Nutrients removal from artificial bathroom greywater using *Botryococcus* sp. strain

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Abstract. The discharge of untreated bathroom greywater directly into drain is a most common practice in the rural area. The uncontrolled discharge of greywater from the village houses escalates the pollution among Malaysian river and provide insanitary environment through mosquito and flies breeding grounds. Therefore, the current work aimed to investigate the potential of *Botryococcus* sp. for removing total nitrogen (TN), total phosphorus (TP) and total organic carbon (TOC) from artificial bathroom greywater and to determine the bio-kinetic removal rate for these parameters. The artificial bathroom greywater was prepared by using regular brands used in the community, the bathroom greywater quality was tested for BOD, COD, SS, pH, and Turbidity. The removal process was conducted in the lab scale with 108 cell mL^{-1} of *Botryococcus* sp. The removal of TN, TP and TOC was measured in interval of 3, 5 and 7 days. The results deduced that *Botryococcus* sp. removed 51.5% of TN, 49.5% of TP and 42.6% of TOC. Moreover, the bio-kinetic model studies, revealed that the specific removal rate of TN, TP and TOC have a significant relationship with initial concentration in the artificial greywater (R^2= 0.63, 0.95 and 0.95 respectively). The kinetic coefficient of greywater parameters removed by *Botryococcus* sp. was determined as k=0.357 mg TN 1 $\log_{10}$ cell mL^{-1} d^{-1} and k_m=31.33 mg L^{-1} (R^2=0.73), k=4.58 mg TP 1 $\log_{10}$ cell mL^{-1} d^{-1} and k_m=283.86 mg L^{-1} (R^2=0.95), k=7.9 mg TOC 1 $\log_{10}$ cell mL^{-1} d^{-1} and k_m=322.32 mg L^{-1} (R^2=0.97). The bio- kinetic model indicated that more than 90% of TN, TP and TOC was taken place as a response for *Botryococcus* sp.

1. Introduction

It is truisms nowadays to recognize that pollution associated problems are a major concern of society. Environmental laws are given general applicability and their enforcement has been increasingly stricter. So, in terms of health, environment and economy, the fight against pollution has become a major issue [1]. There are still a number of cases whereby greywater is discharged directly into water ways, often without treatment. Greywater is the main 'product' of pollutants produce by society daily from residential area. Greywater is defined as wastewater without any input from toilets, which means that is corresponds to the wastewater produced in bathtubs, shower, hand basins and kitchen sinks, in households, office building, school and others [2]. The combination greywater from bathroom, shower and hand basin for the average family accounts for about 26% of total household consumption. Greywater from shower and hand basins normally contains soaps, shampoos. Microalgae have been

petroposed as an alternative biological treatment to remove nutrient [3]. It has faster growth rate and more than 50 times higher photosynthetic efficiency compared terrestrial plants [4]. Several authors have reported that use of microalgae for the removal of main wastewater pollutants such as nutrients [5,6].

The present study aimed to investigate the potential of *Botryococcus* sp. for removing total nitrogen (TN), total phosphorus (TP) and total organic carbon (TOC) from artificial bathroom greywater and to determine the bio-kinetic removal rate for these parameters

2. Materials and Methods

2.1. Preparation of artificial greywater

The synthetic greywater formulations were developed as described by Toifil *et al* (2006), it was prepared by mixing 25 g of hair shampoo (Sunsilk), 30 g of shower gel (Lifebouy), 16 g of toothpaste (Colgate), 20 g of soap (Palmolive) and 15 g of washing powder (K1000). The wastewater characteristics which including Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS), pH, and Turbidity were determined according to APHA [7].

2.2. Experimental design

Botryococcus sp. was prepared as described in previous work [8], with concentrations of 108cell mL^{-1} (8 $\log_{10}$ cell mL^{-1}). It was inoculated into 250 mL of the artificial greywater at the beginning of the experimental periods and the phycoremediation process was conducted to 7 days at room temperature. The concentration of TN, TP and TOC were determined before and after the phycoremediation process for 3, 5 and 7 days according to APHA [7]. The removal percentage of TN, TP and TOC was calculated according to equation 1.

$$\text{Removal percentage} = \frac{C_i - C_f}{C_i} \times 100 \qquad (1)$$

2.3. Determination of removal rate of TN, TP and TOC and batch kinetic coefficient of Botryococcus sp.

The coefficient removal rate of TN, TP and TOC from greywater by *Botryococcus* sp. was investigated in batch reactor inoculated with 8 $\log_{10}$ cell mL^{-1} of *Botryococcus* sp. The coefficient removal rate of TN, TP and TOC was calculated as described by Aslan and Kapdan [9].

3. Results and discussions

3.1. Artificial greywater characteristics

The characteristics of artificial bathroom greywater is illustrated in table 1. It can be noted that the concentrations of COD is 247 mg L^{-1} while was 156 mg L^{-1} for BOD. In comparison with previous studies, the value of COD was between 445 - 621 mg L^{-1} and 40 -105mg L^{-1} for BOD [10]. Jefferson [11] revealed that COD was 367 mg L^{-1} and BOD was 129 mg L^{-1}.

Table 1. The characteristic of artificial bathroom greywater as compared with the researcher.

Parameter	Artificial Bathroom Greywater (this study)	Bathroom Greywater Mohamed *et al* [10]	Bathroom Greywater Jefferson [11]
pH	6.5 – 7.52	6.1 ± 0.06 – 6.5 ± 0.21	7.6
BOD_5	156 ± 7.21	40 ± 0.25 - 105 ± 0.42	129
COD	247 ± 8.37	445 ± 2.52 - 621 ± 4.02	367
Turbidity	612 ± 9.64	NA	59.8
Suspended Solid (SS)	429 ± 7.32	78±4.44 - 163±7.12	NA
Total Nitrogen (TN)	23.6 ± 0.41	10 ± 2.90 - 38 ± 0.56	NA
Total Phosphorus (TP)	16.03 ± 0.02	3 ± 0.87 - 20 ± 1.76	NA
TOC	275.9	NA	NA

All parameters are expressed in mg L^{-1} except for pH and Turbidity (NTU); NA (non-available)

The range of pH for bathroom greywater for this study was in the range of 6.5 to 7.52, while pH in the study conducted by Jefferson [11] and Mohamed *et al* [10] was 7.6 and 6.1 – 6.5 respectively. The SS for this study was 429 mg L^{-1}. The high concentration sources are normally related to either clothes washing operations such as washing machine or hand washing [2]. The value of turbidity was higher (612 NTU) than Jefferson, (2004) with 59.8 NTU. The result for TN and TP in this study was 23.6 mg L^{-1} and 16.03 mg L^{-1}, respectively. These results are within the range reported by Mohamed *et al* [10], where TN and TP was 10 – 38 mg L^{-1} and 3 – 20 mg L^{-1}, respectively. It has indicated that the use of regular personal cleaners resulted in a high Phosphorus load in bathroom greywater [10].

3.2. Removal efficiency of nutrients by Botryococcus sp.

The removal efficiency of nutrients using *Botryococcus* sp. is depicted in figure 1. The percentage removal of TN was 38.6%, 41.4% and 51.4% after 3, 5 and 7 days, respectively, while was 15.3, 29.9% and 49.5% for TP after 3, 5 and 7 days respectively. In comparison the percentage removals of TOC were 35.6%, 28.9% and 42.6% after 3, 5 and 7 days. These findings showed that the maximum removal was recorded after 7 days.

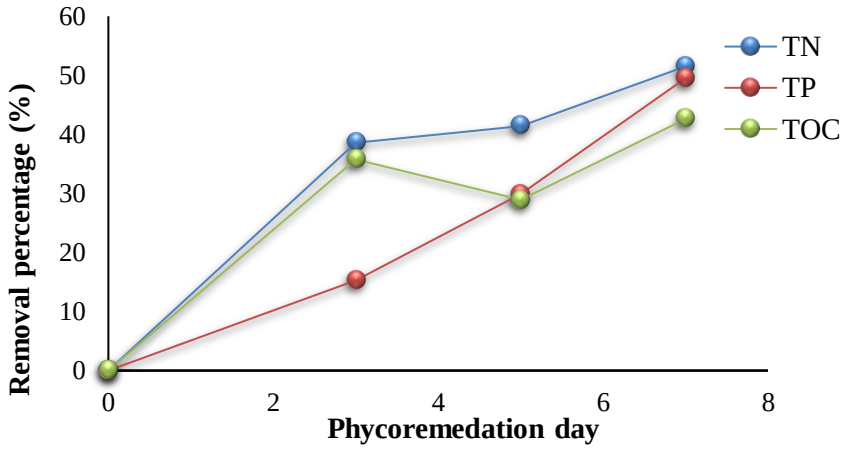

Figure 1. Removal of TN, TP and TOC from Artificial Bathroom Greywater by *Botryococcus* sp

Phosphorus can be eliminated through both biotic phosphorus assimilations into the biomass and abiotic phosphorus precipitation [12]. Jing *et al* [13] demonstrated that about 90% of the phosphate was removed using *C. vulgaris* and *Scenedesmus rubescens*. The removal of total phosphorus was a slower

process compare to that observed for nitrogen. This is due to the fact that nitrogen was the limiting nutrients, not phosphorus [14,15].

3.2.1. Removal rate of TN, TP and TOC and batch kinetic coefficient of Botryococcus sp. The specific removal rate of TN, TP and TOC from artificial greywater samples by *Botryococcus* sp. is presented in figure 2.

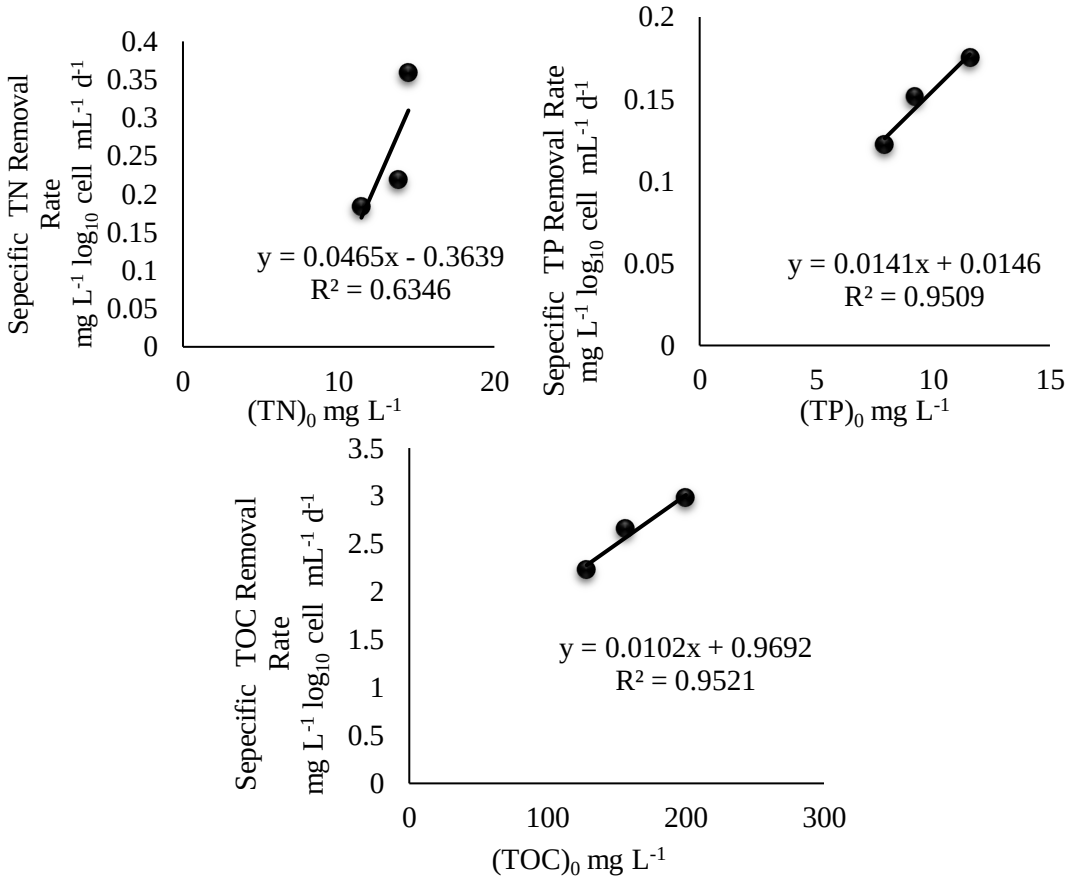

Figure 2. Effect of initial concentration of TN, TP and TOC on the specific removal by *Botryococcus* sp. with initial concentrations of 8 $\log 1_0$ cell mL^{-1}.

The results revealed that the removal of TN associated with their initial concentrations in the greywater samples (R^2=0.83). The maximum removal rate was recorded at 14.48 mg L^{-1}, where 0.35 mg TN 1 $\log_{10}$ cell mL^{-1} d^{-1} were removed. The removal rate of TP exhibited strong relationship with *Botryococcus* sp. growth rate (R^2 = 0.95). The maximum removal was 0.17 mg TP 1 $\log_{10}$ cell mL^{-1} d^{-1} at 11 mg L^{-1}. Similar findings were recorded for TOC, where the removal rate exhibited strong relationship with *Botryococcus* sp. growth rate (R^2 = 0.95). The maximum removal was 3 mg TOC 1 $\log_{10}$ cell mL^{-1} d^{-1} at 200 mg L^{-1}. These findings are similar to that reported by Aslan & Kapdan [9] as well as by Lau *et al* [16], in which the specific removal of nitrogen from synthetic wastewater samples by *C. vulgaris* was more than that for PO_4^{3-}. Jimenez-Perez *et al* [17] revealed that *Scenedesmus* sp. achieved more removal for nitrogen than that for phosphorus. These findings are explained based on the biosorptive capacity (Q_{max}) of the *Botryococcus* sp. cells which represent the amount of pollutant removed by microalgae cells and increase with the increasing of substrate concentrations as reported in previous work by Mohamed *et al* [18].

The slop of specific growth rate of *Botryococcus* sp. versus the remaining concentrations of TN, TP and TOC are presented in figure 3, which indicated that the growth rate has correlated with the removal

of TN (R^2= 0.89), TP (R^2=0.98) and TOC (R^2=0.99). The values of R^2 in specific growth rate of *Botryococcus* sp. was more than that presented with the specific removal rate of TN, TP and TOC. Roleda *et al* [19] who claimed that the microalgae cells have the potential to sustain metabolic activity even without cell division. Moreover, the present results confirmed that the presence of nutrients and organic matter in the greywater induced the growth rate of *Botryococcus* spp. and thus reflect the potential of phycoremediation process in the treatment of greywater.

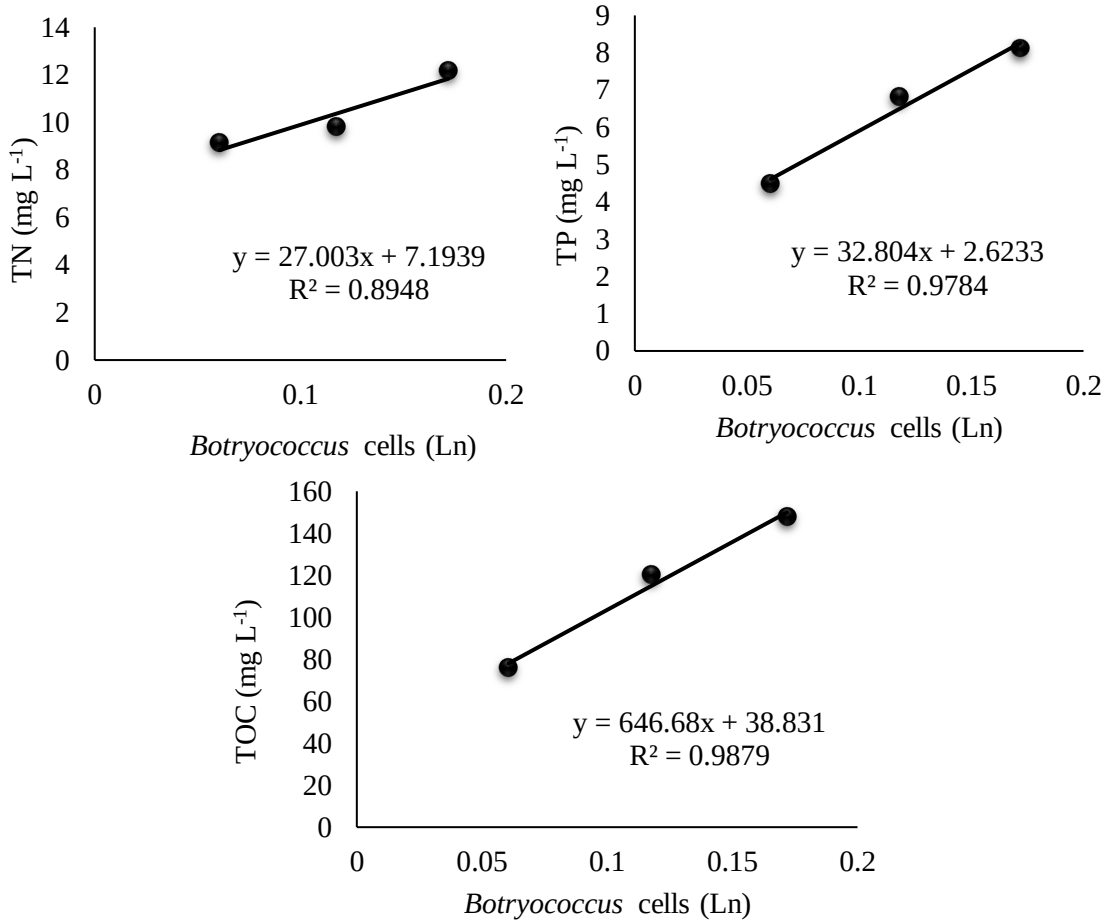

Figure 3. Determination of coefficient relationship between TN, TP and TOC concentrations and *Botryococcus* sp. specific growth rate

The kinetic coefficient of NH_{4-} removal by *Botryococcus* sp. was determined as k=0.357 mg TN 1 log_{10} cell mL^{-1} d^{-1} and k_m=31.33 mg L^{-1} (R^2=0.73), k=4.58 mg TP 1 log_{10} cell mL^{-1} d^{-1} and k_m=283.86 mg L^{-1} (R^2=0.95), k=7.9 mg TOC 1 log_{10} cell mL^{-1} d^{-1} and k_m=322.32 mg L^{-1} (R^2=0.97) (figure 4). The low values of k_m in the kinetic coefficient of TN in comparison to that of TP indicated that *Botryococcus* sp. exhibited high efficiency for TN removal more than TP.

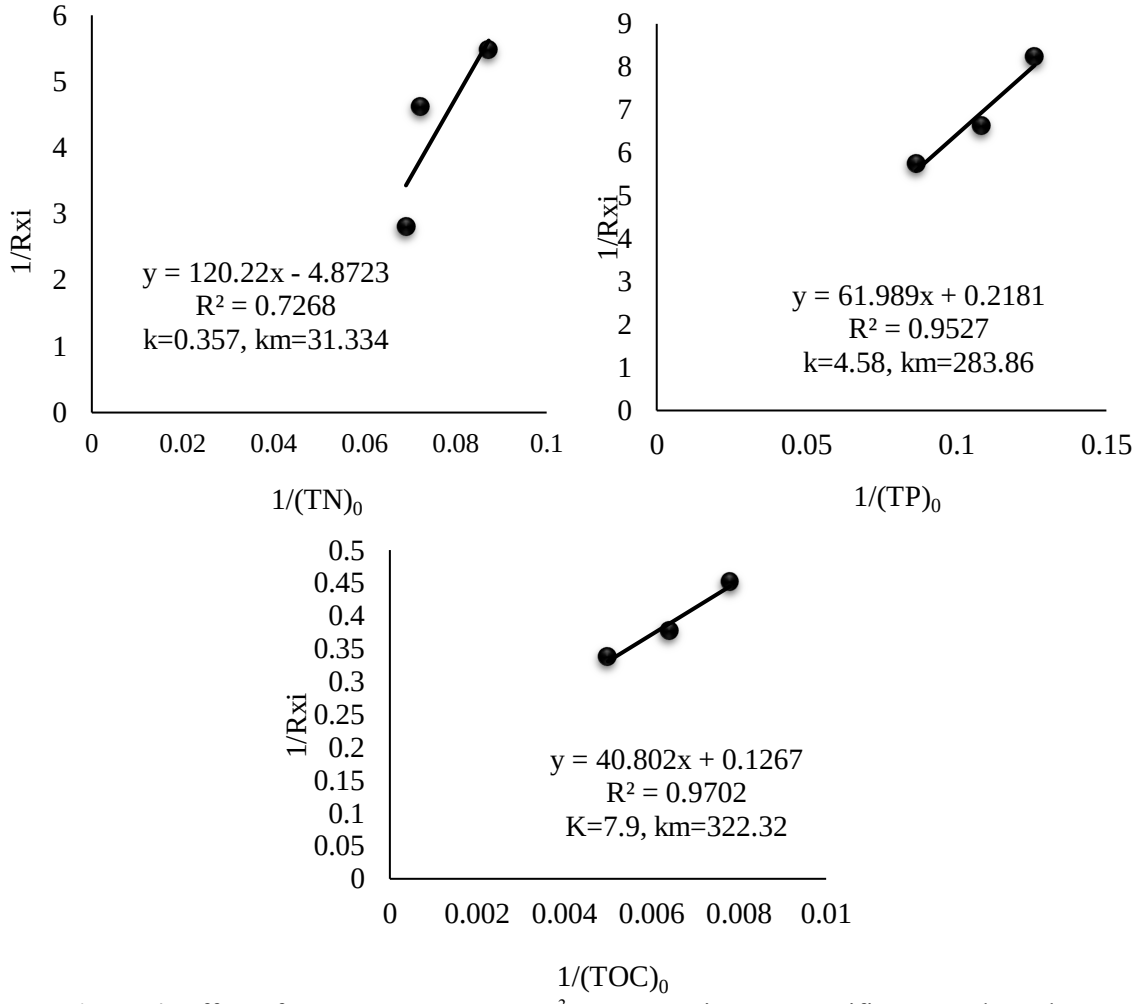

Figure 4. Effect of COD, BOD, NO_3, PO_4^{3-} concentrations on specific removal rate by *Botryococcus* sp

4. Conclusions

The application of *Botryococcus* sp. microalgae in treating artificial bathroom greywater has been studied through their potential to remove nutrients. It is concluded that *Botryococcus* sp. has a good potential for nutrient removal, the maximum removal percentage was 51.5%, 49.5% and 42.6% for TN, TP and TOC, respectively after 7 days of phycoremediation process.

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