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L. ABDULLAH[1], N.M. APANDI[1*], N.M. SUNAR[1,2*], S.N.H. ARIFIN[2], M. HARON[1], R. NAGARAJAH[1], K. CHEONG[1], C. HENG[3], C.F. SOON[4], M. NABIAŁEK[5]

THE PROCESSING PROPAGATION OF MICROALGAE *BOTRYOCOCCUS* SP. CULTIVATION IN PALM OIL MILL EFFLUENT MATERIALS

Palm Oil Mill Effluent Materials (POME) are significantly more contaminated than municipal sewage due to their high chemical and biological oxygen demand (BOD and COD). This study examines the properties of POME wastewater under typical physical settings to track the growth conditions of microalgae, namely *Botryococcus* sp., in various volumes at varying POME dilutions. Begin with analyzing POME's water quality measurements and conclude the growing conditions of microalgae. *Botryococcus* sp. microalgae could not flourish in diluted raw POME. However, it was well propagated in diluted anaerobic POME under adequate light and oxygen conditions. The finding shows that diluted anaerobic POME 70% is the ideal dilution for microalgae *Botryococcus* sp. to proliferate. Raw POME is physically described as a thick, brownish liquid with a high total solids and turbidity concentration that is contained in water. The study explores the use of *Botryococcus* sp. culture and propagation in POME materials for sustainable bioenergy production, highlighting the potential of microalgae for future economic benefits.

Keyword: POME; microalgae *Botryococcus* sp.; microalgae cultivation; wastewater

1. Introduction

Palm oil mill effluent (POME) is one of the major waste streams from the palm oil industry and contains a high proportion of organic material. The palm oil industry generates huge amounts of waste [1]. POME has a high biological oxygen demand (BOD) and chemical oxygen demand (COD), making it more contaminated than municipal sewage. POME, whether fully or partially treated, is still released into adjacent waterways and land with high concentrations of phosphorus, organic nitrogen, and other constituents with the potential to be utilized as a medium for microalgae cultivation [2]. Inorganic fertilizers containing phosphorus and nitrogen are widely used in agriculture due to their high demand. However, due to nitrogen and phosphorus loss through denitrification, leaching, runoff, and retention in organic matter pools, only a small portion of these nutrients can be effectively utilized by plants [3-5].

The application of POME for microalgae cultivation has extensive advantages based on the fact utilization of microalgae for POME treatment leads to a twofold benefit: a reduction in its nutrient levels and the generation of biomass, which can serve as bulk biomass or be transformed into a value-added product. POME-cultivated microalgae exhibit abundant levels of lipids, proteins, and carbohydrates, making them highly valuable for applications in animal feed and fuel production [2,6]. In addition, microalgae growth is an alternative medium for microalgae biomass production [7]. Hence, these microalgae can serve as an excellent fertilizer for further enhancing the propagation and advancement of microalgae technology [2,8-9]. This fact is referred to as microalgae rich in nutrients like phosphorus and nitrogen which help to promote root development to improve overall plant health via the release of hormones, enzymes, and vitamins for growth and stress tolerance.

Microalgae can be used to mitigate atmospheric Carbon Dioxide (CO^2) and clean wastewater. It has been proposed as a good biological platform that can reduce CO^2, a greenhouse gas, and also potentially enhance the high-value companies such as pharmaceutical, cosmetic, food, and biofuel industries [10].

[1] UNIVERSITI TUN HUSSEIN ONN MALAYSIA, FACULTY OF ENGINEERING TECHNOLOGY, DEPARTMENT OF CIVIL ENGINEERING TECHNOLOGY, PAGOH EDUCATION HUB, 84600 PAGOH, MUAR, JOHOR, MALAYSIA
[2] UNIVERSITI TUN HUSSEIN ONN MALAYSIA, RESEARCH CENTRE FOR SOFT SOIL (RECESS), INSTITUTE OF INTEGRATED ENGINEERING, PARIT RAJA, 86400 BATU PAHAT, JOHOR, MALAYSIA
[3] GREEN INNOVATION AND ENERGY SDN BHD., NO. 159A, JALAN SRI PELANGI, TAMAN PELANGI, JOHOR BAHRU, MALAYSIA
[4] UNIVERSITI TUN HUSSEIN ONN MALAYSIA, MICROELECTRONICS & NANOTECHNOLOGY-SHAMSUDDIN RESEARCH CENTRE (MINT-SRC), PARIT RAJA, 86400 BATU PAHAT, JOHOR, MALAYSIA
[5] CZESTOCHOWA UNIVERSITY OF TECHNOLOGY, FACULTY OF PRODUCTION ENGINEERING AND MATERIALS TECHNOLOGY, DEPARTMENT OF PHYSICS, 19 ARMII KRAJOWEJ AV., 42-200 CZĘSTOCHOWA, POLAND

* Coressponding author: shuhaila@uthm.edu.my

It has been demonstrated that microalgae can degrade COD, phosphate, and ammonium under both agitation and aeration cultivation conditions [2,6]. However, microalgae performance in removing the components may vary in different types of cultivation conditions. Among these microalgae species, *Botryococcus* sp. stands out as a promising candidate due to its ability to secrete and accumulate high quantities of hydrocarbon-rich substances, making it a valuable source of biofuel and other high-value compounds [6].

Therefore, this study aims to investigate the growth conditions of microalgae, specifically *Botryococcus* sp., in different volumes of POME dilutions by examining the characteristics of POME wastewater under physical settings. The use of microalgae grown in POME is significant initiative to degrade and convert almost all organic materials in effluent [11]. By exploring the cultivation and propagation of *Botryococcus* sp. in POME materials, the present study can establish a sustainable platform for bioenergy production while addressing POME-related environmental challenges. Microalgae, grown through photosynthesis, have the potential to treat POME by converting them into biomass, which can generate profit by utilizing nitrogen and phosphorous compounds found in POME [12]. Besides, by it, microalgae hold great potential for sustainable biofuel production and various other commercial applications for better future application and generation, also [13] mentioned microalgae as promising solution for environmental issues.

2. Materials and methods

2.1. Material and sampling preparations

This study investigated the POME wastewater characteristics in normal physical conditions and observed the microalgae growth condition in different volumes of microalgae with different dilutions of POME together with the analysis of water quality parameters of POME propagated with microalgae. The POME wastewater samples were collected from the industry; 5 liters of raw POME and 10 liters of anaerobic POME. Each sample was kept in a sample holding drum and labeled with date as shown in Fig. 1(a) whilst the lid of the holding drum was not tightly closed to maintain air exchange to sustain micro-flora in the samples. Before the lab experiment, both samples were transferred into a fine mesh-covered (to maintain air exchange) glass container as shown in Fig. 1 (b). POME characteristics were tested with the HI98194 Multiparameter before preparing the diluted samples. According to APHA [14-15], measurements of POME sample characteristics included pH, dissolved oxygen (DO), total dissolved solids (TDS), temperature, total phosphate, total hardness, total alkalinity, and turbidity. All the mentioned characteristics were determined by following APHA standards and guildelines [14-15].

2.3. POME-microalgae sample preparation

The freshwater microalgae *Botryococcus* sp. was used as a genus of green microalgae. Microalgae *Botryococcus* sp. are a type of unicellular photosynthetic organism with a variety of useful properties that can be exploited to solve problems like wastewater treatment and biomass production [16].

The mixture of raw POME, anaerobic POME, microalgae *Botryococcus* sp., and distilled water was mixed in a 500 mL conical flask with the dilution as illustrated in Fig. 1(a & b). The ratio for the sample preparations is as follows:

- 20000 mL tank: 1000 mL microalgae: 1000 mL POME waste,
- The ratio for the dilution is 5 : 1 : 1.

For 100% Diluted Sample:

- Tank: 20000/500 = 40×2
- POME Waste: 1000/40 = 25 mL
- Microalgae: 1000/40 = 25 mL

2.4. Microalgae cultivation preparation

Temperature and light are the most important parameters in regulating the growth of microalgae. Microalgae need constant sunlight and oxygen to breed. Therefore, 10 samples propagated with microalgae were placed in a box with a supply of artificial

(a) (b)

Fig. 1. (a) POME samples in holding drums (b) POME samples in glass container

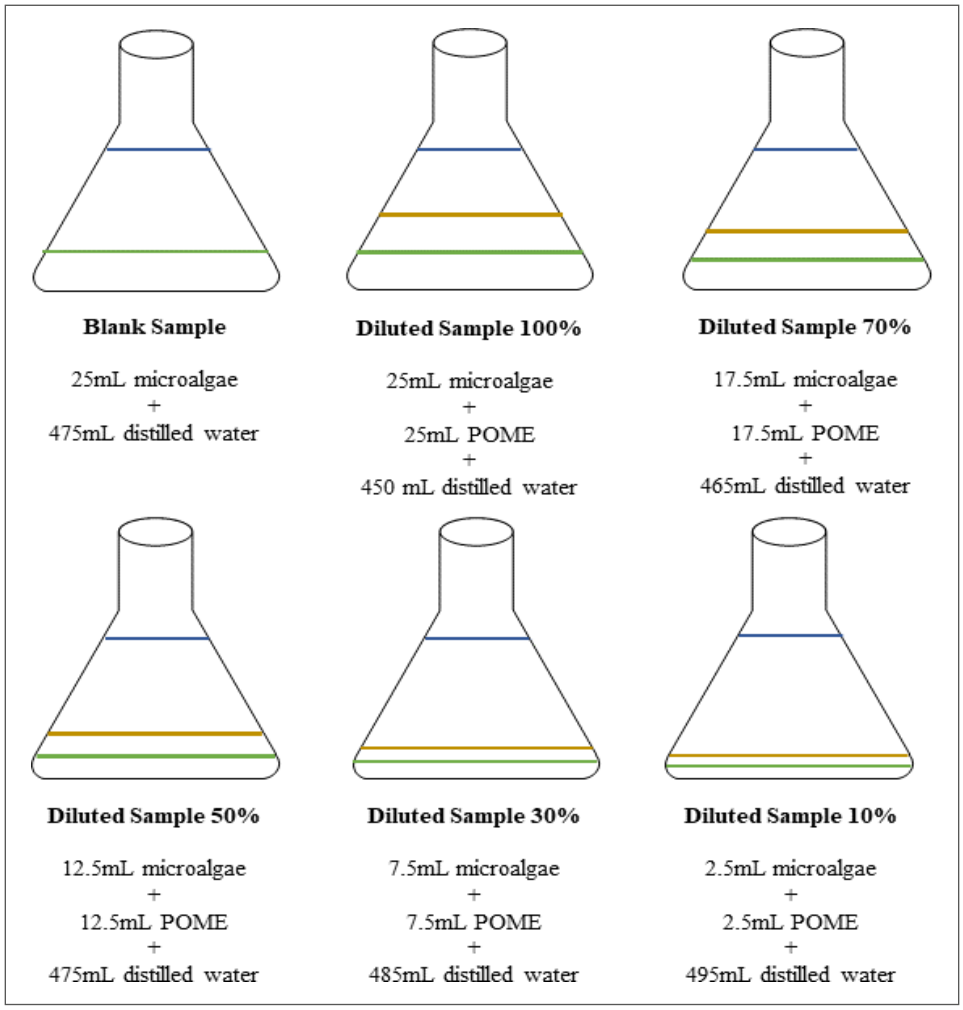

Fig. 2. Dilution of samples

light and an oxygen pump for 24 hours a day. The equipment setup for the cultivation of microalgae is shown in Fig. 3 and Fig. 4. Each sample such as raw and anaerobic sample has five types of dilution in five different conical flasks with 1 conical flask for blank sample. A total of ten conical flasks containing POME, microalgae and distilled water was prepared and put in the box. Each conical flask was equipped with an oxygen pump and oxygen stone to provide enough oxygen for the cultivation process. The experimental was carried out for 5 days.

Fig. 3. Solar lamp for constant light and temperature rod for maintaining temperature

Fig. 4. Full setup for cultivation of microalgae with pumps and oxygen stones

2.4. Growth rate of microalgae

Microalgae growth was determined daily by optical density. The optical density was measured at 680 nm using a spectrophotometer. Wavelength 680 nm was chosen for calculation because this wavelength was selected due to the maximum absorbance obtained when a culture sample was scanning between 550 and 800 nm. Growth rate of *Botryococcus* sp. microalgae was calculated using the formula shown below, where N_1 and N_2 represent optical density (OD) readings at 680 nm, while t_1 and t_2 are time intervals [17].

3. Results and discussion

3.1. POME characteristics

Both raw and anaerobic POME samples were retrieved, and the chemical and physical composition of both samples are determined. Then, further analysis was conducted with various water quality parameters testing to observe the characteristics of POME samples and growth of microalgae in POME. Wastewater characterization is required and critical for determining the nutrient supplements needed for microalgae growth during the cultivation process. TABLE 1 shows the comparison on the general characteristics of anaerobic and raw POME with Department of Environment (DOE) discharged limit and Environmental Quality Act (EQA) 1974 standards. According to the analysis, all the parameters examined exceeded the permissible values set by the Standard Limit Environment Quality Act [18]. The elevated levels of total phosphate content in the effluent suggest that if this sample is discharged into a river, it may poses environmental concerns such as algae bloom.

3.2. Microalgae growth rate

Microalgae growth can be divided into four stages: lag phase, log phase or exponential, stationary phase, and finally death phase production [19]. The lag phase is the first stage of cultivation during microalgae adaption to its surroundings such as medium, pH, temperature, and lighting. Subsequently, the microalgae enter a phase of active division, causing the culture's biomass to grow exponentially. Once this growth reaches a certain point, the stationary phase commences, halting any further increase in biomass. This equilibrium is maintained because cell division and cell death occur at an equal rate. The growth rate of *Botryococcus* sp. was illustrated by plotting the graph of growth rate against time in day as shown in Fig. 5 and Fig. 6.

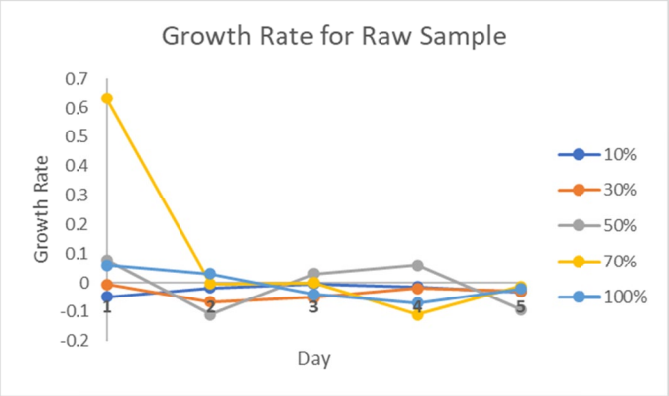

Fig. 5. Growth rate for diluted raw sample

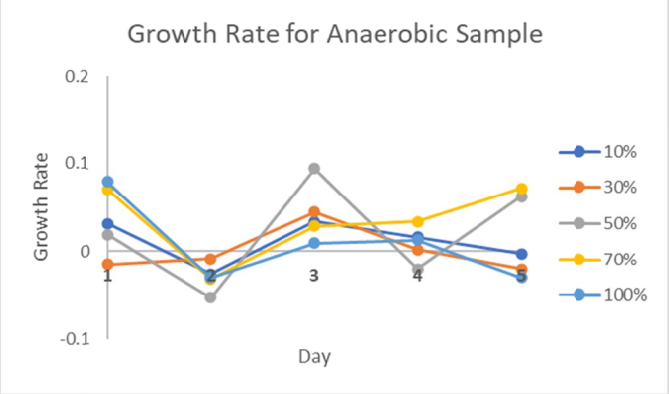

Fig. 6. Growth rate for diluted anaerobic sample

According to Fig. 5, the result obtained shows that *Botryococcus* sp. cannot propagate even in diluted raw POME. Meanwhile according to Fig. 6, it shows that Botryococcus sp. can propagate in anaerobic sample because of its constant positive value in the OD and growth rate.

TABLE 1

Physiochemical compositions for POME characteristics

Parameters	Concentration (mg/L)		DOE discharged Limit	Environmental Quality Act, 1974
	Anaerobic	Raw		
pH	7.734	4.372	5.0	5.5-9.0
DO Conc. (mg/L)	0.172	0.232	—	—
TDS (mg/L)	6058.6	7450.8	1500	1500
Temperature (°C)	26.204	26.424	45	45
Total Phosphate (mg/L)	193.5	2230	5	10
Total Hardness (mg/L)	25	50	—	—
Total Alkalinity (mg/L)	240	80	—	—
Turbidity (NTU)	376	3789.2		

It has been reported that POME influenced growth as a result of the condition that produced the mixotrophic cultivation condition and provided organic carbon. POME, on the other hand, has a high turbidity that inhibits light penetration and acts as an auto-inhibitor. It is found that using undiluted POME as a substrate inhibits the growth of phototrophs due to reduction of light penetration into the fermentation system [19]. Kamyab [20] also stated that a high POME concentration inhibited the growth rate of microalgae due to lact of light intensity and oxygen. One of the most important factors influencing growth rate is light intensity and oxygen [21]. *Botryococcus* sp. biomass has the potential to be a biofuel source due to its ability to produce a large amount of hydrocarbon and could be converted into a variety of chemical products [21]. The ability of *Botryococcus* sp. biomass to produce large amount of hydrocarbon has great potential for biofuel production. A sustainable strategy for the growth of renewable energy generation is the cultivation of microalgae for value-added compounds and as feedstock for biofuels. Cultivating *Botryococcus* sp. has the ability to produce hydrocarbons and could provide a sustainable feedstock for biofuels and chemical components used in many other sectors [21]. However,the cultivation of *Botryococcus* sp. on POME wastewater has not been thoroughly investigated.

4. Conclusion

In conclusion, the POME characteristics as waste that is acidic but not a threat to the microalgae. The physical condition of raw POME is a brownish thick liquid containing water, have high total solids and high turbidity. Based on our findings, it is evident that microalgae cannot propagate in raw POME since the physical characteristics of POME avoid the supply of oxygen and sunlight for the cultivation process of microalgae *Botryococcus* sp. This proves that raw POME waste inhibits the growth of microalgae *Botryococcus* sp. However, it can propagate in anaerobic POME since the diluted anaerobic POME characteristics enable light to penetrate in the cultivation and enough oxygen was supplied for microalgae *Botryococcus* sp. to propagate in it. The most suitable dilution for microalgae *Botryococcus* sp. to propagate is diluted anaerobic POME 70%. Further observation and analysis needed to be conducted with diluted anaerobic POME in order to investigate more significant growth rate of microalgae *Botryococcus* sp. for rapid production of hydrocarbons and biofuels extractions. The current work addressed the environmental issues associated with POME while establishing a sustainable platform for bioenergy production through the investigation of *Botryococcus* sp. culture and propagation in POME materials. As a result of this, microalgae have a lot of potential for producing biofuel sustainably as well as for a variety of other economic uses that will benefit future generations.

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