

PHYCOREMEDICATION USING *BOTRYOCOCCUS* SP. AS NUTRIENTS  
REMOVAL IN ORGANIC WASTEWATERS COUPLED WITH  
HYDROCARBON PRODUCTION

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## **DEDICATION**

Especially to my beloved family, my supervisors and friends.

For giving me infinite care and blessing.

Thank you for your endless support to me.



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## ABSTRACT

Rapid population growth and industrial development are expected to contribute extremely to the world environmental crisis due to the excessive wastewater generation, global warming, climate change and increased use of petroleum fuels. In response to the problems, new technology via phytoremediation to reduce the wastewater contamination coupled with production of sustainable hydrocarbon has received much interest worldwide. Thus, the aim of the study is to produce the hydrocarbon from microalgae, *Botryococcus* sp. combined with phytoremediation of domestic wastewater (DW) and food processing wastewater (FW). The *Botryococcus* sp. locally isolated from the tropical rainforest. The optimisation study proved that the *Botryococcus* sp. grew well in the temperature of 23-33°C, the light intensity of 243  $\mu\text{mol m}^{-2}\text{s}^{-1}$  and 24 hours of light exposure. In fact, this *Botryococcus* sp. much more tolerated with the outdoor condition when integrated with wastewater phytoremediation in term of biomass productivity and wastewaters bioremediation. The best microalgae concentration was performed at  $10^6$  cells/mL for both wastewaters. The highest removal of nutrients (TP, TN and TOC) in DW and FW up to 100% and 92.8%, respectively under outdoor condition; while 95.4% and 76.4%, respectively under indoor condition. Selected heavy metal (Zn, Fe, Cd, Mn) study showed a very significant reduction ( $p<0.05$ ) for both wastewaters as influenced by culture conditions. In flocculation harvesting, alum indicated the best coagulant to recover microalgae biomass from DW with efficiency up to 99.3% while chitosan showed a good candidate to harvest *Botryococcus* sp. from FW with efficiency about 94.9%. This study notably found that different culture media used in cultivation produced difference kinds of hydrocarbon compounds. As known, the biggest contribution of this algae oil as biofuel feedstock that potentially contributes to the development of renewable energy technology. Moreover, the hydrocarbon compounds obtained also have bright perspective to be used as a chemical value added in any related industry.

## ABSTRAK

Pertambahan penduduk yang pesat dan pembangunan industri menyumbang kepada krisis alam sekitar akibat daripada penjanaan air sisa berlebihan, pemanasan global, perubahan iklim serta peningkatan penggunaan bahan api. Dengan itu, teknologi baru melalui *phycoremediation* untuk pemulihan pencemaran air sisa disamping berpotensi mengeluarkan biojisim untuk hidrokarbon amatlah diperlukan. Oleh itu, tujuan kajian ini adalah untuk menghasilkan hidrokarbon dari mikroalga, *Botryococcus* sp. yang di intergrasikan dengan *phycoremediation* air sisa domestic (DW) dan air sisa pemprosesan makanan (FW). *Botryococcus* sp. diperolehi daripada hutan tempatan. Kajian membuktikan bahawa *Botryococcus* sp. membiak dengan baik pada suhu 23-33°C, keamatan cahaya  $243 \mu\text{mol m}^{-2}\text{s}^{-1}$  dan 24 jam pendedahan cahaya. *Botryococcus* sp. lebih mengemari keadaan luar apabila digabungkan dengan air sisa dari segi produktiviti biojisim dan rawatan air sisa. Kepekatan mikroalga yang terbaik adalah pada  $10^6$  sel/mL. Penyingkiran tertinggi nutrien (TP, TN dan TOC) dalam DW dan FW sehingga 100% dan 92.8% dalam keadaan luar; manakala 95.4% dan 76.4% dalam keadaan tertutup. Penyingkiran logam berat (Zn, Fe, Cd, Mn) menunjukkan pengurangan yang ketara ( $p<0.05$ ) untuk kedua-dua air sisa tersebut. Darisegi penuaian, alum menunjukkan agen terbaik untuk menuai mikroalga daripada DW dengan kecekapan sehingga 99.3% manakala chitosan bagus untuk menuai daripada FW dengan kecekapan pada 94.9%. Kajian ini mendapati bahawa terutamanya perbezaan media yang digunakan dalam penanaman menghasilkan sebatian hidrokarbon yang berlainan. Seperti yang diketahui, sumbangan terbesar minyak alga ini adalah sebagai bahan mentah untuk bahan api bio yang berpotensi menyumbang kepada pembangunan teknologi tenaga boleh diperbaharui. Selain itu, sebatian hidrokarbon yang diperolehi juga mempunyai potensi yang besar untuk digunakan sebagai bahan kimia tambahan dalam mana-mana industri yang berkaitan.

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PTTA UTHM  
PERPUSTAKAAN TUNKU TUN AMINAH

## LIST OF SYMBOLS AND ABBREVIATIONS

|              |   |                            |
|--------------|---|----------------------------|
| $\mu_{\max}$ | - | Maximum growth rate        |
| $D_d$        | - | Division per day           |
| $t_d$        | - | Doubling time              |
| <i>sp.</i>   | - | Species                    |
| $H$          | - | Hydrogen                   |
| $C$          | - | Carbon                     |
| $GHG$        | - | Greenhouse gases           |
| $P$          | - | Phycoremediation           |
| $X_0$        | - | Initial cell concentration |
| $X_m$        | - | Maximum cell concentration |
| $Mn$         | - | Manganese                  |
| $Zn$         | - | Zinc                       |
| $Cd$         | - | Cadmium                    |
| $Fe$         | - | Iron                       |
| $^{\circ}C$  | - | Degree Celsius             |
| $M$          | - | Mole                       |
| $H$          | - | Hour                       |
| $N$          | - | North                      |
| $E$          | - | East                       |
| $k$          | - | Coefficient rate constant  |
| $t$          | - | Time                       |
| $BOD$        | - | Biochemical oxygen demand  |
| $COD$        | - | Chemical oxygen demand     |
| $TP$         | - | Total phosphorus           |
| $TN$         | - | Total nitrogen             |
| $DW$         | - | Domestic wastewater        |
| $FW$         | - | Food processing wastewater |

|               |   |  |
|---------------|---|--|
| <i>TOC</i>    | - | Total organic carbon                         |
| <i>TSS</i>    | - | Total Suspended Solid                        |
| <i>IC</i>     | - | Inorganic carbon                             |
| <i>TC</i>     | - | Total carbon                                 |
| <i>ICP-MS</i> | - | Inductively coupled plasma mass spectrometry |
| <i>BBM</i>    | - | Bold's Basal Medium                          |
| <i>GC-MS</i>  | - | Gas chromatography-mass spectrometry         |
| <i>FT-IR</i>  | - | Fourier transform infrared spectroscopy      |
| <i>RSM</i>    | - | Response surface methodology                 |
| <i>DNA</i>    | - | Deoxyribonucleic acid                        |
| <i>PCR</i>    | - | Polymerase chain reaction                    |
| <i>OD</i>     | - | Optical density                              |
| <i>APHA</i>   | - | American Public Health Association           |
| <i>ASTM</i>   | - | American Society for Testing and Materials   |
| <i>UTHM</i>   | - | Universiti Tun Hussein Onn Malaysia          |



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# **CHAPTER 1**

## **INTRODUCTION**

### **1.1 Background of the study**

Wastewater is well known to contain contaminants that can negatively impact the environment if not controlled in terms of pollutant load removal. This is because wastewater containing nutrients such as nitrogen and phosphorus can affect the natural ecosystem particularly the aquatic lives. Large amounts of water used for domestic and industrial purposes result in the generation of large volume of wastewater loaded with nutrients (Danilović *et al.*, 2013). In addition, wastewater may also contain heavy metal pollutants, especially in industrial wastewater eg. Iron, cadmium, zinc, ammonia etc. (Danilović *et al.*, 2013). Furthermore, the presence of heavy metal and organic compound in wastewater can cause long-term problems (Chan, Salsali & McBean, 2014; Onalo, Matias-Peralta & Sunar, 2014; Travieso *et al.*, 1999). Considering all these facts on wastewater, issues as such need to be solved correctly without contributing other problems.

Meanwhile, Sriram & Seenivasan (2012) stated that the wastewater is a word to represent the water with poor or low quality that contains a high amount of pollutants and microbes. Thus, discharging wastewater directly into the water body may lead to the serious environment and human health problems. Without any compromises, pollutant loads in wastewater should be removed to ensure compliance with the local effluent standard before discharging into the environment. Nowadays, selection of treatment method is one interesting topic among the researchers either conventional, bioremediation or advanced method. Phycoremediation is a branch of bioremediation technique in wastewater treatment. According to Phang, Chu &

Rabiei (2015), phycoremediation is the use of either macro or microalgae for the removal or biotransformation of pollutants including nutrients and toxic chemical from wastewater. In the past, microalgae have attracted much attention as an alternative to the conventional treatment method. Microalgae wastewater treatment is an eco-friendly approach that offers the advantages of a cost effective way of removing pollutant loads (Sriram & Seenivasan, 2012). The previous study has reported that the use of algae to treat wastewater has been in practice for over 40 years (Ahmad, Khan & Yasar, 2013) and the first description of this application was reported by Oswald in 1957. Wastewater bioremediation, (phycoremediation) technology can also be combined with hydrocarbon production. Since the hydrocarbon was obtained from biological plant or algae, then it's called as bio-hydrocarbon. In the terminology of its chemical, the hydrocarbon is an organic compound consisting entirely of hydrogen (H) and carbon (C). Hydrocarbon production from microalgae refers to the lipid or oil content that can be obtained from algae biomass. The most popular hydrocarbon product that has been investigated from microalgae recently is biofuel (Rawat *et al.*, 2011). These algae do not only produce biofuel but also great potential to create other bio-based product such as fertiliser, animal foods and bioactive chemicals (Sivakumar & Rajendran, 2013). According to Amaro, Guedes, & Malcata (2011), microalgae such as *Botryococcus* sp. produced highest up to 75% of oil in dry weight basis, which has high potential as a new bio-based product for renewable energy development. Therefore, this species was chosen as the microalgae that conducted in this study.

As the demand for energy continues to increase globally, fossil fuel usage will likewise continue to rise. There is still a plentiful supply of fossil fuels at reasonably low cost, although this is likely to change in the future. More critically, though, rising use of fossil fuel is unlikely to be sustainable in the longer term, principally due to the attributed increase in greenhouse gases emission and the environmental impact of this emission on global warming (Hill *et al.*, 2006).

Compared with other forms of renewable energy (e.g. the wind, tidal and solar), biofuels allow energy to be chemically stored and conveniently be used in existing engines and transportation infrastructures after blending to various degrees with petroleum diesel (Singh & Gu, 2010). This biodiesel is, in essence, a set of monoalkyl esters of long-chain fatty acids – and at present is derived chiefly from the acylglycerols of plant oils. Besides being renewable, biofuel is also non-toxic and

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