THE OPTIMISATION AND SCALE-UP OF MICROALGAE CULTIVATION FOR HIGH LEVEL OIL CONTENT FOR BIODIESEL

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ABSTRACT

Biodiesel is the mono-alkyl ester of long-chain fatty acids derived from renewable feedstock. It is one of the most renewable fuels that is also non-toxic and biodegradable. The microalgae biomass with high oil content is significant as a sustainable resource for biodiesel production. Production of biodiesel using microalgae biomass appears to be a viable alternative because there is no conflict with food supply compared with the first generation biofuels, such as oil crops and animal fat. This thesis deals with the optimisation of the levels of the variables pH and concentration of ferric chloride for harvesting marine microalgae by flocculation, marine microalgae wild strains limited selection for high level of oil, optimisation of biomass growth and oil content in aseptic sparged flasks, and scale-up of marine microalgae cultivation from flasks to non-aseptic tubular photobioreactor based on the attainment of turbulent flow at both scales. The 2^2 Factorial Design and the Method of the Path of Steepest Ascent are used in the optimisation of the levels of the variables for harvesting microalgae by flocculation. Sedimentation efficiency would reach to the top 99% when the volume of added ferric chloride solution (concentration 1 mol/L) is 0.44ml per litre and pH value is 8.45. For the microalgae wild strains limited selection, *Tetraselmis sp, Nannochloropsis Palau Sara, Nannochloropsis Somalia, Nannochloropsis sp, Chlorella sp, Chetoceros sp* strains of microalgae are cultivated aseptically in sea water at the same conditions for 7 days, the biomass are collected and lipid content are measured with GC-MS (Gas Chromatography-Mass Spectrometer Detector). The result shows that *Nannochloropsis sp* give the highest lipid content of 6.32 mg/L. In the optimisation of biomass growth and oil content in aseptic sparged flasks experiments, the 2^3 Factorial Design is used to investigate the effects of the variables nitrogen and phosphorus concentrations, % (v/v) of CO_2 in the sparging air mixture, and illumination intensity. The Factorial Experiments at the area containing the maximum biomass concentration are complemented with the Composite Design. Analysis of the Response Surface indicated that at the theoretical point of maximum biomass concentration nitrogen and phosphorus concentration (N+P) are at 71.3+4.75mg/L, % (v/v) of CO_2 is at 0.98% and illumination intensity (L) is at 781.25 lx, with the predicted biomass concentration at 143.09 mg/L. Experiments conducted at these optimised levels of experimental variables gave the biomass concentration of 136.67 mg/L and lipid concentration of 2.99 mg/L. In the scale-up of marine microalgae cultivation, marine microalgae are grown non-aseptically in the tubular photobioreactor which consisted of a vertical air-lift and a horizontal receiver. At the same light intensity and with the culture in turbulent flow resulting from sparging at 4.0L/min with air, and sparging with 1% (v/v) of CO_2, a biomass concentration of 155 mg/L and a lipid content of 3.15 mg/L were achieved. This non-aseptically grown marine microalgae biomass will be used as the inoculum for a future large-scale open raceway pond cultivation of the marine microalgae grown on sewage-contaminated sea water sparged with industrial waste CO_2.

ABSTRAK

Biodisel adalah ester mono-alkyl daripada asid-asid lemak berantai panjang yang didapati daripada bahan suapan boleh diperbaharui. Ia merupakan antara bahan api yang paling boleh diperbaharui yang juga tidak toksik dan lebih-lebih lagi ia boleh diurai secara biologi. Biojisim microalgae yang mengandungi minyak yang banyak merupakan sumber lestari yang agak penting bagi penghasilan biodisel. Penghasilan biodisel menggunakan minyak microalgae merupakan satu pilihan yang boleh jaya kerana tiada konflik dengan bekalan makanan berbanding dengan biobahanapi generasi pertama, seperti minyak sayuran dan lemak binatang. Tesis ini memperihalkan pengoptimuman aras-aras pemboleubah pH dan kepekatan ferric klorida dalam penuaian microalgae dengan pengflokan, seleksi terhad strain liar bagi penghasilan minyak yang tinggi, pengoptimuman penghasilan biojisim dan kandungan minyaknya dalam sistem aseptik kelalang yang disembur campuran udara dan CO_2, dan skala-naik penghasilan microalgae dan kandungan minyaknya daripada skala kelalang 1.0L yang aseptik kepada skala fotobioreaktor tiub 390L yang tidak aseptik, berdasarkan pencapaian aliran bercampur pada kedua-dua skala. Rekabentuk Faktorial 2^2 dan Kaedah Pendakian Paling Curam digunakan dalam pengoptimuman aras-aras pembolehubah untuk penuaian microalgae dengan pengflokan. Kecekapan sedimentasi mencapai ketinggian lebih daripada 99% bila isipadu larutan ferric klorida (kepekatan 1 mol/L) yang dicampurkan ialah 0.44mL per litre dan pH berada pada nilai 8.45. Dalam seleksi terhad strain liar microalgae, strain-strain *Tetraselmis sp, Nannochloropsis Palau Sara, Nannochloropsis Somalia, Nannochloropsis sp, Chlorella sp, Chetoceros sp*. dibiakkan secara aseptik dala air laut dalam kadaan yang serupa untuk 7 hari, biojisim dituai dan kandungan lipid ditentukan menggunakan GC-MS (Gas Chromatography-Mass Spectrometer Detector). Hasil menunjukkan *Nannochloropsis sp*. memberi kandungan lipid tertinggi pada 6.32 mg/L. Dalam pengoptimuman penghasilan biojisim dan kandungan minyak dalam experiment menggunakan sistem aseptik kelalang yang disembur campuran udara dan CO_2, Rekabentuk Faktorial 2^3 digunakan untuk menyelideki kesan pembolehubah -pembolehubah kepekatan nitrogen dan phosphorus, %(v/v) CO_2 dalam semburan udara, dan keamatan pencahayaan. Ujikaji Faktorial dikawasan yang mengandungi kepekatan biojisim maksimum telah dilengkapi dengan Rekabentuk Komposit. Analisis Permukaan Rangsangan menunjukkan bahawa pada titik maksimum teori bagi kepekatan biojisim, kepekatan nitrogen dan phosphorus (N+P) adalah pada 71.3+4.75mg/L, % (v/v) CO_2 dalam semburan udara adalah pada 0.98% dan keamatan pencahayaan (L) adalah pada 781.25 lx, dengan kepekatan biojisim diramalkan pada 143.09 mg/L. Experimen yang dijalankan menggunakan aras-aras optimum ini memberi kepekatan biojisim pada 136.67 mg/L dan kepekatan lipid pada 2.99 mg/L. Dalam skala-naik pembiakan microalgae, microalgae dibiakkan secara non-aseptik dalam fotobioreaktor tiub yang terdiri daripada bahagian pengangkat-udara yang menegak dan bahagian penerima cahaya yang mengufuk. Pada keamatan pencahayaan yang sama dan dengan kultur berada dalam aliran bercampur yang terhasil akibat

semburan udara pada 4.0L/min, dan dengan semburan CO_2 pada 1%(v/v), kepekatan biojisim 155mg/L dengan kandungan lipid 3.15 mg/L telah dicapai. Mikroalgae yang dibiakkan secara non-aseptik ini akan digunakan sebagai inokulum dalam satu pembiakan microalgae skala besar menggunakan air laut yang tercemar dengan kumbahan dalam kolam lumba terbuka yang disembur CO_2 buangan industri dimasa depan.

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LIST OF ABBREVIATIONS

cm	Centimetre
CO_2	Carbon Dioxide
FAME	Fatty Acid Methyl Ester
Fe	Iron
$FeCl_3$	Ferric Chloride
GC	Gas Chromatography
GHG	Greenhouse Gases
GT	Grand Total
HCO_3^-	Bicarbonate
hr	Hour
L	Litre
m	Metre
mg	Milligram
ml	Millilitre
min	Minute
N	Nitrogen
NaOH	Sodium hydroxide
Kg	Kilogram
P	Phosphorus
PFS	Poly-ferric Sulphate

pH	Hydrogen Ion Concentration
PUFAs	Polyunsaturated Fatty Acid
r	Error
rpm	Revolutions per minute
s	Second
Si	Silicon
TAGs	Triglycerides
V	Volume
µE	microEinsteins
%	Percentage
℃	Celsius Degree
α	alpha

CHAPTER 1

INTRODUCTION

This chapter gives the ideas and the rationale of the research formulation, and covers the subtopics of background of study, problem statements, research objectives and scope of study.

1.1 Background of Study

Researchers in the field of biodiesel are unanimous that oil from microalgae will become the favoured feedstock of the biodiesel plant in the future (Mata *et al.*, 2010; Rosenberg *et al.*, 2008; Chisti, 2008; Dismukes *et al.*, 2008; Gressel, 2008; Chisti, 2007). This is mainly because of its high productivity per unit area of farm and because it does not compete with food crops for arable lands, since it can be grown in lakes or at sea (Howell, 2009; Gressel, 2008).

Presently the estimated cost of producing 1.0 kg (dry wt.) of microalgae in a plant with 10,000 ton per yr capacity is estimated at USD 0.47. Assuming the biomass contains 30% of oil by weight, the cost of biomass to provide 1 liter of oil will be USD1.40. Assuming the oil recovery process contributes 50% to the cost of the final recovered oil, the oil will cost USD2.80 per liter (Chisti, 2008). Assuming that conversion to biodiesel is 100% (Mata *et al.,* 2010), and that conversion process contributes 30% to the cost of biodiesel, then the present cost-price of biodiesel from

microalgae will be around USD4.00 or RM14.00. There is thus a lot more that needs to be done to bring it down to the price of mineral diesel of RM1.80 at the pump in Malaysia. As oil reserves continue to deplete, such improvements become a race against time.

The microalgae biodiesel value chain comprise of:

(a) the microalgae strain,

(b) the microalgae cultivation unit,

(c) the microalgae cultivation,

(d) the site selection,

(e) the microalgae harvesting and biomass concentration,

(g) the microalgae processing and components extraction,

(h) biodiesel production.

Improvements in any or all of these value chain components will contribute towards lowering the cost-price of microalgae biodiesel.

1.2 Statement of Problem

The main area of improvement for the geneticists is in genetic engineering, which is to produce a high-yielding transgenic microalgae strain with the selective advantage which would enable it to grow in highly selective environments so that it can be grown in open-culture systems whilst still remaining relatively free of contamination by other algae and protozoa (Mata *et al.*, 2010). Until the geneticists succeeded in doing that, the main problems facing the biochemical engineers in large-scale commercial production of microalgae for biodiesel are the need for closed-culture systems and the fact that these are very capital intensive (Borowitzka,1999).

The choice for the biochemical engineers in the microalgae cultivation unit is between the closed-culture systems of photobioreactors and open-culture systems such as lakes or ponds, (Borowitzka, 1999; Chen, 1996; Del Campo *et al.*, 2007; Canela *et al.*, 2002; Piccolo, 2008). Closed-culture bioreactors support up to five-fold higher productivity with respect to reactor volume compared to open-culture systems, but they also cost ten times as much to build compared to open-culture systems (Khan *et al.*, 2009). Open ponds are a very proficient and lucrative method of cultivating microalgae, but they become contaminated with superfluous species very quickly, while closed-culture bioreactors permit single-species culture for prolonged durations (Khan *et al.,* 2009).

For microalgae cultivation, the ultimate aim is to use local sewage as nutrients and CO_2 recycled from industrial stacks for sparging it. This is similar in aims with The National Aeronautics and Space Administration (NASA) program of developing an algae fuel by growing the algae in wastewater with the aim of (a) generating a high quality liquid fuel, (b) creating an inexpensive method of treating sewage and (c) systematizing an effective way eliminate carbon dioxide (Howell, 2009), although the design concept will be different. Response surface methodology analysis of CO_2 sequestering by microalgae grown in medium at different C and N concentrations (approximating nutrients in secondary effluents from municipal treatment plants) by Bilanovic *et al*. (2009) have shown that the process can be optimised for maximal biomass production and maximal CO_2 removal.

Poor sewage treatment has been blamed as being one of the causes of corals slowly dying in the sea off the east coast of peninsular Malaysia, as algae was found to have smothered some reefs, indicating nutrient pollution (Tan, 2008). The power sector in Malaysia is polluting too, with Malaysia ranked 24^{th} among the top-50 countries with the highest CO_2 – emitting power sectors, emitting 61,100,000 tons of CO_2 per year. There are many industrial sites (for CO_2) near the sea on the outskirts of major

towns (for sewage) along the east coast of Peninsular Malaysia. For the ultimate site selection of the open-culture system, the sea off the coast of Kuantan in the East Coast of Peninsular Malaysia presents many good options, as it avoids competing with crops for agricultural land.

However, most marine algae do not have the selective advantage which would have enabled them to grow in highly selective environments so that they can be grown in open-culture systems and still remain relatively free of contamination by other algae and protozoa (Borowitzka, 1999). Hence a hybrid system (Khan *et al*., 2009) will need to be tried in this case. In hybrid systems, both open-culture ponds as well as closed-culture bioreactors are used in combination to get better results. The open-culture ponds are inoculated with the desired strain that was cultivated in a closed-culture bioreactor. This inoculum needs to be large enough for the desired species to establish in the open-culture ponds before contamination by other algae and protozoa (Khan *et al*., 2009).

1.3 Research Objectives

The research objectives of this research are as follows:

- To optimise a method for harvesting microalgae.
- To conduct a limited strain selection among wild strains of marine microalgae in order to select a high-yielding strain for oil production.
- To optimise the production of the algal biomass and oil of the selected strain with respect to the levels of the relevant variables in the aseptic batch process.
- To scale up the production of the algal biomass and oil of the selected strain in a non-aseptic batch photobioreactor.

1.4 Scope of Study

The study area of this research was in Kuantan with latitude of 3° 29′24″ N and longitude of 103° 12′0″ E.

The scope in the development of a method for harvesting microalgae will be limited to flocculation, as it is one of the effective and inexpensive methods for harvesting microalgae in large scale. In this research, ferric chloride is chosen as the flocculant agent for treating the microalgae culture, with volume of ferric chloride and pH being the two independent variables to be optimised.

The scope in the development of a method for determining the amount of oil in the microalgae biomass will be limited to separation and identification of fatty acids in the microalgae. They will be analysed in Agilent 6890 Gas Chromatograph equipped with a HP-88 (100m×0.25mm ID, 0.2μm film thickness, J&W 112-88A7), using helium as carrier gas at 1.0ml/min. Fatty acids methyl esters will be identified by comparison with known standard mixture FAME Mix C4-C24 (Cat.no.18919-1AMP, SUPELCO, USA) and quantified by area percents of total fatty acids. The other analysis method is Agilent 7890A Gas Chromatography (GC) system equipped with a DB-1MS (30m×0.25mm ID, 0.25μm film thickness, Agilent 122-0132) and Agilent 5975C Mass Spectrometer Detector (MSD), using helium as carrier gas at 1.0ml/min. The standard methyl nonadecanoate (C19) was added into the sample to quantify the fatty acid.

The scope of the strain selection among wild strains of marine microalgae in order to select a high-yielding strain for biomass and oil production will be limited to the strains obtained from Institute of Tropical Aquaculture, Universiti Malaysia Terengganu and from Marine Finfish Production and Research Centre, Tanjong Demong, 22200 Besut, Terengganu.

The scope of the optimisation of the production of the algal biomass and oil of the selected strain with respect to the levels of the relevant variables in the aseptic batch process will be limited only to the variables which ultimately can practically be controlled at the large scale open-culture process.

The scale up the production of the algal biomass and oil of the selected strain from the aseptic batch process in sparged 2L Scott Bottles to non-aseptic 390L volume batch photobioreactor will be based on the achievement turbulent flow by airlift at the large scale.

CHPTER 2

LITERATURE REVIEW

2.1 Introduction

Economic growth combined with a rising population has led to a steady increase in the global energy demand. The enormity of the energy crisis has multifolded dramatically over the past decade (Varma and Behera, 2003), and together with the adverse environmental consequences of exhaust gases from fossil fuels, have made modern bioenergy gain increased public and scientific attention.

Biomass is one the best sources of energy (Kulkarni and Dalai, 2006). Large-scale production of biomass energy could contribute to sustainable development on several fronts, environmentally, socially and economically (Goldemberg, 2000).

Biodiesel is the monoalkyl ester of long-chain fatty acids derived from renewable feedstocks (Meher *et al*., 2006). The suitability of biodiesel obtained from canola, soybean, palm, sunflower, and algal oil as a diesel fuel substitute have been well-reported (Lang *et al*., 2002; Spolaore *et al*., 2006). The primary advantages of biodiesel are that it is one of the most renewable fuels which are also non-toxic and biodegradable (Gerpen, 2005). Compared to many other alternative transportation fuels, biodiesel can be used in existing diesel engines without modification, and it is suitable for blending in any ratio with petroleum diesel (Singh and Gu, 2010).

Global climate change requires immediate and substantial reductions in anthropogenic greenhouse gases (GHG) emissions, particularly fossil CO_2. Carbon sequestration could be a major tool for reducing atmospheric CO_2 emissions from fossil fuel usage (Khan *et al*., 2009). Microalgae, a group of fast-growing unicellular or simple multi-cellular microorganisms, have the ability to fix CO_2 while capturing solar energy with efficiencies 10-50 times greater than that of terrestrial plants (Li *et al*., 2008) and produces biomass for the subsequent production of biofuels (Khan *et al*., 2009). Microalgae-based carbon sequestration technologies can, in principle, not only cover the cost of carbon capture and sequestration but also produce environmentally friendly biodiesel. Carbon sequestration offers an opportunity for reducing greenhouse gas emission that can complement the current strategies of improving the energy efficiency and increasing the use of non-fossil energy resources (Bilanovic *et al*., 2009; Khan *et al*., 2009).

The viability of microalgae for biodiesel production has been studied by a number of researchers (Mata *et al.*, 2010; Singh and Gu, 2010). As microalgae can grow in lakes or in the sea and do not compete with cereal crops for agricultural land, the possibility of producing biodiesel from microalgae will make it potentially competitive against biodiesel from canola, soybean, palm, and sunflower oil.

2.2 Potentials and economics of microalgae biodiesel

2.2.1 Advantage of the microalgae biodiesel

Microalgae are *very* important from an ecological point of view. Their important role as a food source is due to their content of minerals, vitamins and oils, and their richness in polyunsaturated fatty acids (PUFAs). PUFAs such as α-linolenic, eicosapentaenoic and docosaesaenoic acids, belongs to the ω-3 group (Metting and Pyne, 1986; Schwartz, 1990; Kay, 1991; Shimizu, 1996; Shimizu, 2003).