International Journal of Engineering & Technology, 7 (4.30) (2018) 553-556

International Journal of Engineering & Technology

Website: www.sciencepubco.com/index.php/IJET

Research paper

Batch Kinetics of Nutrients Removal from Synthetic Meat Processing Wastewater by using Microalgae Botryococcus Sp.

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Abstract

Disposed meat processing wastewater contains high range of nutrients such as ammonia nitrogen and orthophosphate which will cause eutrophication and lead to destruction of ecosystem. Therefore, batch experiments were conducted to explore the influence of the range of initial concentration of ammonia nitrogen and orthophosphate found in meat processing wastewater in the removal of those nutrients during phycoremediation of synthetic wastewater by using microalgae Botryococcus sp. Michaelis-Menten rate expression was applied to generate biokinetic coefficients k, reaction rate constant, Km, half saturation constant and Y, yield coefficients. The experiment was conducted using synthetic wastewater with initial NH_4-N concentration varying between 30-480 mg/l and PO_4^{3-} concentrations varying between 14-239 mg/l. The results demonstrate removal efficiency of NH_4-N between 42-100 % and PO_4^{3-} between 63-96 %. Biokinetic coefficients were established as k = 1.72 mg NH_4-N /mg chl *a*/day, Km = 52.29 mg/L and Y_N = 0.027 mg chl *a*/mg NH_4-N for ammonia nitrogen and k = 1.13 mg PO_4^{3-}/mg chl *a*/day, Km = 44.45 mg/L and Y_P = 0.038 mg chl *a*/mg PO_4^{3-} for orthophosphate.

Keywords: *batch kinetics; microalgae; nutrients; phycoremediation; synthetic meat processing wastewater.*

1. Introduction

The water and wastewater treatment has become more vital due to the escalating world population growth which causes poor wastewater disposal (Feng et al., 2009). As the result of population and climate change freshwater resources has been rapidly decreasing. Besides, the accelerating stringentness in the international effluent discharge standards is requiring development in wastewater treatment technologies and demands new inventions in wastewater treatment field (US EPA, 2004; World Bank Group, 2007).

The nutrient content in meat factory wastewater by Cristian, 2010 were total nitrogen, 2743.6 mg/L and total phosphorous, 328.4 mg/L with the N:P ratio of 8:1. While, the nutrient content in meat processing wastewater by Lecompte et al., 2015 were TN, ranges from 60-339 mg/L and PO_4^{3-} ranges from 30.1-77.3 mg/L. NH_4-N and TP content from provincially inspected slaughterhouse wastewater in Ontario, Canada were 63.66 mg/L and 48.4 mg/L respectively in a study by Wu et al., 2012. General characteristic of wastewater from meat related activities (Lecompte *et al.*, 2015) reveals that TN and PO_4^{3-} ranges from 50-841 mg/L and 25-200 mg/L respectively. As the concentration of nutrients in meat processing wastewater varies, the effect of the initial concentration of nutrients on removal efficiency of nutrients has to be crucially investigated to give objective scientific support to the use of microalgae in the wastewater treatment for wastewater from meat processing food industry.

Objective of this study is to evaluate the effects of initial nutrient concentration on nutrients removal efficiency through removal mechanism of biokinetic coefficients k, reaction rate constant, Km, half saturation constant and Y, yield coefficients by using Michaelis–Menten rate expression.

2. Materials and methods

Wastewater sample was collected at one of small meat processing industry located in Batu Pahat, Johor. The samples were taken at 9.00 a.m. and 12.00 p. m. through grab sampling method. These time were selected based on the research done by wastewater (Latiffi et al., 2016) for the highest pollutant discharge for meat processing wastewater. The wastewater samples were tested for NH_4-N and PO_4^{3-} concentration content using BUCHI Distillation Unit K-355 and Ion Chromatography apparatus (Dionex ASE 200). Range of the nutrient concentrations identified.

Table 1: Range of Nutrients Concentration in Meat Processing Wastewater

Nutrients	Unit	Samples taken at 9.00 a.m.	Samples taken at 12.00 p.m.
Ammonia Nitrogen (NH_4-N)	mg/L	99 ± 3.3 - 492 ± 17.1	35 ± 2.8 - 389 ± 8.5
Orthophosphate (PO_4^{3-})	mg/L	39.54 ± 2.1- 236.54 ± 7.8	14.99 ± 3.4- 95.68 ± 6.2

Microalgae sampling, isolation and identification are done prior inoculation process. These microalgae were found and obtained from Endau Rompin National Park, Johor. Sample of isolated algae culture was examined under a light microscope for initial identification and confirmation that the culture is unialgal was done by using a NIKON Eclipse E600 microscope with an at-

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tached camera DXM 1200F. Selected microalgae were cultivated in Bold's Basal Medium, BBM (Nichols and Bold, 1965) and place under natural conditions for growth.

Synthetic wastewater was prepared by using ultrapure water mixed with NH_4Cl and KH_2PO_4 as ammonia nitrogen and orthophosphate sources, respectively. 11 sets 800 ml mixture of ultrapure water with 10 sets of different initial concentrations of ammonia nitrogen and orthophosphate which are 14 and 30; 39 and 80; 64 and 130; 89 and 180; 114 and 230; 139 and 280; 164 and 330; 189 and 380; 214 and 430; and 239 and 480 mg/L respectively and one set with no initial concentrations of ammonia and orthophosphate used as control. The initial Chl *a* content controlled within the range of 2.5mg/L to 6.7 mg/L. pH is controlled at around 6 to 8 pH, N:P ratio is maintained at 2:1 and the microalgae cell concentration used is $1x10^6$ cell concentration. Other factors like light intensity, photoperiod, and temperature are left natural. Variations of nutrient concentrations and Chl *a* content were monitored daily for 18 days and the utilization rates of specific substrate were computed.

NH_4-N and PO_4^{3-} concentration contents were established using BUCHI Distillation Unit K-355 using Method 4500 NH_3 and Ion Chromatography apparatus (Dionex ASE 200) using Method 4110B respectively. To determine the Chl *a* content, 10 ml of algal suspension is centrifuged at 3000rpm for half an hour and the supernatant was disposed. The algae were mixed with 3ml of methanol and the suspension heated in a water bath for 5 minutes. The samples were left to be chilled to room temperature. Then, methanol is added to increase the volume to 5ml. A spectrophotometer is used to determine the Chl *a* concentration in the pigment extract.

3. Results and discussion

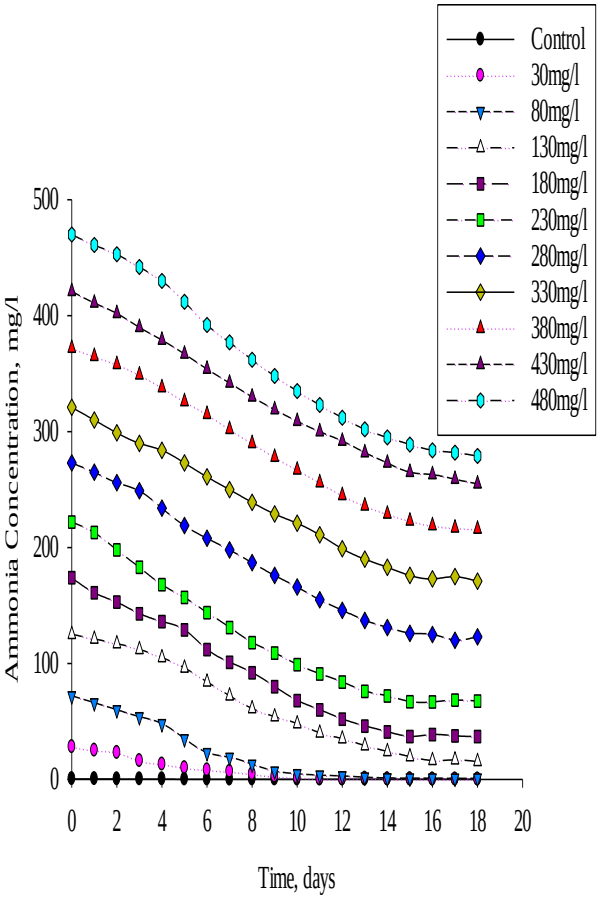

Fig. 1: Differences in NH_4-N Concentrations in the Medium versus Time throughout Batch Cultivation under Multiple Initial NH_4-N Concentrations

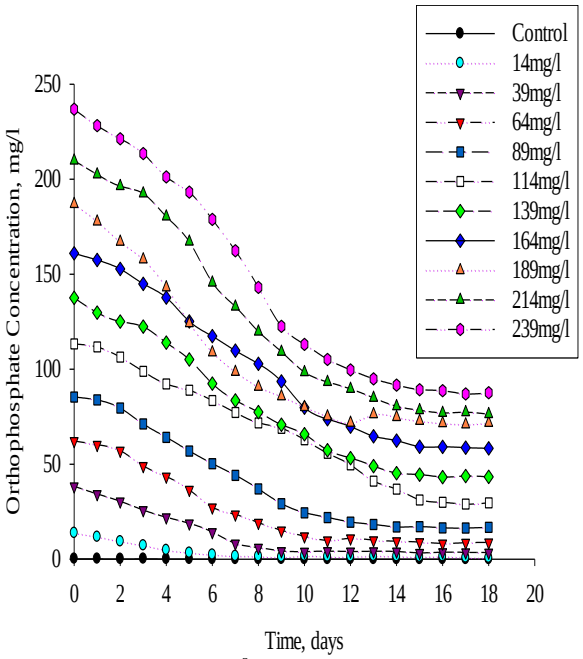

Fig. 2: Differences in PO_4^{3-} Concentrations in the Medium versus Time throughout Batch Cultivation under Multiple Initial PO_4^{3-} Concentrations

Fig. 1 portrays the elimination of NH_4-N concentration against time with multiple initial NH_4-N concentration for the period of 18 days of batch experiment. In the media with lowest initial concentration which is 30 mg/L, NH_4-N was entirely eliminated and 99 % removed from the second lowest initial concentration, 80 mg/L. In spite of that, the NH_4-N removal efficiency further decreases from 88 % to 42 % as the initial concentrations increases from 130 mg/L to 480 mg/L. Fig. 2 illustrates the removal of PO_4^{3-} concentration with time at different initial PO_4^{3-} concentration. The highest PO_4^{3-} removal is 96 % from the medium with the lowest initial concentration 14 mg/L and the second highest is 91 % from the second lowest initial concentration, 39 mg/L. Despite that, the PO_4^{3-} removal efficiency further decreases from 86 % to 63 % as the initial concentrations increases from 64 mg/L to 239 mg/L.

Removal efficiency of NH_4-N and PO_4^{3-} in this study is consistent and comparatively better than the study by Aslan *et al.* (2006) where the highest of 100% and lowest of lesser than 24% of NH_4-N removal efficiency and highest of 78% and lowest of less than 30% removal efficiency of PO_4-P from synthetic wastewater with 10 different initial concentrations of NH_4-N and PO_4-P using microalgae *Cholorella Vulgaris* (Aslan *et al.*, 2006). In the study of microalgae Botryococcus sp. ability to reduce nutrient concentrations in livestock wastewater, achieved TN removal efficiency rate of 88% and TP removal efficiency rate of 98% (Shen *et al.*, 2008). The removal efficiency of nitrate is 99.7-99.8 % and of phosphate is 98.8- 99.1% by using Botryococcus sp. on industrial wastewater (Chinnasamy *et al.*, 2010). In the study by Rinna *et al.*, 2014, two strains of Botryococcus braunii (*UTEX LB 572* and *LABIOMAR/IB/UFBA IBL C115*) were used and the TN removal percentage at the effluent were 62 % and 65% respectively and TP removal percentage at the effluent for both strains are 100%.

Ri, is the initial rates of substrate removal, are applied to establish the coefficients. Rate of initial substrate removal is computed using (1).

$$R_i = -(S_i - S_t)/(t_i - t_t) \quad (1)$$

Where, R_i is the substrate removal rate, S_i represents the initial concentrations of the substrate which is NH_4-N or PO_4^{3-}, S_t represents the substrate concentration tally with "t_t" which represents the time when there is no notable change in substrate concentration.

By dividing R_i to the initial chl *a* content (2), specific rate of substrate removal (R_{Xi}) is established.

$$R_{Xi} = R_{Si} / X_i = kS_i / K_m + S_i \quad (2)$$

Equation 2 can be linearized in double reciprocal form as in Equation 3 and a plot of $1/R_{Xi}$ against $1/S_i$ generates a linear line producing a slope of K_m/k and y-axis intercept of 1/k.

$$1 / R_{Xi} = (1 / k) + [(K_m / k) \times (1/S_i)] \quad (3)$$

From the experimental data regression lines were plotted in form of $1/R_{xi}$ versus $1/ (NH_4\text{-}N)_0$ and $1/R_{xi}$ against $1/(PO_4^{3-})_0$ for removal of respective substrates. Kinetic coefficients of ammonia nitrogen removal and orthophosphate removal by Botryococcus sp. as k and Km were computed from the slope and intercept of this plot's best fit line.

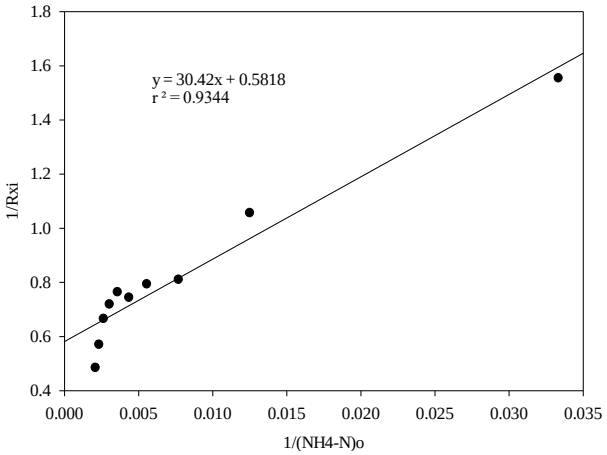

Fig. 3: Kinetic coefficients, K_m and k, for NH_4-N removal established.

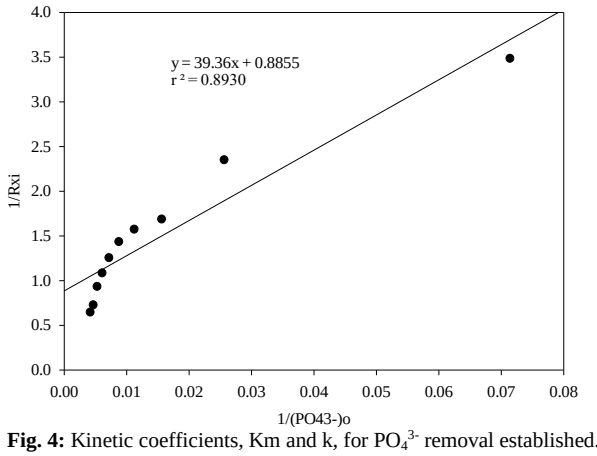

Fig. 4: Kinetic coefficients, Km and k, for PO_4^{3-} removal established.

From the experimental data yielded by Fig. 3 and 4 were plotted in form of $1/R_{xi}$ versus $1/ (NH_4\text{-}N)_0$ and $1/R_{xi}$ versus $1/ (PO_4^{3-})_0$ as depicted in Fig. 5 and 6. Slope and intercept of best fit line of this plot generates kinetic coefficients of ammonia nitrogen elimination by *Botryococcus sp.* were established as k = 1.72 mg NH_4-N/ mg chl *a*/day and Km = 52.29 mg/L. The correlation for inverse specific NH_4-N removal rate and inverse initial NH_4-N concentration from the regression line is R^2=0.98 which means a strong positive linear relationship. Similarly, the coefficients for PO_4^{3-} elimination by *Botryococcus sp.* were generated as k = 1.13 mg PO_4^{3-}/mg chl *a*/day and Km = 44.45 mg/L according to the intercept and the slope of best fit line of 1/Rxi versus $1/ (PO_4^{3-})o$. The correlation for inverse specific PO_4^{3-} removal rate and inverse initial PO_4^{3-} concentration from the regression line is R^2= 0.93 which means a perfect positive linear relationship.

The biokinetic coefficient values reveal that ammonia nitrogen removal rate is higher than that of orthophosphate during the phycoremediation. The reaction rate constant, k and half saturated constant, Km values for both NH_4-N and PO_4^{3-} removal are in agreement with study by Aslan *et al*. (2006), where k = 1.5mg NH_4-N/mg chl *a*/day and Km = 31.5mg/L and k = 0.5mg PO_4-P/mg chl *a*/day and Km = 10.5mg/L.

Equations 4 and 5 were used to generate yield coefficient for NH_4-N and PO_4^{3-} removal, respectively

$$(\text{chl } a)_f - (\text{chl } a)_i = Y_N[(NH_4\text{-}N)_0 - (NH_4\text{-}N)_f] \quad (4)$$

Or

$$(\text{chl } a)_f - (\text{chl } a)_i = Y_P[(PO_4^{3-})_0 - (PO_4^{3-})_f] \quad (5)$$

Where, the final chl *a* concentration (mg/L) and initial chl *a* concentration (mg/L) at the beginning of the experiments is represented by $(\text{chl } a)_f$ and $(\text{chl } a)_i$ respectively. $(NH_4\text{-}N)_0$ represents the initial NH_4-N concentration (mg/L) and $(NH_4\text{-}N)_f$ represents final NH_4-N concentration (mg/L). Yield coefficient for NH_4-N, Y_N (mg chl *a*/mg NH_4-N) is established by slope of $((\text{chl } a)_f - (\text{chl } a)_i)$ against $((NH_4\text{-}N)_0 - (NH_4\text{-}N)_f)$. In the same way yield coefficient for PO_4^{3-}, Y_P is calculated.

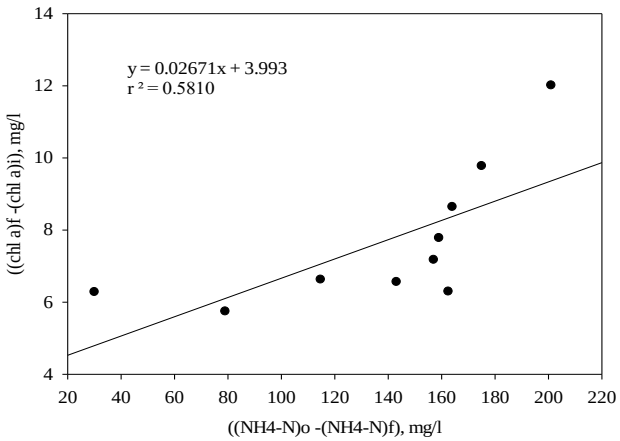

Fig. 5: Yield coefficient for ammonia nitrogen (NH_4-N) removal by *Botryococcus sp.* established

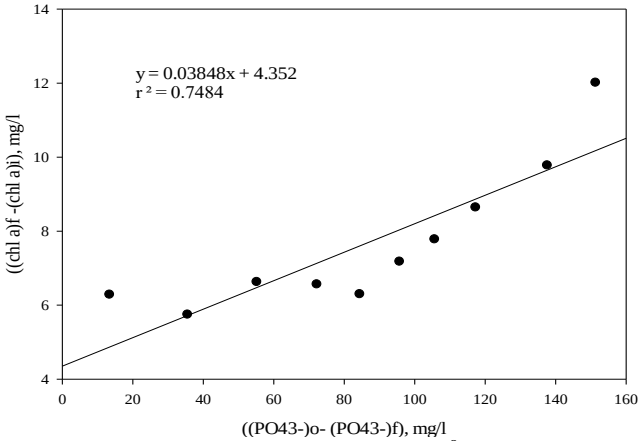

Fig. 6: Yield coefficient for orthophosphate (PO_4^{3-}) removal by *Botryococcus sp.* established

From Fig. 5 it was determined that yield coefficient for NH_4-N removal, Y_N = 0.027 mg chl *a*/mg NH_4-N. The correlation for difference between final and initial chl *a* $((\text{chl } a)_f - (\text{chl } a)_i)$ and difference between final and initial ammonia nitrogen $((NH_4\text{-}N)_0 - (NH_4\text{-}N)_f)$ concentration from the regression line is R^2=0.58 which means a positive linear relationship. The same method used to determine yield coefficient for PO_4^{3-} removal from Fig. 6 re-

sulted in Y_P = 0.038 mg chl *a*/mg PO_4^{3-}. These yield coefficient values are in accordant with study by Aslan *et al*. (2006), where yield coefficient for NH_4-N was Y_N = 0.15mg chl *a*/mg NH_4-N and Y_P = 0.14mg chl *a*/mg PO_4-P for phosphorus.

4. Conclusion

The high reaction rate constant, k and half saturated constant, Km values reveal that complying to the conditions of this research with excessive nutrient concentrations, competitiveness of *Botryococcus sp.* is lower as the existence of sufficient nutrients. Hence, these values indicate that *Botryococcus sp.* can effectively uptake nutrients at high concentrations. And this evinces that *Botryococcus sp.* can be used for phycoremediation at larger scale. The result reveals that Y_P is higher than Y_N. Therefore, this result signifies that orthophosphate produce more chl *a* from *Botryococcus sp*. compared to ammonia nitrogen during phycoremediation process in this study.

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