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Biomass quality of *Scenedesmus* sp. cultivated in wet market wastewater

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ABSTRACT

Aim: This study aims to assess the wet market wastewater efficiency as *Scenedesmus* sp. biomass production medium based on the physical and chemical characteristic as well as the biomass compositions.
Methodology and results: The experiments were set up in transparent glass tank (40 L) containing 20 L of wastewater. The efficiency of different concentrations (10, 15, 20 and 25%) of wet market wastewater diluted with distilled water was compared to Bold Basal Medium (BBM). The biomass was harvested after 12 days of the incubation period by centrifugation. The quantity of biomass yielded was estimated based on the microalgae cell concentrations, while quality of biomass yield was determined by GC-MS. The nutrient contents of the raw wet market wastewater were in the range required for microalgae growth. The wastewater with 20% of dilution exhibited high efficiency for *Scenedesmus* sp. growth in comparison to BBM (4×10^7 vs. 1×10^6 cell/mL respectively). The main compounds in the microalgae biomass included cycloheptane, cyclododecanol, 1-ethenyl-acetate, 2-trifluoroacetoxypentadecane and 9-octadecenoic acid (Z)-, methyl ester.
Conclusion, significance and impact of study: *Scenedesmus* sp. biomass can be produced using the wet market wastewater.

Keywords: Microalgae, production medium, wastewater, wet market

INTRODUCTION

Wet market wastewater contains high contents of nutrients (i.e. nitrogen and phosphorus) because they come from food wastes such as seafood scraps and fish entrails (Plumber and Kiepper, 2011). Direct discharge of these types of wastes into the environment and water system may contribute to pollution. It is a common practice in Malaysia to dispose the wastewaters into the drainage which will flow into the river. This phenomenon is become unacceptable because it escalates the pollution among Malaysian rivers.

Given that the microalgae can absorb the high nutrients of nitrogen and phosphorous that present in the wet market wastewater by assimilation process, the production of valuable microalgae biomass can be enhanced (Zhang *et al*., 2008; Jais *et al*., 2017). Microalgae biomass exhibit significant promise in industrial application such as aquaculture feeds and fertilizer. This is because they contain pigments (e.g. β-carotene, luten, and astaxanthin), carbohydrate, lipids, protein, and vitamins (Badwy *et al*., 2008; Macias-Sancho *et al*., 2014).

The microalgae are now among the most important resources of biomass because they can be utilised in various applications including biodiesel production (Jena *et al*., 2012; Zhu *et al*., 2013). However, one of the main limitation in the microalgae biomass production is the cost of production medium. Reports on the reuse of wastewater for production of microalgae biomass can be found in literature (Pahazri *et al*., 2016). However, the study on utilization of wet market wastewater is still shallow. Therefore, this study aims to assess the wet market wastewater potential to be *Scenedesmus* sp. biomass production medium. The quantity and quality of microalgae growth in terms of growth cell concentrations and microalgae biomass composition was tested.

MATERIALS AND METHODS

Microalgae strains and wet market wastewater (WMW) medium

Scenedesmus sp. was obtained from the culture collection of Faculty of Science, Technology and Human Development, Universiti Tun Hussein Onn Malaysia. The inoculum of *Scenedesmus* sp. was prepared by the sub-culturing in Bold basal medium (BBM). The culture was incubated and continuously aerated for seven days under the sunlight. The raw wet market wastewater (WMW) samples were collected from Pasar Borong Rengit, Batu Pahat, Johor. The ice box was used to preserve the

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collected samples before being sent to the laboratory and then subjected for the chemical and physical analysis within 24 h. Characterisation of the WMW samples were carried out according to the water and wastewater standard methods test, American Public Health Association American Works Association and Water Environment Federation USA, APHA (2002).

In order to prepare wet market wastewater (WMW) medium for microalgae biomass production, the samples were autoclaved at 121 °C for 15 min. The sterilized samples were filtered using GF/C (Whatman) filter to remove suspended solids and then diluted with distilled water into four concentrations including 10, 15, 20 and 25%. The parameters of wet market wastewater media used in the present study are presented in Table 1. BBM medium was used as control media.

Cultivations of *Scenedesmus* sp. in wet market wastewater (WMW) medium

The cultivation experiments were conducted in transparent glass tank (40 L of capacity) containing 20L of autoclaved wet market wastewater medium. The production medium was inoculated with 10^6 cell/mL of *Scenedesmus* sp. and air pump was used to aerate it continuously to ensure that the nutrients were distributed evenly. The cultivations batch were carried out in duplicate and incubated at room temperature for two weeks. The cell concentration of *Scenedesmus* sp. was determined daily using haemocytometer. Addition of formalin (2-3 drops) was used to preserve a fixed volume (10 mL) of the sample. Approximately 1 mL of sample was carefully filled in haemocytometer and covered with glass slide. Equation 1 is used to calculate the microalgae cells:

Spores/mL (in original) = (mean of spores / square)$^{1/5}$ × (25) × (10^4) (Dilution Factor) --- ***(1)***

A growth curve was plotted between or cell concentration and time. Specific growth rate (μ) was calculated with the Equation 2.

$$Growth\ rate;\ K = ln\,(X2 - X1)/T2 - T1 \quad \textbf{(2)}$$

Where, X_2 = final algal concentration, X_1= initial concentration, T_2= final time, T_1= initial time

Scenedesmus sp. biomass grown on 20% of WMW was harvested by using centrifugation (4000 rpm) for 5 min. The quality of produced biomass was determined by GC-MS analyses.

RESULTS AND DISCUSSION

***Scenedesmus* sp. in the wet market wastewater (WMW) media**

The growth curves and specific growth rate (SGR) of *Scenedesmus* sp. in the WMW media during the incubation periods for two weeks are presented in Figures 1 and 2. It can be noted that the maximum growth of *Scenedesmus* sp. in WMW was determined after 12 days of cultivation. Among the different dilution the highest growth was detected with the medium contained 20% of the WMW (3.7×10^7 cell/mL) with a specific growth rate of 1.05 μ/day. The increasing of WMW concentration in the production medium for 25% effected negatively, the concentration of *Scenedesmus* sp. was 2.2×10^7 cell/mL. In comparison the maximum growth in BBM detected after 10 days (1.4×10^7 cell/mL).

Table 1: Physical and chemical characteristic of wet market wastewater used for the preparation of the growth media.

Parameter (mg/L)	Wet market wastewater (WMW) sample			
	10% WMW	15% WMW	20% WMW	25% WMW
pH	7.27 ± 0.01	7.24 ± 0.03	7.56 ± 0.02	7.64 ± 0.01
Turbidity (NTU)	88 ± 0.03	160 ± 0.01	199 ± 0.03	306 ± 0.05
BOD	574 ± 1.0	639 ± 1.0	647 ± 2.0	709 ± 3.51
COD	349 ± 2.65	375 ± 3.0	512 ± 2.52	566 ± 2.52
TSS	98 ± 2.52	126 ± 1.53	87 ± 1.53	104 ± 1.53
NH_3	45.98 ± 0.03	48.94 ± 0.02	53.48 ± 0.02	66.52 ± 0.07
TN	146.67 ± 0.58	214.67 ± 1.53	295.67 ± 1.15	332.33 ± 1.53
TP	1.33 ± 0.01	1.65 ± 0.02	2.35 ± 0.04	2.89 ± 0.03
Zn	0.049 ± 0.002	0.069 ± 0.002	0.091 ± 0.002	0.093 ± 0.002
Fe	0.068 ± 0.002	0.072 ± 0.002	0.083 ± 0.002	0.086 ± 0.001
Mg	32.37 ± 0.059	49.93 ± 0.02	92.81 ± 0.021	101.2 ± 0.04
Cd	0.01 ± 0.0015	0.009 ± 0.003	0.009 ± 0.002	0.01 ± 0.001

(Values given are the average ± SD of number of sample, n=3)
Biochemical oxygen demand (BOD); Chemical oxygen demand (COD); Total suspended solids (TSS); Ammonia (NH_3); Total Phosphorus (TP); Total nitrogen (TN); Zinc (Zn); Ferum (Fe); Magnesium (Mg); Cadmium (Cd); All the parameters are in mg/L, except pH and turbidity.

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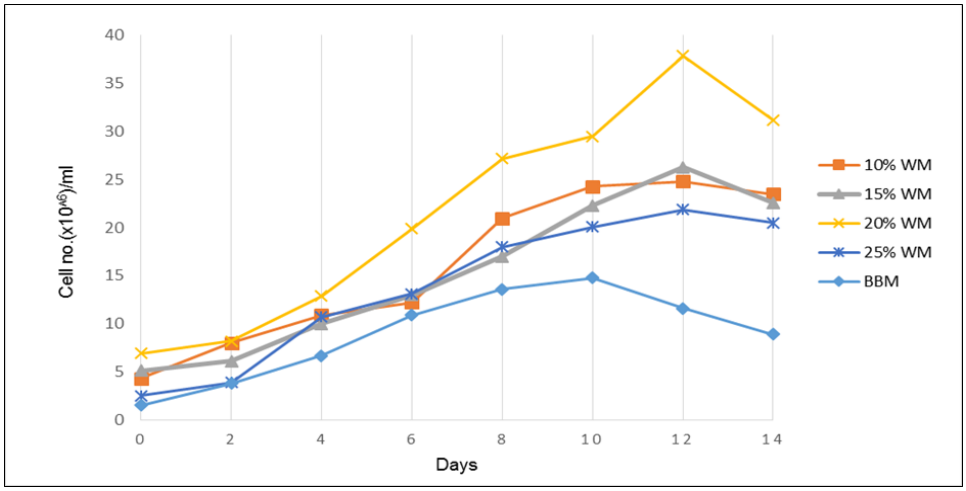

Figure 1: *Scenedesmus* sp. cell concentrations (×10^6)/mL cultivated in different concentration of wet market wastewater (WMW) and BBM.

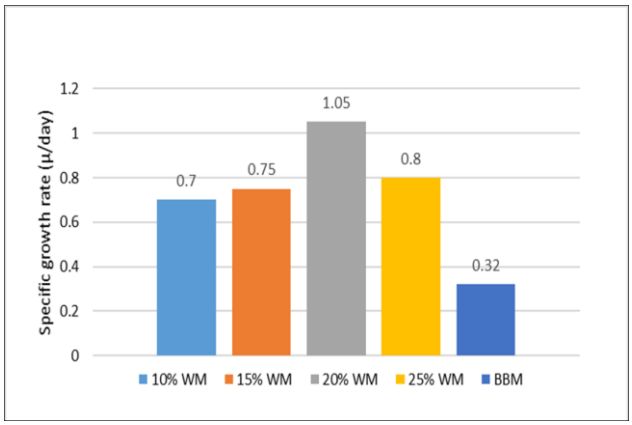

Figure 2: Specific growth rate of *Scenedesmus* sp. cultivated in different concentration of wet market wastewater (WMW) and BBM.

The high growth with 20% of WMW might be due to appropriate amount of nitrogen, phosphorus and COD level compare with other WMW concentration sample (Wang *et al*., 2010). At 15% WMW, *Scenedesmus* sp. grew very slowly at the beginning. However, after 10 days of adaptation the microalgae growth rate began to increase and finally the microalgae had almost the same maximum microalgae cells with the lowest concentration (10% WMW), which indicates that the growth potential of this microalgae under different concentration in the low range (10%-25%) of WMW are effective. Since the growth of *Scenedesmus* sp. are remarkable with WMW compare to BBM which already provide the required nutrient for microalgae, these WMW media proved not only can be treated, but also can generate a high ability potential and sustainable media for algal feedstock as other wastewater; meat processing wastewater, aquaculture wastewater, instant noodle wastewater, industrial wastewater that had been done by previous researchers (Jais *et al*., 2017).

Biomass composition

The GC-MS analyses of *Scenedesmus* sp. biomass generated in the wet market wastewater are depicted in Figure 3. The results revealed that the *Scenedesmus* sp. biomass grown on wet market wastewater have four main compounds included Cycloheptane, Cyclododecanol, 1-ethenyl-acetate, 2-Trifluoroacetoxypentadecane and 9-Octadecenoic acid (Z)-, methyl ester. Cycloheptane (C_7H_{14}) with 98.189 g/mol of Molecular Weight is most commonly used as a nonpolar solvent in the chemical industry and pharmaceutical drugs. 2-Trifluoroacetoxypentadecane ($C_{17}H_{31}F_3O_2$) has been revealed by GC/MS in black gram plant and has revealed to show antimicrobial activity (Anbuselvi and Rebecca, 2012). Hussein *et al*. (2016) proved that Trifluoroacetoxypentadecane extract from *Adiantum Capillus-Veneris* contained antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Salmonella typhi* and *Staphylococcus aureus*. It has to mention that the compounds detected in *Scenedesmus* sp. biomass might be synthesized by the microalgae or accumulated from the wet market wastewater by *Scenedesmus* sp. cells. Kepler *et al*. (1966) reported that 9-Octadecenoic acid (Z)-, methyl ester is an intermediates products of linoleic acid biohydrogenation by *Butyrivibrio fibrisolvens*.

CONCLUSION

It can be concluded that the wet market wastewater is a cheap media to cultivate microalgae *Scenedesmus* sp. for biomass production. Above all, the medium with 20% WMW showed the best performance in respect of cell number and specific growth rate.

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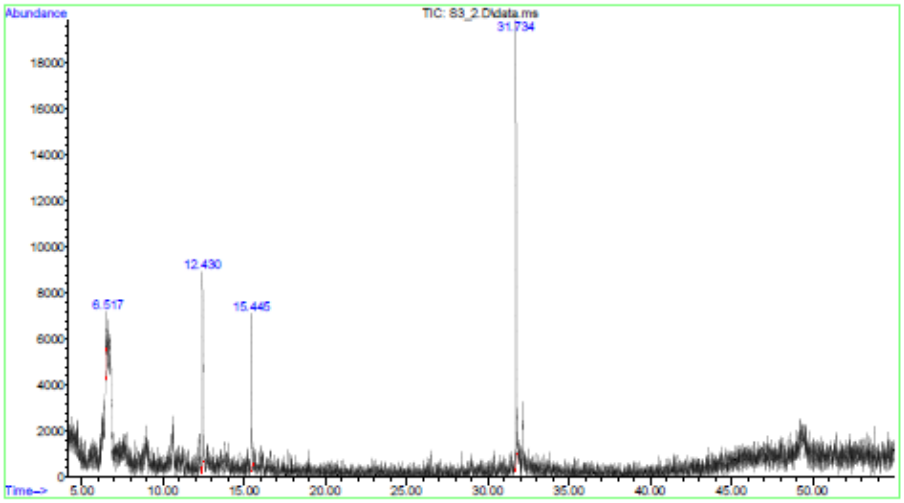

Figure 3: GC-MS analyses of *Scenedesmus* sp. biomass grown on wet market wastewater.

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REFERENCES

Anbuselvi, S. and Rebecca, L. J. (2012). GC-MS study of phytochemicals in black gram using two different organic manures. *Trends in Cognitive* (TIC) Sciences, **5, 17-42.**

APHA, (2002). Standard methods for the examination of water and wastewater. 20th edn. American Public Health Association American Works Association and Water Environment Federation USA.

Badwy, T. M., Ibrahim, E. M. and Zeinhom, M. M. (2008). Partial replacement of fish meal with dried microalga (*Chlorella* spp. and *Scenedesmus* spp.) in Nile tilapia (*Oreochromisniloticus*) diets. *In:* 8th International Symposium on Tilapia in Aquaculture. **pp. 801-811.**

Hussein, H. M., Hameed, I. H. and Ibraheem, O. A. (2016). Antimicrobial activity and spectral chemical analysis of methanolic leaves extract of *Adiantum capillus-veneris* using GC-MS and FT-IR spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research* **8, 369-385.**

Jais, N. M., Mohamed, R. M., Al-Gheethi, A. A. and Amir, H. (2017). Dual role of phycoremediation of wet market wastewater for nutrients and heavy metals removal and microalgae biomass production. *Clean Technology Environment Policy* **19, 37-52.**

Jena, J., Nayak, M., Sekhar Panda, H., Pradhan, N., Sarika, C., Ku. Panda, P. and Behari Sukla, L. (2012). Microalgae of Odisha Coast as a potential source for biodiesel production. *World Environment* **2, 12-17**.

Kepler, C. R., Hirons, K. P., McNeill, J. J. and Tove, S. B. (1966). Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*. *Journal of Biological Chemistry* **241, 1350-1354.**

Macias-Sancho, J., Poersch, L. H., Bauer, W., Romano, L. A., Wasielesky, W. and Tesser, M. B. (2014). Fishmeal substitution with Arthrospira (*Spirulina platensis*) in a practical diet for *Litopenaeus vannamei*: effects on growth and immunological parameters. *Aquaculture* **426, 120-125.**

Pahazri, N. F., Mohamed, R. M., Al-Gheethi A. A. and Amir, H. (2016). Production and harvesting of microalgae biomass from wastewater: A Critical Review. *Environmental Technology Review* **5(1), 39-56.**

Plumber, H. S. and Brain H. K. (2011). Impact of poultry processing by-products on wastewater generation, treatment, and discharges. Proceedings of the 2011 Georgia Water Resources Conference, Georgia Institute of Technology. **pp. 1-5.**

Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y. and Ruan, R. (2010). Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry and Biotechnology* **162, 1174-1186.**

Zhang, E., Wang, B., Wang, Q., Zhang, S. and Zhao, B. (2008). Ammonia-nitrogen and orthophosphate removal by immobilized Scenedesmus sp. isolated from municipal wastewater for potential use in tertiary treatment. *Bioresource Technology* **99, 3787-3793.**

Zhu, L., Wang, Z., Shu, Q. Takala, J., Hiltunen, E., Feng, P. and Yuan, Z. (2013). Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Research* **47, 4294-4302.**

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