UNIVERSITI PUTRA MALAYSIA

GROWTH POTENTIAL OF MIXED MICROALGAE CULTIVATED IN DOMESTIC WASTEWATER WITH NUTRIENT SUPPLEMENTATION UNDER MALAYSIAN WEATHER CONDITIONS

SOHANA SHABNAM

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By

SOHANA SHABNAM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

November 2017

DEDICATION

This thesis is dedicated to all the respected teachers who relentlessly thrive at creating an insight within young minds to see the unseen and help discovering things beyond the horizon

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

GROWTH POTENTIAL OF MIXED MICROALGAE CULTIVATED IN DOMESTIC WASTEWATER WITH NUTRIENT SUPPLEMENTATION UNDER MALAYSIAN WEATHER CONDITIONS

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November 2017

Chairman : Professor Ahmad Ismail, PhD
Faculty : Science

Microalgae are considered to be potential biocatalyst for the production of renewable biofuel along with other valuable products. For sustainable algal production, optimization of the algal growth with cost effective harvesting is crucial. An attempt was made to evaluate the effect of nutrients on biomass production of photoautotrophic mixed microalgae using domestic wastewater as feed-stock under varying weather conditions in open pond system. The objectives were to - i) study the effects of concentration and ratio of nitrogen and phosphorus on growth and biomass production, ii) find out the most efficient flocculating agent for effective harvesting, iii) examine the proximate composition of harvested biomass and iv) evaluate the microalgae composition and diversity prevailed under nutrient adjustments while culturing microalgae in wastewater under weather variations. In order to achieve the objectives, microalgae were collected from wastewater pond and cultured in three different weather conditions (dry, wet and mixed). Nitrogen and phosphorus treatments in wastewater were taken in two different ratios (0.77:1 and 5:1) with increasing concentrations (N and P concentration ranges from 10.30 to 41.20 ppm and 2.06 to 53.12 ppm respectively). Bold Basal Medium (BBM) and original wastewater were taken as control. Meanwhile, four flocculating agents namely $FeCl_3$, alum, $Ca(OH)_2$ and Aflok® (organic flocculant) were investigated to find out the most efficient one for harvesting microalgae considering different pH condition, dosage, algal concentration and sedimentation time. The mixed microalgae diversity under given treatments and weather variations was also closely observed during the culture periods. It was found that, the increase of nutrients concentration in the medium increased the final algal biomass regardless the weather condition. Whereas, significantly higher biomass (>0.60 g/L) obtained where the N : P ratio was greater (5:1). The results from harvesting efficiency of flocculants indicated that, $FeCl_3$ and Aflok® at their minimal dosage (<0.08 g/L and <0.112 g/L respectively) were more effective compared to alum

and $Ca(OH)_2$ under wide environmental variations of the medium. In proximate analysis, it was found that protein content increased with increasing nutrient concentration but lipid and carbohydrate increased when nutrients are in depleted condition. The highest protein, lipid and carbohydrate content were found to be 26.89%, 32.07% and 37.47% respectively. In species composition, the Chlorophyta division was observed to be the largest (62.5% - 90%) microalgae group encountered both in terms of abundance and frequency occurrence regardless of weather variations. The genus *Chlorella vulgaris* was the most dominant species that sustained in all treatments culture due to their resiliency to the media conditions. The species richness was observed to be higher in treatments with higher nutrient content than in treatments with lower value. Meanwhile, diversity indices were found to be lower in both low and high nutrient treatments. However, higher diversity indices were found in treatments with comparably moderate nutrients content, which indicated uneven distribution of microalgae species in lower and higher nutrient treatments. It can be concluded that, when mixed algae is cultivated in open pond system, both the nutrient concentration and ratio are dominating factors that can highly influence the microalgae biomass growth, their biochemical composition as well as their community structure. Therefore, by regulating the nutrients and applying effective harvesting technique sustainable microalgae biomass production is possible from mixed algae culture in wastewater.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

POTENSI PERTUMBUHAN MIKROALGA CAMPURAN YANG DIKULTUR DALAM AIR KUMBAHAN DOMESTIK YANG DIPERKAYAKAN DENGAN NUTRIEN DALAM CUACA MALAYSIA

Oleh

SOHANA SHABNAM

November 2017

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Mikroalga mempunyai potensi sebagai biomangkin untuk pengeluaran biofuel boleh diperbaharui bersama dengan produk lain yang bernilai tinggi. Untuk pengeluaran alga secara mapan, mengoptimumkan pertumbuhan alga dengan penuaian kos efektif adalah keperluan yang amat penting. Sehubungan dengan itu satu kajian telah dibuat untuk menilai kesan nutrien kepada pertumbuhan biojisim mikroalga bercampur secara photoautotrophik menggunakan air kumbahan domestik sebagai asas media pengkulturan di bawah keadaan cuaca yang berbeza-beza menggunakan sistem terbuka. Objektif kajian adalah untuk- i) mengkaji kesan kepekatan dan nisbah nitrogen dan fosforus terhadap pertumbuhan dan penghasilan biojisim, ii) mencari ejen pengumpalan paling berkesan untuk penuaian yang cekap, iii) menilai komposisi proksimat biojisim yang dituai dan iv) menilai komposisi dan kepelbagaian alga yang dikultur dalam air sisa di bawah pelarasan nutrien dalam cuaca berbeza. Dalam usaha untuk mencapai objektif, mikroalga diperolehi dari takungan air sisa domestik dan dikultur dalam tiga keadaan cuaca yang berbeza (kering, basah dan campuran). Rawatan nitrogen dan fosforus dalam air sisa menggunakan dua nisbah yang berbeza (0.77:1 dan 5:1) dengan meningkat kepekatan (N dan P) dalam julat kepekatan 10.30-41.20 ppm dan 2.06-53.12 ppm masing-masing. Media Bold Basal (BBM) dan air sisa yang asal digunakan sebagai kawalan. Sementara itu, empat ejen pengumpalan iaitu $FeCl_3$, alum, $Ca(OH)_2$ dan Aflok® (agen pengumpulan organik) telah dikaji untuk mengetahui ejen yang paling berkesan untuk menuai mikroalga dengan mengambil kira keadaan pH, dos, kepekatan alga dan masa pemendapan yang berbeza. Kepelbagaian mikroalga campuran dalam setiap rawatan dan dalam variasi cuaca dicerap sepanjang tempoh kultur. Keputusan kajian mendapati peningkatan kepekatan nutrien dalam medium meningkat biojisim akhir alga tanpa mengira keadaan cuaca. Didapati biojisim yang diperolehi adalah lebih tinggi (> 0.60 g/L) apabila Nisbah N : P adalah besar (5:1). Penilaian kecekapan penuaian menggunakan bahan penggumpalan

menunjukkan bahawa, $FeCl_3$ dan Aflok® pada dos yang minimum (<0.08 g/L dan <0.12 g/L) masing-masing adalah lebih berkesan berbanding dengan tawas (alum) dan $Ca(OH)_2$ dalam variasi persekitaran media pengkulturan yang besar. Analisis proksimat mendapati kandungan protein meningkat mengikut peningkatan kepekatan nutrien tetapi lipid dan karbohidrat meningkat apabila paras nutrien berkurangan. Peratusan kandungan protein, lipid dan karbohidrat yang paling tinggi adalah 26.89%, 32.07% dan 37.47% masing-masing. Berdasarkan komposisi spesies, divisi Chlorophyta merupakan kelompok divisi mikroalga yang terbesar (62.5% - 90%) dari segi bilangan dan kekerapannya dalam apa jua variasi cuaca. Genus *Chlorella vulgaris* merupakan spesies yang paling dominan dalam kultur semua jenis rawatan kerana daya tahan alga ini terhadap kepada keadaan media yang berbeza. Kekayaan spesies (species richness) adalah lebih tinggi dalam rawatan yang mengandungi paras nutrien yang lebih tinggi berbanding rawatan yang mempunyai paras nutrien yang lebih rendah. Namun begitu indeks kepelbagaian adalah lebih rendah dalam rawatan yang mengandungi paras nutrien yang rendah dan tinggi. Walaupun begitu, indeks kepelbagaian yang lebih tinggi ditemui dalam rawatan yang mengandungi paras nutrien yang sederhana. Ini menunjukkan taburan spesies mikroalga yang tidak sekata dalam rawatan paras nutrien yang lebih rendah dan lebih tinggi. Sebagai kesimpulan, apabila alga bercampur dikultur dalam sistem terbuka, didapati faktor kepekatan nutrien dan nisbah N:P adalah faktor penentu yang sangat penting yang mempengaruhi pertumbuhan dan biojisim mikroalga, komposisi biokimia serta struktur komunitinya. Oleh itu, dengan mengawal kepekatan nutrien, nisbah nutrien dan applikasi teknik penuaian yang berkesan mempunyai potensi untuk menghasilkan biojisim mikroalga secara mapan dengan mengkultur mikroalga campuran dalam air sisa.

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I certify that a Thesis Examination Committee has met on 24 November 2017 to conduct the final examination of Sohana Shabnam on her thesis entitled "Growth Potential of Mixed Microalgae Cultivated in Domestic Wastewater with Nutrient Supplementation under Malaysian Weather Conditions" 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 Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrophotometer
ABS	Absorbance
AIWPS	Advanced integrated wastewater pond systems
ANOVA	One-way independent analysis of variance
ATP	Adenosine triphosphate
BBM	Bold's basal medium
BOD	Biochemical oxygen demand
COP	Conference of the parties
DHA	Docosahexaenoic Acid
DO	Dissolved oxygen
EC	Electrical conductivity
EPA	Eicosapentaenoic acid
GHG	Greenhouse gas
IEA	International energy agency
LCA	Life cycle assessment
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
OD	Optical density
PBR	Photobioreactor
PUFA	Polyunsaturated fatty acid
RCBD	Randomized complete block design
SPSS	Statistical Package for the Social Sciences
TDS	Total dissolved solids

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Environmentally concerned modern world is recently facing major challenges from increasing energy demand, pollution and global warming leading to climate change. The continued use of fossil fuel is considered unsustainable, principally due to rapid consumption rate of its finite source and its contribution to greenhouse gas emission. On December, 2015, Paris Climate Conference (COP21) was established with the prime aim to lower down the greenhouse gas emission and encouraged accelerated adoption of promising environment-friendly low-emission technologies and practices (Stern et al., 2016). Combined with geopolitical and environmental impacts regarding pollution and greenhouse gas emission (Pittman et al., 2011), renewable biofuels have received a significant attention that are conceivably carbon neutral (Demirbas, 2009).

There are variety of sources of feedstock for renewable biofuels that scientists and researchers are working on, for example: ethanol from the fermentation of cane sugar (Goldemberg et al., 2008), methane from the anaerobic digestion of organic waste (Gomez et al., 2008) or biodiesel (alkyl esters) from the transesterification of plantation oil or animal fat (Li et al., 2011). Even though some of these fuels appear to be attractive alternatives to fossil fuels, producing in large scale with strong infrastructures are facing some common complications, like high cost of development and implementation and most importantly, these biofuel feedstocks generate competition with food crops for arable land and available fresh water (Collet et al., 2011).

Microalgae have recently received enormous interest on utilization of their biomass as a resource for fuels and valuable chemicals. Microalgae are photosynthesizing organisms, exist in almost all earth ecosystems and can flourish under a wide range of environmental conditions (Abu-Shanab et al., 2011). These aquatic organisms embrace several advantages over terrestrial biomass, with higher photosynthetic efficiency (khan, et al., 2009), higher biomass and lipid production with faster growth rate (Tredici, 2010), all-year round production, able to grow in wastewater bodies as well as sequestering carbon dioxide (CO_2) from industrial processes (Menetrez, 2012). For these reasons, microalgae are capable of producing more oil per unit area of land compared to terrestrial oilseed crops (Chisti, 2008). Additionally, microalgae cells possess essential proteins, pigments (e.g., chlorophylls, carotenoids and phycobiliproteins) and other metabolites that have high demand in aquaculture and animal feed, nutraceutics and in pharmaceutics (Solimeno et al., 2015).

1.2 Problem Statement

Despite the high potentiality of microalgal biomass however, many challenges have impeded the commercialization of algae culture technology. Limited source of fresh water, costly inorganic nutrients for mass cultivation and cost-effective harvesting of microalgae are some of these troublesome issues. Microalgae life cycle assessment (LCA) results indicated that use of freshwater and fertilizers for microalgae cultivation can lead to more energy inputs, higher emissions of greenhouse gas and huge water utilization than other feed-stocks (Clarens et al., 2010; Lardon et al., 2009). Such excessive consumption could be counterbalanced by using wastewater for cultivating microalgae and thereby reducing at least 90% water demand and cut off almost all nutrients in need (Hu et al., 2011). Lundquist et al. (2010) and Pittman et al. (2011) remarked after reviewing several algae based wastewater treatment scenarios coupled with biofuel production, that in near future only those cases that utilize wastewater for large scale algae production for biofuel would be economically viable.

However, the success of culturing specially selected microalgae strains (fast growing and high lipid containing) in different wastewaters profoundly depends on the implementation of favorable conditions under small scale controlled environment (Lam & lee, 2012; Wu et al., 2014). Consequently, these results are not applicable for low value biofuel production in industrial large scale due to excessive cost in establishment of full controlled infrastructures that eventually raise the pricing of algae derived biofuel (US DOE, 2010; Waltz, 2009). The semi-controlled open raceway pond system however, less likely to be appreciated as these systems are widely open to environmental fluctuations and are more prone to contamination by other microalgae (Mata et al., 2010). Unfortunately, till now this is the only available low cost system for large-scale cultivation (Norsker et al., 2011).

Taking into account the cost effectiveness and sustainability issue, cultivation of mixed indigenous microalgal species may be one of the appropriate solutions that meet all the requirements. The native microalgae species perform better than most other selectively inoculated microalgae species in commercial scale cultivation with wastewaters (Zhou et al., 2011). Adaptation in harmony with the local environment of wastewater not only facilitate the growth of these native species (Wang et al., 2012; Wu et al., 2014), but also physiological acclimatization of algae cells in wastewater improves their nutrient uptake efficiency (Chen et al., 2015). In recent researches it was found that high diversity native microalgae system in wastewater result in more robust operation, have higher growth rate, biomass productivity and even lipid content (Cheng & Tian, 2013; Chinnasamy et al., 2010). Moreover, open pond mass cultivation of mixed microalgae would be reasonably cheap and easy to operate and maintain, making the wastewater bioremediation and algae cultivation more cost-effective and efficient (Su et al., 2012).

In addition to system setup, there are other challenges associated with the nutrients composition (especially nitrogen and phosphorus) necessary for production success.

The nutrient status of wastewater can vary according to types of wastewater and their sources. In the same time, the availability and composition of the nutrients is extremely important for species dominance, biomass productivity as well as biomass chemical composition (Chen et al., 2015). Furthermore, culturing algae requires consideration of numerous environmental influences. Environmental factors such as temperature, light, pH and their fluctuations that influencing algal photosynthesis and growth rate may also affect the cellular metabolism and composition (Juneja, et al., 2013). Not the less, lack of cost-effective and efficient harvesting technique is another key limiting factor impeding the production commercialization, which in general accounts for at least 20-30% of the total production cost due to the tiny size (<70µm) and strong negative surface charge of microalgae cells (Zhou et al., 2014).

1.3 Justification

The over stated critical issues associated with microalgae cultivation in open pond system for biomass production, have been identified and approached individually by many researchers (Chen et al., 2015; Pittman et al., 2011; Wu et al., 2014). However, no cumulative experimental study on the effects of these factors on algae is available and the real potential of using wastewater to culture mixed microalgae is still uncertain and yet more to be explored. Understanding how the concentration and the ratio of major nutrients and environmental changes influence algal growth and in broader sense, the biochemical composition are crucial for successful scale-up of algae cultures in commercial systems for algal biofuels and bio-products production. Efficient harvesting technique is however, adding the final success to the whole production system.

Considering above, this present study, was addressing the hypothesis that culturing the native mixed microalgae in wastewater under open pond system will provide appreciate yield of biomass without sudden crash of culture and artificially altered major nutrients' concentration and ratio will stimulate the growth and biochemical composition. The aims of the research are

i) To measure the effects of concentration and ratio of major nutrients (nitrogen and phosphorus) on mixed microalgae biomass production,

ii) To determine the suitable flocculation technique for efficient harvesting,

iii) To asses the biochemical composition of harvested mixed microalgae after end of the cycles, and

iv) To evaluate the mixed microalgae diversity prevailed under nutrient adjustments while culturing the algae in wastewater in open pond system under different weather conditions.

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