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Preliminary Assessment of Growth Rates on Different Concentration of Microalgae Scenedesmus sp. in Industrial Meat Food Processing Wastewater

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Abstract. This study is aimed to evaluate and access the growth rates and biomass productivity in different concentrations of microalgae *Scenedesmus sp.* using Industrial Meat Food Processing Wastewater as a media. The focus of this study is to determine the best concentrations of microalgae *Scenedesmus sp.* in raw wastewater in terms of kinetics of cells growth rates. The study verified that concentration of $1x10^6$ cells/ml of microalgae gives the highest specific growth rates of biomass at 0.4488 day-1 and 1720 cells/ml/day compare to the other concentrations, while the lowest occurred at concentration of $1x10^3$ cells/ml at 0.4108 day^{-1} and 14.9 cells/ml/day. The result shows the different concentration of microalgae *Scenedesmus sp.* culturing in Industrial Food Processing Wastewater influence the cells growth of biomass and the optimum were obtained at concentration of $1x10^6$ cells/ml which suggested use for Industrial Meat Food Processing Wastewater Treatment purposed. With this finding, it should be seemly to adopt and applied efficiently in treating the wastewater especially for *Scenedesmus sp.* type of microalgae.

1 Introduction

Microalgae are known as a survival microorganism which can grow and adapt well in an extreme environmental conditions such as in various type of wastewater. However, there are some species who can only survive in certain type of conditions [1]. There are more 50,000 species of microalgae has been found which varies from types, species, size and organism groups [2-4]. The structure of algae typically comprises of plastids chlorophyll which ensuing photosynthesis process that devours light as energy and converts inorganic substance into sugar [5-6]. This expressions indicates that microalgae has absorption ability indirectly can restore and reduce substances from wastewater [7].

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Wastewater normally contains high amount of organic matter as well as inorganic nutrients and according to [8] cultivation of microalgae for biomass productions by using wastewater can significantly reduce the water usage up to 90%. This can be achieved by assimilating all the nutrients present in wastewater. Industrial Meat Food Processing wastewater is known to be highly biodegradable, non-toxic and rich in nitrogen and phosphorus elements. Its characteristics is differ comparing with typical municipal wastewater that managed by public treatment plants as it is difficult to predict due to the seasonal and demand nature of business [9]. Activities involving in the meat processing industries known to be high in water usage up to 62 Mm^3/year, as only a small amount from this total are fully utilized into the final products, subsequently the rest of wastewater been discharge into the water bodies [10] and according to [11], meat food processing facility itself are using clean water up to 40m^3/metric tons of meat products which is the highest consumers compare with other food and beverage industries by 24% [11-12]. All the steps involved in the process of production until packaging indirectly gives an impact to the environmental as the waste generates from all of these process will eventually initiate several environmental problems to human beings and aquatic organism as they both rely on water to sustained [9, 12]. Furthermore, nitrogen and phosphorus are an ideal and essentials nutrients that can stimulate a fast growth of algae in freshwater, wastewater streams from varies of sources such as from agricultural run-off, industrial, municipal wastewater and marine ecosystem [13-14]. Even though wastewater could possibly offer some essentials goods for microalgae cultivation activity, however, the raw wastewater compositions are differ compare to the culture medium prepared in the laboratory. Concentration of nitrogen and phosphorus as well as the existent wide range of other microorganism, presence of heavy metals and other toxics comes from wastewater may interfere the growth and uptake of nutrients by microalgae [15]. On the other hand, nitrogen presents in wastewater normally in ammonia forms which can restrain microalgae growth if high in concentration [1, 16, 17]. Therefore, the most significant key in selecting the best concentration of microalgae is it can strongly adapt and have high specific growth rates interpretation in selected wastewater.

2 Materials and methods

2.1 Microalgae *Scenedesmus sp.*

Three (3) steps of procedure on preparations of microalgae i.e. microalgae sampling, isolation and identification need to be done prior culturing. Microalgae of *Scenedesmus sp.* was obtained from plankton located in Endau Rompin National Park, Johor. This location is selected due to well preserved national park that has less environmental pollution which contributes to a better colonies and growth. Many previous studies by [18-19] have been used and applied microalgae from this location for treatments and growth studies that portrayed effective results and outcome. A sterilize glass bottle was used for collection and seal with cotton wool for preservations to allowed air flow and photosynthesis. A small amount of algae were isolated and isolation process was done under microscope by using platinum needles. The isolated microalgae then will be examined for initial identification and confirmation using NIKON Eclipse E600 microscope. For culturing, the isolated microalgae is cultivate in Basal Bold Medium, BBM [20] and later were place under natural condition of 12h: 12h intensity lights with temperature ±35^{o}C for growth observations.

2.2 Wastewater Sampling Preparations

Sample was collected at one of small and medium food industries located in Johor. Sampling was done once a week for 8 weeks duration to get the average values and range. The collected wastewater was preserve in plastic bottle of Polyethylene Terephthalate (PET) and was stored at 4^0C without any acid preservation. Wastewater that was collected from the study site were filtered before inoculation process takes place to minimized all unwanted microorganism or bacteria that may exists in the wastewater.

2.3 Inoculations of different microalgae *Scenedesmus sp.* concentration for laboratory batch optimizations

Six (6) different concentration of microalgae *Scenedesmus sp.* was prepared and use in this batch study i.e. concentration $1x10^3$ cells/ml, $1x10^4$ cells/ml, $1x10^5$ cells/ml, $1x10^6$ cells/ml, and $1x10^7$ cells/ml respectively. The concentration was calculated using haemocytometer together with equations by Stephenson [21] i.e. $C_1V_1 = C_2V_2$ where by C_1 is the initial concentration of the stocks solutions, V_1 is the amount of stock solution required, C_2 is the concentration of the wanted stock concentration and V_2 is the volume of the stock solutions. This equation is suitable for solution, mixture and media as stated by Stephenson [21]. The growth rates data were collected and counted for every 24hours until the growth enter the death or decline stage [22].

3 Cells Concentration Counting

Microalgae cell concentration was determined through cell counting using haemocytometer [23-24]. The haemocytometer is a slide glass size 30 mm x 70 mm x 4 mm thick. It has 3 chambers and cells counts are performed at the center part. The specific growth rates of microalgae were calculated using Eq.1 derives formula from Andersen R.A. [24] i.e. $\mu / day = (Ln(N_t/N_o)/(t_f - t_i))$. Where N_t is Final algae concentrations, N_0 is Initial algae concentrations and Δ_t are subtraction of Final time with the Initial time (day).

4 Data Analysis

4.1 Microalgae *Scenedesmus sp*. characteristics

Characteristics of microalgae *Scenedesmus sp.* is shown in Table 1 and its physical characteristics is shown in Fig. 1 by using SEM micrograph.

Table 1. Characteristics of microalgae *Scenedesmus sp.*

Parameters	Values
pH	6.38
Total Suspended Solid, TSS (mg/L)	375
Turbidity (NTU)	77.8
Sulfate, SO_4^{2-} (mg/L)	35
Dissolved Oxygen, DO (mg/L)	16.38
Total Nitrogen, TN (mg/L)	308.9
Total Phosphorus, TP (mg/L)	0.75
Total Organic Carbon, TOC (mg/L)	38.01
Ammonia Nitrogen, NH_3-N (mg/L)	1.95
Zinc, Zn (mg/L)	0.47
Copper, Cu (mg/L)	1.24

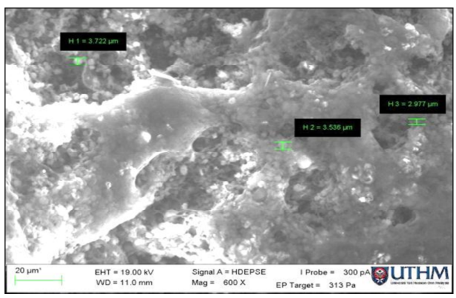

Fig. 1. SEM micrgraph (600X) of *Scenedesmus sp.*

4.2 Industrial Meat Food Processing Wastewater Characteristics

Wastewater characterization is significantly important as it is to determine the availability of nutrients in the wastewater for microalgae growth. Table 2 shows the physical and chemical parameter of meat processing wastewater in comparison with standard discharge effluent allowable by local authorities. Chemical Oxygen Demand (COD) generally defined as the total quantity of oxygen required for complete oxidation of organic compound to carbon dioxide and water and it is significantly to continually measure to determine the degree of pollution in an effluents [25]. Concentration of COD is 1643 mg/L, which is over the allowable limit permit by the local authorities. BOD_5 is known to be the basic of parameter in finding the degree of pollutions in water. As for Biological oxygen demand, (BOD), the concentration of the wastewater is 895 mg/L which also above the limits of standard A and Standard B. This wastewater also contains other parameter such as Total Nitrogen, Ammonia Nitrogen, Total Phosphorus, Orthophosphate, Total Organic Carbon, Total Suspended Solids as well as Total Dissolved Solids with concentration of 151 mg/L, 10 mg/L, 45 mg/L, 35 mg/L, 333 mg/L, 333 mg/L and 1120 mg/L respectively. All of the mentions parameters are way above limits line as indicate in the [26]. In terms of pH, the wastewater pH value is acceptable and fit for microalgae cultivation as refer to [27]. There have been few researchers who have done and shown some potentials in cultivating microalgae in other different types of raw wastewater [28-31], or phycoremediation and bio transform those pollutants to essentials biomass as well as cleaned the wastewater at the same time. Moreover, growth efficiency of the cells is subjected to several of environmental factors and nutrients availability. Therefore, this study is to utilized raw

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industrial meat food processing wastewater into cultivating the s*cenedesmus sp.* for the growth cells efficiency.

4.3 Cell Growth of *Scenedesmus sp. in* Industrial Meat Food Processing Wastewater

The growth of *Scenedesmus sp*. cells curve in the industrial meat food processing wastewater are showing positive growing over time for all concentration except for $1x10^7$ cells/ml. A typical growth of curve for microorganism as stated by Shuler [22] consist of five (5) phase; the lag, exponential, deceleration, stationary and death or decline phase. As for these experimental growth (Fig. 2), cells of $1x10^3$ cells/ml, $1x10^4$ cells/ml, $1x10^5$ cells/ml and $1x10^6$ cells/ml shows an increasing of biomass concentration over time except for $1x10^7$ cells/ml. The results of growth are similar to the growth of *Botryococcus sp*.in domestic wastewater that has been reported by Gani [28], whereby, too much of populations of microalgae will cause suffocation of nutrients available in the wastewater to sustained the microalgae growth, resulting no increasing of biomass concentrations over time and based [22], at high concentration of microalgae in the medium, the growth rates will inhibited.

Table 2. Characteristics of the Industrial Meat Food Processing Wastewater used as growth media for *Scenedesmus sp.* Sampling date: January 2015 ~ February 2015.

Physiochemical parameters	**Average Concentrations Mg/L**	***Standard A**	***Standard B**
Biological Oxygen Demand, BOD	1070±350.913	20	50
Chemical Oxygen Demand, COD	2350±835.427	50	100
Total Nitrogen	317.22±171.147	-	-
Total Phosphorus	62.86±36.525	5	10
Orthophosphate	47.37±27.530	-	-
Total Organic Carbon	493.82±267.881	-	-
Total Suspended Solids, TSS	1400±299.100	50	100
pH	6.5-8.0	6.0 – 9.0	5.5 – 9.0

*EQA, 1974

Fig. 2 shows the comparison between the collected experimental data in comparing with mathematical modeling [32]. Each of specific growth rates of the concentrations can be calculated and obtained during the exponential phase of the microalgae growth by drawing a straight line that touched at least three or more points in Fig. 3 as accordingly to Andersen [24] methods. The growing curve between experimental data and modeling data is considerably different as during experiments works, lots of other factors such as environmental factors, equipment's error and human errors contribute during the analysis.

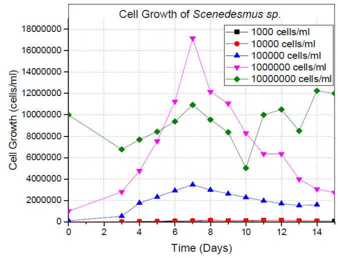

Fig. 2. Scenedesmus sp. growth curve in different concentrations.

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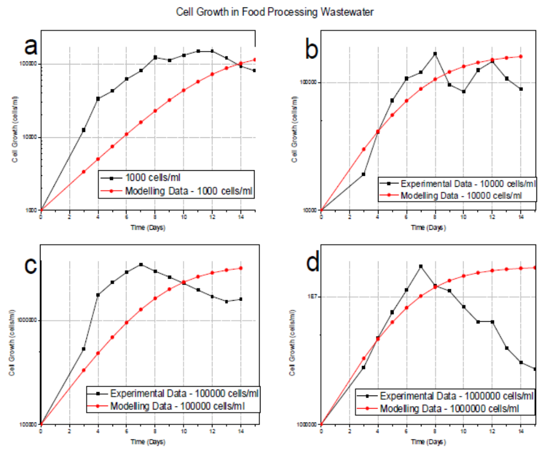

Fig. 3. a) Cell growth of Scenedesmus sp. for $1x10^3$ cells/ml concentration; b) Cell growth of Scenedesmus sp. for $1x10^4$ cells/ml concentrations; c) Cell growth of Scenedesmus sp. for $1x10^5$ cells/ml concentration; d) Cell growth of Scenedesmus sp. for $1x10^6$ cells/ml

Table 3. Comparison of growth parameters between experimental data and mathematical modelling for *Scenedesmus sp.* in different initial of concentrations.

Concentration (cells/ml)	Experimental Data				
	Max Specific Growth Rates µmax/day	**Division per day (Dd)**	**Doubling Time, td (day)**	**Biomass productivity (cells/ml/day) 10^4**	**R^2**
$1x10^3$	0.4108±0.0425	0.5927	1.687	14.9	0.9393
$1x10^4$	0.4125±0.0583	0.5951	1.680	16.8	0.9321
$1x10^5$	0.4250±0.0308	0.6131	1.631	348.0	0.8187
$1x10^6$	0.4483±0.0244	0.6468	1.546	1720.0	0.9967
$1x10^7$	0	0	0	0	0

* Experiments conducted in triplicate manner (*n =3)*

The specific growth rates of biomass increases with microalgae concentration until they reached a maximum value that associate with optimal concentrations, concentration $1x10^6$ cells/ml shows the highest specific growth of biomass increment followed by $1x10^5$ cells/ml, $1x10^4$ cells/ml and $1x10^3$ cells/ml with specific growth rates of 0.4483 day^{-1}, 0.4250 day^{-1}, 0.4125 day^{-1} and 0.4108 day^{-1} respectively. Beyond these concentrations, it can be considered as saturated phase where the growth rapidly decreased or no growth occurred [33]. As per $1x10^6$ cells/ml, the exponential curve starts from day 3rd until day 7th, similarly to $1x10^5$ cells/ml while $1x10^4$ cells/ml and $1x10^3$ cells/ml starts their exponential curve at day 3rd until day 8th correspondingly. In identifying specific growth rates, can be directly obtained by applying Equation (1). Biomass productivity on the other hand shows some significant increments for all concentrations, particularly for $1x10^6$ cells/ml with maximum biomass productivity and specific growth rates of 1720 cells/ml/day and 0.4483 day^{-1}. This results are slightly higher than the results obtained by [34] studies with specific growth rates of 0.103 day^{-1} when they cultivate *Scenedesmus sp.* in municipal wastewater and biomass productivity up to 50.75 mg/L/day (dried weight) [35]. The specific growth rate obtained in this study is slightly higher than study done by [36] with maximum specific growth rate of 0.17 day^{-1}, and biomass production of 253.45

mg/l in terms of dry weight by using BG-11 medium media during cultivation of *Scenedesmus SDEC-8.* Moreover, *chrollella SDEC 4* on the other hand obtained 0.18 day^{-1} of maximum specific growth rate with 157.30 mg/l in terms dry weight of biomass production. Different species will give different growth rates due to its algometric relationship between growth and cell size as well as metabolic process, resulting smaller size of species tends to grew faster than the bigger size [33], same goes with different media or medium used.

5 Conclusion

The series of experiments conducted here are aimed to access and verified the best concentration of microalgae *scenedesmus sp.* cultivating in Industrial Meat Food Processing Wastewater. The results shows concentration of $1x10^6$ cells/ml of microalgae *scenedesmus sp.* gives the highest specific growth rates biomass of 0.4488 day-1. Thus, from these findings, the most adequate population that suitable for sustaining the microalgae *scenedesmus sp.* growth in Industrial Meat Food Processing Wastewater is verified. This study is another small chapter on accessing the effectiveness of cultivating microalgae *Scenedesmus sp.* in industrial meat food processing wastewater for wastewater purification and biomass productivity, as the culturing microalgae in industrial meat food processing wastewater are highly dependable on wastewater nutrients availability as well as initial cell concentrations. Therefore, more research on microalgae *Scenedesmus sp.* would be essentials for phycoremediation and potentially biomass.

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