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SCREENING OF SUSTAINABLE HYDROCARBON EXTRACTED FROM MICROALGAE VIA PHYCOREMEDIATION

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ABSTRACT

A preliminary study of extracted hydrocarbon from microalgae biomass including nutrients removal is presented. The aim of this work is to determine the removal of nutrients from domestic wastewater by *Botryococcus* sp. and to identify the qualitative hydrocarbon from extracted biomass. The results showed that *Botryococcus* sp. is capable to remove total nitrogen 60.83% and total phosphorus 36.17% from domestic wastewater. Since the best result was found in wastewater treatment, lipid content was performed with solvent extraction using soxhlet extractor. From GC-MS analysis, Phthalic acid, 2-ethylhexyl tridecylester was obtained with the largest peak area of 71.56%. This study proved that *Botryococcus* sp. from domestic wastewater treatment phycoremediation could produce biomass with suitable amount of lipids and chemical compound.

Keywords: *botryococcus* sp., domestic wastewater, nutrients, biomass.

INTRODUCTION

Domestic wastewater is a main source of contaminations and polluted to water bodies as well as aquatic life in marine ecosystem. Current treatment technologies are expensive and partially effective to remove excessive nutrients [33]. Therefore, a lot of wastewater researcher had introduced biological treatment using microalgae to deal with the excess nutrients present in wastewater [1, 4, 5, 6, 8, 9, 10, 13, 15, 16, 18, 22, 23, 24].

Furthermore, growing microalgae in wastewater is not only for treatment purposed, but successfully had been investigated be able to produce bio-oil from microalgae biomass such as hydrocarbon, lipid, biodiesel and biofuel [3, 11, 14, 26, 29, 31, 32, 34, 35, 36, 37, 39, 45, 47, 48, 50]. Microalgae have high potential as alternative source of biodiesel because it is a sustainable lipid extracted from biological photosynthetic plant. Other than that, microalgae biodiesel also assumed as ecofriendly approach and be able to increase the production of renewable energy to compete with conventional biofuel source [14, 17, 21, 25, 38, 43].

Moreover, [12] stated that microalgae gained enormous consideration from scientific community worldwide emerging as a viable feedstock for a renewable energy source virtually being carbon neutral, high lipid content and comparatively more advantageous to other sources of biofuels. Accordingly, wastewaters were chosen to grow microalgae due to high concentration of nutrients and simultaneously effectively to produce biomass for hydrocarbon production. As stated by [48] where *Botryococcusbraunii* able to produce microalgae lipid content up to 25-80% of dry biomass, *Spirulina maxima* about 6-7% and *Nannochloropsis* sp. up to 68%. Meanwhile for nutrient removal, Sahu 2014 found that total nitrogen could be reduce about 71% and 67% of total phosphorus when he used *Chlorella vulgaris* to treating the municipal wastewater. Other study done by [28] using *Scenedesmusobliquus* in piggery wastewater found that total nitrogen reduced around 23-58% while for total phosphorus was about 48-69%. In addition, *Botryococcus* sp. reduced total nitrogen 67% in greywater while increased 8.1% in dairy wastewater (Gani *et al.* 2015a, b). It can be seen that the effective of microalgae in treating wastewater and hydrocarbon mostly depending on the media condition and also selection of microalgae species.

Hence, aim of this paper was to establish the steps for the development of microalgae cultivation system in wastewater to produce sustainable extracted lipid from microalgae biomass. The removal of excessive nutrients such as total nitrogen and total phosphorus present in wastewater were determined. Beside, preliminary hydrocarbon qualitative composition was also performed.

MATERIALS AND METHODS

Microalgae preparation

The *Botryococcus* sp. was collected, isolated and purified prior to the experiment. The microalgae was identified based on the morphology characteristics and confirmed by DNA molecular test.Upon confirmation of the purity of the isolated, the inoculum was kept in modified Bolds basal media (both in agar slant and broth) inside an incubator at ambient temperature (27 °C-30 °C) for 14 days. The microalgae were subsequently cultured in 2L capacity flask prior to the experiment. After that, the microalgal culture was harvested by centrifugation at low speed (3500rpm) for ten minutes with two times washing with sterile distilled water for prior to cell count. The cell density was counted using haemocytometer at an initial cell according to Andersen (2005). After which starting initial concentration of 10^3cell/mL were inoculated in the wastewater to commence the treatment (APHA, 2012).

Wastewater sampling

Domestic wastewater was obtained from influent of sewage treatment plant located inside BatuPahat, Johor, Malaysia. The samples, which were collected using acid washed 10L bottles (APHA, 2012) and nutrient such as total phosphorus (DR6000, Method 8190) and total nitrogen (TOC Analyzer, Brand: TOC-VCSH, Japan, Shimadzu) were tested immediately. Then, wastewater kept in a cold room (4°C) before commencement the experiment. Prior to the microalgae cultivation process, the wastewater was filtered first with a 0.45µm membrane filter (Whatman).

Cultivation process

The photobioreactor (Figure-1) made of acrylic glass used in this study. The construction of photobioreactor according to [49] with some modifications. The microalgae cultivated in wastewaters based on the outdoor natural condition. In this concept, wastewater will be circulated and homogenized using a water pump. The total volume of the photobioreactor is approximately 27 L and allowable working volume limited to 25 L only to give air space of photobioreactor. This type of photobioreactor designed to avoid the contamination if compared to the open pond. By that, the purity of the product or hydrocarbon produced can be maintained. Also, some of the advantages using this bioreactor is as more cost-effectively for the growth of microalgae for nutrient removal in wastewater for biomass production, minimal power consumption and easy to handling.

Hydrocarbon screening examination

Flocculation method using aluminium sulfate, Al2(SO4)3 as coagulant was employed to harvest the microalgal biomass. Upon concentration of the biomass, oven drying at 60°C for 24 hours followed. After that, extraction procedure was conducted based on EPA Method 9071B (n-Hexane Extractablematerial). This method is applicable for the extraction of non-volatile hydrocarbon, vegetable oils, animal fats, waxes, soaps, grease, biological lipids and related materials.The oil sample was analyzed using DB 5 MS column (30 m x 0.32 mm ID x 0.25 µm film thickness) using GC–MS. The conditions are used as per [20]. The initial temperature of the oven is at 130°C for 5 min thatwas increased to 200°C at the rate of 8°C per minute. After maintaining at 200°C for 2 min, the temperature was increased to 280°C at the rate of 5°C/min and maintained for 15 min. The injector port and the detector temperatures are 240°C and 250°C respectively. The peak was tentatively identified based on library search report or NIST (National Institute of Standard and Technology) database.

RESULT AND DISCUSSIONS

Nutrients removal

The results shown in Figure 2 clearly indicated a good removal effect of total nitrogen and total phosphorus at final stage of cultivation by *Botryococcus* sp. The removal of total nitrogen was 36.33% on the Day 10 of cultivation and increased up to 60.83% on the Day 20 from initial concentration of 19.9 mg/L. It shown that *Botryococcus* sp. was able to remove more than half of initial concentration the total nitrogen. This result inline with findings of [41, 46] who reported 71% and 58% removal in total nitrogen using *Chlorella vulgaris* and microalgal bacterial flocs. Meanwhile, total phosphorus removal by *Botryococcus sp.* (Figure-2) slightly lower than total nitrogen where total phosphorus able to be reduce about 16% on the Day 10 and 36.17% on the day 20. However, [42] were observed different removal percentage of total phosphorus in which their *Chlorella sp*. could remove up to 70% on primary treated wastewater. Also, [28] found that *Scenedesmusobliquus* capable to remove total phosphorus about 48-69% from piggery wastewater.

There are several factors that had been discussed could give effect to the differential value of removal for both nutrients such as microalgae species and cell concentration used, type of wastewater in term of pollutant load and sampling time, environmental factors like light, photoperiod, temperature, salinity and pH and pretreatment of wastewater before commence the experiment [15, 29, 44]. Furthermore, [12] had stated that microalgae remove significant amount of nitrogen and phosphorus for protein, nucleic acid and phospholipid synthesis of their cell accumulation.

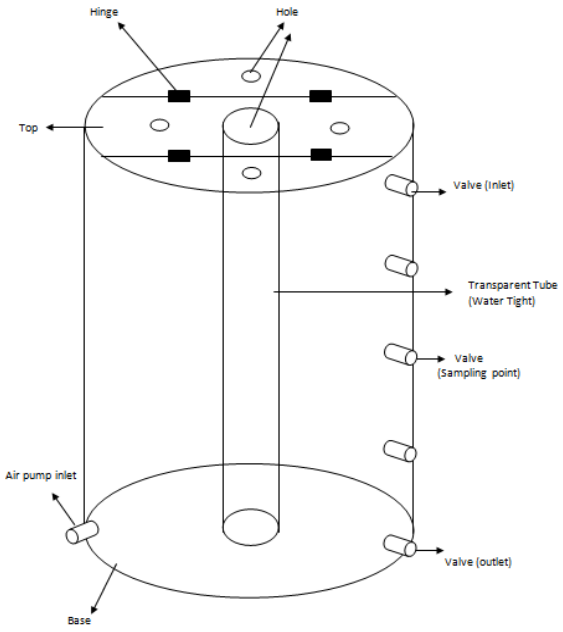

Figure-1. Schematic diagram of developed photobioreactor system.

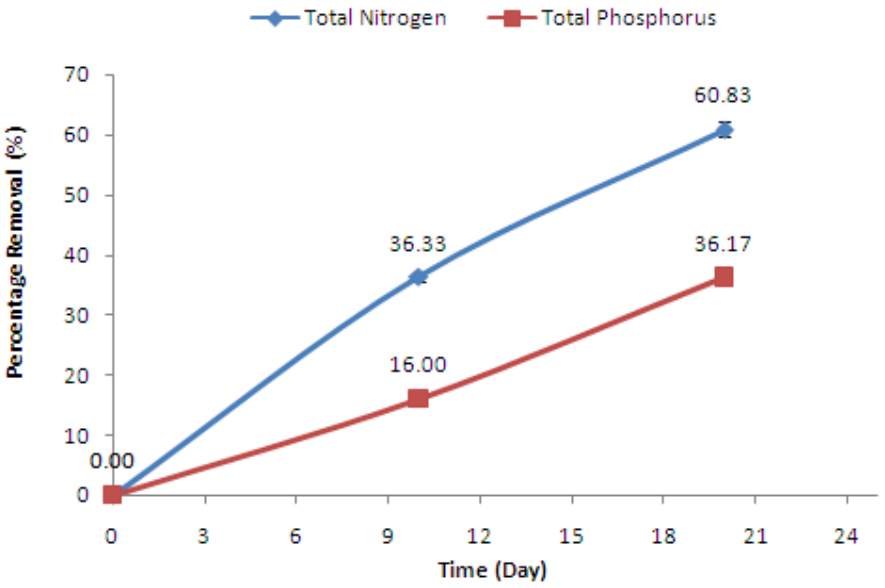

Figure-2. Nutrients removal by *Botryococcus*sp. phycoremediation.

Hydrocarbon screening

The qualitative analysis of extracted hydrocarbon from *Botryococcus* sp. grown on domestic wastewater is tabulated in Table-1 below. Initially, there were 5 compounds were identified in n-Hexane extract by GC-MS. The largest peak area was 71.56% namely phthalic acid, 2-ethylhexyl tridecyl ester carrying 460.68 g/mol o molecular weight. The second largest peak area 7.59% is due to the presence of 1-Diphenyl (tert-butyl) silyloxy-4-methoxybenzene. The third peak was at 6.85% which is properties of 2-(Acetoxymethyl)-3-(methoxycarbon) biphenylene. The other two less prominent peaks at different area and molecular weight are given in Table-1. This result showed us that the potential of *Botryococcus*sp hydrocarbon in many industry such as biofuel, bio-plastic and bio-based product.Evidence supporting that this oil can be used as abioplastic with the existence of the largest peak area of Phthalic acid. According to [27] report which is clearly have indicated the high potential of this oil to be used in plastic industry as additive materials.

Table-1. Hydrocarbon composition from extracted *Botryococcus* sp. Biomass.

Compounds name	Molecular formula	Molecular weight (g/mol)	% Area
Phthalic acid, 2-ethylhexyl tridecyl ester	$C_{29}H_{48}O_4$	460.68	71.56
1-Diphenyl (tert-butyl) silyloxy-4-methoxybenzene	$C_{23}H_{26}O_2Si$	362.53	7.59
2-(Acetoxymethyl)-3-(methoxycarbon) biphenylene	$C_{12}H_8$	152.19	6.85
Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15 - hexamethyl-	$C_{16}H_{48}O_7Si_8$	577.23	4.78
Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368.63	3.03

CONCLUSIONS

Preliminary qualitative screening of hydrocarbon from microalgae is proposed including consecutive steps of phycoremediation, biomass harvesting and lipid extraction by growing *Botryococcus* sp. in photo bioreactor. The study proved that *Botryococcus* sp. is effective for the reduction of nutrients from domestic wastewater. Considering that *Botryococcus* sp. have been successfully produced an amount of lipid via phycoremediation in this study, it is enough to suggest that this microalgae is a suitable candidate for combination of hydrocarbon production and wastewater treatment for sustainable development of our environmental protection.

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