroflocculation cell can be designed into four types with monopolar electrodes, monopolar electrodes in series connection, monopolar electrodes in parallel connection, bipolar electrodes. N. Daneshvar et al (2004), has described a working setup for all the four cell types used in the decolourization of dye solution, and concluded that monopolar electrodes were more effective than bipolar electrodes and electrocoagulation cell with series connection of monopolar electrodes was most effective (Daneshvar *et al.*, 2004). However, few research studies have suggested the use of bipolar electrodes as more

effective (Mameri & Yeddou, 1998; Mameri *et al.*, 2001; Chin-Jung *et al.*, 2005), although the use of electroflocculation cell with monopolar electrodes in series and parallel connection was not compared. Therefore from these studies we can definitely conclude that use of multiple electrodes in different combinations (bipolar, monopolar series, monopolar parallel) is always more effective than simple electroflocculation cell with two monopolar electrodes.

Figures below show the arrangement of electrodes in all the four cell types. Figure 5.4 shows simple monopolar orientation with anode and cathode on the opposite walls of the electroflocculation cell and the circuit diagram.

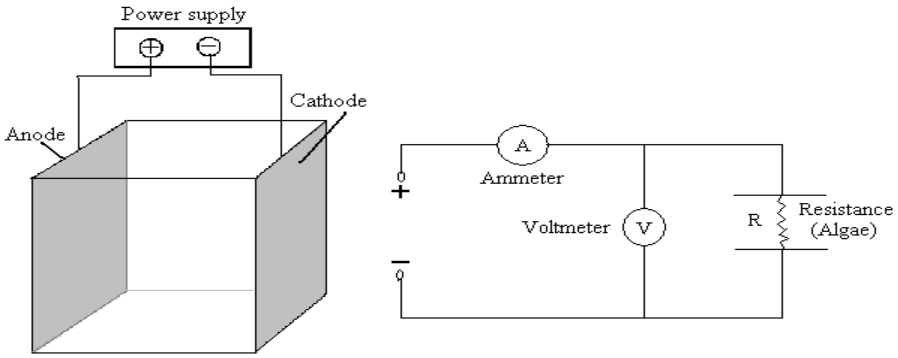

Figure 5.4 Electroflocculation cell with two monopolar electrodes

Figure 5.5 shows monopolar orientation with multiple electrodes in series connection with the circuit diagram. The internal adjacent electrodes are connected to form a series of anodes and cathodes. These internal electrodes are not connected directly to the D.C supply.

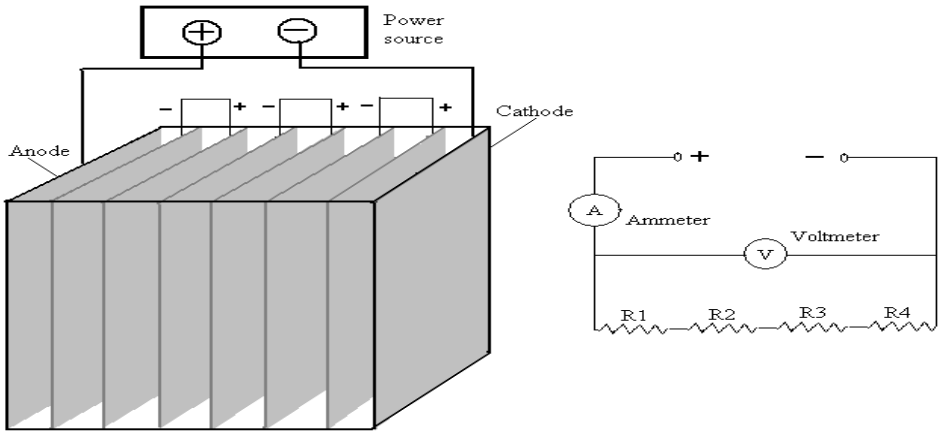

Figure 5.5 Electroflocculation cell with monopolar electrodes in series connection

Figure 5.6 shows monopolar orientation with multiple electrodes in parallel connection and the circuit diagram. The internal electrodes are connected with the D.C supply in such a fashion that anodes and cathodes are formed alternatively.

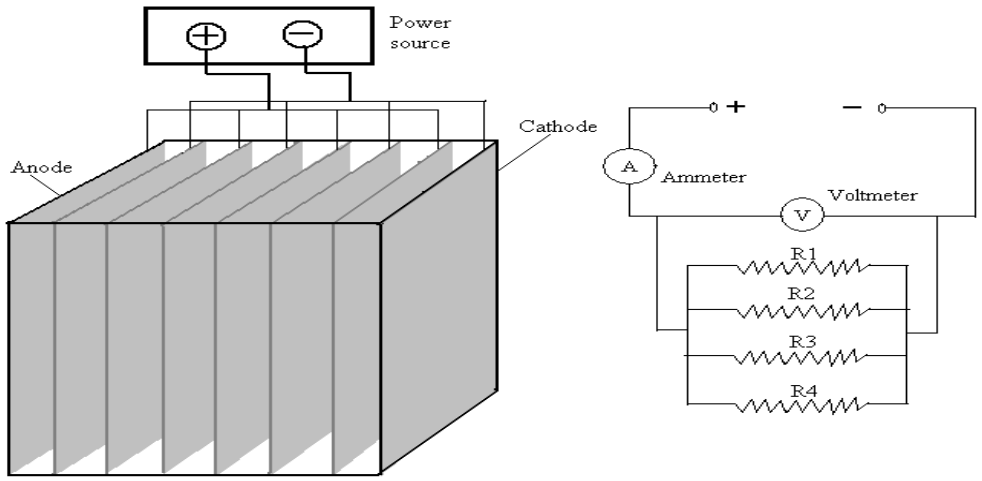

Figure 5.6 Electroflocculation cell with monopolar electrodes in parallel connection

Figure 5.7 shows bipolar orientation with multiple electrodes. The gap between two electrodes forms a resistance as shown in the circuit diagram. The two external electrodes form an anode and cathode. Each electrode in between these two electrodes acquires +ve and –ve charge on its opposite surface. The internal electrodes are not connected to the D.C supply.

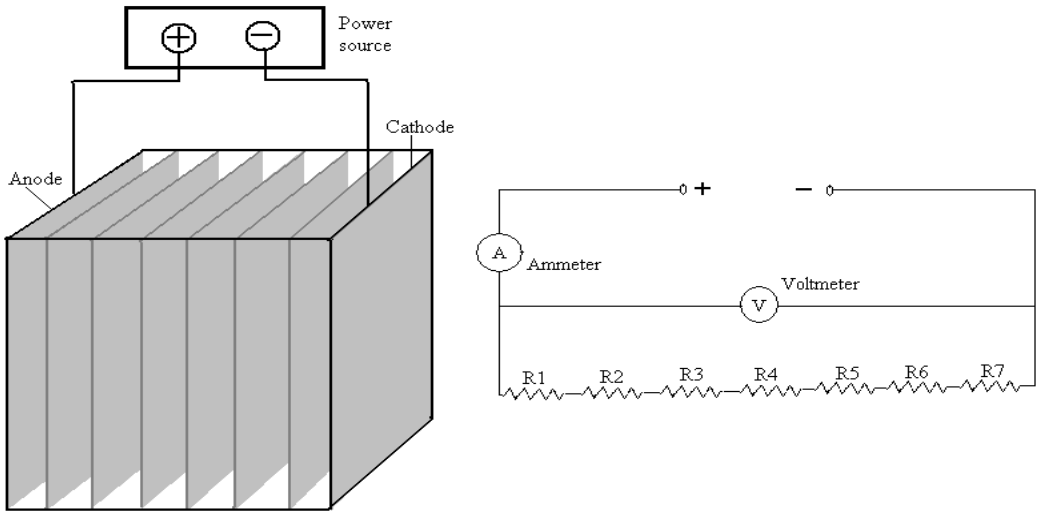

Figure 5.7 Electroflocculation cell with bipolar electrodes

5.4 Cost Analysis

Studies in previous chapters concluded that electroflocculation is a low cost harvesting technique. The major costs for electroflocculation include the costs of electricity and cost for the replacement of the electrodes. From experiments it was concluded that aluminium was better material of electrode for the flocculation of MUR230 and MUR232 cultures, however equation 5.4 presented in this section is generalised and can be used for any material of electrodes.

5.4.1 Power Costs

Power is the product of current, voltage and time. In the process of electroflocculation for a given optimised current density the voltage is controlled by the resistance of the culture solution. Equation 5.1 shows the cost required for flocculation of $1m^3$ of microalgae culture.

$$\frac{\$}{m^3} = \left(\frac{\$}{kWh}\right) \times \left(\frac{Voltage(V) \times Current(A) \times Time(h)}{1000}\right) \times \left(\frac{1}{m^3}\right) \quad (5.1)$$

5.4.2 Cost of Electrode Replacement

To calculate the cost of material required for the electrode to be replaced (anode) it is necessary to calculate the mass of metal deposited in a required amount of time at given current density. The calculation of amount of metal deposited is one of the applications of Faraday's laws. The following equations show a systematic approach of calculating the amount of metal deposited in the electrolyte or culture solution, modified from B. Gaida (1970).

The mass of metal dispersed in the culture solution/electrolyte can be calculated from the following formula:

$$m = c \times I \times t \quad (5.2)$$

Where,

m= mass of metal (g)

c= electrochemical equivalent (g/Ah); this value is constant and is different for different for different metals

I= current (A)

t = time (h)

The accuracy of equation 5.2 depends on the assumption that current is completely effective in releasing the metal ions from the anode, however, in practice side effects like evolution of hydrogen may use some of the current (Gaida, 1970). Moreover the corrosion of metal electrode in marine environment could increase the current efficiency.

Modifying equation 5.2 gives

$$m = c \times I \times t \times \eta \quad (5.3)$$

Where,

η= current efficiency in %

Now that we know the metal mass lost by the anode the cost required can be calculated from equation 5.4.

$$\frac{\$}{m(Kg)} = \left(\frac{\$}{Kg}\right) \times \left(\frac{c \times I \times t \times \eta}{1000}\right) \quad (5.4)$$

5.4.3 Additional Costs

The additional cost includes material cost for the design of electroflocculation cell (usually non-conductive material like fibre glass is recommended), cost of an AC/DC rectifier and pumping costs (for continuous process).

Thus, in electroflocculation the major costs involve costs for power consumed and anode material costs.

5.5 Process Drawbacks

Although electroflocculation process cost effective, there are few process drawbacks that need further study and solutions to categorize this technique as a unique harvesting technique. The process drawbacks include periodic replacement of anodes, fouling of electrodes, instability of floccs while floating and corrosion in marine environment. This section will discuss briefly about these process drawbacks and possible solutions to the problems.

5.5.1 Periodic Replacement of Anodes

The process of electroflocculation is based on the principle of bonding of cations released from the anode with the negatively charged microalgae. When the ions are released from the anode the weight and size of anodes starts reducing. At some stage in the process the anodes need to be replaced. And for this a continuous process needs to be shutdown and dismantling of the electroflocculation cell may be necessary.

Using high initial thickness of the anodes will increase the time period of replacing the electrodes. Modern design techniques should be used while designing the electroflocculation cell for the ease of removal of old electrodes and replacement.

5.5.2 Fouling of Electrodes

From the experiments performed and literature review it has been seen that fouling of electrodes is one of the most common problems in the electroflocculation/electrocoagulation processes (Nikolaev *et al.*, 1982; Azarian *et al.*, 2007). The fouling of electrodes is due to formation of metal precipitates including calcium carbonate layers on the cathode and oxide layer formation at the anode. The increase in thickness of this layer gradually reduces the efficiency of the process as well as the current efficiency as it offers more resistance to the current path.

Changing the polarity of the electrodes could be one of the effective solutions to this problem (Mao *et al.*, 2008). Mechanical cleaning of the electrodes is another way of removing the layers and cleaning electrodes however this is not a good option for continuous electroflocculation process. Also, addition of inhibiting agents could prevent fouling.

5.5.3 Instability of the Floccs

In the process of electroflocculation of marine microalgae the cations released from the anode attach with the negatively charged microalgae and forms floccs. The gases released during the process of electrolysis of the culture attach with the floccs and raise them to the surface. It is beneficial to have the floccs floating for a longer period of time because it is easier to scrap them off the surface. However when the gas bubbles attached to the floccs are released the floccs become unstable and fall to the bottom under gravity. Figure 5.8 shows the floating floccs immediately after flocculation and settled floccs some time after flocculation. Moreover when the floccs are floating at the surface a slight vibration can disturb the stability of the floccs and start mixing with the supernatant.

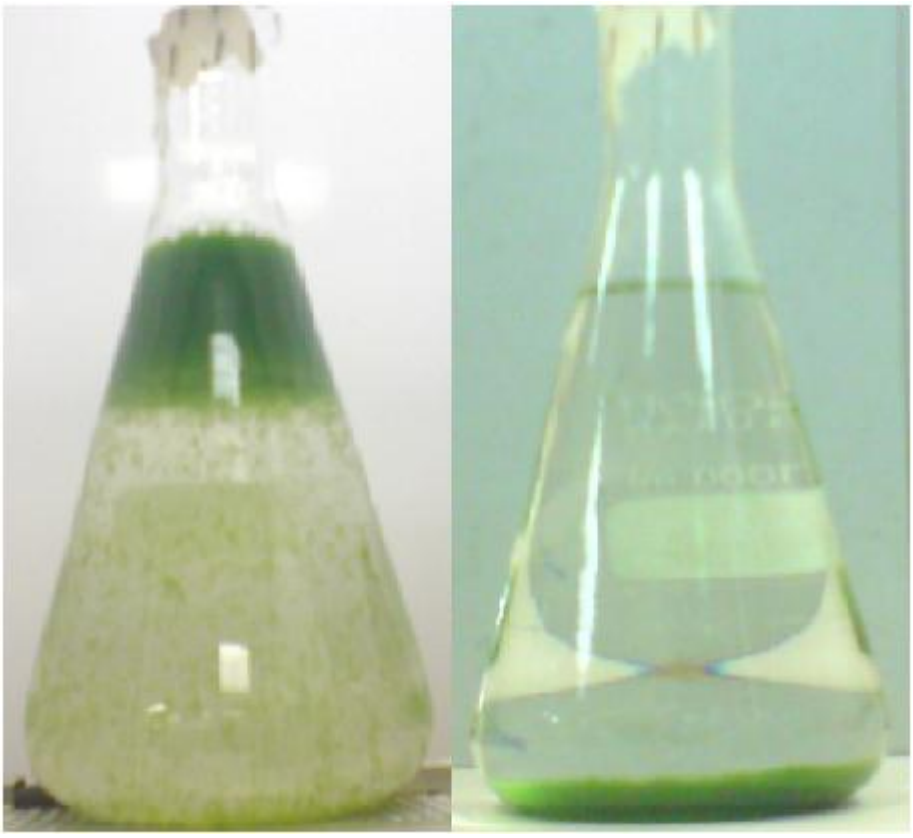

Figure 5.8 Left-floated floccs after electroflocculation, Right-settled floccs after few minutes

Higher current densities are responsible for higher density of air bubbles to keep the floccs floating for longer time. To take advantage of the floating floccs it is necessary to design a scrapper to remove the floccs as soon as they rise to the surface.

Alternatively to avoid the costs for operating a scrapper in a continuous system the inlet and outlet flowrates can be adjusted so that the floated floccs flow over a weir at the outlet. More experiments are required to be studied to compare the concentration factor and overall cost effectiveness using the scrapper and weir system.

5.5.4 Corrosion in Marine Environment

Using electroflocculation for harvesting marine microalgae could be disadvantageous because of the corrosion occurring at the electrodes, as well with any metal coming in contact with the saline water.

Corrosion at anodes is unavoidable but can be reduced by addition of inhibiting agents; however corrosion at cathode can be prevented by using highly corrosion resistant material like SS-316. Using fibre glass for the electroflocculation equipment is beneficial for corrosion resistance.

Chapter 6. Conclusions

6.1 Electroflocculation

The study of electroflocculation as a harvesting technique was successfully commenced on the marine microalgae. Results for lab-scale studies show that recovery efficiencies of more than 90% and concentration factor of 20-25 times can be easily achieved with power requirement of 1.83kWh/m^3 using the electroflocculation as a harvesting technique. However at pilot-scale a minimum power requirement of 0.168kWh/m^3 was noted with more than 95% removal efficiency and concentration factor of 25 times at pilot-scale with specially designed aluminium electrodes and high salinity cultures of MUR230 and MUR232. Proper optimization of the electroflocculation process can still reduce the power requirements and thus the total cost of harvesting.

From the lab-scale experiments it was concluded that a minimal current of 0.1 A is enough to flocculate MUR230 and MUR232 however the time taken would be very inefficient (several hours). The current tolerated by the microalgae was 50A; however, the maximum current tolerance limit was not determined due to the limit of the D.C power supply.

From eight different electrode materials, aluminium and galvanized steel gave the best results of flocculation with a clear supernatant and good floccs. However, the life of galvanized steel electrodes is finished as soon as the zinc layer is deteriorated. Therefore, Aluminium is the best option for electroflocculation at large scale.

The minimum distance of 1cm to 3cm will provide an optimal power requirement, however this optimal distance range was based on lab-scale experiments and further study is required at large scale with multiple electrodes.

In summary electroflocculation as technique for harvesting marine microalgae is promising and cost effective. However tremendous amount of research and a more rigorous and consistent approach is required for optimization, reducing costs and to establish a set of design parameters for the electroflocculation process to be

universally accepted as a reliable, efficient and cost effective technique of harvesting microalgae, in the large scale production of biodiesel.

6.2 Culturing Marine Microalgae Species MUR230 and MUR232

For the commercial large-scale culturing of the microalgae it is essential to check the reliability and stability of the microalgae over a long term period. The major success was maintaining healthy cultures of MUR230 and MUR232 in outdoor raceway ponds for 9 months under daily changing climatic conditions. Results show that the marine microalgae species of MUR230 and MUR232 were reliable and stable under these conditions.

The marine microalgae species of MUR230 and MUR232 can tolerate temperatures from 5°C to 45°C. The growth rate and productivities decreased considerably during low temperature regime, which shows that these species grow better at higher temperatures between 30-40°C. An average doubling time of approximately one week and two weeks was observed during summer and winter respectively and an average AFDW of 0.3-0.6g/L was recorded for both the species. The average productivities of 1-5$gm^{-2}day^{-1}$ was recorded, indicating the species MUR230 and MUR232 as good renewable source of lipids for biodiesel production.

Nomenclatures

Abbreviations

AFDW	Ash Free Dry Weight
CF	Concentration factor
DC	Direct Current
DO	Dissolved Oxygen
DOE	Department of Energy
EDTA	Ethylenediaminetetraacetic acid
PAR	Photosynthetically Active Irradiation
RE	Recovery efficiency
SERI	Solar Energy Research Institute
SS	Stainless Steel

Symbols

C	Concentration, gL^{-1}
c	electrochemical equivalent, g/Ah
C_0	Initial concentration of cells in the culture, cells/ml
C_f	Concentration of cells in the supernatant, cells/ml
I	Current, A
m	mass, g
P	Power, kWh
S/V	Surface area to volume ratio, m^2/m^3
t	time, h
V	Voltage, V
V	Volume, L
V_0	Initial volume of culture, L
V_f	Volume of supernatant, L
V_b	Bed volume of the floccs, L
x	Cell concentration, cells/ml
η	current efficiency

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