

## NUTRIENTS ASSESSMENT OF ANAEROBIC PALM OIL MILL EFFLUENT (AnPOME) AS AN ALTERNATIVE MEDIA FOR MICROALGAE CULTURE

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### ABSTRACT

The main aim of the present study is to investigate the availability of macronutrient and micronutrient in AnPOME. All collected samples were analyzed using HACH method. The results reveal that the C/N and N/P ratio of the AnPOME is six times more than the theoretical value. Concentration of micronutrients also shown higher concentration range compared to that of the Bold Basal Medium (BBM). However, the concentration of phosphorus in AnPOME six times lower than nitrogen indicates that phosphorus limitation in AnPOME. Hence, this finding suggested the best microalgae to be grown in AnPOME is mixotrophic or heterotrophic mode. Overall, reuse of AnPOME replacing synthetic growth is highly recommended considering that enough composition of macronutrient and micronutrient in AnPOME and inexpensive alternative to synthetic media.

**Keywords:** microalgae; Anaerobic Palm Oil Mill Effluent (AnPOME); BBM; macronutrient; micronutrient.

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## 1. INTRODUCTION

Malaysia is the world's second-largest palm oil producer after Indonesia. The average Malaysian crude palm oil production in 2016 was 17.32 million tonnes and is expected to be increased to 19.85 million tonnes in 2017. Therefore, its status as one of the key players within the country's agro-industry is recognized, solidifying itself as the fourth largest contributor to domestic economy growth. Even though palm oil industry is a major source of financial revenue domestically, it is also a major agro-industrial wastewater producer. Various types of waste produced by the palm oil industry such as oil palm trunks (OPT), oil palm fronds (OPF), empty fruit bunches (EFB), palm pressed fibres (PPF), palm shells and palm oil mill effluent (POME). According to [1], among all the waste produced, POME is said to be the most difficult waste to be handled due to high volume generated. For each tonne of crude palm oil (CPO) production necessitate for 5-7.5 tonnes of water, which 50% of them converted to POME [2]. Additionally, POME also contains high organic content (COD 50,000 mg/L, BOD 25,000 mg/L), Nitrogen, Phosphate, Potassium, Magnesium and Calcium which can cause eutrophication phenomenon [3]. Therefore, efficient treatment of POME is necessary in order to encourage sustainability of palm oil industry as promoted by the Malaysian Palm Oil Board (MPOB) and the Malaysian Palm Oil Council (MPOC) [4].

Most of the microalgae industry uses commercial media as a culture medium. In order to increase the production rate, supplementary nutrients and fertilizers such as NPK are added during algae cultivation. However, this method has led to the increases of operational costs and occasionally surpass its biomass production profit [5]. Thus, this makes commercial media less suitable for the mass production of microalgae. Alternatively, application of POME as a culture medium provides lower operational cost and beneficial for both industries in a long term. A large-scale outdoor cultivation can be carried out as there are abundant of nutrient sources in POME. Besides, this microalgae cultivation treatment able to overcome the limitation of conventional effluent treatment [6] practiced by most of the palm oil miller. Currently, zero waste technology is introduced to the Malaysian palm oil industry with the aim of no pollutant go to air, land and water. This technology comprises of EPB biomass pretreatment for biohydrogen production, biohydrogen fermentation and biomethane production from POME and pretreated EFB, heterogeneous catalysis for biomethane

reforming, biohydrogen purification for steam and power generation, membrane treatment for water recycling and reuse, and bioconversion of EFB agrowaste into organic fertilizers. Cultivation of microalgae through CO<sub>2</sub> sequestration also implemented. This will prevent the emission of greenhouse gas (GHG) to the atmosphere. Finally, to ensure the success of zero waste technology, the anaerobic POME (AnPOME) resulting from POME fermentation is reused for algae cultivation. Therefore, the characteristic of AnPOME is important before it can be used as a growth medium of algae. This is because nutrient concentrations are the main factors that need to be addressed to ensure that algae growth is at a maximum level. Hence, the purpose of this study is to identify the macronutrient and micronutrient content of an AnPOME sample taken from various sampling times.

## 2. METHODOLOGY

### 2.1. Sampling Site and Collection

The AnPOME were collected from the anaerobic digester tank from Sime Darby Tenammaram Palm Oil Mill, Bestari Jaya, Selangor, Malaysia GPS location at latitude 3°23'44.30"N longitude 101°25'03.41"E. Samples collected is stored in closed containers and directly transported to the laboratory without any delay. All samples were kept in a cold room at temperature less than 4°C, but over the freezing point in order to prevent the wastewater from undergoing biodegradation due to microbial activity [7].

### 2.2. AnPOME Characteristics

AnPOME sample was pre-treated by centrifuged at 8000rpm for 10 minutes. Afterward, supernatant of the sample was collected and filtered using syringe filter (0.45µm) to remove the solids and kept for further analysis. Hach DR 2800 spectrophotometer has been used for analysis of COD, BOD<sub>5</sub>, TN, NH<sub>4</sub><sup>+</sup>-N and TP by using Hach reagents accordingly. Whereas, the analysis of turbidity was measured by the Hach 2100 AN Turbidimeter and pH was determined by Cyberscan pH 300m (Eutech Instrument). For total suspended solid (TSS), AnPOME are filtered through Whatman glass microfibers (Grade GF/C 1.2 µm). The filters are dried and weighed to determine the amount of total suspended solids in mg L<sup>-1</sup> of sample. The determination of micronutrient content is performed using Agilent 7700 inductively coupled plasma mass spectrometry (ICP-MS).

### 3. RESULTS AND DISCUSSION

The properties of pre-treated anaerobic digestive POME (AnPOME) analysed and summarized in Table 1. Results obtained in this study were compared with literature and standard media (BBM). As shown in Table 1, pH value of this study is 7.82 almost the same as the study conducted by other researchers as well as the pH of standard media (BBM). The initial pH of the media is an important for the growth of microalgae [8]. In general, as observed from prior studies, initial pH of the media affects the solubility and the presence of the carbon dioxide which ultimately controls the growth of the cells [8-9]. The optimum pH most of the microalgae is between 7 and 8 while pH lower than 5 or higher than 9 are not tolerable [11]. Thus, the present finding support [11] study which concluded that AnPOME is suitable media for microalgae culture and offers an inexpensive alternative to synthetic media. Total suspended solid (TSS) concentration in the study was  $343.33 \text{ mg L}^{-1}$  lower than the regulatory limit of  $400 \text{ mg L}^{-1}$ . While, the concentration of COD and BOD were  $4066.67 \text{ mg L}^{-1}$  and  $1146 \text{ mg L}^{-1}$  respectively. These values exceed the regulatory limit of  $20 \text{ mg L}^{-1}$  BOD discharge. Thus, this indirectly implies AnPOME needs to be treated before releasing into the body of water. Besides, the large different characteristics of COD and BOD of AnPOME in this study with [12-13] may cause by many factors. As stated by [14], the characteristics of waste produced by palm oil mill is mainly affected by different oil extraction techniques, quality of oil palm fruit, cropping season and efficiency of wastewater treatment plant.

Theoretically, molecular formula of an average algae cell is  $\text{C}_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}\text{P}$  which consist of carbon, nitrogen and phosphorus as the main elements. Thus, these macronutrients need to be supplied to the maximum level for the optimum and productive growth. Nevertheless, the unbalanced nutrients inhibit growth of microalgae. In this study, the C/N ratio of the AnPOME is six times more than the theoretical value. N/P ratio also shows the same trend. Besides concentrations of nitrogen in AnPOME is  $330 \text{ mg L}^{-1}$ , significantly higher than in BBM. Meanwhile concentration of phosphorus is similar to BBM. In [15] stated 1 g of ammoniacal nitrogen or nitrate nitrogen consumption yielded approximately 15.8g of microalgae biomass. In another study, Woertz et al proposed a moderate concentration of nitrogen ( $30 \text{ mg L}^{-1}$ ) effectively increase the growth rate and lipid production of microalgae [16]. Thus, it is apparent from this study that AnPOME able to provide enough carbon and

nitrogen sources for microalgae growth.

In addition, another essential macronutrient required by microalgae is phosphorus. It plays major roles in multiple biological functions that involves energy metabolism. For example, association of ATP (adenosine triphosphate) with phosphorus, nucleic acids metabolism (DNA and RNA), maintenance of cell membrane structure (phospholipids) and cell signalling (phosphoprotein) [17]. During phosphorus deficiency in growth media, rate of respiration will be reduced. Consequently, the rate of photosynthesis  $\text{CO}_2$  decreases while darker  $\text{CO}_2$  fixation will increase and thereby increase the activity of two enzyme phosphoenolpyruvate carboxylase and phosphoenolpyruvate phosphatase [11]. This study shows that the concentration of phosphorus six times lower than nitrogen indicate that phosphorus limitation in AnPOME. Hence, these findings suggest that in general the best microalgae to be grown in AnPOME is mixotrophic or heterotrophic mode.

**Table 1.** Characteristics of AnPOME compared to literature and BBM

Parameter	AnPOME (This study)	[18]	[12]	[13]	BBM
pH	7.80±0.02	7.10	7.40	7.24	7.00
BOD (mg L <sup>-1</sup> )	1146.00±44.72	1520.00	1355.00	1938.00	-
COD (mg L <sup>-1</sup> )	4066.67±136.63	3040.00	13,650.00	20,314.00	-
Turbidity (NTU)	257.17±18.21	-	-	-	-
NH <sub>4</sub> <sup>+</sup> -N (mg L <sup>-1</sup> )	117.83±5.67	-	-	32.00	-
TSS (mg L <sup>-1</sup> )	343.33±85.24	334.00	12,750.00	14,686.00	-
Total Phosphorus, TP (mg L <sup>-1</sup> )	57.52±10.13	-	-	164.00	53.35
Total Nitrogen, TN (mg L <sup>-1</sup> )	330.00±30.00	320.00	320.00	-	41.16

The distribution of micronutrients in AnPOME and BBM is shown in Table 2. It can be seen that concentration of Na, Ca, Mg and K were approximately 350 mg L<sup>-1</sup>, 25.40 mg L<sup>-1</sup>, 374.08 mg L<sup>-1</sup> and 2436.43 mg L<sup>-1</sup> respectively are in the high ranges compared to that of the BBM. The results also show that, concentration of sodium lower than potassium. Sodium and potassium plays an important role in the transition of molecular nitrogen into ammonia during

the nitrogen fixing process in microalgae cell [19]. Since sodium and potassium categorized in the same group of the Periodic Table thus it has same chemical properties. Indeed, sodium ion can replace potassium during deficient conditions. The present findings also indicate concentration of magnesium is fifteen times more than calcium. The result is in the lines of earlier literature [20] that found the minimal requirements of magnesium of microalgae reported always to be more than that for calcium. The deficiency of magnesium nutrients in the media will inhibit cell division, resultant in unusually large etiolated cells [20].

**Table 2.** Distribution of micronutrients in AnPOME and BBM

Elements	AnPOME	BBM
Na	350.00±12.52	77.46
Ca	25.40±3.48	6.80
Mg	374.08±8.33	10.47
K	2436.43±44.87	105.50
Fe	2.35±0.91	1.01
B	0.97±0.08	2.00
Zn	1.00±0.65	2.00
Mn	1.56±0.26	0.40
Cu	0.42±0.02	0.40
Co	0.01±0.00	0.10

\*Concentrations of each element are in units of mg L<sup>-1</sup>

Moreover, Table 2 indicates that concentration of Fe, B, Zn, Mn, Cu and Co are in the similar level with that in BBM. As reported from previous studied inorganic elements such as N, P, K, Mg, Ca, S, Fe, Cu, Mn, Zn, Mo, Na, Co, V, Si, Se, Cr, Cd, Cl, B and I found in microalgae. Of these, N, P, Mg, Fe, Cu, Mn, Zn and Mo were essential micronutrients for all microalgae [21]. The roles of each of the micronutrients shown in Table 3.

**Table 3.** Role of micronutrients in microalgae cell

<b>Elements</b>	<b>Functions</b>
Mg	Central atom of chlorophyll molecule, aggregation of ribosomes into function units, for formation of catalyzed.
Fe	Used in physiological processes such electron transfer reaction in plant photosynthesis and nitrogen assimilation, respiration, protein and nucleic acid synthesis.
Cu	Electron transport (photosynthesis and respiration) enzymes, disproportionation of O <sub>2</sub> radicals to O <sub>2</sub> and H <sub>2</sub> O <sub>2</sub> in reaction.
Mn	Electron transport in PSII, maintenance of chloroplast membrane structure, breakdown reactions and those involving halogens.
Zn	Important as a metabolic function in enzymes, element in ribosome structure, nucleic acid replication and polymerization, hydration and dehydration of CO <sub>2</sub> .
Mo	Used in nitrogen reduction reaction (nitrate and nitrite reduction to ammonium) and absorption of ion.

This study has found that generally concentration of micronutrients in AnPOME is sufficient to be used as a source of nutrient for microalgae growth.

#### 4. CONCLUSION

In this study, the aim was to assess amount of macronutrients and micronutrients in AnPOME. Overall, the results indicate that compositions of both nutrients in AnPOME were greater or equal than its amount in synthetic media. Therefore, it is proven AnPOME contain adequate macronutrient and micronutrient source for culturing microalgae. Besides, zero waste sustainability concept also can be applied since all waste, including AnPOME from the palm oil production can be reused. The second major finding was that the concentration of phosphorus in AnPOME six times lower than nitrogen resulting slow rate of respiration, increase darker CO<sub>2</sub> fixation and reduce photosynthetic CO<sub>2</sub> fixation. Significantly, the most suitable microalgae to be cultured in AnPOME were mixotrophic or heterotrophic types.

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