UNIVERSITI PUTRA MALAYSIA

GROWTH AND QUALITY OF *Chlorella vulgaris* AND *Chaetoceros calcitrans* IN PHOTOBIOREACTORS USING LOW COST MEDIA FORMULATED FROM FERTILISER

ALEXANDER ZACHARIAH DE SILVA

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By

ALEXANDER ZACHARIAH DE SILVA

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfilment of the requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

GROWTH AND QUALITY OF *Chlorella vulgaris* AND *Chaetoceros calcitrans* IN PHOTOBIOREACTORS USING LOW COST MEDIA FORMULATED FROM FERTILISER

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February 2019

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Faculty : Veterinary Medicine

Microalgae are gaining interest by numerous industries such as the aquaculture, health sciences and pharmaceutical. However, microalgae cultivation is constantly linked to high investment due to the cost of culture media. Therefore, formulation of low-cost media for high quality microalgae cultivation would increase the interest of microalgae usage in many industries. The current study aims to formulate a simple and cost-effective media for the cultivation of *Chlorella vulgaris* and *Chaetoceros calcitrans.* Locally available common fertiliser 'Serbajadi 63' containing 21:21:21 of nitrogen: phosphorus: potassium and 'Serbajadi 46' containing 46% of urea were used as the base formulae for the study.

Twelve media were formulated for *C. vulgaris* and *C. calcitrans* with various concentrations and elements. The formulations were tested in two phases. In the first phase, *C. vulgaris* and *C. calcitrans* were cultured in 2 L flasks and were compared with the conventional media, i.e., Bold's basal medium (BBM) and Conway, respectively. Formulations that showed comparable or better results were selected for phase two where the microalgae were mass cultured in 70 L photobioreactors (PBR) and analysed.

During phase one, NPKFM which was formulated from 0.8 g/L of 'Serbajadi 63' with addition of $MgSO_4$ (0.317 g/L) and acidified $FeSO_4.7H_2O$ (0.02 g/L) produced favourable results for *C. vulgaris* culture. The formulation produced similar amounts ($p > 0.05$) of biomass and proteins as the control. Significantly higher amounts ($p < 0.05$) of

chlorophyll-b (chl-b), carotenoids and carbohydrates were produced in NPKFM compared to BBM. Thus, NPKFM was used to mass culture *C. vulgaris*.

Mass cultivation of *C. vulgaris* in NPKFM produced similar amounts ($p > 0.05$) of biomass, proteins and carbohydrates compared to BBM. Higher amount ($p < 0.05$) of lipids and polyunsaturated fatty acids (PUFA) were produced in NPKFM as well.

In the case of *C. calcitrans*, the cultivation in 2 L flask revealed that NCFM produced the most favourable results compared to other formulations. NCFM was formulated from 0.476 g/L of 'Serbajadi 63' added with $FeCl_3.H_2O$ (0.0013 g/L), $MnCl_2.4H_2O$ (0.00036 g/L) and $NaSiO_3$ (0.02 g/L) produced comparable ($p > 0.05$) optical density (OD) and cell density (CD) to Conway. Higher amounts ($p < 0.05$) of proteins and lipids were also produced in NCFM, while not significantly different ($p > 0.05$) amount of chlorophyll-a (chl-a) and chl-b were produced in both media. Thus, NCFM was selected for phase two.

In the mass cultivation of *C. calcitrans*, NCFM produced significantly higher ($p < 0.05$) CD, proteins and lipids compared to the Conway medium. Additionally, similar ($p > 0.05$) amounts of carbohydrates, chl-a, chl-b, carotenoids and omega-6 were produced in both media as well.

Economically, producing 1 L of NPKFM and 1 kg of *C. vulgaris* in NPKFM was found to be 59.09% and 49.93% cheaper respectively compared to BBM. Likewise, producing 1 L of NCFM and 1 kg of *C. calcitrans* in NCFM was 71.67% and 24.09% cheaper compared to Conway, respectively. Thus, both NPKFM and NCFM were suitable formulations for cost-effective mass cultivation of *C. vulgaris* and *C. calcitrans*, respectively.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KUALITI DAN PERTUMBUHAN *Chlorella vulgaris* DAN *Chaetoceros calcitrans* MENGGUNAKAN MEDIA MURAH YANG DIPERBUAT DARI BAJA DALAM FOTOBIOREAKTOR

Oleh

ALEXANDER ZACHARIAH DE SILVA

Februari 2019

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Pada masa kini, mikroalga adalah suatu permintaan yang tinggi untuk pelbagai industri seperti industri akuakultur, sains kesihatan dan farmaseutikal. Walau bagaimanapun, pengkulturan mikroalga sering kali dikaitkan dengan kos yang tinggi. Maka, perumusan suatu media kos rendah untuk pengkulturan mikroalga akan menambah minat pelbagai pihak terhadap mikroalga. Kajian ini adalah bertujuan untuk merumuskan media yang kos efektif untuk pengkulturan *Chlorella vulgaris* dan *Chaetoceros calcitrans*. Baja tempatan 'Serbajadi 63' dan 'Serbajadi 46' yang masing-masing mengandungi 21:21:21 nitrogen:fosforus:kalium dan 46% urea digunakan sebagai formula asas untuk kajian ini.

Sebanyak 12 media telah dirumuskan dan diuji untuk kedua-dua *C. vulgaris* dan *C. calcitrans.* Setiap rumusan diuji melalui dua fasa. Di fasa pertama, *C. vulgaris* dan *C. calcitrans* telah dikulturkan di dalam kelalang kon 2 L dan dibandingkan dengan media konvensional iaitu Bold's basal medium (BBM) dan Conway. Rumusan yang menunjukkan hasil yang sama atau lebih baik dari media kawalan, dipilih untuk ujian fasa kedua dimana pengkulturan berskala besar dalam fotobioreaktor 70 L dan dianalisis.

Pada fasa pertama untuk *C. vulgaris*, NPKFM yang telah dirumuskan daripada 0.8 g/L 'Serbajadi 63' dengan penambahan 0.317 g/L $MgSO_4$ dan 0.02 g/L $FeSO_4.7H_2O$ berasid telah menghasilkan keputusan yang memberangsangkan. Rumusan tersebut telah menghasilkan biojisim dan jumlah protein yang sama ($p > 0.05$) berbanding dengan kawalan ujian tersebut. Selain itu, jumlah klorofil-b, karotenoid dan karbohidrat adalah

lebih tinggi ($p < 0.05$) di dalam NPKFM berbanding dengan BBM. Maka, NPKFM telah dipilih untuk digunakan untuk pengkulturan besaran *C. vulgaris.*

Pengkulturan secara besaran *C. vulgaris* menunjukkan bahawa NPKFM menghasilkan biojsim, protein dan karbohidrat yang sama ($p > 0.05$) berbanding dengan BBM. NPKFM juga telah mengandungi lipid dan asid lemak tak tepu (PUFA) yang lebih tinggi ($p < 0.05$) berbanding dengan BBM.

Untuk kajian *C. calcitrans* pula, pengkulturan mikroalga tersebut di dalam balang 2 L telah menunjukkan bahawa rumusan NCFM mempunyai hasil yang paling menguntungkan berbanding rumusan lain. NCFM yang terdiri daripada 0.476 g/L 'Serbajadi 63' yang ditambah dengan 0.0013 g/L $FeCl_3.H_2O$, 0.00036 g/L $MnCl_2.4H_2O$ dan 0.02 g/L $NaSiO_3$ menghasilkan kepadatan optik (OD) dan kepadatan sel (CD) yang sama ($p > 0.05$) dengan Conway. Jumlah biojisim, protein and lipid yang lebih tinggi ($p < 0.05$) juga dihasilkan di dalam NCFM. Jumlah klorofil-a dan b yang sama ($p > 0.05$) telah dihasilkan di dalam NCFM dan Conway. Oleh itu, NCFM telah digunakan untuk pengkulturan besaran fasa dua *C. calcitrans*.

Pengkulturan berskala besar *C. calcitrans* menggunakan NCFM menghasilkan CD, protein dan lipid yang lebih tinggi ($p < 0.05$) berbanding dengan Conway. Selain itu, jumlah karbohidrat, klorofil-a, b, karotenoid dan Omega-6 yang sama ($p > 0.05$) ditemui di kedua-dua NCFM dan Conway.

Dari segi ekonomi, untuk menghasilkan 1 L NPKFM dan 1 kg *C. vulgaris* dalam NPKFM adalah masing-masing 59.09% dan 49.93% lebih murah daripada BBM. Pada masa yang sama, untuk menghasilkan 1 L NCFM dan 1 kg *C. calcitrans* dalam NCFM adalah masing-masing 71.67% dan 24.09% lebih murah daripada Conway. Maka, kedua-dua NPKFM dan NCFM adalah rumusan yang sesuai untuk pengkulturan secara besar-besaran yang murah dan kos efektif untuk *C. vulgaris* dan *C. calcitrans* masing-masing.

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I certify that a Thesis Examination Committee has met on 14 February 2019 to conduct the final examination of Alexander Zachariah De Silva on his thesis entitled "Quality and growth of *Chlorella vulgaris* and *Chaetoceros calcitrans* in photobioreactors using low cost media formulated from fertiliser" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBRIEVATIONS

BBM	Bold's basal medium
CD	Cell density
chl-a	chlorophyll-a
chl-b	chlorophyll-b
dw	dry weight
Fe	Iron
kwh	kilowatt hour
L	litre
Mg	Magnesium
Mn	Manganese
mL	mililitre
OD	Optical density
PBR	Photobioreactor
×g	relative centrifugal force
rpm	revolutions per minute

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CHAPTER 1

GENERAL INTRODUCTION

1.1. Background of Study

Microalgae are unicellular microscopic algae that grow by performing photosynthesis. Microalgae are considered to be the primary food source for many aquatic life forms and play a key role in the development of aquaculture. Live microalgae are generally used as larval diets in aquaculture but spray-dried biomass is utilized for health foods, food additives and feed supplements for human consumption (Yamaguchi 1997). Industrially, microalgae offer a vast array of valuable compounds such as its biomass, lipids, carbohydrates, proteins, and pigments. A number of factors affect the growth and proximate composition of microalgae such as light intensity, temperature, media and salinity (Thompson *et al.* 1992). According to Sharma *et al*. (2011) the growth and proximate of microalgae are highly influenced by its culture media.

One of widely cultivated microalga is *Chlorella vulgaris*. The microalga has been widely used in the food, pharmaceutical (Sankar & Ramasubramanian 2012) and aquaculture industry (Safi *et al*. 2014). These industries are interested in *Chlorella* because of its many properties of being antitumor, anticarcinogenic, antiviral, anticataract, antiulcer and even antioxidative (Shibata *et al*. 2003). It has also been reported to be able to have protective abilities towards viral and bacterial infections in normal and also immunosuppressed mice (Tanaka *et al*. 1986; Queiroz *et al*. 2003). A review by Safi *et al.* (2014) stated that consumption of *C. vulgaris* by fish showed better pigmentation on the fish and increased life expectancy.

Chaetoceros calcitrans is commonly grown in bivalve hatcheries (Tredici *et al.* 2009) and as feed for shrimp larvae (Raghavan *et al*. 2008). It is considered to be suitable to be fed to most marine filter feeders due to its nutritional value (Chotipuntu 2005). Thus, the demand for this microalga is high in many countries that emphasize on marine hatcheries such as shrimp larvae (Krichnavaruk *et al*. 2005).

As more than 90% of the world's aquaculture is located in Asia (FAO 2010) thus there is a high demand of food in the aquaculture industry. Hence, quality cultivation of *C. vulgaris* and *C. calcitrans* benefits the aquaculture industry tremendously. However, microalgae cultivation is always seen as a high cost investment (Hemaiswarya *et al.* 2011). Conventional media needed for the cultivation of microalgae consist of 10–20 different chemicals thus contribute to its high cost. Therefore, if a cost-effective medium was made for the cultivation of *C. vulgaris* and *C. calcitrans*, it would benefit the aquaculture industry in the long run.

1.2. Problem Statement

Media used for the cultivation highly influences the growth and quality of microalgae (Sharma *et al*. 2011). There are numerous conventional media such as BBM, Walne, Conway and f/2 medium just to name a few, that have been formulated to cultivate different types of microalgae. However, each media contains a variety of inorganic salts that are expensive and tedious to prepare (Kaladharan *et al*. 2002). Therefore, the large amount of chemicals for the preparation of conventional media for mass cultivation in photobioreactors is not feasible. Hence, an alternative cheaper option would be the use of agricultural or plant fertiliser (López-Ruíz *et al*. 1995). However, using agricultural fertiliser does not yield optimum cell growth rate and quality compared to conventional media. Thus, mass cultivation of quality microalgae becomes daunting to farmers and unattractive to numerous industries.

1.3. Purpose of study

The present study investigates the formulation of a more simple and cost-effective media for the cultivation of *C. vulgaris* and *C. calcitrans.* The low-cost media formulated must be able to produce favourable or at par quality of the microalgae compared to conventional media. Fertiliser was used as a base formula for the formulation of the media. The growth and quality of *C. vulgaris* and *C. calcitrans* were analysed to ensure the effectiveness of the media formulated.

1.4. Objectives

This study was conducted to address the following objectives:

i. To determine the growth of *Chlorella vulgaris* and *Chaetoceros calcitrans* cultured in photobioreactors using low cost media formulated from fertilisers.

ii. To evaluate the proximate & biochemical composition of *Chlorella vulgaris* and *Chaetoceros calcitrans* cultured in photobioreactors using low cost media formulated from fertilisers.

iii. To calculate the cost for the production of *Chlorella vulgaris* and *Chaetoceros calcitrans* in photobioreactos using low cost media formulated from fertilisers.

Null hypotheses: The growth rate and proximate quality of microalgae cultured in the low cost media would not be comparable or better than the conventional medium.

Alternative hypotheses: The growth rate and proximate quality of microalgae cultured in formulated media would be comparable or better than the conventional medium.

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BIODATA OF STUDENT

My name is Alexander Zachariah De Silva and I am a Master of Science postgraduate in Universitit Putra Malaysia since 2014. I was born in Kedah Darul Aman, Malaysia.

I pursed my bachelor's Degree in Microbiology in UPM on 2008 and graduated on 2011. I've taken courses on Virology, Mycology, Bacteriology, Biochemistry and Microbial Physiology, just to name a few. During my final year project of my degree I've developed an expertise in HPLC working with *Lactobacillus* and *Bifidobacterium*.

After attaining my bachelor's degree, I went off to work in the private sector. I worked in two different companies as a Quality Assurance Executive from 2011 until 2013. I've obtained profound understanding on the HACCP, ISO and Halal certification during those times. However, after several years of working, I've decided to continue to purse my degree in Master of Science.

I joined UPM as a postgraduate in 2014 under the supervision of Dato' Dr. Mohamed Shariff Mohamed Din in the Institute of Bioscience. He gave me an opportunity to work with developing low cost media for microalgae. Thus, we including his fellow researcher, Dr. Sanjoy Banerjee came up with the project titled 'Growth and quality of *Chlorella vulgaris* and *Chaetoceros calcitrans* in photobioreactors using low cost media formulated from fertiliser.' I've successfully fulfilled the objectives of the project which I ended on 2018.

PUBLICATIONS

De Silva, A. Z., Shariff, M., Banerjee, S. and Yusoff, F. M. (2018). Growth and quality enhancement of *Chlorella vulgaris* Beyerinck (Beijerinck) 1890 using simple cost-effective medium. *Asian Fisheries Science* 31: 61–72.