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## Nutrient Removal from Anaerobically Treated Palm Oil Mill Effluent by *Spirulinaplatensis* and *Scenedesmusdimorphus*

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### ABSTRACT

Large quantities of nutrients present in palm oil mill effluent (POME) are one of the main causes of environmental pollution that negatively change natural water bodies. It is desirable that effluent treatment facilities remove nutrients from the POME prior to discharge to the environment in order to meet regulatory limits. Bio-treatment of nutrients in bioreactors is, by definition, achieved by microalgae. In the present study, microalgae *Spirulina platensis* and *Scenedesmus dimorphus* during bio treatment of anaerobically treated POME was evaluated. Experiment was conducted in outdoor raceway reactors to evaluate nutrient uptake using field relevant reactor designs. Results showed that these algae were very effective in reduction of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Nitrogen (TN), ammonia Nitrogen ( $\text{NH}_4^+ - \text{N}$ ) and phosphorus (P) in POME. Further, it has been observed that *S. platensis* was having best nutrient removal efficiency compare than *S. dimorphus*. Therefore, the nutrient removal efficiency from POME using *S. platensis* and *S. dimorphus* offer an alternative strategy for POME treatment.

**Keywords:** *Spirulinaplatensis*, *Scenedesmusdimorphus*, Palm Oil Mill Effluent, nutrient removal

### INTRODUCTION

The generation of palm oil results in the huge amounts of highly polluting wastewater referred as palm oil mill effluent (POME). If the POME is discharged without treatment, it can certainly cause considerable water pollution problems [1]. A wide range of approaches have been developed for the treatment of POME [2]. Biological treatment is the method used most frequently, which includes of anaerobic and facultative pond systems. Whilst anaerobic pond treatment system has been already adopted to treat highly polluted POME, anaerobic treatment of POME only could scarcely produce effluents to a level meet with the DOE standard discharge limit [2]. It is essential to subject the POME to an appropriate bio-treatment method before releasing in order to meet regulatory limits. Bio-treatment processes are considered the most environmentally companionable and the inexpensive wastewater treatment methods [3].

Palm oil mill has reached a new dynamic era with the option of culturing microalgae using POME. The large production of microalgae has traditionally been for use as a wastewater treatment [4]. Microalgae are proficient in the elimination of nutrients from wastewater. For this reason, the utilization of wastewaters to cultivate microalgae could concurrently solve the problems of: (1) demand of freshwater (2) high price of nutrients and (3) require remediating wastes. The importance of microalgae for nutrient removal is known, but not for the type of POME wastewater declared in this article. A significant biological method for wastewater treatment is through growth of algae. There are several designs that incorporate algae based treatments. An inexpensive biological

method for algae based wastewater treatment method is through use of raceway ponds. This wastewater treatment method was first proposed by Oswald in the 1950's [5, 6] and the concept was later expanded to propose use of this system for energy production through harvesting and utilization of algal biomass [7].

Studies have shown the feasibility to use microalgae to remove nutrients from different types of wastewaters [8]. However, no knowledge has yet been made available regarding the potential of coupling microalgae cultivation with the treatment of anaerobically treated POME. From a theoretical point of view, high cell density cultivation is required to afford high nutrient removal efficiencies from high strength wastewaters such as POME. This can only be achieved with short light path open pond raceway pond reactor systems that effectively supply sun light to all of the cells encapsulated inside the microalgal culture. The aim of this study was to estimate the possibility of nutrients removal from POME and to screen a potential strain for the development of biomass energy from microalgae.

## MATERIALS AND METHODS

### Microalgae and culture conditions

The strain of *Spirulina platensis* and *Scenedesmus dimorphus* used in the study were obtained from Algaetech Sdn. Bhd, Malaysia. *S. dimorphus* were suspended in Bold's Basal Medium (BBM) while *S. platensis* was grown in Zarrouk's medium. Cells used for the inoculums were grown separately in a plastic container of constant working volume 20 L, which was maintained at ambient temperature, bubbled with air (5 L min<sup>-1</sup>) and continuously illuminated with fluorescent lamps (30 μEm<sup>-2</sup>s<sup>-1</sup>). The cell density of *S. platensis* and *S. dimorphus* were raised up to 1.0 optical density (600 nm).

### Wastewater resource

Anaerobically treated POME was collected from Sime Darby East Mill, Carey Island, Selangor, Malaysia. Five hundred Liters of POME sample was collected in plastic container and stored at 4°C in the coldroom prior to the experiments. The effluent was subjected to pre-treatment via sedimentation and filtration to eliminate large, non-soluble particulate solids.

### Experimental set-up and analysis

The outdoor raceway reactor was made of ¼" acrylic sheeting and the reactors have 2 channels. A length to width ratio of 2:1 and a volume of approximately 500 L. Mixing was accomplished by a paddlewheel set-up. An electric motor (Variable Low Speed Motor) was used to rotate paddle wheels at approximately 10 rpm. Transparent acrylic was used to allow light penetration for photosynthesis to occur in an open environment.

Ten percentages of cultivated inoculums were aseptically transferred into the outdoor raceway pond reactor containing POME. The influence of initial cell density on *S. platensis* and *S. dimorphus* growth and nutrient removal was studied separately in the outdoor raceway reactor under the same experimental set up with the same operational conditions. Samples were taken every 2 days interval in 50 mL volumes and analyzed for BOD, COD, Total Nitrogen (TN), ammonia Nitrogen (NH<sub>4</sub><sup>+</sup>-N) and phosphorus (P) according to standard methods [9]. The cellular concentration growths were determined by numbers of cell using Haemocytometer. All the experiments were performed in duplicate and average values were recorded.

## RESULTS AND DISCUSSION

### Growth profile of *S. Platensis* and *S. dimorphus* on anaerobically treated POME

The growth characteristics of *S. platensis* and *S. dimorphus* in POME within 20 days were investigated as shown in Fig. 1. *S. platensis* density in samples ranged from 1.50×10<sup>4</sup> cells mL<sup>-1</sup> to 7.43×10<sup>4</sup> cells mL<sup>-1</sup> during the study period. The maximum cell density was recorded on 18<sup>th</sup> day. The density of *S. dimorphus* ranged from 0.5×10<sup>4</sup> cells mL<sup>-1</sup> to 5.2×10<sup>4</sup> cells mL<sup>-1</sup>. The maximum cell density was observed on 18<sup>th</sup> day for both microalgae.

In this study, the cell numbers were low, because of shifting in environment when moved from the commercial medium to POME. The microalgae were able to adapt step by step and significantly utilize the nutrient source from the POME. Factors inhibiting that involve microalgae growth were expressed by the slow growth and the cells concentration kept stable after the 18<sup>th</sup> day cultivation. Involving factors on algae growth are numerous [10].

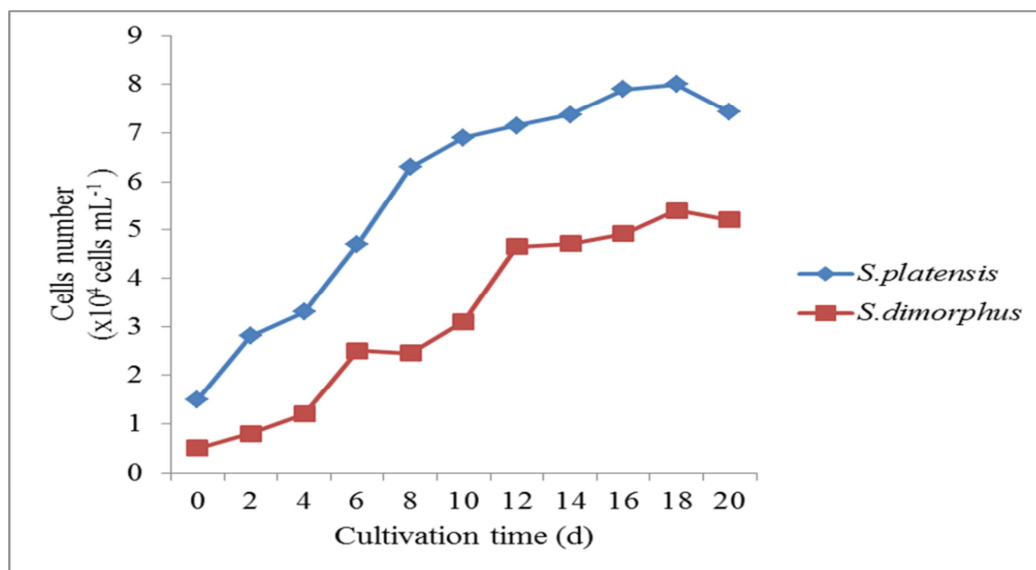


Fig.1. Growth profile for *S. platensis* and *S. dimorphus* cultivated in anaerobically treated POME.

The major factors are limited nutrients supply and light penetration. It was proved that *S. platensis* and *S. dimorphus* could tolerate high nutrients availability, suggesting that the nutrient deviation was not one of the limiting factors in this experiment. Thus, it is necessary to examine the cultivation of microalgae in POME using open raceway pond reactor, in which nutrient sources are utilized timely, with different retention time in scale up systems in the following observations.

#### Dynamics of nutrient removal by *S. platensis* and *S. dimorphus*

The profiles of nutrient removal by *S. platensis* and *S. dimorphus* in the outdoor raceway pond reactor were studied. The amounts of  $\text{NH}_4\text{-N}$  removed were 19.8 mg L<sup>-1</sup> (93.8%) and 37 mg L<sup>-1</sup> (88.5%) after 18 days by *S. platensis* and *S. dimorphus* respectively (Fig. 2).

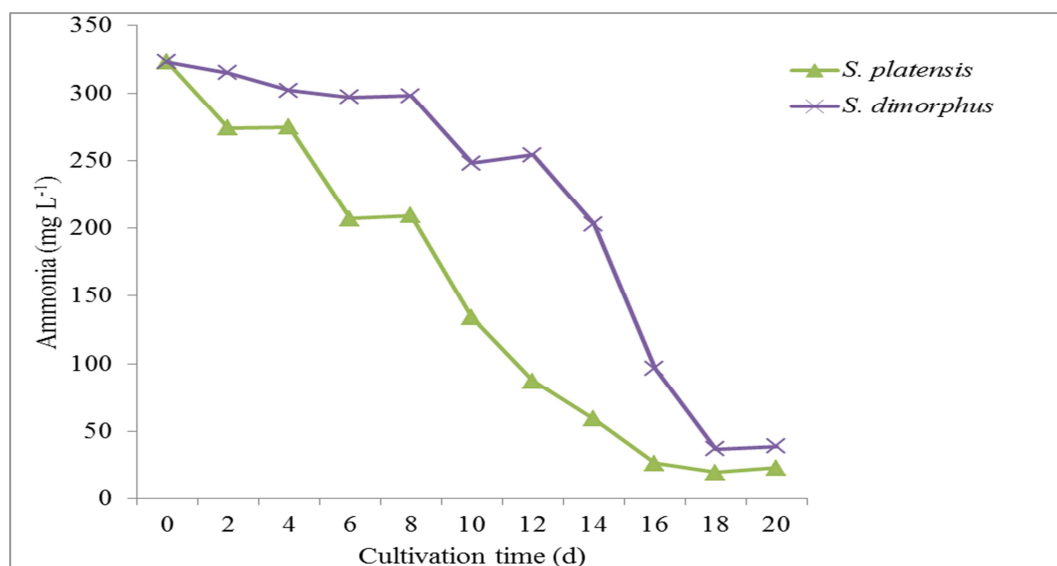
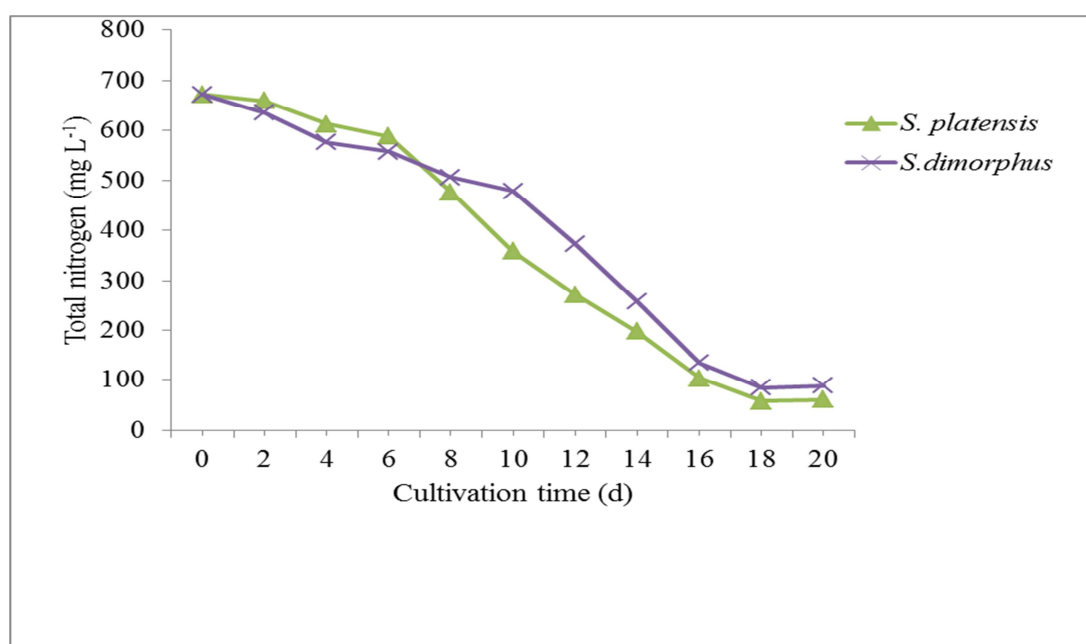


Fig. 2. Removal of ammonia nitrogen ( $\text{NH}_4\text{-N}$ ) of *S. platensis* and *S. dimorphus* inoculated in anaerobically treated POME.

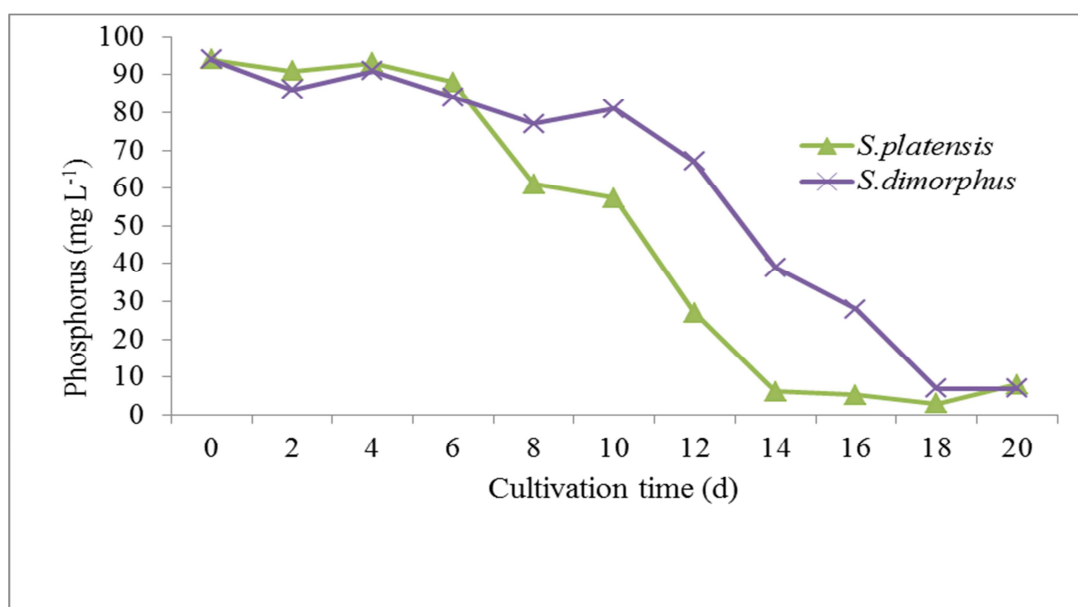
As indicated in Fig. 3, the TN content was greatly reduced from 672 to 57.9 mg/L after 18 days, resulting in a TN removal efficiency of above 91% by *S. platensis*.



**Fig. 3. Removal of total nitrogen of *S. platensis* and *S. dimorphus* inoculated in anaerobically treated POME.**

On the other hand, *S. dimorphus* were capable of removing up to 87.5% of the TN from POME. The same trend was reported by Cheunbarn and Peerapornpisal, [11]. Microalgae use nitrogen sources to build nucleic acids, amino acids, pigments and proteins. Algae could directly uptake some forms inorganic nitrogen such as  $\text{NH}_4$ ,  $\text{NO}_3$ , and  $\text{NO}_2$  for cell growth [12]. Ammonium is required versus the oxidized compounds, because its absorption has less energetic cost [13].

The trend of phosphorus removal was similar to TN removal. In the first twelve days P amount dropped significantly from 94 to 27 mg/L and regular removal was observed even after the cease of *S. platensis* growth, till the end of the experiment (Fig.4).



**Fig. 4. Removal of phosphorus of *S. platensis* and *S. dimorphus* inoculated in anaerobically treated POME.**

The phosphorus removal rate was reached maximum at 96.8%, which indicated higher removal efficiency compared with *S. dimorphus*. The concentration of phosphorus was reduced significantly by *S. dimorphus* with the removal efficiency of 92.5% after 18 days. The same trends had been reported previously using other type of wastewaters [14, 15]. Voltolina *et al.* [16] reported that the strain *Scenedesmus*, grown in artificial wastewater, also reduced more than 50% of the phosphates. Removal in phosphate contents of the digested effluent reached over 99% [17]. In

addition, Ogbonna *et al.*[18] studied that *S. platensis* could efficiently reduce the content of nitrates, ammonia, and phosphates from synthetic wastewater. The main reason for high P reduction efficiency was indicated in previous studies that both of microalgae uptake and mainly phosphate precipitation by coagulation with metal ion in concentrated municipal wastewater resulting in high P removal efficiency [19, 20, 21, 22].

Moreover 84.9% and 79 % of COD were removed (Fig.5) by *S. platensis* and *S. dimorphus*, respectively, suggesting that the microalgal strain could consume various organic compounds as carbon resources. It was coincided with the earlier findings [10].

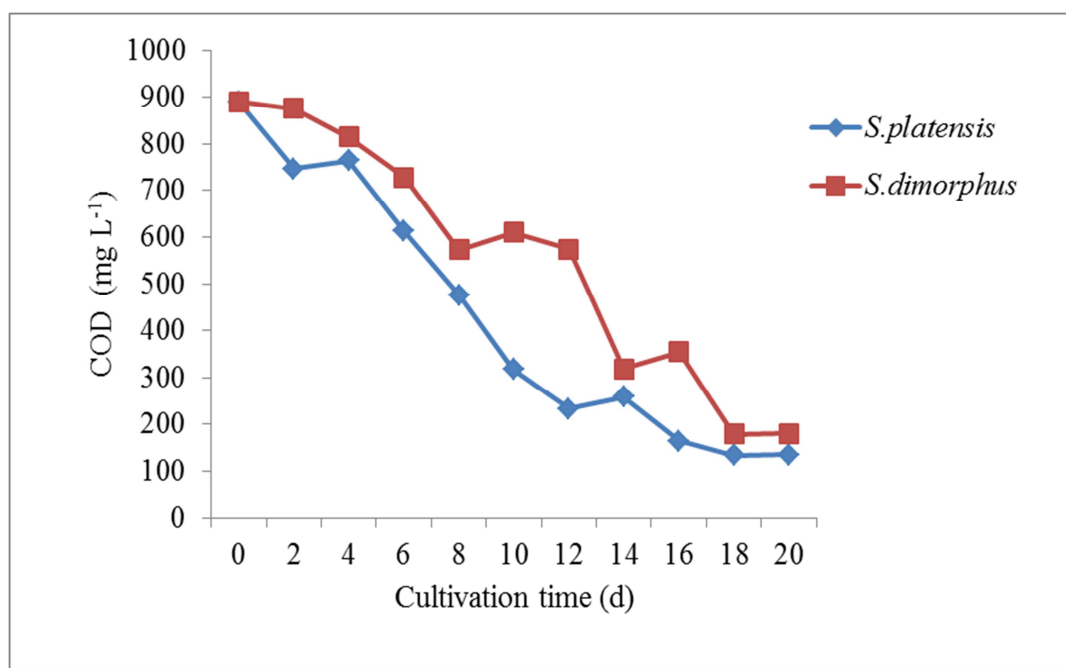


Fig. 5. COD removal by *S. platensis* and *S. dimorphus* in anaerobically treated POME.

In addition, this is comparable to the study using *S. platensis* for biotreatment of Swine wastewater the maximum 84.3% of reduction with the initial COD was  $8740 \pm 362$  mg/L [23]. BOD value of anaerobic POME was decreased significantly with a removal efficiency of more than 78.3% by *S. platensis*. *S. dimorphus* was able to reduce the BOD content in anaerobic POME from their initial value of  $523 \text{ mg L}^{-1}$  to  $148.8 \text{ mg L}^{-1}$  (71.5%) as shown in Fig.6.

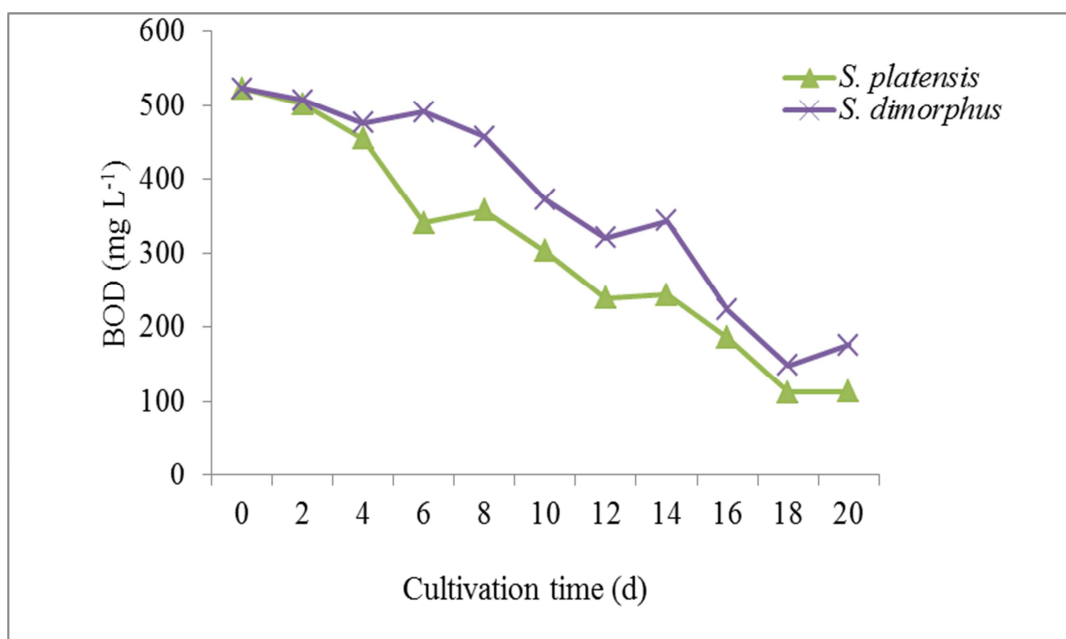


Fig. 6. BOD removal by *S. platensis* and *S. dimorphus* in anaerobically treated POME.

As COD reduction, symbiotic relationship between microalgae and anaerobic/aerobic bacteria play a major role in reducing the BOD also. As a result, treatment is more efficient and thereby less land intense. Further, short period on the nutrient removal efficiency can be achieved. Results showed that algae to the open conditions still grow at high pollutants just at a slower growth rate and uptake N and P concentrations. Therefore, bioremediation using microalgae offer an alternative strategy for POME treatment, and consequently reduce the impact of palm oil industry to the environment. Further experiments need to be done to determine the potential of algae biomass. This integrated wastewater treatment and biomass production system can thus benefit the community as well as the environment.

### CONCLUSION

The strain *S. platensis* and *S. dimorphus* were proven to be an ideal candidate for nutrient removal in POME based on its growth ability as well as high nutrient removal efficiency. The selected microalgal strain has great potential to be used in other nutrient rich wastewater resources to perform dual purpose of effective wastewater treatment and economically viable and environmentally friendly production of biobased products in the near future.

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