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Performance and kinetics of algal-bacterial photobioreactor (AB-PBR) treating septic tank effluent

Chawalit Chaiwong, Thammarat Koottatep, Nawatch Surinkul and Chongrak Polprasert

ABSTRACT

Septic tank effluent contains high organic and nutrient contents. This study aimed to evaluate treatment performance of an algal-bacterial photobioreactor (AB-PBR) treating the septic tank effluent. The experimental unit employed a transparent plastic medium made from recycled drinking water bottles for attached-growth biofilm. Red LED lamp (light intensity ~100 μmol/m^2/s) was applied as an energy source for the growth of algal-bacterial biofilm in the AB-PBR. The experimental results showed that AB-PBR operated at the hydraulic retention time (HRT) of 3 days gave the highest chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) removal efficiencies of 64, 45 and 35%, respectively, by which the effluent COD concentrations could meet the effluent standards of Thailand, but the effluent TN and TP concentrations needed to be further removed. The Stover–Kincannon model was applied to determine the kinetic values of COD and TN removals with R^2 values greater than 0.8. Microbiological examinations indicated *Chlorella* sp. is the predominant algal species growing in the AB-PBR, while the amplicon sequencing information analytical results revealed the bacterial phylum of Proteobacteria to be the predominant bacterial group.

Key words | algal-bacterial photobioreactor, kinetic model, on-site sanitation system, septic tank effluent

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ABBREVIATIONS

A	surface area
AB-PBR	algal-bacterial photobioreactor
AIT	Asian Institute of Technology
COD	chemical oxygen demand
DNA	deoxyribonucleic acid
DO	dissolved oxygen
HRT	hydraulic retention time
K_S	saturation value constant
NH_3-N	ammonia nitrogen
NLR	nitrogen loading rate
NRR	nitrogen removal rate
OLR	organic loading rate
ORR	organic removal rate
OSS	on-site sanitation system
OTU	operational taxonomic unit
PBR	photobioreactor
PCD	Pollution Control Department
PETE	polyethylene terephthalate
PLR	phosphorus loading rate
PRR	phosphorus removal rate
PW	algal pond water
Q	flow rate
rRNA	ribosomal ribonucleic acid
S	substrate concentration
TKN	total Kjeldahl nitrogen
TN	total nitrogen
TP	total phosphorus
TSS	total suspended solids
U_{max}	maximum substrate removal rate
V	volume of reactor
WW	influent wastewater

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INTRODUCTION

In many developing countries, the use of septic tanks as an on-site sanitation system (OSS) is widely practiced to treat domestic wastewater, especially wastewater from toilets or blackwater. However, effluent from the on-site treatment systems still contains large amount of environmental pollutants, especially nutrients and organic matters. For instance, Nam *et al.* (2006) reported the chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) concentrations in effluent from septic tanks in developing countries to be 300–1,500, 200–700 and 15–50 mg/L, respectively, higher than the characteristics of typical domestic wastewater (Metcalf & Eddy 2014). Hence, performance of the conventional septic tank effluent seem not capable of discharging its effluent to the environment.

The algal-bacterial photobioreactor (AB-PBR) system is a technology that can be applied to treat both organic and nutrient matters in wastewaters, offering low cost in investment, and simple operation and maintenance (Craggs *et al.* 2011; Posadas *et al.* 2014). Moreover, separation of the biomass from wastewater is not required because the biomass can be retained in the reactor with a short operating hydraulic retention time (HRT) (Boelee *et al.* 2011). The AB-PBR system functions via algal-bacterial symbiotic reactions. Oxygen (O_2) from the photosynthetic reactions of algae is used by heterotrophic and autotrophic bacteria (nitrifying bacteria) to oxidize organics and ammonia, respectively, in the wastewater, while nutrients in the wastewater can be biologically removed by assimilation into algal cells (Munoz & Guieysse 2006; Boelee *et al.* 2011; Posadas *et al.* 2014). Currently, many types of AB-PBR are being applied to treat pollutants in various types of wastewater. For example, Muñoz *et al.* (2009) reported application of the AB-PBR in treating organic matters and nitrogen in the industrial wastewater, while an algal turf scrubber, another type of AB-PBR, was designed to treat nutrients from polluted surface water (Mulbry *et al.* 2010). Flow cell PBR (Boelee *et al.* 2011) and Tubular PBR (Posadas *et al.* 2014) were also used to treat municipal and domestic wastewater, respectively. However, application of an AB-PBR system for treating septic tank effluent, which contains high concentration of COD, TN and TP, has been not reported.

This study aimed to evaluate treatment performance and kinetics of the AB-PBR employing attached-growth media recycled from polyethylene terephthalate (PETE) water bottles to treat the effluent from septic tanks. Moreover, algal and bacterial species were also investigated in the AB-PBR operated at the optimum conditions.

METHODOLOGY

Algal-bacterial inoculums and source of wastewater sample

Algal inoculums were obtained from an algal pond water (PW) in the Asian Institute of Technology (AIT) campus, Phathumtani, Thailand. Activated sludge from the AIT wastewater treatment plant was used as bacterial inoculum in this study. The septic tank effluent collected from septic tank of a household located in Phathumtani province, Thailand, was used as influent in this study.

Experimental set-up

The AB-PBR reactor was made of transparent acrylic plastic to allow for light penetration. Configuration of the reactor was $0.10 \times 0.50 \times 0.6$ m (width: depth: length), which was equipped with five acrylic baffles inside and was designed as a closed system (Figure 1(a)). Attached growth media of 180 pieces applied in the AB-PBR was prepared from recycled PETE plastic (Figure 1(b)), each with a surface area of 0.022 m^2/piece. The working volume and specific surface area of the AB-PBR were 24 L and 162 m^2/m^3, respectively. To allow for the algal biofilm to perform photosynthesis throughout the whole reactor depth, the AB-PBR was continuously illuminated by a red LED (Bogdan®, Bogdan LED – J&Y Trading, Thailand) with peak wavelength of 635 nm and light intensity of 100 μmol/m^2/s (measured by MQ-200 Apogee® quantum meter).

Algal-bacterial biofilm culture

The method of algal-bacterial biofilm culture was modified from the previous studies of Krustok *et al.* (2015a) and Babu *et al.* (2010). The PW collected from the upper layer (depth of about 0.2 m) was mixed with the WW ratio of 3/7 to achieve the N/P ratio of 16/1 (Whitton *et al.* 2016). About 14 L of the mixture and 10 L of the activated sludge were then put into the AB-PBR. After that, the AB-PBR was continuously lit (24 hours) by the red LED lamp under ambient temperature (≈30 °C) for about 15 days to allow biofilm development. Then, the AB-PBR was continuously fed with the WW to achieve steady-state conditions based on relatively constant effluent COD concentrations.

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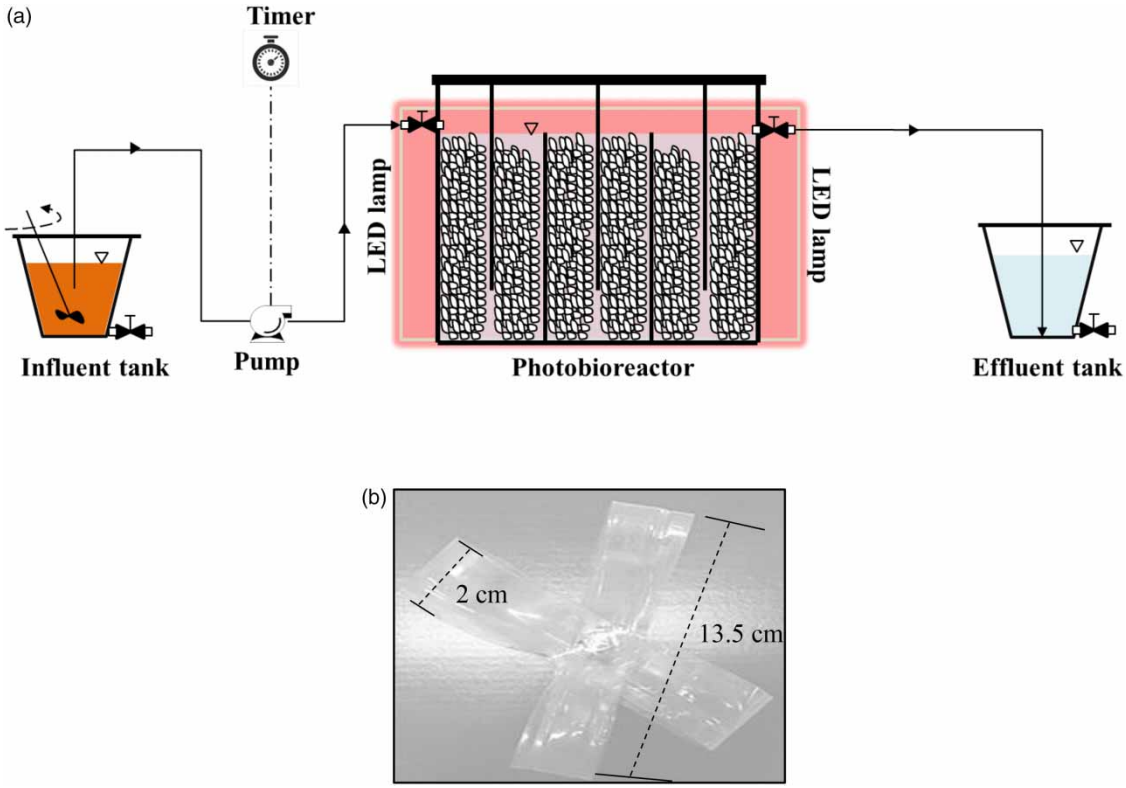

Figure 1 | Experimental set-up (a) and attached-growth media (b).

Wastewater characteristics and operating conditions

The wastewater characteristics (Table 1) showed the COD, total suspended solids (TSS), TN, NH_3-N, and TP concentrations to be 227, 97, 122, 104, 15 mg/L, respectively. The WW was fed intermittently into the AB-PBR according to typical toilet flushing times of Mayer *et al.* (1999). The experiments were divided into three stages depending on HRT, organic loading rate (OLR), nitrogen loading rate (NLR) and phosphorus loading rate (PLR) (Table 2).

Table 1 | Characteristics of influent wastewater

Parameters	Unit	Value
pH	–	7.78 ± 0.27
DO	mg/L	0.42 ± 0.26
Temperature	°C	29.4 ± 4.26
COD	mg/L	227 ± 20
TSS	mg/L	97 ± 28
TN*	mg/L	122 ± 10
NH_3-N	mg/L	104 ± 9
TP	mg/L	15 ± 2

*NO_3-N and NO_2-N concentrations in the wastewater were less than 1.0 mg/L.

Table 2 | Operational conditions

		Operating conditions			
Experimental stages	Duration (day)	HRT (day)	OLR mgCOD/(L·d)	NLR mgN/(L·d)	PLR mgP/(L·d)
I	26	1	220 ± 30	122 ± 11	15 ± 1.9
II	23	2	125 ± 26	59 ± 3	7.3 ± 0.7
III	22	3	73 ± 11	42 ± 3	5.0 ± 0.3

Analytical methods

Wastewater analysis

Influent and effluent were sampled three times a week for analysis of COD, TSS, TKN, NH_3-N, NO_3-N, NO_2-N and TP concentrations, according to *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association (APHA) American Water Works Association (AWWA) & Water Environment Federation (WEF) 2012). DO and pH were monitored twice a week by using a portable DO meter (Mettler Toledo S4-Standard Kit Seven2Go) and pH meter (©Mettler Toledo AG 2009), respectively.

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Algal-bacterial biomass

The attached growth media were randomly collected from six positions in the AB-PBR at the end of stage III, and the biofilms were then harvested using an ultrasonic cleaner (500 W, 40 KHz, 30 min) from the media and dried at 105 °C for 24 h in an oven for analysing dried weight biomass. The harvested biofilm samples were further analyzed to determine the algal and bacterial species and their relative abundances in the AB-PBR using the techniques described below.

Algal species

Determination of algal species in the AB-PBR was done by microscopic method (APHA-AWWA-WEF 2012). After finishing the stage III experiment, biofilms on the media were carefully scraped from 5 cm, 25 cm and 40 cm depths of the reactor, then diluted in 1 L of distilled water and fixed with lugol acid and stored at 4 °C before analysis at the laboratory of Thailand Institute of Scientific and Technological Research, Phathumtani, Thailand.

Bacterial species

The bacterial species of the biofilms after the stage III experiment were analysed by amplicon sequencing technique (Liu *et al.* 2012; Herbold *et al.* 2015). The DNA firstly was extracted from the sample by targeting bacterial 16S rRNA-encoding genes (D'Amore *et al.* 2016) for amplicon sequencing information analysis by Illumina MiSeq and HiSeq 2000 DNA sequencing (Beijing Genomics Institute, BGI, and Shenzhen, China). A quality test of the DNA samples was done first, then the entire qualified DNA was used to construct libraries. The libraries were then filtered and clustered to Operational Taxonomic Unit (OTU) with 97% sequence similarity. The OTU in different samples were summarized in a profiling table or histograms, which were drawn with the software R (v3.0.3).

Kinetic models

Stover–Kincannon model

Stover–Kincannon is one of the mathematical models used to determine the kinetic constants for substrate removal in biofilm systems. Previously, the model was used to model many biofilm reactors, namely the integrated rotating biological contactor-activated sludge system (Akhbari *et al.* 2012), the immobilized PBR system (Kapdan & Aslan 2008), the up-flow anaerobic filter (Padilla-Gasca & López 2010), the trickling filter (Raj & Murthy 1999), the submerged aerobic biofilm (Gonzalez-Martinez *et al.* 2000) and the static granular bed reactor (Debik & Coskun 2009). In this model, the substrate utilization rate is expressed as a function of the organic loading rate. The model considers the substrate removal rate as a function of substrate loading rate at steady state as in Equation (1)

$$\frac{dS}{dt} = \frac{QS_0 - QS}{V} \tag{1}$$

The original Stover–Kincannon model was expressed as:

$$\frac{dS}{dt} = \frac{U_{max}\left(\frac{QS_0}{A}\right)}{K_s + \left(\frac{QS_0}{A}\right)} \tag{2}$$

In which A represents the total surface area (m^2) where biomass is immobilized, U_{max} is the maximum substrate removal rate (mg/L/d) and K_S is a saturation value constant (mg/L/day). Nevertheless, due to difficulties in measuring the active surface area of the biofilm growth in the system, the original model has been modified for the immobilized reactor by introducing the effective volume of the reactor. Hence, a modified Stover–Kincannon model can be re-written by arranging Equations (1) and (2) as:

$$\frac{dS}{dt} = \frac{QS_0 - QS}{V} = \frac{U_{max}\left(\frac{QS_0}{V}\right)}{K_s + \left(\frac{QS_0}{V}\right)} \tag{3}$$

The linearizing equation was re-arranged to be:

$$\frac{V}{Q(S_0 - S)} = \frac{K_s}{U_{max}}\left(\frac{V}{QS_0}\right) + \frac{1}{U_{max}} \tag{4}$$

Therefore, K_S and U_{max} can be defined by plotting V/Q (S_0-S) and V/QS_0.

RESULTS AND DISCUSSION

At the steady- state conditions determined by effluent COD concentrations, the DO concentrations and pH and temperature in the AB-PBR system were recorded to be 6.8 ± 1.7 mg/L, 8.4 ± 0.4 and 28.9 ± 1.8 °C, respectively. Since

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this PBR was a closed system, it could be expected that DO in the reactor was generated from the algal photosynthesis. Typically, DO concentrations of more than 2 mg/L are suitable for aerobically oxidizing organic matters and ammonia in the wastewater by aerobic bacteria (Metcalf & Eddy 2014).

Treatment performance of AB-PBR

The effluent COD concentrations were found to be 105 ± 9, 101 ± 16 and 79 ± 10 mgCOD/L, corresponding to the COD removal efficiencies of 50 ± 5, 59 ± 4 and 64 ± 4% at OLR of 220 ±30, 125 ±26 and 73 ±11 mgCOD/(L·d), respectively (Figure 2). Due to having the lowest OLR, the highest COD removal was observed in the stage III experiment ($P < 0.05$). However, the range of effluent COD concentration of 79 to 105 mg/L of the three stages is within the discharge standard of Thailand (PCD, 2010). Figure 3 shows the results of TN removal efficiencies which were 20 ± 2, 35 ± 6 and 47 ± 6% for the stages I, II and III, respectively, resulting in the effluent TN concentrations of 100 ± 13, 78 ± 5 and 69 ± 6 mg/L. Due to the high DO concentration of 6.8 ± 1.7 mg/L, most of the TN should be removed by algal-bacterial biomass assimilation and nitrification reactions. The effluent NO_3-N concentrations were found to be 17 ± 5, 18 ± 6 and 25 ± 4 mg/L for the stages I, II and III, respectively. The highest effluent NO_3-N concentration was observed in the stage III ($P < 0.05$) probably because of its lowest OLR, which enhanced the nitrifying bacteria to convert more NH_3-N to become NO_3-N (Muñoz Sierra *et al.* 2014).

The OLR range employed in this study did not appear to have significant effects on the TP removed ($P > 0.05$). As shown in Figure 4, the average TP removal efficiency for the three stages was 29 ± 10%, resulting in the effluent TP concentration of 10 ± 2 mg/L. Based on the operating conditions, the TP might be removed mainly by the algal-bacterial biomass assimilation and some by precipitation.

Similarly, there were not significant differences among TSS removal efficiencies of all operating stages with the average effluent TSS concentration being 24 ± 9 mg/L, corresponding to the average TSS removal efficiency of 73 ± 10%. About 9 L of accumulated TSS as sludge was observed and removed at the end of stage III.

Kinetic model

As the data of effluent COD and TN concentrations shown in Figures 2 and 3, respectively, were considered to be at steady-state conditions, they were applied to Equation (4), as illustrated in Figure 5, with R^2 values of 0.95 and 0.81, respectively. The kinetic constants of COD removal were found to be 0.41 gCOD/(L·d) and 0.58 gCOD/(L·d) of U_{max} and K_S, respectively, which were greater than the previous study AB-PBR system under sunlight condition having 0.19 and 0.17 gCOD/(L·d) of U_{max} and K_S, respectively (Surinkul *et al.* 2017). Meanwhile, the kinetic values in this study were relatively low when compared with other aerobic biofilm systems, namely the trickling filter system having 4.0 and 3.8 gCOD/(L·d) of U_{max} and K_S (Raj & Murthy 1999)

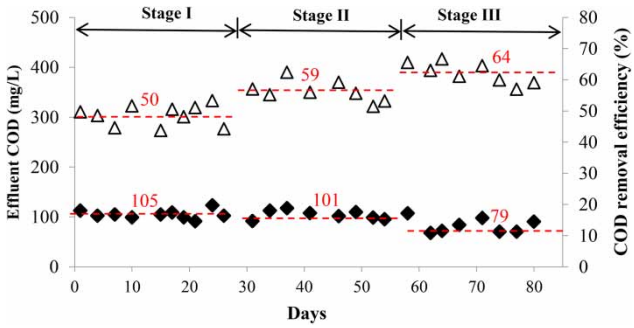

Figure 2 | Effluent COD concentration (♦), COD removal efficiency (△) and average value (---).

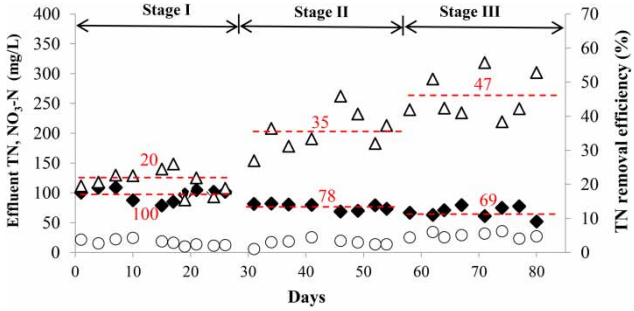

Figure 3 | Effluent TN concentration (♦), effluent NO_3-N concentration (○), TN removal efficiency (△) and average value (---).

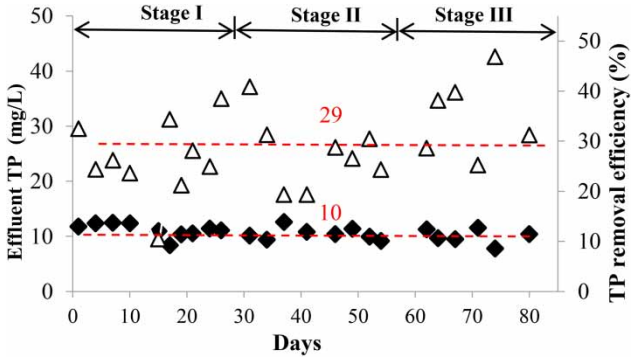

Figure 4 | Effluent TP concentration (♦), TP removal efficiency (△) and average value (---).

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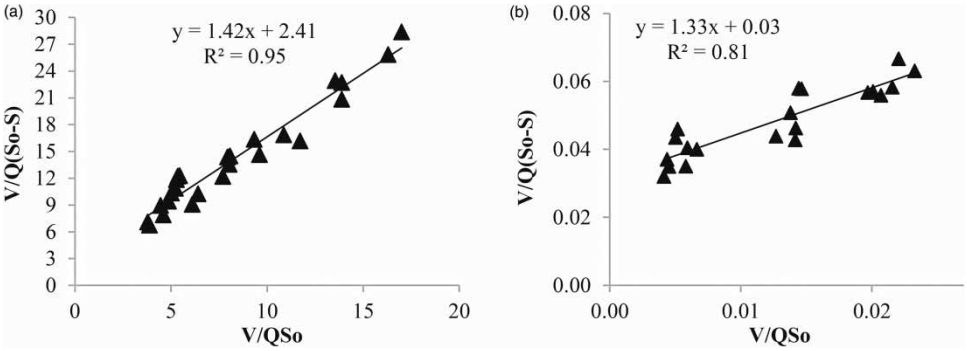

Figure 5 | Stover–Kincannon models for COD (a) and TN (b) removals in the AB-PBR.

and the integrated rotating biological contactor-activated sludge system having 15.2 and 14.7 gCOD/(L·d) of U_{max} and K_S, respectively (Akhbari *et al.* 2012). The trickling filter system of Raj & Murthy (1999) had the specific surface area of 243 m^2/m^3, higher than the employed 162 m^2/m^3 of specific surface area in the AB-PBR system in this study, which suggested the presence of more bacterial biomass responsible for COD degradation. Similarly, the integrated rotating biological contactor-activated sludge system of Akhbari *et al.* (2012) was operated with the heterotrophic bacteria concentration of 5.8 g/L, higher than the AB-PBR system of this study, which was operated with the combined algal-bacterial biomass of 4.6 g/L. It could be hypothesized that the presence of low biomass in the AB-PBR system was responsible for the relatively low U_{max} and K_S values.

The kinetic constants for TN removal in the AB-PBR were found to be 33.3 mg/(L·d) and 43.9 mg/(L·d) of U_{max} and K_S, respectively, which were higher than that of other biofilm bioreactors including the integrated rotating biological contactor-activated sludge system having 10.9 and 7.1 mgN/(L·d) of U_{max} and K_S, respectively (Akhbari *et al.* 2012) and the immobilized PBR system having 13.0 and 10.3 mgN/(L·d) of U_{max} and K_S, respectively (Kapdan & Aslan 2008). The relatively high kinetic constants for TN removal were hypothesized to be due to algal biomass assimilation and nitrification reactions occurring in the AB-PBR.

Hence, from Equation (4), the obtained U_{max} and K_S values for COD and TN removal could be used to determine the volume of the AB-PBR reactor (V) to achieve the desired effluent substrate concentration (S).

Algal species in AB-PBR

A microscopic picture of the algal species is shown in Figure 6. *Chlorella* sp. was found to be the predominant

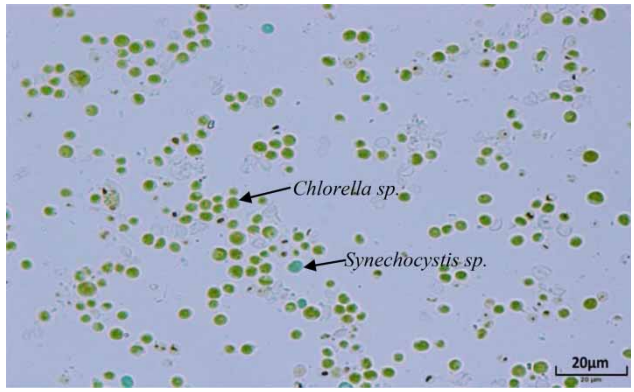

Figure 6 | Algal species in the AB-PBR under times 400 magnification.

algal species in the AB-PBR, which constituted 95.6% (1.12×10^7 cells/mL) of the total algal population. Other algal species of the biofilm found in the reactor were *Synechocystis* sp. (2.3% or 2.64×10^5 cells/mL), *Phormidium* sp. (1.8% or 2.05×10^5 filaments/mL and *Monoraphidium* sp. (0.1% or 2.66×10^3 cells/mL). The results of the predominant algal population in this AB-PBR were similar to the previous studies of Lakaniemi *et al.* (2012), Krustok *et al.* (2015a) and (Kouzuma & Watanabe 2015). *Chlorella* sp. is ranked as the most pollution tolerant algal group in the PBR system for treating wastewater (Posadas *et al.* 2014). Its growth was responsible for photosynthesis that produced DO for the biofilm bacteria to degrade COD matters (Figure 2) and for biomass assimilation of TN and TP (Figures 3 and 4).

Bacterial species in the AB-PBR

Bacterial profiles of the biofilm are presented in phylum (Figure 7(a)) and class (Figure 7(b)) levels. The most predominant bacteria in the AB-PBR belonged to the phylum of Proteobacteria (~40% of total population).

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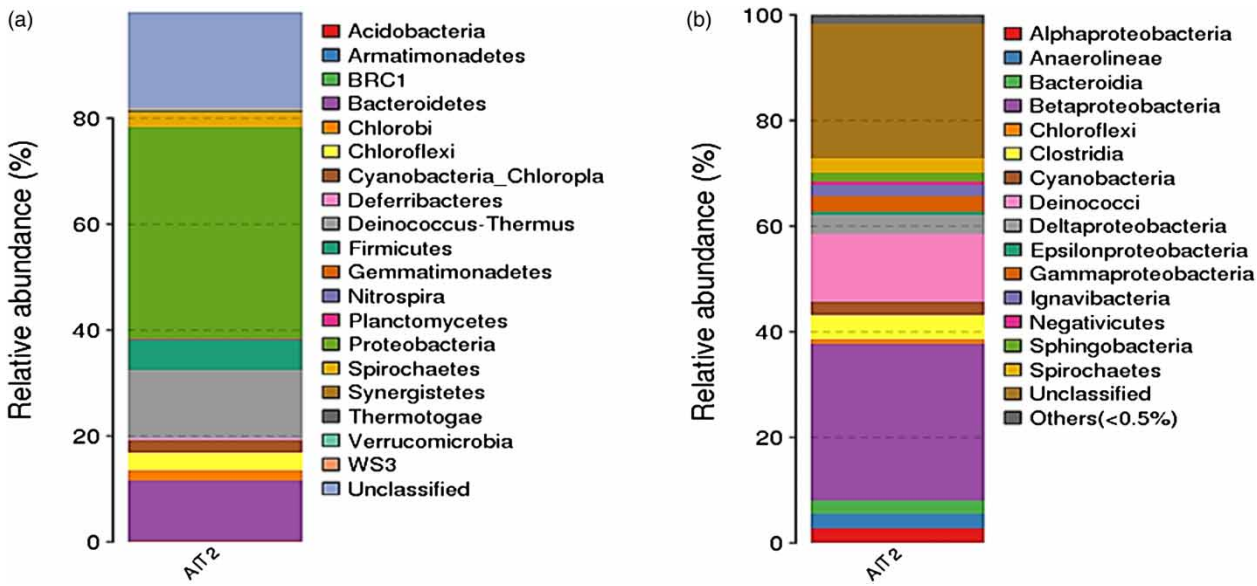

Figure 7 | Bacterial community of the AB-PBR in Phylum (a) and Class (b) levels.

Other bacteria in the AB-PBR were determined to be phyla of Bacteroidetes, Deinococcus–Thermus and Firmicutes. For class levels, the bacterial population was predominated by Betaproteobacteria and Deinococci. Predomination of the bacterial groups in this study, responsible for COD removal (Figure 2), were found to be similar to the other

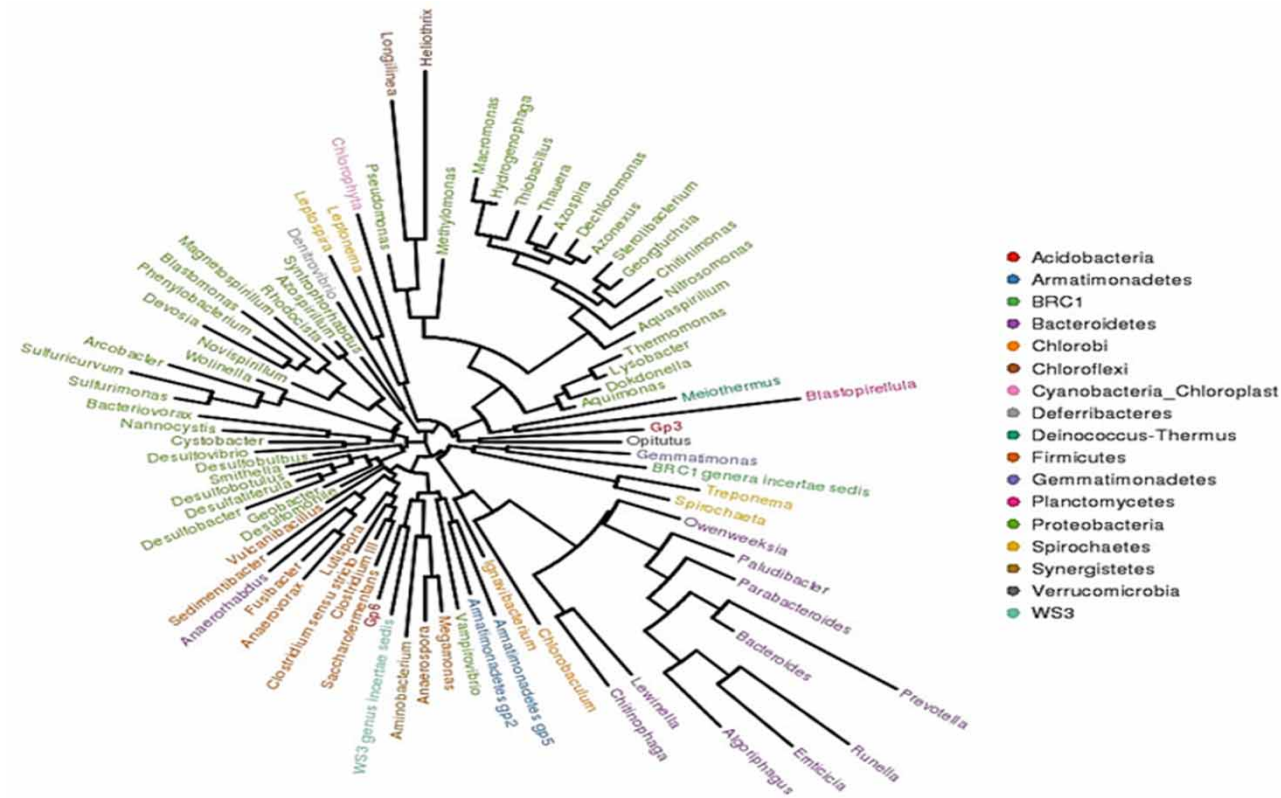

Figure 8 | Genus level phylogenetic tree (the same phylum is shown as the same color). The full color version of this figure is available in the online version of this paper, at http://dx.doi.org/10.2166/wst.2018.519.

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PBR systems of Lakaniemi *et al.* (2012), Krustok *et al.* (2015b), Su *et al.* (2011), Ibekwe *et al.* (2017) and Ryu *et al.* (2015). Additionally, Chen *et al.* (2017) stated that groups of *Deinococcus-Thermus*, *Firmicutes* and *Actinobacteria* could play some roles on organic degradation in the aerobic biofilm bioreactor. Furthermore, *Nitrosomonas* sp., one of most important ammonia oxidizing bacteria (AOB) being in class of Betaproteobacteria, was also observed in the AB-PBR as shown genus level phylogenetic tree (Figure 8). Moreover, *Nitrospira*, a group of nitrite oxidizing bacteria (NOB) commonly observed in biofilm in the natural environment (Metcalf & Eddy 2014), was also found in the biofilm. Hence, these results of bacterial profile indicate the occurrence of nitrification processes in the AB-PBR, responsible for TN removal and NO_3-N production (Figure 3). In addition, *Pseudomonas* sp., a well known denitrifying bacterial species (Miyahara *et al.* 2010), was also detected in the AB-PBR. Hence, some part of the produced NO_3-N could be removed via the denitrification process.

It should be noted that the data of algal and bacterial species reported above were obtained from one biofilm sample collected at the completion of Stage III. Further studies of this aspect are recommended by collecting more biofilm samples and applying the PCoA, PCA and RDA methods to analyzing the species and abundance of the algal and bacterial species.

CONCLUSIONS

Based on the results obtained from the study, the following conclusions are made:

- AB-PBR process could be applied to treat septic tank effluent to meet the discharge standards of Thailand.
- The highest COD, TN and TP removal efficiencies of 64, 45 and 35%, respectively, were achieved at the HRT and OLR of 3 days and 73 mgCOD/(L·d), respectively.
- COD and TN removal efficiencies were found to follow the Stover–Kincannon model, and the determined kinetic constants could be used to design the AB-PBR to treat septic tank effluent.
- *Chlorella* sp. was found to be the predominant algal species growing in the AB-PBR, while Proteobacteria was the most predominant bacterial group. The occurrence of the nitrifying bacteria such as *Nitrosomonas* and *Nitrospira* groups were also observed, which were responsible for nitrification reactions in the AB-PBR.

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