Study the Growth of Microalgae in Palm Oil Mill Effluent Waste Water

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Abstract. This paper emphasizes mainly on the biomass productivity and lipids content of two microalgae strains known by their high lipids content namely: Botryoccoccus sudeticus and Chlorella vulgaris. These strains were first screened for the highest biomass and lipids content, then Plackett–Burman design was used to evaluate the significant media for the growth when using POME waste water as culture medium. Results show that Botryoccus sudeticus contains high content of biomass and lipids yield. Moreover, all the three factors have positive effect on the biomass productivity, while using one nutrient factor gives much lower biomass. These results can be used further as an insight for optimizing the biomass and the oil productivity of the microalgae.

1. Introduction
Microalgae are prokaryotic or eukaryotic, unicellular or simple multicellular structure, photosynthetic microorganisms which can grow rapidly in suspension and live in difficult conditions [1]. Moreover, these microorganisms are useful for bioremediation applications [2] and [3]; nitrogen fixing biofertilizer [4] and they can provide several types of renewable biofuels.

Biodiesel production from food supplies is reported to need more land for the crop cultivation. However, researchers have estimated that only 2.5% of the whole lands used to produce the crops can be used for algae cultivation, which has 30% of its total mass oil [5]. Many algae species have been identified and reported to contain over than 30% of its weight as oil [6]. Generally, the growth medium must provide with all the nutrients necessary for the algae growth. Those nutrients range from essential nutrients like: nitrogen (N), phosphorus (P) and carbon(C) to facultative nutrients depending on the algae strain and on the final product that we are targeting to produce. The overall nutrients can be summarized in the following formula given by Chisti [5]: CO$_{0.48}$ H$_{1.83}$ N$_{0.11}$ P$_{0.01}$. Besides that, the sources of each essential nutrient also play an important role in term of the final biomass produced and their lipids yield. It was reported in many studies that the combination of glucose with light gave better results in terms of both growth rate and lipids productivity. Moreover, sodium nitrate is considered to be less expensive which will make as a source of interests in the industrial practices [7]. Another essential nutrient is phosphorous; Researchers have found that algae bloom was mainly resulted from the elevation of nitrogen and phosphorus concentration in water, especially phosphorus concentration.

The combination of the microalgae production with the treatment of the waste water might be very promising green technology. The waste water would assist with their organic pollutant as fertilizer during the growth cycle of those valuable microorganisms. Many countries are struggling in finding permanent solution for the discharge of the waste waters especially the ones coming from the
industrial sector. Malaysia is facing the same problem with the palm oil industry, which is considered to be one of the greatest sponsors for Malaysian economy. For the last period of time, this industry has well increased to contribute with almost the half of the whole world palm production [8]. Instead of the value added products that it can be recycled from the POME. The aim of this study is to compare the lipids content of two microalgae strains *Chlorella vulgaris* and *Botryoccocus sudeticus* know by their high common occurrence, easy growth characteristics and significant lipids content from one hand. Then, to study the nutrient limiting factor for the biomass and lipids production when using the waste water as culture medium.

2. Material and methods

Two strains of microalgae namely *Chlorella Vulgaris* and *Botryoccocus Sudeticus* are used in this experiment. The first step consists of screening of the potential strain for the high biomass and lipids productivity. Finally Plackett–Burman design used to screen the important variables of media component. ‘Table 1’ presents the design matrix of the variables under investigation as well as levels of each variable used in the experimental design. The parameters for media components selected for the experiment are: carbon source or glucose, nitrogen source or sodium nitrate and phosphorus source or potassium di-hydrogen phosphate.

| Run | Factor A | Factor B | Factor C | Biomass mg/l |
|-----|----------|----------|----------|--------------|
| 1   | +        | -        | -        | 403.3        |
| 2   | -        | +        | -        | 386.85       |
| 3   | -        | -        | +        | 471.88       |
| 4   | +        | +        | -        | 898.26       |
| 5   | +        | -        | +        | 528.66       |
| 6   | -        | +        | +        | 508.15       |
| 7   | +        | +        | +        | 1478         |
| 8   | -        | -        | -        | 13.33        |

Factor A: Glucose, Factor B: Sodium nitrate, Factor C: potassium di-hydrogen phosphate.

3. Results and discussion

*Screening of potential strain used for biomass and lipids production*: Two strains known by their ability of producing high biomass and lipids content namely *Chlorella vulgaris* and *Botryoccocus sudeticus* were used in order to select the one having the highest biomass and lipids yield, so that it will be used further in our experiment. The results are presented in ‘figure 1’.

‘Figure 1’ shows that both *C vulgaris* and *B sudeticus* have produced more than 1g/l of dried biomass. However, there is a great difference in term of lipids yield. And from those results we can say that for 1.47g/l of biomass from *B sudeticus*, about 38% of lipids can be obtained from, which is making it good candidate for algae biofuel production. In many previous studies, *Botryoccucus sudeticus* gives higher lipids content ranging from 25-75% of dry weight while for *C vulgaris* the lipids content can be lower ranging from 14-40% [5]. Increased lipid content also occurred in many microalgae as a response to different culture condition [9]. However, lipid production by microalgae is regulated by environmental factors, and to optimize them, a control is needed [10].
3.1. Evaluation of the media components and process parameters for biomass production by Botryococcus sudeticus using Plackett–Burman design

The main effect of variables on biomass production namely: glucose, sodium nitrate and potassium dihydrogen phosphate was found to be 510.33, 488.51, 296.31 respectively. The results indicated that all the factors have positive effects on the growth and biomass productivity.

‘Figure 2’ shows that the factor A or carbon has more effects on the biomass growth while this effect is decreasing with factor B to be the lowest with factor C or phosphorus. These results are well agreeing with the literature that confirms that carbon, nitrogen and phosphorus are considered to be the main essential nutrients which are very essential for the biomass growth. Chen stated that in order to develop balanced media for optimal microalgae growth, growth medium must be provided with adequate nutrients, most frequently: carbon, nitrogen and phosphorus are the main nutrients causative for biomass production [11]. Organic carbon is essential due to the composition of the half of the microalgae which is made with carbon [12]. Nitrogen is also considered to be the 2nd essential nutrient factor. Most of the microorganisms use nitrogen in form of nitrate, nitrite, ammonia and urea. Those forms can be responsible of some differences in terms of microalgae growth and biomass. Last but not least phosphorus is used to store phospholipids or other molecules related to energy such as ATP, NAD. Furthermore, it is used to produce further biological constituents like coenzymes, proteins, DNA, RNA [13].
4. Conclusions
In this study biomass production from microalgae was carried out using liquid state fermentation with two strains *Chlorella vulgaris* and *Botryococcus sudeticus*. The results have shown that *Botryococcus sudeticus* offers better lipids percentage and biomass productivity. It was then used in the second experiment in order to show the essential nutrient needed when using the POMSE as culture medium for the algae growth. Organic carbon, nitrogen and phosphorus have a positive effect on the biomass growth.

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