TROUBLESHOOTING ON SCALE-UP PROCESS OF MICROALGAE GROWTH FROM FLASK TO PHOTOBIOREACTOR

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ABSTRACT

Cultivation of microalgae in the photobioreactor at the optimum growth needs many considerations to be put in. The growth of microalgae will be based on CO_2 supply, temperature and mixing. Another consideration is about the lighting supply. Thus, photobioreactor is designed based on the best criteria to produce the optimum growth. Based on the best criteria, scale-up process is done. To be successful in the scale-up process has many challenges and difficulties. Some of challenges with the growth of microalgae for oil production are that only a very thin layer of suspended algae in a few centimeters deep, is actually active in photosynthesis and. The objective of the research is to do the troubleshooting on scale-up process by using tubular photobioreactor. In this experiment, the microalgae used is *Nannochloropsis* sp. There are two parts of microalgae cultivation which are in lab scale (2L) flask and in 390L tubular photobioreactor. The reconstituted medium using sea salt and F/2 are prepared. Four runs with different condition in inoculum volume and sparging air flow rate have been done. Because of the unsuccessful scale-up run, two monitor experiments has been done in flasks based on insufficient inoculum volume and addition of carbon dioxide as causes of failure. From the monitor experiment on insufficient inoculum volume, it can be concluded that the failure of scale-up process is because of inoculum volume which is too dilute; 2.5%, 3.5%, 5% and also 10%. To ensure the scale-up process is successful, the inoculum volume need to be increased as well as denser the concentration of microalgae. In the monitor experiment on the addition of carbon dioxide, with the supplying of carbon dioxide at low flow rate, the growth of microalgae is increased day by day. It is proved that the addition of carbon dioxide at low flow rate is not the cause of failure of the scale-up process. Other causes of failure maybe because of the lower level in sparging air flow rate and photoperiod cycle. As a conclusion, the objectives is achieved and the troubleshooting process is done.

ABSTRAK

Pembiakan mikroalga di dalam photobioreaktor pada pertumbuhan yang optimum memerlukan banyak pertimbangan. Pertumbuhan mikroalga bergantung kepada pembekalan karbon dioksida dan oksigen, suhu dan percampuran. Antara pertimbangan lain adalah melalui pembekalan cahaya. Oleh itu, photobioreaktor di reka bentuk berdasarkan kriteria terbaik untuk menghasilkan pertumbuhan yang optimum. Maka, proses penskalaan dilakukan. Untuk berjaya di dalam proses penskalaan mempunyai banyak halangan dan kepayahan. Sesetengah halangan dalam pertumbuhan mikroalga untuk penghasilan minyak adalah hanya lapisan nipis alga yang terampai di dalam beberapa centimeter kedalaman mengalami fotosintesis yang aktif. Objektif kajian ini adalah melakukan penyelesaian masalah dalam proses penskalaan di dalam tubular photobioreaktor. Di dalam eksperimen ini, mikroalga yang digunakan adalah spesis *Nannochloropsis*. Terdapat dua bahagian dalam penanaman mikroalga dimana satu di dalam skala makmal di dalam kelalang 2 liter dan di dalam tubular photobioreaktor. Air laut dan F/2 media disediakan. 4 eksperimen dilakukan dengan membezakan keadaan isipadu inokulum dan kadar aliran udara. Namun disebabkan eksperimen penskalaan tidak berjaya, 2 eksperimen pemantauan dilakukan berdasarkan kekurangan isipadu inokulum dan penambahan karbon dioksida sebagai penyebab kepada kegagalan. Daripada eksperimen pemantauan berdasarkan kekurangan isipadu inokulum, ianya boleh disimpulkan bahawa proses penskalaan tidak berjaya kerana isipadu inokulum yang terlalu cair; 2.5%, 3.5%, 5% dan juga 10%. Untuk memastikan proses penskalaan berjaya, isipadu inokulum perlu ditambah sama bagi memekatkan lagi kepekatan mikroalga di dalam photobioreaktor. Pada eksperimen pemantauan diatas penambahan karbon dioksida, dengan membekalkan karbon dioksida pada kadar aliran yang rendah, pertumbuhan mikroalga bertambah dari hari ke hari. Ini menunjukkan yang penambahan karbon dioksida bukan penyebab kepada kegagalan proses penskalaan. Penyebab lain kepada kegagalan tersebut mungkin kerana kadar aliran udara yang rendah dan kitaran photoperiod. Kesimpulannya, objektif tercapai dan proses penyelesaian masalah telah dilakukan.

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ρ	fluid's density
$\emptyset$	diameter of the tube
μ_l	viscosity of fluid
R_e	reynold number
%	Percentage
L	Liter
Lx	Lux
min	Minute
mg	Milligram
ml	Mililiter
U_l	velocity of the liquid in the tubes
v/v	volume over volume

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Recently, the biodiesel demand is increasing as the current used diesel is now depleting. Biodiesel is seen as an attractive renewable energy because of the environmental process and the ability in enhancing public health and also the beneficial to environment.

Biodiesel can be referred to formation of biodegradable oil and fats, such as animal fats and vegetables oil to a renewable energy to be used daily. Biodiesel is produced through the chemical reaction between lipids and alcohol. The world is now moving to a 'greener' fuel as this biodiesel fuel is predicted to be applied all over the world in future.

About hundred years ago, a German mechanical engineer, Rudolf Christian Karl Diesel had done a testing on vegetable oil as fuel for his engine (Shay, 1993). Began with cheap petroleum, suitable crude oil fractions were transformed to fuel and diesel fuels. Vegetables oils were used in late 1930s and early 1940 as diesel only in urgent situation (M. Fangrui, A. H. Milford, 1999). Limited resources of fossil oil and alarming common concern on environmental matter are the catalyst in hunting for new feasible alternatives, to meet the future requirements.

The society of Planning Commission of India in 2003 had come out with a valuable report, focusing on the improvement of biodiesel from Jatropha curcas. Starting from that, researches are done from time to time by the multi-disciplinary communities to generate

biodiesel from variety of feedstocks. Includes in the studies were also algae, a second generation of biodiesel. This photosynthetic organism transforms water, sunlight and carbon dioxide to sugars, which also producing lipids, to be processed in biodiesel production.

Algae are organisms that grow in aquatic environments and use light and carbon dioxide (CO_2) to create biomass. Demands competition between foods and other biodiesel feedstock and high oil prices had been ignited interest in farming algae in order to convert it into biodiesel (Wen & Johnson, 2009). Furthermore, algae are seen as non-food feedstock to avoid the food-fuel conflict.

Algal biomass contains three main components: carbohydrates, proteins, and lipids. Growths of algae are quickly compared to global crops. Every 24 hours, their size is commonly double. Some algae can double every 3.5 hours during the peak growth phase (Wen & Johnson, 2009).

Some of algae characteristics that are they do not affect fresh water resources which they can be produced through ocean and waste water, and are biodegradable and relatively harmless to the environment if spilled (Biosphere Farms, 2006). Oil content of algae is usually between 20 percent and 50 percent, while some strains can reach as high as 80 percent (Metting 1996; Spolaore et al. 2006). Compared with terrestrial crops, which take a season to grow and only contain a maximum of about 5 percent dry weight of oil, algae grow quickly and contain high oil content. Algae permits harvests in a very short time frame as it has harvesting cycle of 1-10 days.

Algae can be cultivated in two different ways. One is by using photobioreactors. Most of the companies are pursuing algae as source of biofuels are pumping nutrient-laden water through plastic or borosilicate glass tubes that are exposed to the sun. Some disadvantages were identified when using the photobioreactors, such as difficult to handle than open pond and costly. Algae can be cultivated on marginal lands, the land which has no use on food or plants.

Photobioreactors are defined as reactors that do not allow direct exchange of gases or contaminants between culture and atmosphere. John Pirt et al. (1980) were the first to document a tubular bioreactor for the production of Chlorella. The topic of photobioreactors has been reviewed by a number of authors, most notably Borowitzka (1996), Tredici (1999), Pulz (2001) Richmond (2004), Janssen et al. (2002), and in addition to the classic document by Burlew (1953). Several types of closed reactors have been introduced that are classified by Tredici (1999) as (1) flat or tubular; (2) horizontal, inclined, vertical or spiral; and (3) manifold or serpentine. Reactors may also be considered based on their lighting source, whether solar or artificial, internally versus externally illuminated, constant or flashing light source and by the pumping mechanism, either airlift or electric pump.

1.2 Problem Statement

Microalgae are potentially valuable microorganisms because of having the light-harvesting cell factories which can convert carbon dioxide into biomass. Although many can grow heterotrophically, all microalgae are photoautotrophs, requiring mainly sun, water, and inorganic nutrients for growth (Xu, Weathers, Xiong, & Liu, 2009) . Compared to higher plants, microalgae are simple in structure, being unicellular, filamentous or colonial, and energy is directed via photosynthesis into growth and reproduction; they do not need to establish and maintain complex tissues and organs.

Culture of microalgae has been commersial with some of the species like Spirulina for health food, Dunaliella salina and Haematococcus pluvialis for carotenoid production. While in the past natural waters or artificial ponds were used to grow algae, more recently closed photobioreactors have been employed. Closed photobioreactors, on the other hand, have been used to grow photosynthetic microorganisms such as microalgae, cyanobacteria, plant cells, and photosynthetic bacteria for various research and biotechnological applications.

Production of microalgae in the photobioreactor at the optimum growth needs many considerations to be put in. Thus, photobioreactor is designed based on the best criteria to

produce the optimum growth. As known, to do a scale-up process is difficult. Some challenges and difficulty need to be faced. Some of challenges and risks associated with the growth of microalgae for oil production are listed below (Nexant, 2009);

- Dependence on large streams of continuously fed concentrated CO_2 with need to be relatively uncontaminated.
- Only a very thin layer of suspended algae in a few centimeters deep, is actually active in photosynthesis
- Byproduct hydrogen can be a safety risk in a closed syste,s
- Algae suspensions are extremely dilute and difficult to concentrate to levels where oils can be economically recovered.

From the challenges and risks, the troubleshooting on scale-up process is done and the problem identification is considered based on

- Sparging air flow rate
- Inoculum volume

1.3 Objectives

The objectives of the research are troubleshooting on scale up process in photobioreactor based on parameters;

- Sparging air flow rate
- Inoculum volume

Also identify the problem that cause the failure of the scale-up process using tubular photobioreactor

1.4 Scope Of The Study

To achieve the objective, few scopes have been identified in this research:

- Cultivation of microalgae in the lab scale to be used as inoculum to scale-up process
- Scale-up process of microalgae cultivation in the tubular photobioreactor.

- Study the effect of sparging air flow rate and inoculum volume to the scale-up process.
- Identify the problem on difficulty of scale-up process.
- Discuss the failure of the scale-up process

1.5 Rationale and Significance

Scale-up process of producing biodiesel from microalgae has some challenges and difficulties. Although cultivation of microalgae seems easy, there are many challenges including:

(i) minimizing contamination
(ii) efficient provision of carbon dioxide and light;
(iii) controlling cultivation conditions;
(iv) reducing capital and production costs; and
(v) minimizing space requirements

Based on study of scale-up process, problems that always occurred can be identified. The problems that occured during scale-up process can be discussed. The optimum conditions for the microalgae cultivation in tubular photobioreactor can be determined. By the optimum conditions, production of biodiesel from microalgae using tubular photobioreactor can be done.

CHAPTER 2

LITERATURE REVIEW

2.1 Microalgae

Algae, organisms that grow in aquatic environments and use light and carbon dioxide to create biomass. Classifications of algae can be divided into two which is macroalgae and microalgae. Macroalgae are large, multi-cellular algae often seen growing in ponds. These larger algae can grow in a variety of ways. The largest multi-cellular algae are called seaweed. Microalgae, are tiny and measured in micrometers, unicellular algae that normally grow in suspension within a body of water (Wen & Johnson, 2009).

Microalgae comprise a group of photosynthetic, heterotrophic organisms which have an extraordinary potential for cultivation as energy crops. They can be cultivated under difficult agro-climatic conditions and are able to produce a wide range of commercially interesting byproducts (Oilgae.com, 2007)

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Figure 2.1: Example of macroalgae:seaweed, *Ulva Lacuta*

Figure 2.2 : A single *Spirulina* colony, a form of spiral filamentous blue-green algae

2.2.1 *Nannochloropsis* sp.

Nannochloropsis is algae genus comprising approximately 6 species which is N. gaditana, N. granulate, N. limnetica, N. oceanic, N. oculata and also N. salina. The species have mostly been known from the marine environment but also occur in fresh and brackish water (Sakthivel, Elumalai, & M., 2011). Most of the species are small and cannot be distinguished by either light or electron microscopy. These species is able to build up a high concentration of range of pigments such as astaxanthin and canthaxanthin. They have a very simple structure and have a diameter of 2 micrometers.

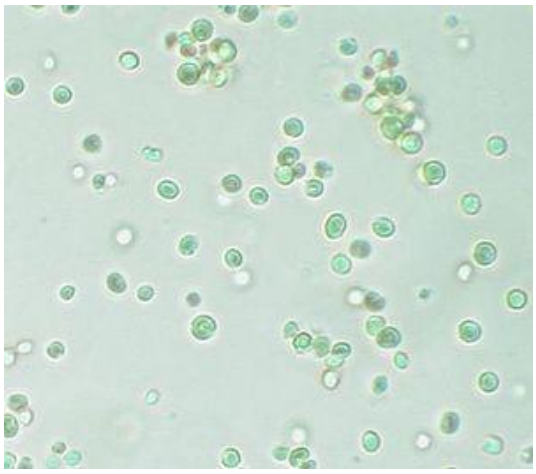

Figure 2.3: The microalgae *Nannochloropsis* sp., viewed under a light microscope

It is considered a promising alga for industrial applications because of its ability to accumulate high levels of polyunsaturated fatty acids and has been investigated for biodiesel production (Darzins, Pienkos, & Edye, 2010). The algae that are used in biodiesel production are usually aquatic unicellular green algae. This type of algae is a photosynthetic eukaryote characterized by high growth rates and high population densities.

Under good conditions, green algae can double its biomass in less than 24 hours (Christi, 2007; Schneider, 2006). Additionally, green algae can have huge lipid contents, frequently over 50% (Christi, 2007; Schneider, 2006). This high yield, high density biomass is ideal for intensive agriculture and may be an excellent source for biodiesel production. Table 2.1 below showed the oil content found in the green algae (Christi, 2007)

Table 2.1 : Oil Content in Some Species Found in Green Algae

Species	**Oil Content (% based on dry weight)**
Chlorella sp.	28-32
Nitzschia sp.	45-47
Nannochloropsis sp.	31-68
Schizochytrium sp.	50-77

2.2 Requirement for Microalgae Growth

In achieved the optimum growth of microalgae, many considerations has to be put on. In the growth of green microalgae, the photosynthesis process is performed. Based on the photosynthesis process, it uses carbon dioxide and water, releasing oxygen as a waste product with the presence of light. So, carbon dioxide supply and light are most of important considerations. Heat, mixing and nutrients also need in the growth process.

2.2.1 Carbon Dioxide

The effect of carbon dioxide on microalgae metabolism is very important. Microalgae cultivation needs CO_2 for photosynthesis. Research indicated that utilizing an atmosphere that contains ample CO_2 not only can help algae cells grow, but also can regulate pH value and carbon balance. Insufficient dissolved CO_2 supply in the culture medium would restrict the algae growth, but oversupply of CO_2 would prevent the cells from conducting photosynthesis and slow down algae growth (Schumpe, 1985; Watanabe *et al.,* 1995; Cogne *et al.,* 2003; Ai *et al.,* 2008).

2.2.2 Light

Light as with all plants, algae photosynthesize, i.e. they convert carbon dioxide into organic compounds, especially sugars, using the energy from light. As light is the source of energy for this process the intensity, spectral quality and photoperiod need to be considered. Light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture (e.g. 1,000 lux is suitable for some small lab flasks, but 5,000-10,000 might be required for larger volumes).

Light may be natural or supplied by fluorescent tubes. Too high light intensity may result in photo-inhibition. Also, overheating due to both natural and artificial illumination should be avoided (Lavens & Sorgeloos, 1996). Fluorescent tubes emitting either in the blue or the red light spectrum should be preferred as these are the most active portions of the light spectrum for photosynthesis. The duration of artificial illumination should be minimum 18 hours of light per day, although cultivated phytoplankton develops normally under constant illumination (Lavens & Sorgeloos, 1996).

2.2.3 Temperature

The optimal temperature for cultures is generally between 20 and 24°C, although this may vary with the composition of the culture medium, the species and strain cultured. Most commonly cultured species of microalgae tolerate temperatures between 16 and 27°C (Chen, et al., 2009). Temperatures lower than 16°C will slow down growth, whereas those higher than 35°C are worse for a number of species. If necessary, algal cultures can be cooled by a flow of cold water over the surface of the culture vessel or by controlling the air temperature with refrigerated air - conditioning units.

2.2.4 Mixing

Mixing is necessary to prevent the sedimentation of algae. Also to ensure that all cells of the population is equally exposed to the light and nutrients. By mixing, thermal stratification can be avoided and gas exchange between the culture medium and air is improved. For very dense cultures, the CO_2 originating from the air bubbled through the culture is limiting the algal growth and pure carbon dioxide may be supplemented to the air supply. CO_2 addition furthermore buffers the water against pH changes as a result of the CO_2/HCO_3^- balance (Lavens & Sorgeloos, 1996).

Mixing is a key parameter for acceptable performance of microalgal bioreactors. Low mixing rates hamper gaseous mass transfer and might even permit biomass settling. In either case, poor mixing leads to emergence of stagnant zones, where light and nutrients are insufficiently available and anoxic/anaerobic conditions will thus prevail, which results in a decrease of productivity (Kumar, Ergas, Yuan, Sahu, & Zhang, 2010)

2.2.5 Nutrients

Nutrients are inorganic salts required for the growth process. In the microalgae growth, nitrogen is one of the important elements. Naturally, nitrogen is mostly found in the form of nitrate and ammonium. Some studies show that increasing nitrogen content in the medium reduces fat production (Grobbelar, 2004). The best source for nitrogen supplement is $NaNO_3$. The amount of nitrate and nitrite intake is strongly related to light intensity. A decrease in ratio of cellular chlorophyll, protein contents and carotenoids to chlorophyll is occured because of nitrate shortage (Young and Beardall,2003). When algae suffers from severe nitrogen shortage, the unsaturated fatty acids content is increased reaching up to 35% of dry weight (Goderberg et al., 2002). Nutrients of microalgae growth can be obtained from F/2 medium.

2.2.6 pH Value

For most cultured microalgae species, the pH ranges is between 7 and 9. And the optimum range being from 8.2-8.7. Complete culture collapse due to the disruption of many cellular processes can result from a failure to maintain an acceptable pH. The latter is accomplished by aerating the culture (Lavens & Sorgeloos, 1996). In the case of high-density microalgae culture, the addition of carbon dioxide allows to correct for increased pH, which may reach limiting values of up to pH 9 during microalgae growth.

2.3 Photobioreactor design

Culture of microalgae in the open systems like ponds and raceways is well developed but only some species can be maintained in that system which is control contamination by using highly alkaline. Great variety of algae in monoseptic culture can be provided by fully closed photobioreactor. The most promising fully closed photobioreactor is tubular photobioreactor which divide into two part; airlift system and tubulant solar receiver (Molina Grima,1999; Tredici,1999). The circulation of culture by using the airlift device has benefits which are circulation is achieved without moving the parts and provides robust culture system with a reduced potential for contamination (E.Molina et al., 2000).

2.3.1 Airlift system

In an airlift driven tubular photobioreactor, the recirculation velocity of the culture and oxygen removal characteristics are closely linked. The culture performance is critically dependent on attaining an optimal design that provides the requisite flow and gas exchange. The airlift column circulates the culture through the solar collector tubing where most of the photosynthesis occurs. The oxygen produced by photosynthesis accumulates in the broth until the fluid returns to the airlift zone where the accumulated oxygen is stripped by air. A gas–liquid separator in the upper part of the airlift column prevents gas bubbles from recirculating into the solar collector. The solar loop is designed to efficiently collect the solar radiation, minimize resistance to flow, and occupy minimal area to reduce the demand for land. In addition, the diameter of the solar tubing is selected so that the volume of the dark zone (i.e. one with light intensity below saturation) is kept to a minimum. Also, the interchange of fluid between the light and the dark zones in the solar loop must be sufficiently rapid that element of fluid does not reside continuously in the dark zone for long (Ogbanna and Tanaka, 1997).

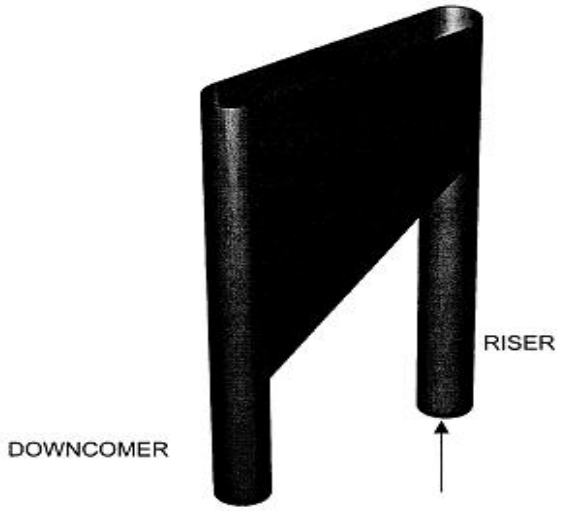

Figure 2.4 : The Gas Liquid Separator

The head zone of the airlift column was designed for almost complete separation of the gas from the liquid, before the broth recirculated into the solar collector. Complete disengagement of gas meant that the driving force for liquid circulation was the maximum attainable for any aeration rate in the airlift riser. To achieve effective separation of gas and liquid, the distance between the entrance and the exit of the degasser should be such that the smallest bubbles have a sufficient time to disengage before the fluid enters the down comer (Chisti and Moo-Young, 1993).

The airlift device fulfills two needs: the circulation of the fluid through the solar loop and stripping of oxygen from the broth. The volume of the broth in the airlift device needs to be small compared to the volume in the solar loop so that the cells spend as much time as possible in the relatively better illuminated loop. In this work, the riser and down comer tubes of the airlift device were vertical extensions of the ends of the solar loop. The volume in the gas liquid separator was minimized by reducing the spacing between the parallel walls to the width of the riser tube.

2.3.2 Tubular Solar Receiver

Tubular photobioreactors that circulate the culture by using an airlift device are especially attractive for several reasons: circulation is achieved without moving parts and this provides a robust culture system with a reduced potential for contamination (Chisti,1989); the cell damage associated with mechanical pumping is avoided (Chisti,1999;Vandanjon et al.,1999); and the airlift device combines the function of a pump and a gas exchanger that removes the oxygen produced by photosynthesis (Camacho Rubio et al.,1999). Continuous removal of oxygen is essential, as excessive dissolved oxygen in the broth inhibits photosynthesis.

Unlike open raceways, photobioreactors permit essentially single-species culture of microalgae for prolonged durations. Photobioreactors have been successfully used for producing large quantities of microalgal biomass (Molina Grima et al., 1999; Tredici, 1999; Pulz, 2001; Carvalho et al., 2006). A tubular photobioreactor consists of straight transparent tubes that are usually made of plastic or glass. This tubular array, or the solar collector,is where the sunlight is captured The solar collector tubes are generally 0.1m or less in diameter. Tube diameter is limited because light does not penetrate too deeply in the dense culture broth that is necessary for ensuring a high biomass productivity of the photobioreactor. The solar collector is oriented to maximize sunlight capture (Molina Grima et al., 1999; Sánchez Mirón et al., 1999). In addition, the design must ensure that the flow in the solar tube is turbulent (i.e. the minimum Reynolds number should exceed 3000) so that the cells do not stagnate in the dark interior of the tube. At the same time, the dimensions of the fluid microeddies should always exceed those of the algal cells. So that turbulence associated damage is prevented.For maximizing the biomass productivity, the irradiance on the surface of the solar tubes must be maximized. This external surface irradiance depends mainly on the solar irradiance, which is a function of the location and the weather conditions (Incropera and Thomas, 1978).