BIOMASS QUALITY OF *Scenedesmus* SP. CULTIVATED IN WET MARKET WASTEWATER

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ABSTRACT

Wastewater has a great potential in producing microalgae biomass which has several applications as feeds for aquaculture, foods, supplement, biodiesel and fertiliser as well as in the industrial applications. The present study aimed to evaluate the efficiency of wet market wastewater as a production medium for Scenedesmus sp. biomass based on the physical and chemical characteristic as well as the biomass compositions. the experiments were set up in a 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 the 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^{-1} respectively). The main compounds in the microalgae biomass included Cycloheptane, Cyclododecanol, 1-ethenyl-acetate, 2-Trifluoroacetoxypentadecane and 9-Octadecenoic acid (Z)-, methyl ester. The wet market wastewater would be used as a production medium for Scenedesmus sp. biomass.

Keywords: Microalgae; production medium; wastewater; wet market.

INTRODUCTION

The direct discharge of wet market wastewaters into the environment and water system represents a source pollution due to the high contents of nutrients (nitrogen and phosphorus) in these wastes which are derived from the fresh foodstuff, waste scraps of seafood and fish entrails [1]. In Malaysia, the disposal of the wastewaters into the drainage and end up flowing into the river is a common practice. This phenomenon becomes unacceptable because it escalates the pollution among Malaysian rivers.

On another hand, the high nutrients available in the wet market wastewater might improve the production of valuable microalgae biomass due to the potential of the microalgae species to absorb nitrogen and phosphorus from the wastewater by assimilation process [2, 3]. Microalgae biomass exhibit significant promise in an industrial application such as aquaculture feeds and fertiliser due to the available their contents of carbohydrate, protein, lipids and vitamins as well as pigments such as β-carotene, luten and astaxanthin [4, 5].

The microalgae have gained great importance in recent years as one of the most important biomass resources, this importance lies in their utilisation for different applications included biodiesel production [6, 7]. However, one of the main limitation in the microalgae biomass production is the cost of production medium. The reuse of wastewater for the production of microalgae biomass has been reported in the literature [8]. However, the utilisation of wet market wastewater has not investigated in depth yet. Therefore, the current work aimed to assess the potential of wet market wastewater as a production medium for Scenedesmus sp. biomass. The quantity and quality of microalgae growth in terms of growth cell concentrations and microalgae biomass composition were 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 under outdoor sunlight with continuous aeration for seven days. The raw wet market wastewater (WMW) samples were collected from Pasar Borong Rengit, Batu Pahat, Johor. The collected samples were transported to the laboratory in an ice box and then subjected to the chemical and physical analysis within 24 hrs. The characteristics of the WMW samples were carried out according to standard methods for the examination of water and wastewater, American Public Health Association American Works Association and Water Environment Federation USA, APHA [9].

In order to prepare wet market wastewater (WMW) medium for microalgae biomass production, the samples were autoclaved at 121ºC for 15 min. The sterilised 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.

Table 1. Physical and chemical characteristic of wet market wastewater used for the preparation of the growth media (Values given are the average ± st. dev (SD) of number of sample, n = 3

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

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.

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

The cultivation experiments were conducted in a transparent glass tank (40 L of capacity) containing 20 L of autoclaved wet market wastewater medium. The production medium was inoculated with 106 cell mL^{-1} of *Scenedesmus* sp. and aerated continuously using an air pump to ensure the distribution of nutrients. 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. A fixed volume (10 mL) of the sample was preserved by adding 2-3 drops of formalin. One mL of sample was carefully filled in haemocytometer and covered with glass slide. Microalgae cells were calculated by the following equation expression:

$$\text{Cells/mL (in original)} = (\text{mean of cells/square})^{1/5} \times (25) \times (10^4)(\text{Dilution Factor}) \quad (1)$$

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

$$\text{Growth rate; } \mu = \text{Ln}\frac{(X_2 - X_1)}{T_2 - T_1} \quad (2)$$

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 (μ) of Scenedesmus sp. in the WMW media during the incubation periods for two weeks are presented in Figure 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 containing 20% of the WMW (3.7×10^7 cell mL^{-1}) with a specific growth rate of 1.05 μ/day. The increasing of WMW concentration in the production medium for 25% affected negatively, the concentration of Scenedesmus sp. was 2.2×10^7 cell mL^{-1}. In comparison, the maximum growth in BBM detected after 10 days (1.4×10^7 cell mL^{-1}).

The high growth with 20% of WMW might be due to the appropriate amount of nitrogen, phosphorus and COD level compare with another WMW concentration sample [10]. 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 compared 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 [3, 9].

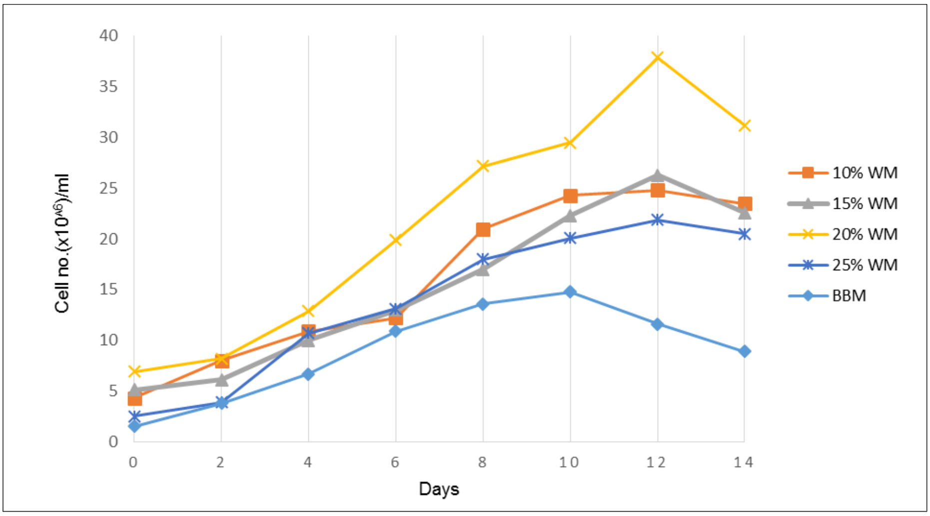

Figure 1. *Scenedesmus* sp. cell concentrations (x10^6) mL^{-1} cultivated in different concentration of wet market wastewater (WMW) and BBM

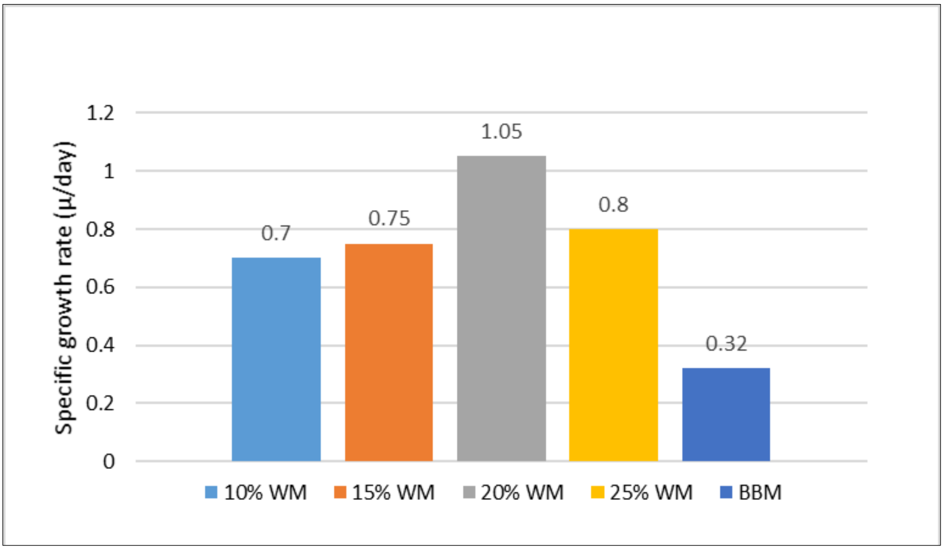

Figure 2. Specific growth rate of *Scenedesmus* sp. cultivated in different concentration of wet market wastewater (WMW) and BBM

Biomass composition

The GC-MS analyses of *Scenedesmus* sp. biomass generated in the wet market wastewater are depicted in Figure 3.

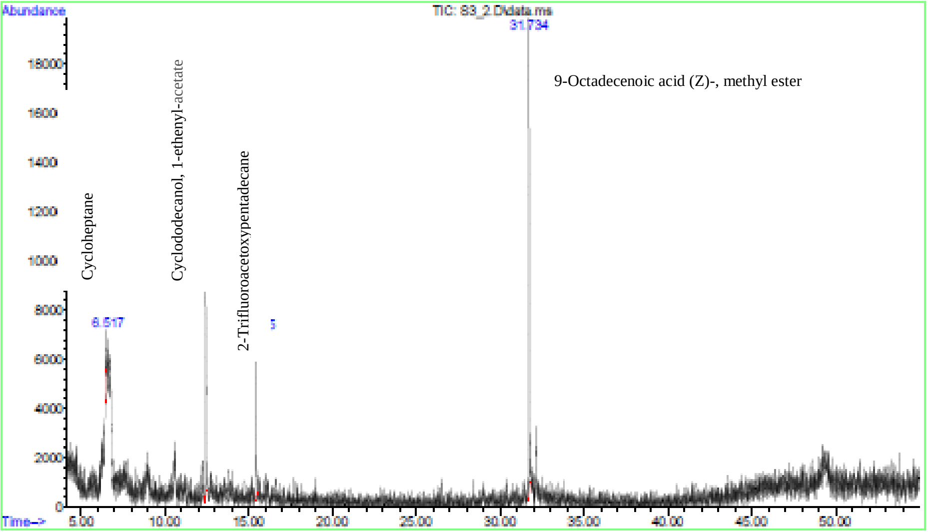

Figure 3. GC-MS analyses of *Scenedesmus* sp. biomass grown on wet market wastewater.

The results revealed that *Scenedesmus* sp. biomass grown in 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 [11]. Hussein et al. [12] extracted Trifluoroacetoxypentadecane from Adiantum Capillus-Veneris and indicated that the compound has antibacterial activity against *Bacillus subtilis*, *Pseudomonas eurogenosa, Streptococcus faecalis, Salmonella typhi* and *Staphylococcus aureus.*

It has to mention that the compounds detected in *Scenedesmus* sp. biomass might be synthesised by the microalgae or accumulated from the wet market wastewater by *Scenedesmus* sp. cells. Kepler et al. [13] indicated that 9-Octadecenoic acid (Z)-, methylester is an intermediates products of the biohydrogenation of linoleic acid by Butyrivibrio *fibrisolvens.*

CONCLUSION

It can be concluded that the wet market wastewater can be used as an inexpensive medium for the cultivation of microalgae *Scenedesmus* sp. for biomass production. Among the four different of concentrations of WMW, 20% WMW showed the best performance in respect of cell number and specific growth rate.

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REFERENCES

[1] Plumber, H. S., & Kiepper, B. H. (2011). Impact of poultry processing by-products on wastewater generation, treatment, and discharges. *Georgia Institute of Technology*.

[2] Zhang, E., Wang, B., Wang, Q., Zhang, S., & 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.

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

[4] Macias-Sancho, J., Poersch, L. H., Bauer, W., Romano, L. A., Wasielesky, W., & 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.

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

[6] Jena, J., Nayak, M., Sekhar Panda, H., Pradhan, N., Sarika, C., Ku. Panda, P., & Behari Sukla, L. (2012). Microalgae of Odisha Coast as a Potential Source for Biodiesel Production. *World Environment*, *2*, 12–17.

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

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

[9] APHA, Standard methods for the examination of water and wastewater 1045 Int.J.Curr. Microbiol. App. Sci (2014) 3(8) 1036-1047 water, 20th edition. American Public Health Association American Works Association and Water Environment Federation USA (2002).

[10] Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., & Ruan, R. (2010). Cultivation of Green Algae Chlorella sp. in Different Wastewaters from Municipal Wastewater Treatment Plant. *Applied Biochemistry and Biotechnology*, *162*,1174–1186.

[11] Anbuselvi, S., & Rebecca, L. J. (2012). GC-MS study of phytochemicals in black gram using two different organic manures. *TIC*, *5*, 17-42.

[12] Hussein, H. M., Hameed, I. H., & 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.

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