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Nutrient removal from artificial bathroom greywater by phycoremediation using *Botryococcus* sp.

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ABSTRACT

Bathroom greywater represent the major portion of the total greywater production from the household activity. These wastes should be subjected to a treatment process before being discharged directly into the environment to avoid the occurrence of the eutrophication phenomenon. Hence, the current work aimed to investigate the potential of phycoremediation process for removing nutrients from artificial bathroom greywater (ABGW) by *Botryococcus* sp. The phycoremediation process was conducted for 30 d, while the microalgae cell growth in ABGW was measured daily. The biokinetic absorption using Michaelis–Menten were determined for NO_3–N and PO_4–P. The results revealed that the maximum microalgae cell growth was recorded on the 10th day with 2.0×10^6 cells/mL. The highest removal of NO_3–N (97%) and PO_4–P (87%) were achieved on the 30th day of phycoremediation. The biokinetic absorption rate using Michaelis–Menten coefficient were K = 0.265 mg NO_3–N mg/Chl-a/d and K_m = 2.38 mg/L, while for PO_4–P were K = 1.057 mg PO_4–P mg/Chl-a/d and K_m = 12.04 mg/L. In conclusion, phycoremediation using *Botryococcus* sp. exhibited a high potential for the nutrients removal from ABGW.

Keywords: Nitrate-N; Phosphate-P; Biokinetics; Absorption; Personal care products

1. Introduction

Bathroom greywater is a type of wastewater derived from the hand basin, shower, bath, and laundry water and represent 30% of household greywater flow [1]. These wastes are rich with the nutrients which lead to cause eutrophication phenomenon in the natural water system after the discharge process. The nutrients in the greywater are originated from the chemical substances used during the bathing. Besides, some references indicated that the nutrients also originate from the urine passed during the showers [2]. Therefore, the removal of nutrients and organic contents is an essential step to avert eutrophication and water bloom [3].

Numerous systems operated for the removal of nutrients from greywater and other pollutions. However, these techniques are expensive and generate elevated thick soft mud [4–6]. Natural treatment systems via primary settling with cascaded water flow, aeration, agitation, and filtration [7,8] are less expensive. Yet, there is a lack of information when it concerns the removal of nutrients especially phycoremediation with microalgae *Botryococcus* sp.

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Greywater management treatment system is a system that allows direct utilization of the water. It can use natural gravity by a hybrid treatment process with the use of natural materials and wetland system. It will facilitate in breaking down the organic compounds and recovery of nutrients [9].

Among several treatment processes for removing nutrients, microalgae through the phycoremediation process is the most potent due to their capacity to assimilate nutrients. Phycoremediation is an attractive method because of their photosynthetic capabilities, changing solar energy into handy biomasses and embracing nutrients such as nitrogen and phosphorus which inflicts eutrophication [9]. Phycoremediation process is a green technique used for removing nutrients from different wastewater as a function of microalgae such as *Botryococcus* sp. Due to the high efficiency of microalgae to assimilate nutrients. Domestic greywater is among different wastewater which is more suitable medium for algal growth due to high content of carbon, nitrogen, and phosphorus as well as trace elements necessary for their growth. Nitrogen and phosphorus are among the most important nutrients in the greywater and are presented in concentrations ranged between 20 and 40 mg/L for total nitrogen and 50 and 70 mg/L for total phosphorus (TP) which lie in the appropriate range for microalgae growth. The source of phosphorus in bathroom greywater is the soap and detergent content used by house occupants [7,9]. The phycoremediation with microalgae is a really acceptable method because of their photosynthetic capabilities, changing solar energy into biomasses yields, and embracing phosphorus and nitrogen contents which inflicts eutrophication [9].

In the current work, the removal of nutrients from artificial bathroom greywater (ABGW) by phycoremediation using *Botryococcus* sp. was investigated. Moreover, the specific removal rate of the nutrients by *Botryococcus* sp. was studied using Michaelis–Menten kinetic model.

2. Materials and methods

The ABGW with commonly used in the present study was prepared according to Wurochekke (Table 1) [2]. *Botryococcus* sp. was selected in this study based on the previous studies which exhibited high efficiency for removing nutrients from meat processing wastewater [10]. The microalgae was sub-cultured in bolts basal medium (BBM) as described by Bischoff and Bold [11]. The medium was incubated at room temperature (25°C–29°C) for 7 d (12 h L:12 h D period with 18–20 MJ/m^2/d of solar irradiance). The microalgae cell biomass was recovered from the culture medium using a centrifuge (4,020 rcf) washed twice using sterilized deionized water to remove the residues and then suspended in 10 mL of sterilized normal saline. The concentrations of microalgae cells were determined using hemocytometer according to APHA [12] and expressed as cell/mL.

2.1. Phycoremediation of ABGW

In order to investigate the efficiency of *Botryococcus* sp. in removing nutrients from ABGW samples, the phycoremediation of ABGW was conducted in Erlenmeyer flasks (1 L). The ABGW were diluted with distilled water by 20%, 40%, 60%, 80%, and 100% and then each dilution was inoculated with 10^6 cells/mL of *Botryococcus* sp. cells (each experiment was conducted in triplicate at a different time). The ABGW sample (without microalgae inoculation) was used as control. The inoculated ABGW samples were covered with sterile cotton plugs immediately after the inoculation and placed under the direction of sunlight (12 h light and 12 h dark) at the ambient temperature for 30 d.

Table 1
Artificial bathroom graywater (ABGW) component

Parameter	Concentrations (mg/L)
Soap	0.13
Detergent	0.97
Shower gel	0.34
Toothpaste	0.37
Shampoo	0.88
pH	6.55

The concentrations of total nitrogen (TN) and total phosphorus (TP) were carried out for each sample at the periods of 0, 1, 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30 d. A fixed volume (100 mL) from each dilution was transferred from the ABGW and used for determining the final concentrations of TN and TP according to APHA [13]. The efficiency of *Botryococcus* sp. in removing TN and TP from ABGW was calculated according to Eq. (1).

$$R\% = \frac{\left(C_0 - C_i\right)}{C_0} \times 100\% \tag{1}$$

where C_0 represents nutrients concentration at the beginning experiment while C_i represents the nutrients concentration on the day which reading is taken during the phycoremediation process.

2.2. Biokinetic absorption rate of microalgae Botryococcus sp.

In this study, ABGW inoculated with microalgae *Botryococcus* sp. was used in determining biokinetic coefficients, K_m saturation constant and reaction rate constant K using Michaelis–Menten kinetic relationship in Eq. (2) [14]. From the experimental data a plot of $\frac{1}{R_{xi}}$ against $\frac{1}{S_0}$ yields a slope and intercept on the y-axis and the biokinetic coefficients were calculated:

$$\frac{1}{R_{xi}} = \frac{1}{K} + \frac{K_m}{K}\frac{1}{S_0} \tag{2}$$

where K is the reaction rate, R_{xi} is a specific rate of substrate removal, and S is initial substrate concentration.

The Chlorophyll-a (Chl-a) was determined using spectrophotometer (HACH DR 6000, United States) at a wavelength of 664, and 647 according to Jeffrey and Humphrey [15] using the following formula:

$$\text{Chl-a}\left(\frac{\text{mg}}{\text{L}}\right) = 11.93\ \ E664 - 1.93\ E647 \tag{3}$$

where *E*664 = value of absorbance at wavelength 664 nm; *E*647 = value of absorbance at wavelength 647 nm.

The N and P removal rate was presented as mg of the nutrient/mg Chl-a/d was used to represent only the live cells, since the counting of microalgae was conducted by the light microscope which cannot detect the live and dead cells.

3. Results and discussion

3.1. Nutrient (nitrate-N and phosphate-P) removal by Botryococcus sp. from ABGW

The phycoremediation of ABGW using *Botryococcus* sp. with the different initial of nitrate-N (NO_3–N) concentrations against time for 30 d of batch operation is shown in Figs. 1a and b. The NO_3–N was removed by 96% and 97% from the ABGW at 2.3 and 1.2 mg/L of initial concentrations in 40% and 20% of ABGW dilution. Similarly, NO_3–N the removal efficiency was 95% at 4.65 and 3.32 mg/L in 80% and 60% of ABGW dilution respectively. However, the removal efficiency further decreased to 78% at 4.99 mg/L in 100% ABGW dilution.

The final phosphate-P concentration was 1.22 mg/L with 87% of the removal efficiency at 9.5 mg/L of the initial concentrations in 20% ABGW. In the 100% ABGW, the removal efficiency dropped to 73% (Figs. 2a and b). The results revealed that the efficiency of *Botryococcus* sp. for removing nitrate-N was more than for removing phosphate-P.

The Chl-a content increased (from 0.8 to 6.1 mg/L) significantly ($P < 0.05$) with the increasing of NO_3–N concentrations and associated with *Botryococcus* sp. growth (Fig. 3). This shows the sign of *Botryococcus* sp. growth in ABGW. The increasing Chl-a in ABGW indicated for the efficiency of phycoremediation process in removing nutrients [16].

These results are consistent with the previous studies, Valderrama et al. [17] revealed that *Chlorella vulgaris* removed 72% of TN and 28% of TP from the diluted ethanol and citric acid production effluent, while removed 80% from the high rate algal ponds (HRAP) after 7 d [18]. The researchers in the literature indicated that the microalgae have less affinity to remove phosphate-P in comparison to nitrate-N. Lee and Lee [19] found that *Chlorella kessleri* removed between 8% and 20% of phosphorus. However, the experimental data obtained in this study revealed that phosphate-P was better removed for 80% ABGW (dropped from 13.5 to 3.19 mg/L in 30 d). Aslan and Kapdan [14] recorded 78% of phosphate-P removal from synthetic wastewater.

3.2. Biokinetic coefficients, nitrate-N, and phosphate-P specific removal rate and yield coefficients from ABGW

The specific removal rate of NO_3–N with varying NO_3–N concentrations is depicted in Fig. 4. The removal

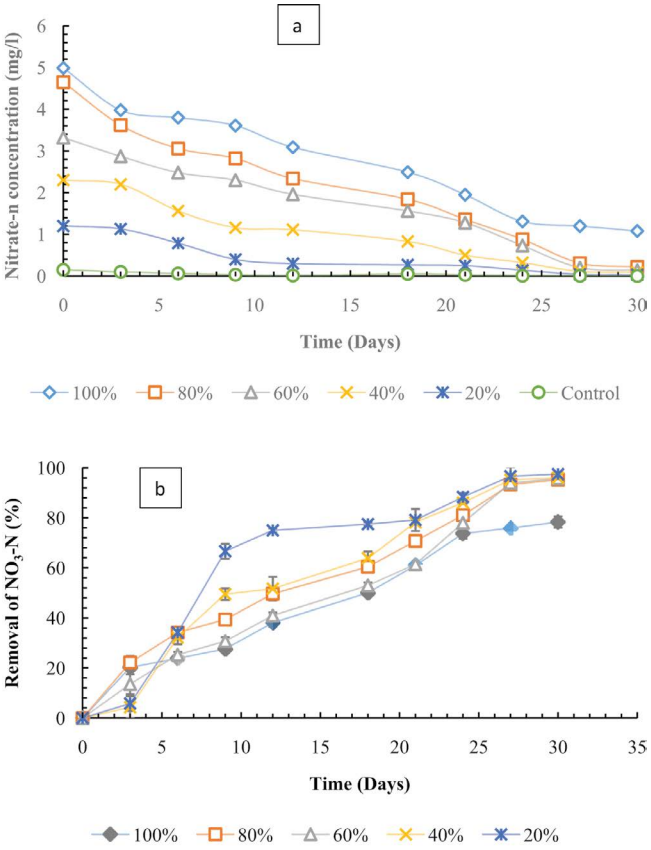

Fig. 1. (a) Nitrate-N final concentration and (b) removal percentage of nitrate-N from ABGW.

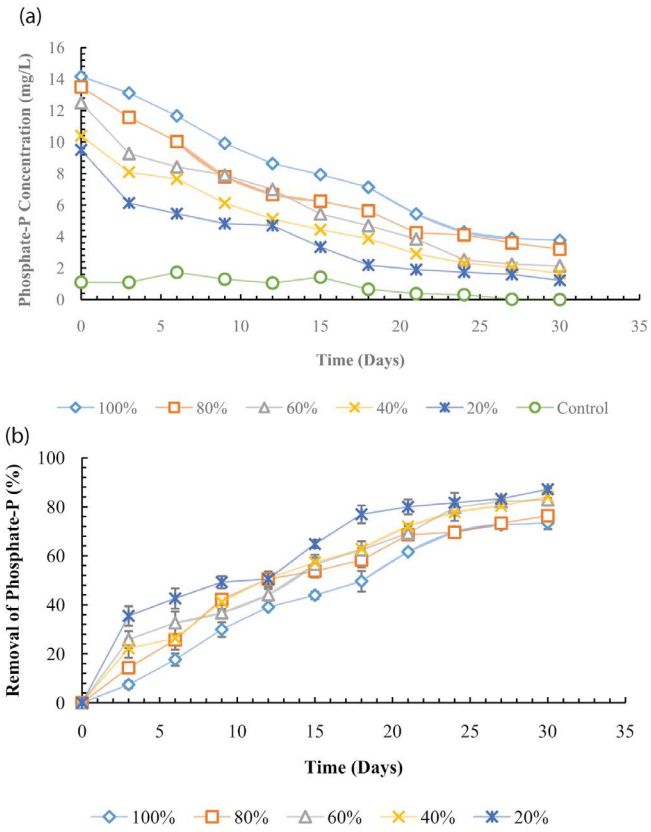

Fig. 2. (a) Final concentrations of phosphate-P in ABGW and (b) removal percentage.

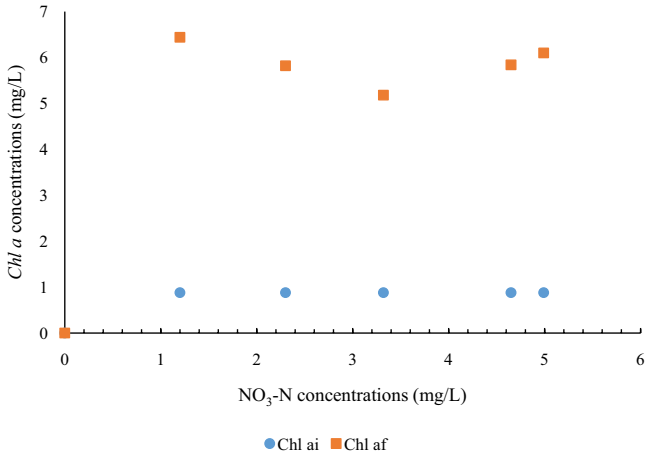

Fig. 3. Variation of Chl-a content with initial nitrate-N concentration.

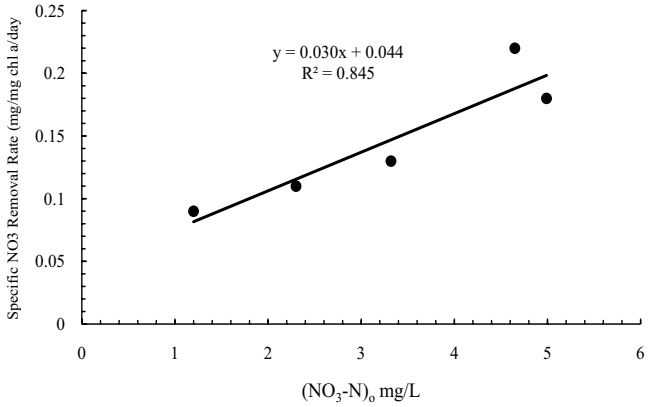

Fig. 4. Effect of initial NO_3–N on specific NO_3–N removal rate.

rate increased by increasing in the initial NO_3–N concentrations. The maximum rate reached was 0.22 mg/mg Chl-a/d in the concentration from 1.2 to 5 mg/L. The specific PO_4–P removal rate increased from 0.02 mg/mg Chl-a/d to around 0.64 mg/mg Chl-a/d for the PO_4–P concentrations between 9.5 and 14.16 mg/L (Fig. 5). The substrate (nutrient) removal rates were reported in previous studies instead of specific removal rates.

The maximum values attained in Aslan and Kapdan [14] study were 3.0 mg/mg Chl-a/L/d and 0.52 mg/mg Chl-a/d for NH_4–N and PO_4–P using *C. vulgaris*. In another study, the nitrogen and phosphorus removal rates by *C. vulgaris* were 5.44 and 1.30 mg/L d, respectively [20]. Wong and Tam [21] reported that the removal rates of *Chlorella pyrenoidosa* as 3.4 mg N/L d and 10.7 mg P/L d. However, significantly higher removal rates of 20.83 mg P/L d and 83 mg N/L d for suspended growth culture of *Scenedesmus intermedius* and 10.15 mg P/L d and 56.06 mg N/L d for *Nannochloris* sp. were reported by Martínez et al. [22]. However, the disparity in the result of this study and previous studies could be due to differences in wastewater strain, there is high pollution in wastewater compared to greywater as well as different microalgae species used.

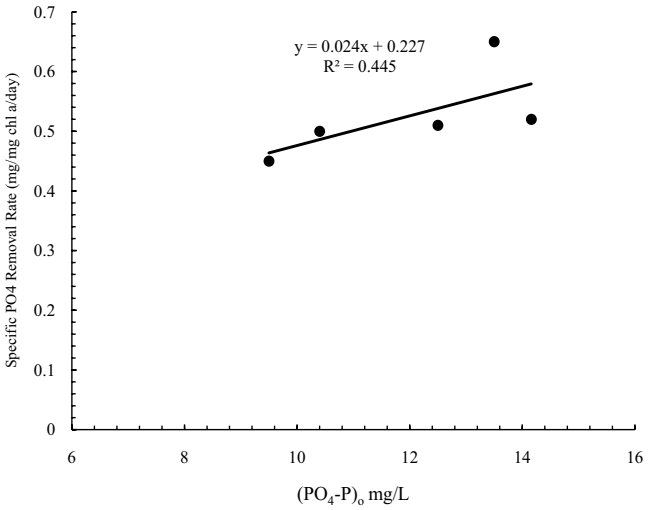

Fig. 5. Effects of initial PO_4–P on specific PO_4–P removal rate.

From the linear lineweaver–Burke plot transformed in Eq. (2), the plot between $1/R_{xi}$ against $\frac{1}{NO-N}$ and $\frac{1}{OP_4-P}$ is shown in Figs. 6 and 7. The equations of the straight line for the slope and intercept $\left(\frac{K_m}{K} \text{ and } \frac{1}{K}\right)$ were determined. The kinetic coefficients for NO_3–N removal in ABGW and uptake (Fig. 6) by *Botryococcus* sp. were determined as a reaction rate constant (K) of 0.256 mg NO_3–N mg/Chl-a/d and a saturated constant (K_m) of 2.38 mg/L (R^2 = 0.83). Similarly, experimental data from PO_4–P removal rate (Fig. 7) from the best fit regression line were determined as a reaction rate constant (K) of 1.057 mg PO_4–P mg/Chl-a/d and saturation constant (K_m) of 12.04 mg/L from the intercept and the slope (R^2 = 0.94). Therefore, the NO_3–N and PO_4–P substrate removal rates in this study were in line with those reported by Aslan and Kapdan [16]. Thus, the low value of K_m in NO_3–N over PO_4–P means the enzyme has a high affinity with the substrate. Hence, if K_m is small, enzymes will reach the maximum catalytic efficiency at low substrate levels. This means optimum nutrient removal or uptake by *Botryococcus* sp. occurs in low strain greywater.

For all NO_3–N and PO_4–P removal, the plot of Chl-a produced against substrate depleted is shown in Figs. 8 and 9. The slope gives a yield coefficient for NO_3–N (Y_N) and PO_4–P (Y_P). The calculated Y_N was 0.36 mg Chl-a/mg NO_3–N (R^2 = 0.8358) and the Y_P was 1.18 mg Chl-a/mg PO_4–P (R^2 = 0.0954). The coefficient (R^2) is located between 80% and 90% not very close to 100, might be due to the distribution of the raw data.

The kinetics of the nutrient uptake model is different from other models that are basically on an exponential growth phase. A kinetic nutrient uptake model is derived using the same data, but focuses on nutrient uptake rates. The kinetic coefficients K and K_m in this study were calculated from the overall uptake measurement that reflects the substrate carrier ability of *Botryococcus* sp. Thus, it demonstrated that NO_3–N and PO_4–P uptake by *Botryococcus* sp. can be described using Michaelis–Menten kinetics [14].

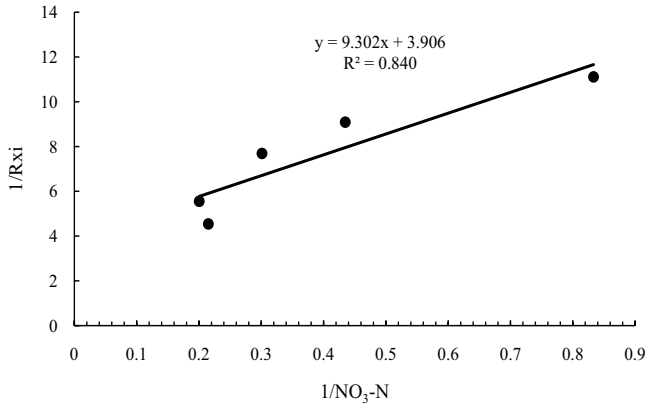

Fig. 6. Determination of kinetic coefficients, K_m and K for NO_3–N removal.

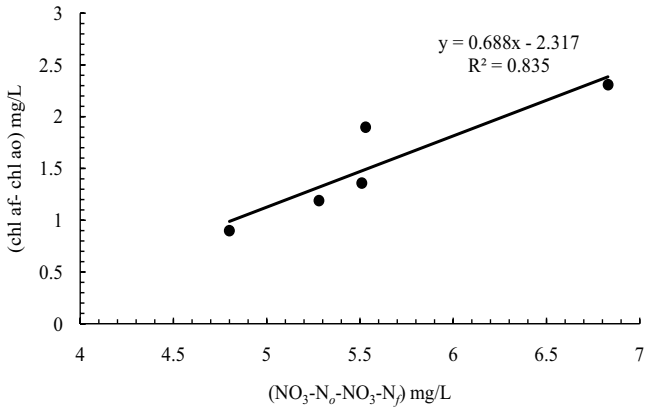

Fig. 8. Determination of yield coefficient for NO_3–N uptake by *Botryococcus* sp.

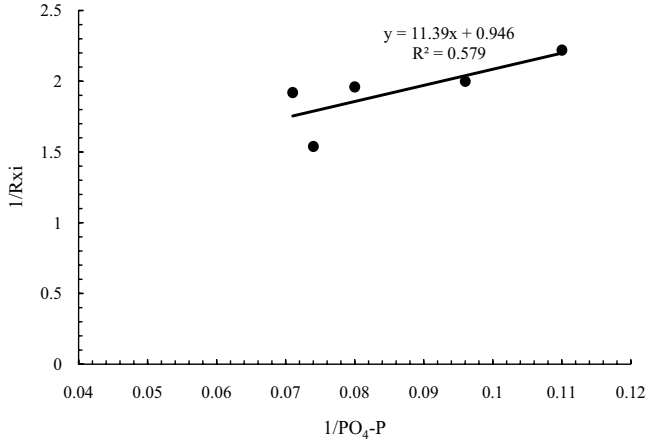

Fig. 7. Determination of kinetic coefficients, K_m and K for PO_4–P removal.

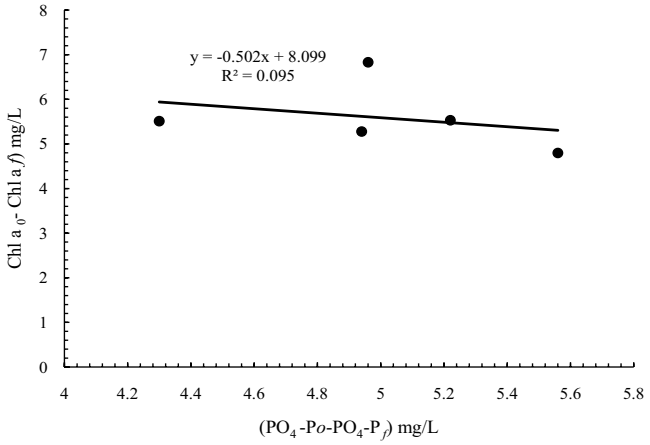

Fig. 9. Determination of yield coefficient for PO_4–P uptake by *Botryococcus* sp.

Furthermore, Michaelis–Menten kinetics was used to describe nitrate in higher plants like rice [22].

The present study established an uptake relationship with NO_3–N, the reaction rate constant K of 0.46 mg/Chl-a/d and a saturation constant or maximum uptake rate or Michaelis–Menten constant K_m of 5.75 mg/L. The uptake rates of PO_4–P are K of 8.53 mg/Chl-a/d and K_m of 176.88 mg/L. It is paramount to note that this model demonstrated a nutrient limited uptake rate and not a nutrient limited growth rate. In addition, Aslan and Kapdan [14] reported a K of 1.5 mg NH_4–N mg Chl-a/d, K_m of 31.5 mg/L and K of 0.5 mg PO_4–P mg Chl-a/d and K_m of 10.5 mg/L. These reported results are different from the results of this study due to substrate difference as ABGW was used in this study. Fiksen et al. [23] stated that a higher K_m value is associated with an increase in competitive ability for nutrient concentration. The microalgae *Botryococcus* sp. need to adapt to the composition of bathroom greywater during the phycoremediation process. Thus, the competitive ability of *Botryococcus* sp. in ABGW might be lower during the early stages of inoculation but will later increase at an exponential rate thereby increasing the uptake of nutrients. This exponential growth is due to the proliferation of the microalgae cells and its size. In this case, microalgae cell size is associated with an increase in K_m value [24]. This would create more uptake sites thereby increasing the rate of nutrient uptake. From this study, the K_m values were relatively high for both NO_3–N and PO_4–P possibly due to *Botryococcus* sp.'s competitive ability with respect to cell size. This implies that *Botryococcus* sp. are better competitors with a good uptake rate (K). Therefore, the model developed from this study is a representation of complex interacting components. The selection of algal species, interspecies differentiation, bioreactor size, type, and media composition of greywater, light source, temperature, pH, and CO_2 concentration will all have an effect on the maximum uptake rate and reaction rate constant developed for nitrate-N and phosphates-P above. Hence, if Chl-a and substrate concentrations were measured, the calculation steps outlined in the results can be easily applied to a larger scale.

4. Conclusion

The biokinetic coefficients of NO_3–N absorption from experimental data were determined as K = 0.256 mg NO_3–N mg/Chl-a/d and K_m = 2.38 mg/L (R^2 = 0.83) and

PO_4–P coefficients were K = 1.057 mg PO_4–P mg/Chl-a/d and K_m = 12.04 mg/L (R^2 = 0.94). According to these results, it proves the potentiality of microalgae *Botryococcus* sp. as a successful technique to eliminate nutrients in bathroom greywater. Moreover, in order to explain the efficiency of *Botryococcus* sp. in removing the nutrients the removal mechanism should be investigated.

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