Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2021.27087

222 (2021) 336–345
May

Efficiency of *Botryococcus* sp. in photobioreactor treatment system for nutrient removal from greywater

Anwaruddin Wurochekke[a,b], Radin Maya Saphira Radin Mohamed[c,*], Adel Al-Gheethi[c,*], Nurina Fitriani[d]

[a]*Jos Electricity Distribution Plc Address: 9 Ahmadu Bello Way Jos, Plateau State, Nigeria*
[b]*Micro-pollutant Research Centre (MPRC), Faculty of Civil Engineering and Built Environment, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia*
[c]*Department of Civil Engineering, Faculty of Civil Engineering & Built Environment, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Johor, Malaysia, Tel. +0607 456 4236; Fax: +607 453 6588; email: maya@uthm.edu.my (R.M.S.R. Mohamed), Tel. +0607 456 4346; Fax: +607 453 6588; email: adel@uthm.edu.my/adelalghithi@gmail.com (A. Al-Gheethi)*
[d]*Biology Department, Faculty of Science and Technology, Universitas Airlangga, Kampus C Jl.Mulyorejo, Surabaya (60115), Indonesia*

Received 9 April 2020; Accepted 16 January 2021

ABSTRACT

Direct discharge of household bathroom greywater into drains is one of the main causes of eutrophication in natural water bodies. The current work aimed to study the removal of nutrients (ammonium, total Kjeldahl nitrogen, and orthophosphate [PO_4^{3+}]) from greywater (collected from four houses) by phycoremediation process using *Botryococcus* sp. in a photobioreactor. A laboratory-scale greywater treatment system was set up by using a photobioreactor tank with *Botryococcus* sp., and the treatment process was conducted at ambient temperature of 25°C–35°C for 21 d. The results reveal that greywater has pH between 6.1 and 8.27, biochemical oxygen demand (BOD_5) and chemical oxygen demand values in the range from 46 to 199 mg/L and from 76 to 438 mg/L respectively, and total suspended solids ranged from 29 to 245 mg/L. NO_3–N ranged from 1.03 to 7.54 mg/L and PO_4^{3+} ranged from 0.12 to 22.7 mg/L. The maximum growth of *Botryococcus* sp. with an initial inoculum of 10^5 cell/mL was between 6 to 8 d (1.96×10^6 cell/mL). Meanwhile, an initial inoculation of 10^6 cell/mL resulted in maximum growth after 7 d (2.89×10^7 cell/mL) in greywater collected from House A. The removal of ammonium by *Botryococcus* sp. reached 87% from greywater in House A after 21 d and 77% from greywater in House D. In contrast, the total Kjeldahl nitrogen removal was 99.7% and the removal of PO_4–P was 78.7% These results prove the efficiency of *Botryococcus* sp. in NO_3–N and PO_4–P removal from greywater. It can be concluded that the photobioreactor with *Botryococcus* sp. used in the present study exhibited an efficiency for removing the nutrients from bathroom greywater.

Keywords: Botryococcus sp.; Microalgae; Photobioreactor; Phycoremediation; Personal care products

1. Introduction

Conventional discharge of greywater into drains gains the least attention in terms of environmental sanitation. Bathroom greywater from individual village houses in many developing countries like Malaysia is often discharged untreated into storm water drains [1]. This discharge can cause unpleasant odours, become a breeding ground for mosquitoes and flies, disturb the aesthetics of the environment, and deposit nutrients (nitrogen [N] and phosphorus

* Corresponding authors.

[P]) in the drain. Bathroom greywater discharged without treatment to nearby drains in village areas may potentially increase eutrophication in water bodies caused by excess N and P contents [2]. Therefore, bathroom greywater ought to be properly treated before being discharged into water bodies.

Greywater is a type of wastewater from the kitchen, bathroom (i.e., discharge from hand basin, shower, and bath), and laundry water [3]. The bathroom contributes more than 50% of the total usable greywater volume in a typical household [4]. Besides that, greywater which originates from bathrooms and showers makes up over 30% of household greywater flow [5]. Water used for washing hands and showers generates about 50%–60% of the total greywater and is considered as the least polluted type of greywater compared to others. Common chemical pollutants include soap, shampoo, hair dye, toothpaste, and cleaning products, while biological pollutants include faecal bacteria.

Numerous systems operate to remove nutrients from greywater, although these are expensive and generate elevated thick, soft mud. Natural treatment systems via primary settling with cascaded water flow, aeration, agitation, and filtration are normally used and are less expensive [6,7]. Yet, there is a lack of information when it concerns the removal of nutrients, especially phycoremediation with microalgae *Botryococcus* sp. A greywater management treatment system is a system that allows direct utilization of the water. It uses natural gravity by a hybrid treatment process using natural materials and the wetland system. It facilitates the breakdown of organic compounds and the recovery of nutrients [7]. Bathroom greywater should preferably be treated anaerobically because of lower treatment costs and the possibility of recovering energy [8].

Technologies such as filters with sand, gravel, limestone, pine bark, activated carbon/charcoal and sponge filters, sedimentation and flocculation, and constructed wetlands are available for greywater treatment. Still, there is no specific treatment available for bathroom greywater. Even though microalgae have been used in wastewater treatment, no sufficient treatment was used for greywater or bathroom greywater. Leal et al. [8] revealed that microalgae could grow by using discharged greywater with a nutrient ratio (N:P) of 3.6:1. Furthermore, Shi et al. [9] demonstrated that phycoremediation of municipal wastewater by using microalgae *Scenedesmus* sp. and *Chlorella* sp. achieved nutrient removal of up to 90% and 80% for N and P, respectively. This shows the high potential of microalgae in the phycoremediation of greywater. In many reported studies, most phycoremediation processes were performed under controlled laboratory conditions. Greywater can contain nutrients such as total phosphorus, total nitrogen from detergents [10], and total organic carbon [11] that benefit algal growth. Therefore, in this study, phycoremediation process of greywater with *Botryococcus* sp. at a laboratory-scale system under ambient environment was investigated.

2. Materials and methods

2.1. Raw bathroom greywater samples

The study was carried out at Parit Haji Salleh, with coordinates 1° 54′ 0″ North, 103° 9′ 0″ East, Parit Raja, Batu Pahat, Johor, Malaysia. An initial visit to the site was carried out to get permission from the community head to study each occupant's practice in discharging bathroom greywater. The area was chosen because the residents discharge the bathroom greywater into stormwater drainage without treatment. Bathroom greywater samples were collected from the individual household effluent pipe through grab sampling then composite these samples. Samples were collected from four different houses. Three liters of bathroom greywater samples were collected hourly through the pipe to get the direct effluent (Fig. 1). The sampling was conducted from 6–9 a.m. and 5–8 p.m. for three consecutive days per week from March 2014 to February 2015. The samples were collected in a plastic bottle and transported to a wastewater laboratory at UTHM Johor Malaysia for characterization. During transportation, the samples were kept at 4°C in an icebox to maintain sample freshness. Samples were stored in a chiller at 4°C before being characterized for pH, turbidity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), NO_3^-, NO_2, NH_4^+, total Kjeldahl nitrogen (TKN), PO_4^{3+}, Na, Ca, and Mg according to APHA [12].

2.2. Culturing microalgae Botryococcus sp.

Botryococcus sp. (JF261263.2) was obtained from a tropical rainforest in the southern region of Peninsular Malaysia. The strain was identified based on the morphology and molecular analysis using 16S rRNA sequencing as described in the previous work [13]. The organism was cultured in Bold's Basal Medium (BBM) [14] and incubated under direct sunlight to enable the algae underneath to get more light (12 h light and 12 h dark) during cultivation for 7 d. The *Botryococcus* sp. cell in the culture medium was determined through cell counting using a light microscope and haemocytometer according to the procedure described in the previous study [15].

The daily cell growth of *Botryococcus* sp. in raw greywater from four houses (A to D) was determined by using different initial cell inoculum concentrations (10^5, 10^6, and 10^7 cell/mL) to obtain the best cell growth. The positive control was BBM medium, while the negative control was tap water.

2.3. Laboratory scale setup for the greywater treatment system

The laboratory-scale greywater treatment system was set up by using a photobioreactor tank for the phycoremediation process of raw bathroom greywater (Fig. 3). The photobioreactor size was 60 × 30 × 30 cm with a capacity of 54 cm. For each experimental treatment run, a fixed inoculum volume (10 mL) having 10^8 cell/mL was added to 990 mL of greywater to a final *Botryococcus* sp. concentration of 10^6 cell/mL. Each 1 L sample was mixed separately to ensure a homogenous mixture of inoculum and greywater in the photobioreactor tank. This procedure was repeated 18 times. Thereafter, the 18 L mixture was transported into the photobioreactor. Two sampling taps of 19.05 mm with a 5 cm gap were placed along with the height of the photobioreactor. The photobioreactor was operated for 21 d as its maximum operation time. The schematic diagram of the photobioreactor is shown in Fig. 2.

Fig. 1. Bathroom greywater discharge from four houses sampling point.

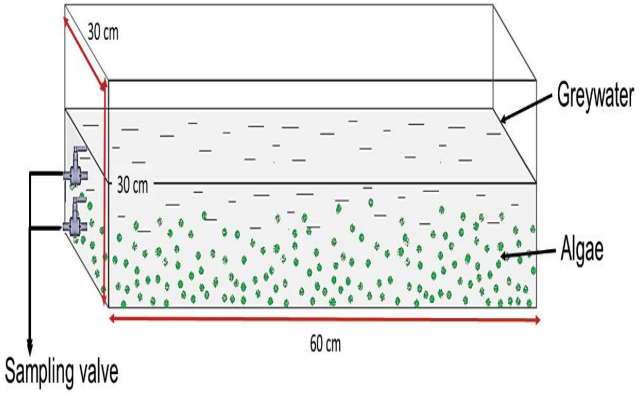

Fig. 2. Bathroom greywater laboratory-scale greywater treatment system (photobioreactor).

The photobioreactor for phycoremediation was filled with the required volume of raw bathroom greywater and microalgae *Botryococcus* sp. as inoculum. The photobioreactor was run under natural conditions (i.e., 12 h light and 12 h dark) with an ambient temperature of 25°C–35°C for 21 d. The optimum cells of microalgae obtained were used to feed on the organic and inorganic nutrients in the bathroom greywater. The bathroom greywater effluent was collected from the tap connected to the photobioreactor in a 500 mL flask to test for parameters such as pH and nutrients. The microalgae growth during the treatment process of the greywater was also determined.

2.4. Statistical analysis

The data were subjected to a one-way analysis of variances with three replicates. The differences between data

were compared using the SNK test (ANOVA). Analysis of variance (ANOVA) was performed to determine the significance of the differences between the collected data. The differences were considered significant at $p < 0.05$ (95% of the confidence level). The data were analyzed using SPSS for Windows, version 20.

3. Results and discussion

3.1. Characteristics of bathroom greywater

The characteristics of the greywater produced by the houses vary extensively, depending on the size of the household and the residents' habits. In this study, higher variation in both the quantity and quality of bathroom greywater was observed, as it would be expected from small houses. The bathroom greywater was characterized according to the procedures described in APHA [12] to measure the level of pollutants. The data on raw bathroom greywater quality are displayed in Table 1. The pH was between 6.1 and 8.27. These values agree with those reported by Christova-Boal et al. [16], which ranged from 6.4 to 8.4. The pH in raw bathroom greywater was relatively neutral (pH 6.5–7.5). This could be due to the release of hydrogen ions from ammonia in urine, bathing, and washing of nappies, if there are toddlers [13]. The pH range for healthy water is 6.5 to 8. Mohamed et al. [17] found that the pH for raw bathroom greywater was 6.1–6.4. Meanwhile, Patrick et al. [18] reported a pH of 7.7 from raw bathroom greywater. These differences might be due to the variation of greywater and the type of products utilized by the household.

The BOD and COD values were 46–199 mg/L and 76–438 mg/L, respectively, for all houses. These values might be contributed by the pollutants present in the bathroom greywater. In contrast, Christova-Boal et al. [16] reported BOD value in the range of 76–200 mg/L. Mohamed et al. [17] reported BOD value at 78–163 mg/L and COD value at 445–621 mg/L, while the range of 367–420 mg/L was reported by Jefferson et al. [19]. The COD concentration in bathroom greywater for this study is lower than those reported by other researchers. The difference in the values between studies could be due to different detergents used. The TSS in the raw bathroom greywater for all houses ranged from 29–245 mg/L, which is slightly high. This value could result from shaving activities, fallen hair, and cloth fibres that detached during washing. However, the TSS values obtained in this study are far less than those reported in two previous studies, which were 23–358 and 633 mg/L, respectively [20,21]. Nevertheless, households using solid soaps and detergents gave higher TSS, COD, and BOD values due to the presence of particles from body and cloth washing [22].

The existence of nitrogen in wastewater during discharge can be undesirable as it has environmental impacts and can affect public health [23]. In this study, nitrate concentration from bathroom greywater in all houses ranged from 1.03–7.54 mg/L. Eriksson et al. [24,25] reported a slightly lower nitrate value (6.3 mg/L) in bathroom greywater. This variation could result from different products used in the bathroom. All forms of nitrogen can be utilized as a nutrient by microalgae, although the most common nitrogen compounds assimilated by microalgae are ammonium and nitrate [26].

Mohamed et al. [17] reported phosphate concentration up to 20 mg/L, which is slightly lesser than the value obtained in this study, which ranged between 0.12 and 22.7 mg/L among all houses. Examination on personal care products used in these households indicates that the sources of the phosphate were toothpaste, detergent, and solid soaps used by the occupants [19].

3.2. Growth of Botryococcus sp. in raw bathroom greywater

The ability of the *Botryococcus* sp. to grow in greywater indicates the presence of nutrients. In this study, *Botryococcus* sp. growth was investigated daily for 14 d of cultivation. The maximum growth of *Botryococcus* sp. with 10^5 cell/mL was between day 6 and 8 (1.96×10^6 cell/mL) in the greywater from Houses B and D, and also in the positive control (Fig. 3a). The highest growth of *Botryococcus* sp. with an initial cell concentration of 10^6 cell/mL was recorded after day 7 (2.89×10^7 cell/mL) in greywater from House A (Fig. 3b). The lowest growth was noted in the negative control, probably due to the absence of the nutrients. The positive control showed the highest growth among others, because of the high nutrient content in the BBM media. In contrast, the growth of the microalgae cell with an initial inoculum of 10^7 cell/mL was recorded in the greywater from House C on day 8 with 1.88×10^6 cell/mL. However, the maximum cell growth was achieved on day 7 for most houses and control samples (Fig. 3c). The growth rate of *Botryococcus* sp. was the highest in the positive control (BBM), with an average density of 3.04×10^6 cell/mL. These findings agreed with Farooq et al. [27] and Munir et al. [28], who reported that the synthetic medium gives good results for algal growth. Generally, since the nutrient required by the algal cell for normal growth are provided in the growth media, the microalga growth rate is expected to be the highest compared to the one in bathroom greywater medium. Although the growth rate of *Botryococcus* sp. in the raw bathroom greywater was lower, the fact that its growth pattern was similar to that of the positive control suggests that it was able to utilize the nutrients present in the raw bathroom greywater. This result proves the potential of using *Botryococcus* sp. in greywater bioremediation.

3.3. Efficiency of Botryococcus sp. for removing nutrients from raw greywater

The removal of ammonium (NH_4^+) from greywater by *Botryococcus* sp. is depicted in Fig. 4a. The NH_4^+ concentration in the raw bathroom greywater in House C dropped from 5.88 to 2.62 mg/L in 6 d, and the removal efficiency was identical for all the houses. The NH_4^+ removal efficiency increased with time to reach 87% in House A after 21 d, while the minimum NH_4^+ removal efficiency was 77% (House D). Aslan and Kapdan [29] reported that NH_4^+ was completely removed from the media by *Chlorella vulgaris* when the initial NH_4^+ were between 132 and 21.2 mg/L. Furthermore, the removal efficiency of NH_4^+ decreased from 50% to less than 24% when the NH_4^+ concentration was higher than 129 mg/L. In the present study, the best removal

Table 1
Range of characteristics from raw bathroom greywater

Parameter	Case study								Average	Range of previous study on bathroom greywater	
In situ analysis	House A		House B		House C		House D				
	am	pm	am	pm	am	pm	am	pm		Mohamed et al. [17]	Eriksson et al. [24]
pH	6.3–8.27	6.27.47	6.4–7.98	6.2–7.85	6.4 ± 0.65	6.3	6.5–7.05	6.2–7.08	6.1–8.27	6.1–6.4	7.6–8.6
Turbidity (NTU)	47–89.2	52–308	39–160	56–287	47–139	50–124	52–229	60–186	39–308	ND	ND
BOD	80–145	76–112	72–127	82–199	85 ± 58	46–101	79–104	99–134	46–199	445–621	77–240
COD	153–310	76–242	164–350	206–438	176 ± 109	93–234	187–234	288–300	76–438	78–163	77–240
TSS	50.8–147	50.2–178	50.4–175	50.7–195	66–190	29–223	65–221	50.7–245	29–245	78–163	7–207
NO_3^-	4.6–21	2.55–4.1	5.2–17.02	1.03–14.2	3.79–5.88	1.81–1.91	5.22–7.54	2.66–2.94	1.03–7.54	ND	0.28–6.3
NO_2	0.03–0.06	0.02–0.03	ND	0.02–0.05	ND	ND	ND	ND	0.02–0.06	ND	ND
NH_4^+	1.49–1.62	1.12–1.74	0.93–17.0	3.03–22.4	0.32–0.93	0.16–1.03	1.21–24	0.84–11.8	0.16–11.8	ND	0.02–0.42
TKN	1.21–3.5	6.12–6.53	2.25–3.97	1.07–3.97	1.46–5.13	0.81–3.97	5.05–5.6	7.01–8.87	0.81–8.87	ND	ND
PO_4^{3+}	0.12–21.9	4.4–69.6	2.86–25	19.1–42.3	2–16.3	3.86–9.9	7.52–37.4	3.9–11.5	0.12–22.7	ND	0.9–49
Na	2.2–2.72	0.38–1.78	0.1–1.44	0.37–2.1	0.33–0.94	0.5–0.58	0.43–1	0.47–2.45	0.1–2.72	ND	44.7–98.5
Ca	0.007–0.3	0.03–0.19	0.04–0.06	0.1–0.18	0.05–0.8	0.06–0.52	0.04–0.54	0.08–0.7	0.007–0.54	ND	99–100
Mg	0.004–0.1	0.01–0.02	0.03–0.05	0–0.01	0–0.005	0–0.008	0–0.004	0.007–0.1	0.004–0.1	ND	20.8–23

ND = Not detected; BOD – biochemical oxygen demand; COD – chemical oxygen demand; TSS – total suspended solids; TKN – total Kjeldahl nitrogen;
Sampling was collected from March 2014 to February 2015, from 6–9 in the morning and 5–8 at night, $n = 16$.

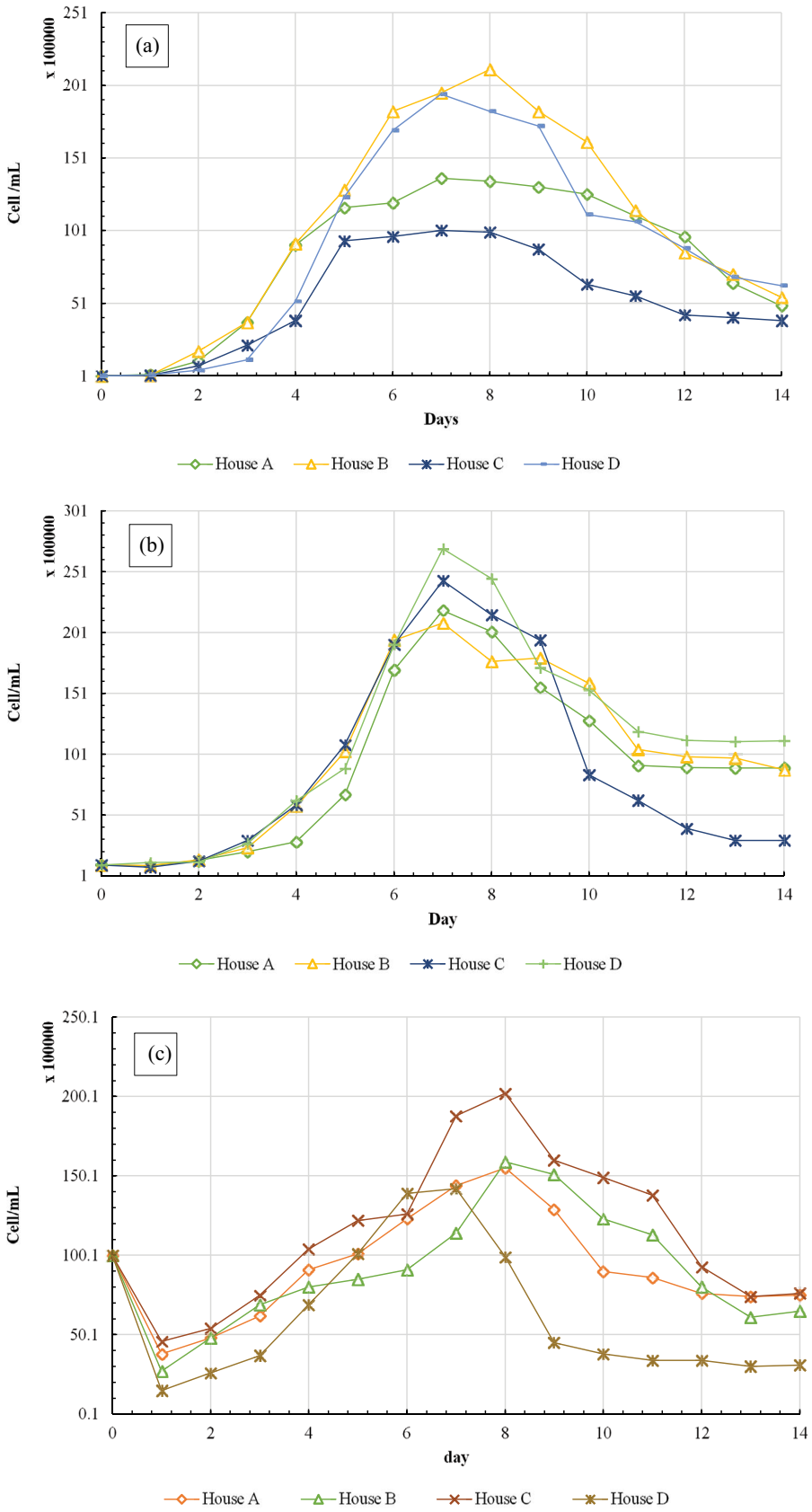

Fig. 3. Growth of *Botryococcus* sp. in raw bathroom greywater: (a) 10^5 cell/mL, (b) 10^6 cell/mL, and (c) 10^7 cell/mL.

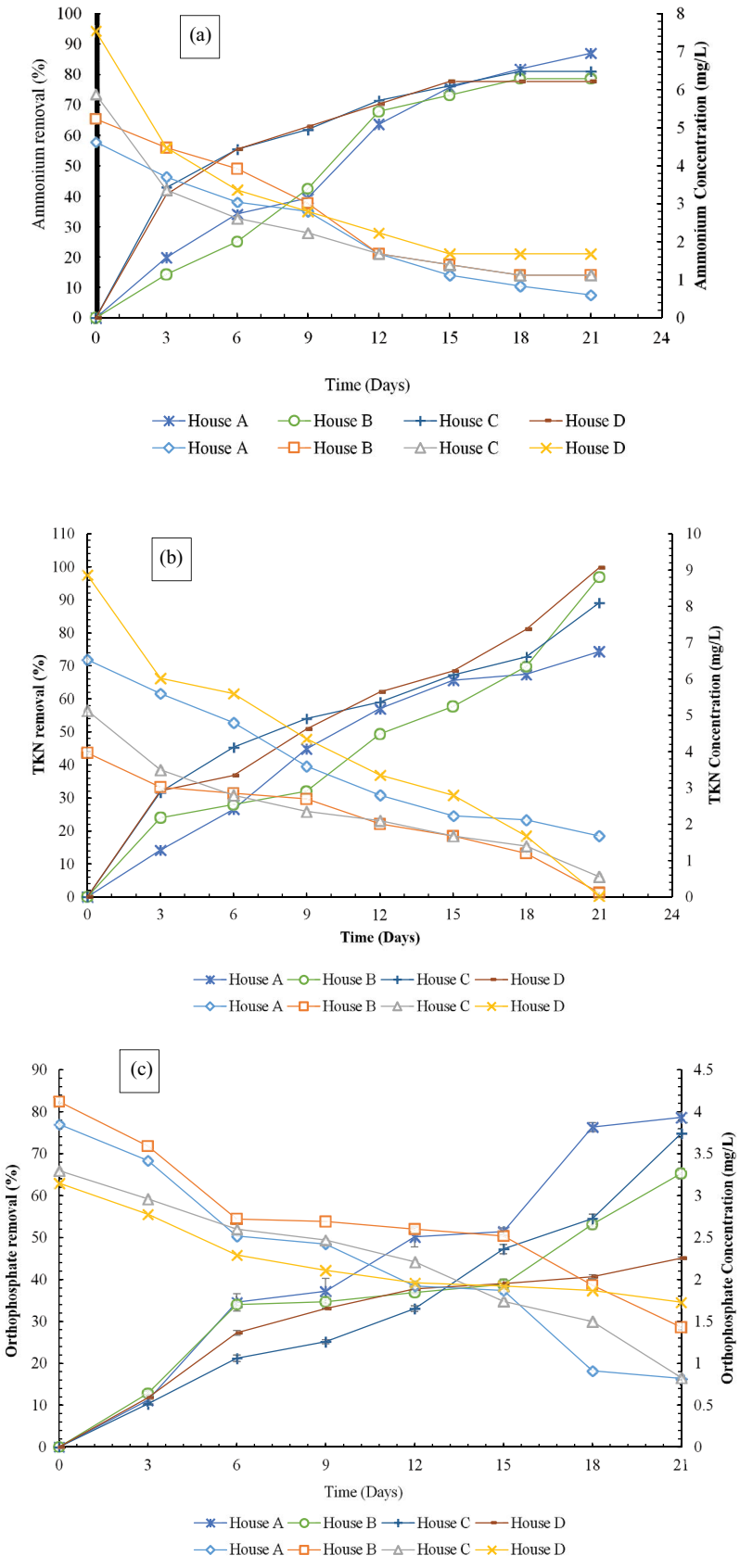

Fig. 4. The removal efficiency of nutrients from different raw greywater samples by *Botryococcus* sp.: (a) NH_4, (b) TKN, and (c) PO_4.

efficiency was 87% when the initial NH_4^+ concentration was 4.62 mg/L. However, the fact that nutrient content was still detectable in the photobioreactor after three weeks of culture suggests that light intensity could be another limitation [30]. It is hypothesized that at a higher light intensity, biomass production increases if nutrients (NH_4 and PO_4^{3-}) are still available for growth. In comparison, this study removes over 80% of NH_4^+ from the photobioreactor (Laboratory Scale Greywater Treatment System) in three weeks with an influent of 6.2 mg/L compared to the study by Ruiz-Marin et al. [30], which achieved 97.8% NH_4^+ removal with an influent of 10.5 mg/L in six weeks using industrial wastewater.

The removal rates of TKN from different greywater samples are shown in Fig. 4b. The best removal and percentage efficiency of TKN by *Botryococcus* sp. was achieved in House D, where TKN was reduced from 8.87 to 0.02 mg/L, attaining 99.7% removal efficiency. Similarly, Chinnasamy et al. [31] also reported a microalgae uptake up to 87% of TKN when grown photoautotrophically in a lab-scale photobioreactor.

The orthophosphate (PO_4^{3-}) uptake from the bathroom greywater medium by *Botryococcus* sp. is shown in Fig. 4c. The removal efficiency of PO_4^{3+} from bathroom greywater was less compared to NH_4^+ removal efficiency. Orthophosphate removal rate was higher in bathroom greywater from House B from 3.3 to 2.6 mg/L in 6 d, removing up to 1.4 mg/L (33.98%). However, the maximum removal efficiency was achieved in the greywater sample from House A (78.7%) in three weeks. The result of this study is comparable with those from Aslan and Kapdan [29], who obtained 78% removal efficiency for orthophosphate. Thus, PO_4^{3+} removal by *Botryococcus* sp. showed the same behaviour with NO_3^- removal, as NO_3^- was rapidly removed in 6 d. Therefore, the limited PO_4^{3+} removal after day 6 was probably due to insufficient NO_3^- in the medium. Hence, NO_3^- presence in bathroom greywater is required for the uptake of orthophosphate. Shi et al. [9] found that about 90% of PO_4^{3+} was removed using microalgae such as *C. vulgaris* and *Scenedesmus rubescens*. Yuan et al. [32] reported removing 23.51% of PO_4 from synthetic wastewater using *Spirulina* sp. in 140 d. However, they used 24 h lighting for the study, whereas only 12 h of natural lighting was used in this study. The short lighting period signifies that the photobioreactor containing microalgae *Botryococcus* sp. shows better removal performance and efficiency compared to other species. PO_4^{3-} uptake is dependent on microalgae growth, which is directly associated with inorganic carbon (CO_2) assimilation during its photosynthesis activity in the photobioreactor. This increases DO, thereby decreasing CO_2 and thus produces less carbonic acid [33]. Therefore, pH is increased and precipitated and incorporated into the microalgae biomass.

3.4. Characteristics of greywater during the removal process

The removal efficiency was influenced by the pH of the greywater samples during phycoremediation due to photosynthetic activity. It was observed that at a minimum pH level (8.5) was tested during the present work. The findings of our work are in agreement with another study that used *Phaeodactylum tricornutum* which can grow at pH 7.5 and above [34]. pH is one of the most significant factors besides temperature and light for algae growth [35].

The average DO concentration attained was 8.5 mg/L and is in agreement with the value obtained by Vargas et al. [36] (7.0 ± 0.5 mg/L), which is less than the DO concentration reported as inhibitory for microalgae of 20 mg/L [33]. DO concentration has a significant effect on the rates of nitrifier growth and nitrification during phycoremediation in a biological treatment system. In this situation, the growth condition of *Botryococcus* sp. becomes favourable as they consume inorganic carbon (CO_2) and use DO to oxidise ammonia to nitrite and later to nitrate [37]. The influence of DO during phycoremediation in photobioreactor is important because oxygen is produced during photosynthesis by *Botryococcus* sp.

Temperature also has a great impact on nitrification within the treatment system. The temperature range to achieve nitrification is 15°C–35°C. Algal growth increased with the increase in temperature ranging from 27°C to 32°C in this study (data not shown). Raw bathroom greywater possessed the best condition for growth at 32.4°C, and the cells recorded a concentration of 6.1×10^6 cell/mL. The algae showed a decrease in growth at temperatures above 33°C, which was 5.1×10^6 cell/mL for the raw bathroom greywater. Similar results were shown for the effect of temperature to algal cells on *N. oculata* and *Chlorella* sp. The growth was visibly affected at temperatures more than 30°C. At 35°C, the microalgae exhibit a 17% growth deterioration, and further temperature escalation to 38°C led to the death of the algal cells [38]. In another study, the optimal temperature for growing most species of algae is recorded between 20°C and 30°C [39]. However, *Botryococcus* sp. in this study is capable of withstanding higher temperatures, which is 32°C. Hence, a better potential is expected with this species of algae, though this may vary from one geographical study area to another.

The *Botryococcus* sp. kept under light and dark condition showed growth with a mean value of 6.02×10^6 cell/mL in this study at a light intensity of 1,500–2,000 Lux (data not shown). Thus, it is obvious from the results that light has a substantial effect on algal growth. The growth decreases due to damage from light pigments at high light intensities that exceed 32,400 Lux [40]. Other essential findings have highlighted the influence of light intensity on algal growth. Thus, light exposure, intensity, and penetration are imperative factors for algal cultivation [41]. Hu et al. [42] reported that microalgae grown at different light intensities showed incredible changes in growth. Therefore, sufficient light influences algal photosynthesis for growth and subsequently, the precipitation of PO_4–P. The quality of effluent (bathroom greywater) from the photobioreactor, especially the raw bathroom greywater, met Malaysia' Standard A of allowable discharge limit to stagnant water bodies, which is 2 mg/L. It also met the specification in the Industrial Wastewater Treatment Plant to Water Bodies by Gerardi [33], which is ≤2 mg/L of discharge to water bodies.

4. Conclusion

Greywater characteristics were described in this work. It has pH between 6.1 and 8.27, BOD_5 values of 46–199 mg/L, COD values between 76 and 438 mg/L, TSS values between

29 and 245 mg/L, nitrate-N levels of 1.03–7.54 mg/L, and phosphate levels between 0.12 and 22.7 mg/L. The removal of ammonium (NH_4^+) from greywater by *Botryococcus* sp. reached 87% in the greywater sample from House A after 21 d and 77% in the sample from House D. The total Kjeldahl nitrogen removal was 99.7%, and the orthophosphate (PO_4^{3+}) removal was 78.7%. The study concludes that algal bioremediation is a viable alternate technology for sustainably treating bathroom greywater. Hence, there will be less/no bathroom greywater pollutants discharged into water bodies, and household occupants might reuse the treated bathroom greywater for outdoor usages.

Acknowledgments

The authors acknowledge the Ph.D. thesis of Wurochekke Anwaruddin Ahmed (2017). Phycoremediation of Artificial Bathroom Greywater in Village Houses using Microalgae *Botryococcus* sp. Ph.D. UTHM.) from which this manuscript is derived. The authors would like to thank the Ministry of Education Malaysia (KPM) for providing the Fundamental Research Grant Scheme (FRGS) with reference code: FRGS/1/2019/WAB05/UTHM/02/5 (Modification of Bead Adsorbents with Ceramic Sanitary Ware Waste (CSWW) and Chitosan for Laundry Greywater (LGW) Safe Disposal) and contract grant H377 as financial support for this research project.

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