

Protein and Lipid Content of Microalgae *Scenedesmus* sp. Biomass Grown in Wet Market Wastewater

Najeeha Mohd Apandi¹, Radin Maya Saphira Radin Mohamed^{1,*}, Nur Atikah Ahmad Latiffi¹, Nur Farahanim Mohd Rozlan¹, and A.A.S. Al-Gheethi¹

¹Department of Water and Environmental Engineering, Faculty of Civil and Environmental Engineering, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia

Abstract. Microalgae biomass is a type of organism that has various advantages and often used for variety of uses not only for wastewater treatment purposes but also serve as bio-based products development. Because of high in protein and lipid accumulation, this freshwater microalgae *Scenedesmus* sp. was selected for the present study. In this study, microalgae *Scenedesmus* sp. was grown in four concentrations of wet market wastewater (10%WM, 15%WM, 20%WM and 25%WM) in comparison with the Bold Basal Medium (BBM) as a control. The result shows that the highest protein content (50.72%) was in 10%WM followed by 15%WM, 20%WM, 25%WM and BBM. As for lipid content, the best concentration was obtained in the 25%WM with the value of 26.7% compared to other concentrations and BBM. Therefore, the result indicates that microalgae *Scenedesmus* sp. biomass cultured using wet market wastewater has a great potential in producing protein-rich and lipid content which significantly could be used as a fish feed product.

1 Introduction

An assortment of microalgae, including *Scenedesmus* sp. have been concentrated on for their conceivable viability as bioresources for application as fish feed, supplement, human consumption due to their nutritious compounds eg; protein, lipids, carbohydrates and other valuable trace element and additionally for the bioremediation of contaminated water [1-3]. In recent years, many researchers have studied the value potential of dual application of microalgae for wastewater treatment and biomass production [4, 5]. Unfortunately, wastewater from wet market contain nutrient and elements derived from the fresh foodstuff, waste scraps of poultry and fish entrails which consists of variety components such as nitrogen and phosphorus commonly discharge directly to the drainage without any treatment [6]. In Malaysia, the disposal of these wastewaters is commonly direct into the drainage and end up flowing into the river. This phenomenon escalates the pollution to obnoxious smell and filthy surrounding environment due to the wastewater discharge

* Corresponding author: maya@uthm.edu.my

freely. This issue may prompt an uncomfortable condition directly to the people and occupants at the wet market business area [7].

Basically, conventional wastewater treatment is highly expensive due to chemicals needed to treat the wastewater. However, biological treatment using microalgae as an alternative to reduce the pollutant with less expensive and at the same time valuable biomass microalgae can be produced. Microalgae based wastewater treatment is eco-friendly and offers the advantage of a cost effective way of nutrient removal and biomass production [8]. Since microalgae have the ability to reduce nitrogen and phosphorus levels in wastewater [9], instead of the nutrients being wasted; this will provide nutrients for the microalgae to grow which in turn become valuable biomass products. Since microalgae has been recognized as a very favourable source of biomass, this can be applied in order to treat wastewater efficiently without involve high production cost.

The idea of using microalgae as a source of finding an alternative fish feed replacement is not something new and it is being considered important as unconventional feed ingredients in replacement of high cost feed stuffs that has been increasing [10,11]. Microalgae has the promising substitution protein-rich and lipid sources for fish, where several of studies had investigate the effect of partial replacement of fish feed with microalgae *Scenedesmus* sp. [12, 13]. The objective of this study was to determine the protein and lipid content of *Scenedesmus* sp. cultivated in different concentration of wet market wastewater under outdoor culture condition. In addition, the morphological of *Scenedesmus* sp. after growing in wet market wastewater also characterised using Scanning Electron micrograph (SEM) accordingly.

2 Materials and methods

2.1 Cultivations of *Scenedesmus* sp. using wet market wastewater

Microalgae *Scenedesmus* sp. was obtained from the culture collection of Faculty of Science, Technology and Human Development, Tun Hussein Onn University of Malaysia. The inoculum of *Scenedesmus* sp. was maintained in Bold basal medium (BBM) prior to experiment. The experiment was set up using 2L of Erlenmeyer flask containing 1.5 L of wastewater and Bold Basal Media (BBM). BBM medium was used as control media. Wastewater was transported to the laboratory and stirred for about 30 minutes and allowed to settle for 1 hour. The supernatant was separated from the remaining suspended solids and diluted with distilled water into four concentrations (10%, 15%, 20% and 25%). Microalgae was cultivated under outdoor sunlight with continuous aeration at 3.2 L/min to ensure the distribution of nutrients using atmospheric air from an aquarium pump through air sparger. The experiment flask was inoculated with the same initial microalgae cells of (10^6 cells/ml) at the initial day of the experiment. The biomass was harvested immediately after 12th days by centrifugation at 4000 rpm for 5 min. The cultivation batches were carried out in duplicate.

2.2 Analysis of proximate composition

The measurement of protein, ash, fiber and moisture content was performed based on AOAC (2002) [14] methods. Lipid content was determined according to Bligh and Dyer [15] and Folch et al. [16]. All biomass samples were kept in an airtight container and were sent for further analysis of Scanning Electron Microscopy (SEM) for cell morphology characterization after cultivation.

3 Results and discussion

3.1 Protein and Lipid Content of *Scenedesmus* sp.

Table 1 shows the chemical composition of *Scenedesmus* sp. biomass which were cultivated in different types of media. The highest protein content was recorded by 10%WM followed by 15%WM, 20%WM, 25%WM and BBM respectively. Low protein content recorded in 25%WM with 37.3% while the highest protein content was recorded at 10%WM with 50.72%. It is to be found that the protein content increased along with the decreasing wet market wastewater ratio added to the cultures. On the other hand, the highest lipid content was found to be in 25%WM with a total value of 26.7% were significantly higher than those obtained from other concentration and BBM. As the concentration goes lower, the lipid content were decreased (Fig. 1). These results show that it may possibly due to micronutrient deficiency or some other stress factor was involved; higher nitrogen involvement achieved higher lipid productivity [17].

Table 1. Proximate composition (% of dry matter)

Composition	10%WM	15%WM	20%WM	25%WM	BBM
Crude potein (%)	50.72 ± 0.64	41.2 ± 0.23	38.6 ± 1.27	37.3 ± 0.10	35.0 ± 1.52
Crude lipid	8.0 ± 0.10	14.7 ± 0.17	19.9 ± 0.18	26.7 ± 0.56	15.1 ± 0.19
Ash (%)	3.1 ± 0.25	7.1 ± 0.04	9.2 ± 0.67	12.0 ± 0.48	10.2 ± 0.29
Moisture (%)	6.4 ± 0.26	8.5 ± 0.25	8.7 ± 0.13	9.0 ± 0.10	7.7 ± 0.29
Crude fiber (%)	6.91 ± 0.21	6.39 ± 0.19	6.72± 0.36	6.24± 0.34	6.50 ± 0.07

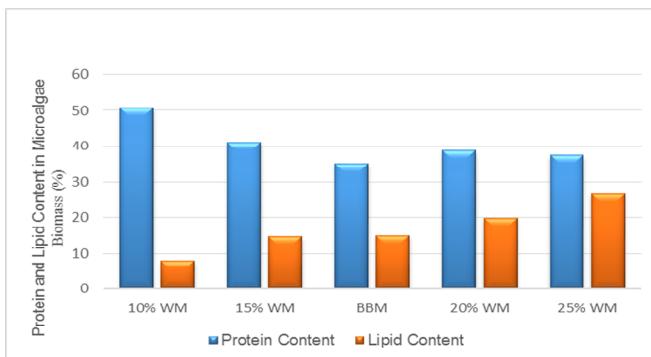


Fig. 1. Protein and lipid content

Protein content was found higher and the lipid content was lower in the 10%WM than in the 25%WM. This measurement indicates that stress conditions in 10%WM were higher than in the 25%WM in the cultures. The stress condition triggered due to the nutrient content of nitrogen and phosphorus as both a particular key for protein and lipid accumulation in microalgae cells. It is likely known that the aspects of this stress conditions are tightly linked to the available amount of nutrients; limitation of nitrogen, phosphorus, and the presence of other microorganisms [18]. Moreover, the trend value of protein and lipid were similar to the values reported by Markou *et al.* [19] who cultivated microalgae using slaughterhouse wastewater and revealed that this phenomenon could be explained to

the fact that the presence of higher nitrate and phosphorus concentration related to the changes in the protein and lipid content. Assuming further used for this microalgae *Scenedesmus* sp., as fish feed, this study is already fulfill the requirement of protein (40%-50%) and lipid (6%-13%) percentage needed for fish in the standard from Food and Agriculture Organization of The United Nations (FAO) [20]. Thus, this proximate quality of microalgae *Scenedesmus* sp. biomass cultivated from wet market wastewater in this study can be the ideal amount to supply in terms of protein and lipid based on the percentage amount to be consumed by fish. However, in order to simplify whether this microalgae *Scenedesmus* sp. biomass grown on wet market wastewater is harmless to be used as fish feed, thoroughly follow-up research is needed.

3.2 Microalgae cell characterization via SEM

The cell morphology of *Scenedesmus* sp. that has been grown on five different of wet market wastewater media was observed under Scanning electron micrograph (SEM) as depicted in Fig. 2. The SEM at the magnification of 1200 \times was used to characterize the biomass for both culture media (WM and BBM). It shows that wet microalgae *Scenedesmus* sp. biomass from each media was characterized by having different length and longer axis for all sample which ranged of 6 μm - 8 μm to 2 μm - 3 μm . In fact, the individual microalgae cells were found to be in spherical and unicellular as typical colonies consisting of numerous cells.

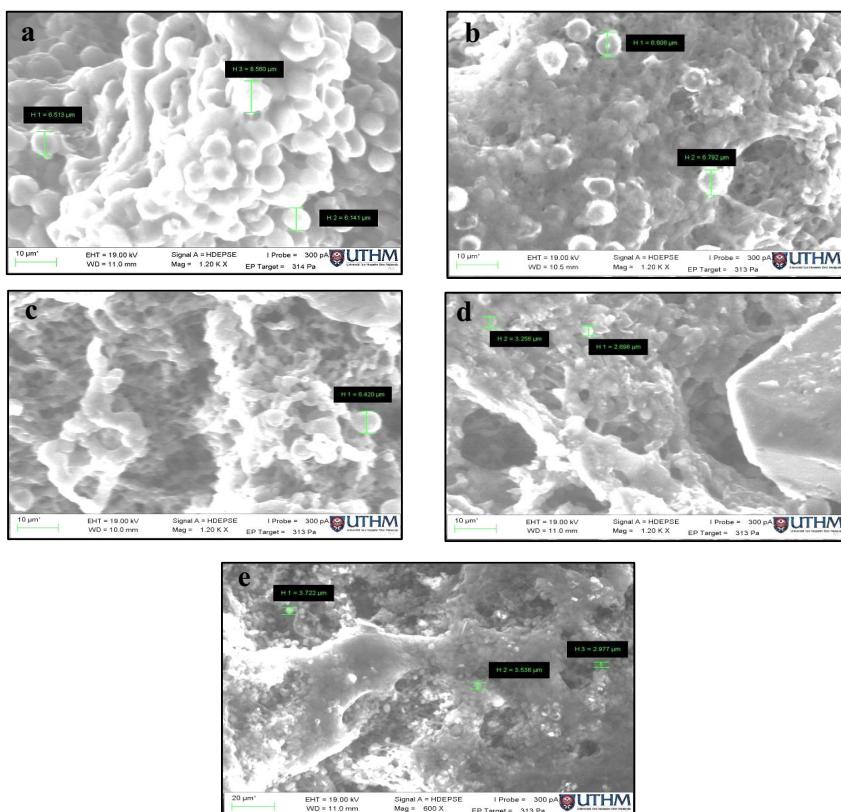


Fig. 2. SEM micrograph of 1200 \times (a) 10%WM; 6 μm - 8 μm (b) 15%WM; 6 μm (c) 20 WM; 6 μm (d) 25%WM; 2 μm - 3 μm (e) BBM; 2 μm - 3 μm

4 Conclusion

This study shows that wet market wastewater can be used as an alternative media for the cultivation of microalgae *Scenedesmus* sp. protein and lipid content production. Among the four different concentrations of WM, 20% WM showed the best performance in respect of protein and lipid content. Besides, a tremendously high of protein content was found in 10%WM with 50.72% while high lipid content was found in 25%WM with 26.7%. This indicates that microalgae *Scenedesmus* sp. biomass was rich in protein but lower in lipid straightly can be a potential application for bio-based product such as fish feedstock. This research is also motivated by the increasing interest in application of microalgae biomass and the implementation of environmentally friendly tools in treating wastewater.

This research was funded under the PRGS (G004) and eScience Fund project no: 02-01-13-SF0135. Special thanks to Dr. Hazel Monica Matias-Peralta for providing the microalgae seed and any parties that involved in this project specifically University Tun Hussein Onn Malaysia for providing the equipment and research facilities to carry out this project. This paper was partly sponsored by the Centre for Graduate Studies UTHM.

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