Performance evaluation of two trickling filters removing LAS and caffeine from wastewater: Light reactor (algal-bacterial consortium) vs dark reactor (bacterial consortium)

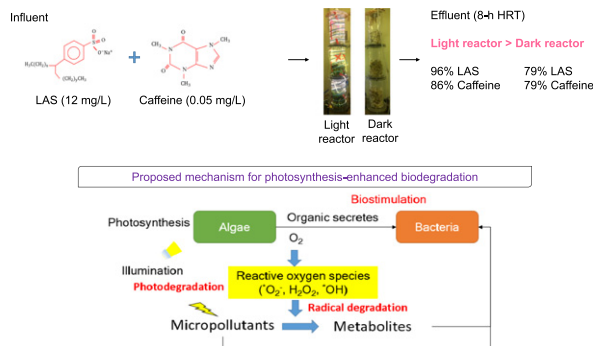Keerthi Katam [a], Toshiyuki Shimizu [b], Satoshi Soda [b,*], Debraj Bhattacharyya [a]

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HIGHLIGHTS

- The Light Reactor showed better removal of linear alkylbenzene sulphonate.
- Hydraulic retention time had played a major role in microbial population dynamics.
- Long retention time is required to retain micropollutant degraders in a reactor.
- Free radicals produced by algal photosynthesis helped in micropollutant removal.

GRAPHICAL ABSTRACT

ABSTRACT

Micropollutant removal efficiencies of two trickling filters – operated under light and dark conditions were studied and compared. Linear alkylbenzene sulphonate (LAS) and caffeine were selected as model micropollutants. Both lab-scale trickling-filter type reactors were packed with polyurethane foam sponge cubes ($2\,cm \times 2\,cm \times 2\,cm$) with 40% occupancy. The trickling filter with the white color LED light was named as Light Reactor (LR), which was operated under light: dark cycle of 12:12 h with a quantum flux of 15 μmoles/m^2/s. No light was provided in the other trickling filter, named Dark Reactor (DR). Synthetic wastewater containing glucose (250 mg/L), LAS (12 mg/L), and caffeine (0.05 mg/L) was fed to the reactors at a hydraulic retention time (HRT) of 12- and 8-h at 25 °C for 2 months. The C, N, and P removal at 12-h HRT were 85%, 15%, and 49%, respectively, in LR, the corresponding values in DR were 88%, 18%, and 43%. Similarly, at 8-h HRT 90%, 24%, and 37% was observed in LR and 84%, 19%, and 37% in DR. However, the LAS and caffeine removal decreased from 99 to 96% and 96 to 86% respectively in LR, and from 96 to 79% in DR with decreasing the HRT from 12 to 8-h. The number of LAS degraders in LR (5.5×10^4 CFU/sponge cube) was higher compared to DR (2.2×10^4 CFU/sponge cube) at 8-h HRT. The above results indicate that algal-bacterial symbiotic relationship in LR was beneficial for carbon and micropollutants removal from domestic wastewater.

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1. Introduction

The use of photobioreactors with algal-bacterial consortia to treat wastewater has gained much attention recently due to significant pollutant removal in small reactor volume (Muñoz and Guieysse, 2006; Petrini et al., 2018; Su et al., 2011). A balanced purification process is achieved by combining the ability of algae to assimilate nutrients with the ability of bacteria to degrade organic matter. There are various types of photobioreactors such as tubular, plate, horizontal, foil, and porous substrate types (Schade and Meier, 2019). Among these, the porous substrate reactor can prevent washout of microorganisms and helps in providing high solid retention time (SRT) which results in a dense biofilm culture. In addition to easily-degradable organic matter and nutrients, various anthropogenic chemicals in wastewater present an important concern. Because of the inefficiency of conventional wastewater treatment processes, new strategies should be explored for removing such pollutants from wastewater (Krzeminski et al., 2019; Rogowska et al., 2019). Interestingly, recent studies have shown that photosynthesis-enhanced biodegradation occurs in an algal-bacterial consortium for detergents, pharmaceuticals, and organic solvents, especially the aromatic compounds (Borde et al., 2003; Subashchandrabose et al., 2011).

Domestic wastewater mainly contains pharmaceutical, pesticides, surfactants, polyaromatic hydrocarbons and flame retarders (Rowsell et al., 2010). Surfactants constitute >13% of the domestic wastewater composition (Rowsell et al., 2010). Linear alkylbenzene sulphonate (LAS) anionic surfactant is used as a key component in household detergents, dishwashing products, and multipurpose cleaner (Escrig-Doménech et al., 2016; Noutsopoulos et al., 2018). After its use, LAS is finally discharged through treated or untreated wastewater into aquatic ecosystems. The toxicity of LAS on aquatic life has been well reported (Lechuga et al., 2016). Another common micropollutant in wastewater is caffeine. Caffeine is a natural stimulant and most commonly used psychoactive drug in the world. In fact, caffeine has been suggested as a chemical marker for urban wastewater contamination in freshwater systems (Gonçalves et al., 2017).

The objective of this study is to evaluate the performance of the trickling filter-type photobioreactor with an algal-bacterial consortium in removing micro-pollutants from wastewater and to compare the performance with that of a similar system in dark condition. LAS and caffeine were selected as model aromatic and non-aromatic pollutants in this research, respectively. Synthetic wastewater containing LAS and caffeine was treated by a laboratory-scale reactor equipped with white LED light (light reactor, LR) and a reactor in dark condition (dark reactor, DR). These trickling filter-type reactors were packed with a semitransparent porous sponge as a substrate for supporting the biofilm growth of the microbial cultures. The trickling filter has a relatively low power requirement just for pumping the water to the top of the filter.

2. Materials and methods

2.1. Reactor configuration

Two identical trickling filters were fabricated. Fig. 1 shows the schematic diagram of the trickling filters. Each reactor had three cylindrical segments – top, middle, and bottom. Each segment was 31.5 cm in height and 14 cm in width (internal diameter). A collection segment (14 cm internal diameter and 15.7 cm height) was placed under the bottom segment to collect the effluents. Two openings (1 cm diameter) were made at the upper and the lower portion on the wall of each segment to allow the airflow inside the reactor. The selection of filter media is key for enhancing the performance of the trickling filter. The porous substrate can prevent washout of algal-bacterial cells and helps in providing high SRT which results in a dense biofilm culture. An aquarium filter made of semitransparent polyurethane (FILTERMAT, Gex Corp., Japan) was used as the sponge media. The void ratio of the filter was >98%. The filter was cut into small cubes 2 cm on each edge. Each piece of the sponge cube has a water-holding capacity of 5 mL. Each cylindrical segment was filled with 344 pieces of the sponge cube. The bulk sponge volume in each reactor was 1.72 L with 40% occupancy in the reactor. White color LED light strips were used for LR as a light
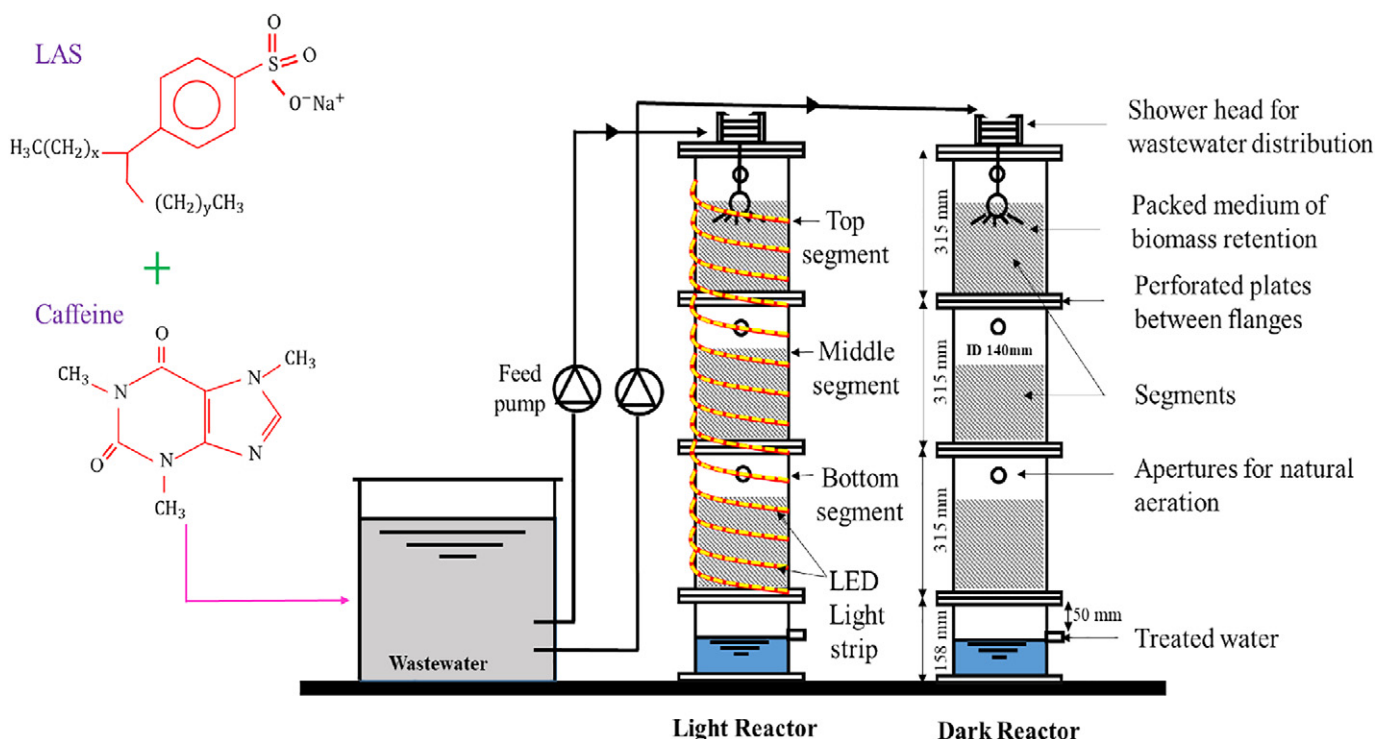

Fig. 1. Schematic diagram of tricking filters (LR and DR).

Table 1
Synthetic wastewater composition.

Compound	Concentration (mg/L)
Glucose	250
Ammonium chloride	190
Dipotassium phosphate	40
Potassium dihydrogen phosphate	15
Sodium bicarbonate	275
Sodium metasilicate nonahydrate	100
Linear alkylbenzene sulphonate	11.7
Caffeine	0.05
Iron(III) chloride hexahydrate	12

source for promoting algal growth. The LR was operated under light:dark cycle of 12:12 h with a quantum flux of 15 µmoles/m^2/s. Both the reactors were maintained at 25 °C and covered to prevent the entry of any external light other than LED for LR. The HRT was calculated based on the sponge volume.

2.2. Microorganisms

2.2.1. Algal-bacterial culture

The mixed algal-bacterial culture used in this study was collected from the surface of stones in a 40 cm deep pond, (N34° 58′ 34.806″ and E135° 58′ 8.1876″) located in Kusatsu, Shiga Prefecture, Japan. The biofilm of the stones was scraped off and washed with tap water. The biofilm sample was observed under a microscope to confirm the presence of green algae and diatoms. The harvested algae culture was centrifuged at 2000 rpm for 15 min and rinsed three times with deionized water. The culture was inoculated in 200 mL of a 1000-fold diluted solution of commercial liquid fertilizer (Hyponex 6–10-5, Hyponex Japan Corp., Japan) with 35 mg/L sodium silicate in a 250-mL Erlenmeyer

flask. After reaching the stationary phase, the algal-bacterial culture was transferred in a 12 L rectangular aquarium tank (10 L culture volume) with aquarium stones on the bottom. The tank was kept under electric light (12:12 light-dark cycle) at room temperature with the quantum flux of 32 µmoles/m^2/s and aeration at 1.5 L/min. The dissolved oxygen (DO) and mixed liquor suspended solids (MLSS) of the algal-bacterial culture was about 7.8 mg/L and 2720 mg/L.
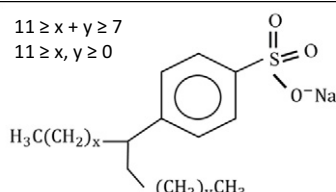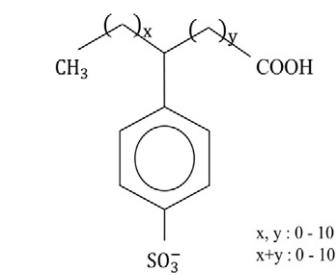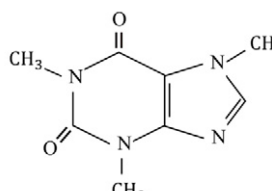
2.2.2. Bacterial culture

The activated sludge was cultured with a peptone stock solution (BOD = 20,000 mg/L; BOD volumetric loading = 0.4 kg/day/m^3) along with nutrients, minerals, and buffer base (Table S1) at room temperature. Aeration was provided in the reactor at 10 L/min using an aerator. The DO concentration in the reactor was between 7 and 8 mg/L. The volatile suspended solids content in mixed liquor was 1668 ± 220 mg/L.

2.3. Synthetic wastewater

Synthetic municipal wastewater (SMW) was prepared with LAS (11.7 ± 3 mg/L) and caffeine (0.05 ± 0.02 mg/L) along with nitrogen, phosphorus, and glucose. The composition of SMW is given in Table 1. The concentrations of organic carbon (TOC), total nitrogen (TN), and phosphorus (P) were 113.6 ± 4.5, 50.4 ± 2.2, and 7.5 ± 2.3 mg/L, respectively. The structural and physicochemical properties of LAS, sulfophenyl carboxylic acid (SPC), and caffeine was shown in Table 2. The distribution of homologues in the standard LAS solution (Fujifilm Wako Pure Chemical Corp., Japan) was as follows: C10 (13%), C11 (38%), C12 (31%) and C13 (18%). The pH of the SMW was adjusted between 7.5 and 7.8. The composition of SMW used in this study ranged from low to high strength municipal wastewater (Tchobanoglous et al., 2014).

Table 2
Structures and physicochemical properties of LAS and caffeine (National Center for Biotechnology Information, 2019a,b).

Compound	Structure		Molecular formula	Molecular weight (g/mol)	Log K_{ow}	pKa
LAS		LAS C10	$C_{16}H_{25}NaO_3S$	320		
		LAS C11	$C_{17}H_{27}NaO_3S$	334		
		LAS C12	$C_{18}H_{29}NaO_3S$	348	0.45	2.554
		LAS C13	$C_{19}H_{31}NaO_3S$	363		
		LAS C14	$C_{20}H_{33}NaO_3S$	377		
SPC (LAS metabolite)		SPC C4	$C_9H_{10}NaO_3SCOOH$	266		
		SPC C5	$C_{10}H_{12}NaO_3SCOOH$	280		
		SPC C6	$C_{11}H_{14}NaO_3SCOOH$	294		
		SPC C7	$C_{12}H_{16}NaO_3SCOOH$	308		
		SPC C8	$C_{13}H_{18}NaO_3SCOOH$	322		
		SPC C9	$C_{14}H_{20}NaO_3SCOOH$	336		
		SPC C10	$C_{15}H_{22}NaO_3SCOOH$	350		
		SPC C11	$C_{16}H_{24}NaO_3SCOOH$	364		
		SPC C12	$C_{17}H_{26}NaO_3SCOOH$	378		
		SPC C13	$C_{18}H_{28}NaO_3SCOOH$	392		
Caffeine			$C_8H_{10}N_4O_2$	194	-0.07	14

2.4. Experimental design (reactor operational conditions)

The reactors were operated under four different phases. In the first phase, for the purpose of seeding, biomass suspension with an equal amount of the activated sludge culture and the algal culture was pumped into the reactor through the recycle line for a week. During the second phase, the SMW without LAS and caffeine was fed to the re-actor at 12-h HRT for 15 days to promote the growth of biomass on sponge media. In the third phase, the SMW with LAS and caffeine was fed at 12-h HRT (34.6 g-LAS/m^3-sponge/day and 0.1 g-caffeine/m^3-sponge/day). The beginning of the third phase was taken as day 0. After the stabilization of removal efficiency in both reactors, the HRT was changed to 8-h (51.9 g-LAS/m^3-sponge/day and 0.15 g-caffeine/m^3-sponge/day) in the fourth phase. The effluent samples were col-lected thrice a week for TOC, TN, P, LAS and caffeine analysis. The sponge samples were collected once for each HRT to analyze the concentrations of retained sludge and chlorophyll.

2.5. Analytical methods

2.5.1. Determination of wastewater parameters (carbon and nutrients)

The collected effluent samples were filtered using a 0.45 μm filter paper. The P content in filtered samples was analyzed by following the Standard Methods for Water and Wastewater (APHA/AWWA/WEF, 2012). TOC and TN were measured using a TOC analyzer (TOC-V, Shimadzu, Kyoto, Japan). The detection limits for TOC, TN and P were 5 mg/L, 5 mg/L, and 10 μg P/L respectively.

2.5.2. Determination of biomass and chlorophyll

The sponge cubes (around 10 pieces) were collected from all seg-ments (top, middle, and bottom) of both the reactors. The biofilm in the sponge cubes was squeezed to measure biomass and chlorophyll concentration according to the Standard Methods (APHA/AWWA/WEF, 2012).
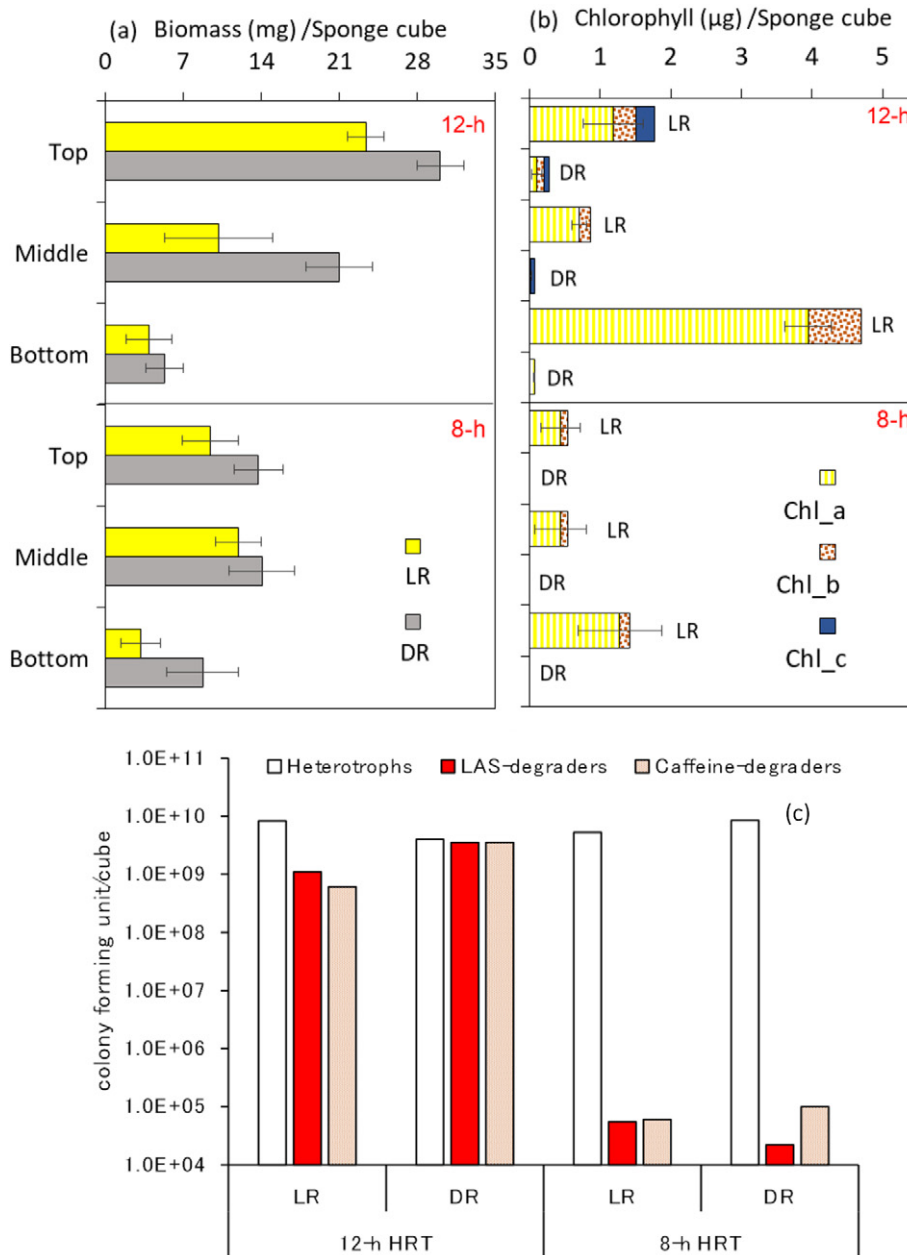

Fig. 2. Biomass profile in Light reactor (LR) and Dark reactor (DR) at 8 and 12-h HRTs (a) Biomass distribution along the height (b) chlorophyll distribution along the height (c) cell count of heterotrophic, LAS, and caffeine degraders.

2.5.3. Determination of LAS and caffeine

The concentrations of LAS (C10-C13 homologues) and caffeine were analyzed by liquid chromatography mass spectrometry using multiple reaction monitoring mode on LC-MS 8030 (Shimadzu; Japan). The LC column (Shim-pack VP-ODS, 2.0 mm i.d. $\times$ 150 mm length, 5 μm) was maintained at 40 °C. The flow rate of the mobile phase was 0.2 mL min^{-1} with isocratic elution (20:80 = 7.5 mM ammonium acetate: methanol). Samples of 10 μL volume were injected. Quantitative analysis was performed by external calibration using LAS and caffeine standards (FUJIFILM Wako Pure Chemical Corp., Japan). The limit of quantification for LAS and caffeine were 0.0001 mg/L and 0.0001 mg/L respectively. For quantifying the effluent LAS, an anionic surfactant mixture standard solution (FUJIFILM Wako Pure Chemical Corp., Japan) was used. Anionic mixture standard contains an equal mix of C10 to C14 LAS compounds. LAS breaks down to SPC during biodegradation. Due to lack of SPC standard, a semi quantification method was used to understand the SPC compounds (LAS metabolites). The peak area obtained for each SPCs (C4–C13) was divided by the LAS standard peak area. The mean area obtained for 0.1 mg/L of C10LAS from the standard mixture was used as the LAS standard peak area.

This value is expressed as the SPC value.

$$SPC\ value = (SPC\ homologue\ area)/(LAS\ standard\ area)$$

$$LAS\ standard\ area = Mean\ peak\ area\ of\ 0.1\,mg/L\ C10\ LAS$$

2.6. Enumeration of LAS and caffeine degraders

R2A agar (Merck and Co. Inc., Darmstadt, Germany) was used to enumerate heterotrophic bacteria. LAS-degrading bacteria were enumerated by a basal medium (BSM; K_2HPO_4, 1.0 g/L; $(NH_4)_2SO_4$, 1.0 g/L; $MgSO_4 \cdot 7H_2O$, 0.2 g/L; $FeCl_3$, 0.01 g/L; NaCl, 0.05 g/L; and $CaCl_2$, 0.05 g/L) containing 50 mg/L LAS as the sole carbon source and 15 g/L agar. Similarly, caffeine-degrading bacteria were enumerated by the BSM containing 500 mg/L of caffeine and 15 g/L agar. The bacteria in the top segment of the reactor were analyzed before changing HRT. The colony-forming units (CFU) were counted after 72 h of incubation at 28–30 °C.

2.7. Statistical analysis

To determine the significant difference between LR and DR during wastewater treatment, Welch's t-test was performed. The micro (LAS and caffeine) and macro (carbon, nitrogen and phosphorous) pollutant removal at different HRTs were analyzed. The p-value (p ≤ 0.05) were considered as significantly different. The statistical analysis was performed using Excel 2013, Data Analysis Tool.

3. Results and discussion

3.1. Algae/bacteria in retained biomass

The vertical distribution of biomass and chlorophyll attached to sponge cubes is shown in Fig. 2(a & b). After 20 days of reactor operation at 12-h HRT the average biomass concentration attached to sponges in the top segment was 23.4 ± 1.6 and 9.5 ± 2.5 mg/sponge cube at 12- and 8-h HRT in LR and 30.1 ± 2.1 and 14 ± 2.5 mg/sponge cube in DR respectively. The biomass in LR was less compared to DR. The biomass concentration in the lower part of the reactors was observed to be less in both the reactors. Whereas the chlorophyll concentration in the retained biomass increased along with the reactor depth.

The chlorophyll concentration in LR was highest in the bottom segment with 4.7 and 3.4 μg/sponge cube at 12 and 8-h HRT respectively. The chlorophyll concentration decreased by 70% in the top segment with a decrease in HRT in LR. Although both reactors were seeded with the same biomass (sludge + algae), the chlorophyll concentration in DR eventually became almost zero. The ratio of chla/chlb or chla/chlc suggests a balance of phytoplankton communities (diatoms and green algae). The initial chla:chlb:chlc concentration of the algal-bacterial culture was 2.9:0.17:0.3. After reaching steady-state, the chlc value was zero in middle and bottom segments at 12-h HRT and zero in all segments at 8-h HRT in LR. This suggests the domination of green algae over diatoms (Maina and Wang, 2015). The algal growth is comparatively less than the bacterial growth and mostly species-dependent. The maximum growth rates for diatoms vary between 2.25 and 2.5 d^{-1} (Park et al., 2005) and chlorella ranged between 0.79 and 5.9 d^{-1} (Huesemann et al., 2016; Sasi et al., 2011; Wu et al., 2013). The decrease in algal ratio mainly in top segments at high organic loading rate suggests the role of bacteria domination in utilizing the macro pollutants to proliferate.

The population of heterotrophic bacteria, LAS and caffeine degraders in the top segment of the reactors are shown in Fig. 2(c). There was no apparent difference in the heterotrophs count in both rectors. However, the number of LAS and caffeine degraders in both reactors drastically decreased by 4–5 orders of magnitude with a decrease in HRT. In LR the number of cells per sponge cube degrading LAS and caffeine decreased from 1.1×10^9 to 5.5×10^4 and 6.1×10^8 to 6×10^4 with a decrease in HRT. Similarly, in DR, with decreasing HRT the LAS and caffeine degrading bacterial count per sponge cube decreased from 3.6×10^9 to 2.2×10^4 and 3.6×10^9 to 1×10^5 respectively. The LAS and caffeine
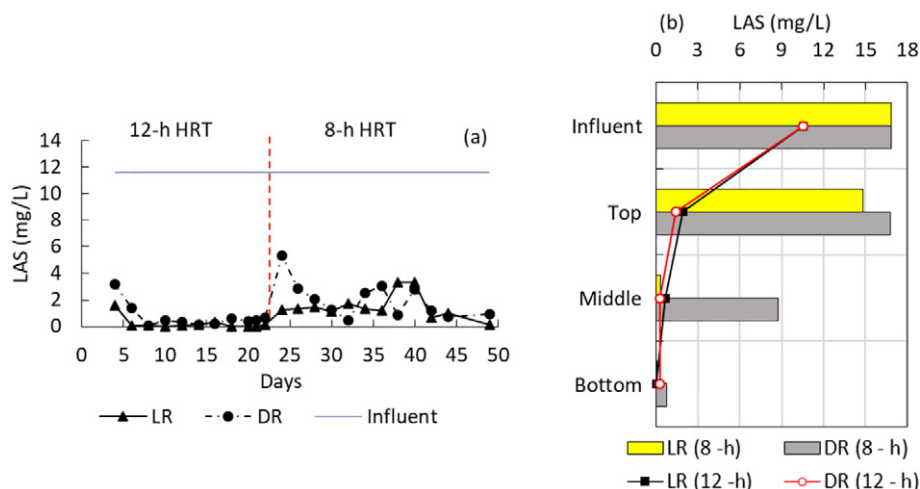

Fig. 3. LAS profile in LR and DR at 8 and 12-h HRTs (a) daily effluent values (b) segment wise distribution.

Table 3
Performance of the reactors in wastewater treatment.

Parameters (removal %)	HRT = 12 h		HRT = 8 h	
	LR	DR	LR	DR
LAS	99 ± 1*	95.6 ± 3.9	95.6 ± 3.4*	78.9 ± 15.1
Caffeine	96.3 ± 1.9	96.0 ± 1.7	86.2 ± 7.1	78.6 ± 10.8
TOC	85.5 ± 1.8	87.8 ± 1.3*	90.5 ± 1.3*	84 ± 3.8
TN	15 ± 4.2	17.6 ± 7.2	24.2 ± 3.6	18.9 ± 6.8
P	48.9 ± 3.1*	43.1 ± 6.0	36.9 ± 1.1	37.3 ± 1.4

* Values with p-value ≤ 0.05 for LR vs DR.

degraders were actually retained in the reactor at 12-h HRT although those bacteria rather than algae were washed out from the reactor at 8-HRT.

3.2. LAS removal

Fig. 3(a) shows the effluent LAS concentrations (sum of C10, C11, C12, C13 LAS) over time of LR and DR. The influent LAS concentration was 11.7 ± 3.0 mg/L, and average LAS effluent concentrations at 12-h HRT in LR was 0.1 ± 0.1 mg/L and in DR was 0.44 ± 0.39 mg/L. At 8-h HRT the average residual LAS concentration was 0.53 ± 0.41 mg/L in LR and 2.56 ± 1.84 mg/L in DR. The decrease in HRT from 12- to 8-h had shown an effect on the LAS removal, there was an increase in effluent LAS values in both the systems. This indicates that both reactors required acclimation time to remove LAS at a short retention time (higher loading rate).

The distribution of LAS along with the reactor depth after reaching the steady-state is shown in Fig. 3(b). The effluent LAS concentration in top, middle, and bottom segments of LR at 12-h HRT was 1.95, 0.65, and 0.03 mg/L and in 8-h HRT was 14.84, 0.31, and 0.10 mg/L, respectively. Similarly, the distribution of LAS in top, middle, and bottom segments of DR at 12-h HRT was 1.41, 0.31, and 0.31 mg/L and in 8-h HRT was 16.78, 8.73, and 0.76 mg/L respectively. At longer HRT (12-h), LAS removal in both LR and DR after the top segment was above 88%. At 8-h HRT, 98% removal was observed below the second segment (depth: 63 cm) in LR but only 49.5% in DR. It was observed that the decrease in biomass and LAS degraders in top segment at 8-h HRT had shown more effect on DR compared to LR emphasizing the role of algae in LAS removal.

The performance of reactors in removing LAS from wastewater is shown in Table 3. The LAS removal in LR at 12- and 8-h HRT was 99 ± 1% and 95.6 ± 3.4% respectively. DR showed 95.6 ± 3.9% removal at 12-h HRT and 78.9 ± 15.1% removal at 8-h HRT. The decrease in

retention time had negatively affected the reactor performance, both the reactors showed better performance at 12-h HRT (p ≤ 0.05) compared to 8-h HRT. Eslami et al. (2017) reported 94% LAS removal with the influent concentration of 4–6 mg/L by the Integrated Fixed Film Activated Sludge reactor. LAS removal of 96.4% was reported by Seyedsalehi et al. (2018) while treating urban wastewater using sequencing batch biofilm reactors with a movable bed at 2.5 h HRT, with influent LAS concentration of 15 mg/L. It was observed that the LR showed better effluent quality compared to DR in LAS removal (p ≤ 0.05) in both the HRTs. In this study, the maximum removal was observed at 12-h HRT in LR with 99% LAS removal.

3.2.1. SPC - intermediate metabolites of LAS

The concentration of LAS homologues in LR and DR is shown in Fig. 4. The mean influent concentration (mg/L) of C10:C11:C12:C13 LAS homologues was 1:4:4:2. The average effluent concentrations (mg/L) of C10:C11:C12:C13 LAS homologues in LR at 12- and 8-h HRT were 0.02:0.05:0.02:0.01 and 0.5:0.8:0.4:0.2 respectively. Similarly, the effluent concentrations (mg/L) of C10:C11:C12:C13 LAS for 12- and 8-h HRT in DR was 0.13:0.21:07:0.02 and 0.5:0.9:0.3:0.1, respectively. A significant difference in the removal of LAS homologues was observed at 12-h HRT with LR showing lower concentrations of C10 and C11 LAS (p ≤ 0.05). At 8-h HRT there was no significant difference in the C10–C13 LAS homologues in both LR and DR. The decrease in HRT had increased the concentration of LAS homologues in the effluent. Both reactors showed lower concentrations of C10–C13 LAS at 12-h HRT compared to 8-h HRT (p ≤ 0.05). The LR showed lower concentrations for C10 and C11 LAS than the DR at 12-h HRT, indicating rapid primary biodegradation (ω-oxidation).

Fig. 5 shows the profile of individual SPC (C4–C13) in the treated water at 12- and 8-h HRT. The initial step in the aerobic degradation of LAS begins with the ω-oxidation of the terminal methyl group of the alkyl chain of LAS (Schoberl, 1989), which generates an SPC with the same number of carbon atoms as the parent molecule. As the initial attack on the alkyl chain can take place on both sides of the phenyl substituent, a broader range of C4–C13 SPC alkyl homologues can be formed, which is a more complex mixture than the parent compound. At 8-h HRT, LR showed lower C4–C9 SPCs and C13 SPC (p ≤ 0.05) compared to 12-h HRT SPCs profile. Similarly, DR showed lower C4–C6, C9, and C12 SPCs (p ≤ 0.05) at 8-h HRT compared to 12-h HRT SPCs profile. The LR showed lower C4, C10, and C11 SPC concentrations at 12-h HRT (p ≤ 0.05). At 8-h HRT LR showed lower C10–C13 SPCs compared to DR (p ≤ 0.05) indicating rapid β-oxidation than the DR. The LR showed rapid primary degradation (ω-oxidation) of LAS and β-oxidation of
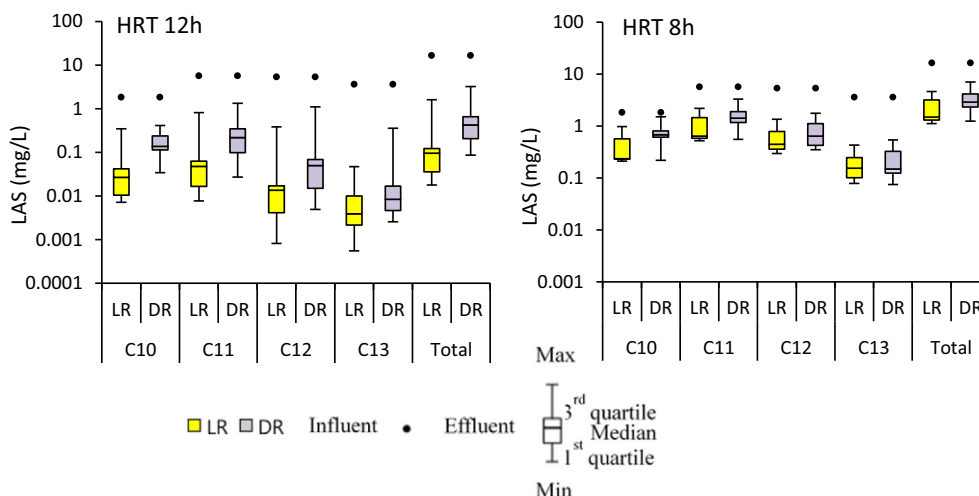

Fig. 4. Individual homologue (C10–C13 LAS) profile in LR and DR at 8 and 12-h HRTs.
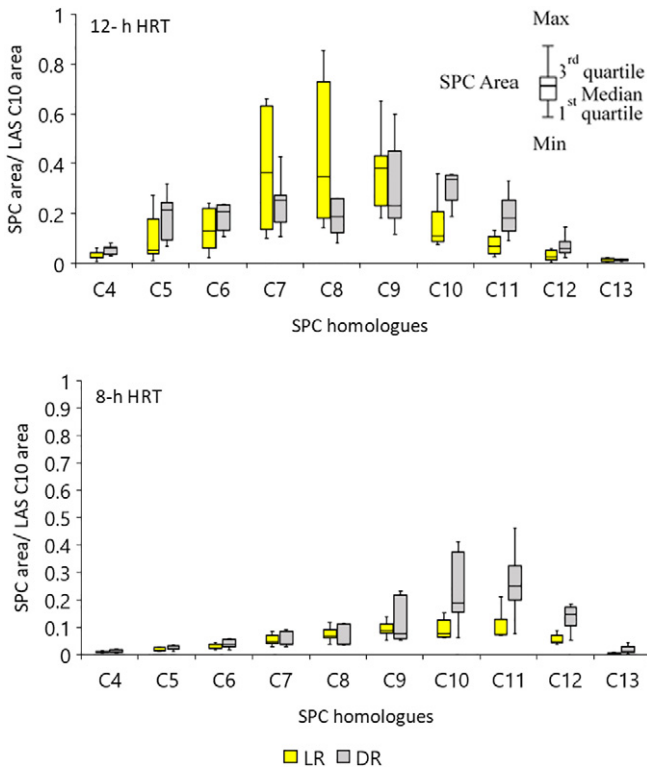
Fig. 5. LAS metabolites (SPC (C4–C13)) profile in the treated water at 8 and 12-h HRTs in LR and DR.

SPCs than the DR. However, with the decrease in HRT there was a significant difference in SPCs profile in both the reactors.

The mechanisms for the micropollutant removal in algal-bacterial systems are biodegradation by bacteria, uptake by algal cells, evaporation, and photodegradation (Bai and Acharya, 2017; Matamoros et al., 2016). Hua et al. (2012) reported that the removal of LAS was found to be dominated by the photosynthetic activities in biofilms, with lesser roles attributed to biodegradation and adsorption by the biofilms, and the role of direct photolysis was negligible. During photochemical reactions, free radicals formed by light energy or through algal photosynthesis play a major role in exciting the chemical species (Marshall et al., 2002). The most common free radicals observed in both the mechanisms are hydroxyl ($\cdot$OH), carbonate ($\cdot CO_3^-$), and peroxide (Abargues et al., 2018). These oxygen species were found to be highly reactive

and have the ability to decompose the oxygen-consuming organics effectively (Peng et al., 2006; Zepp and Schlotzhauer, 1983). Thus, there might be some role played by the reactive oxygen species in the breakdown of LAS. The LAS degraders cell count for LR at 12-h HRT was less than DR; however, with an increase in loading rate (at 8-h HRT) the LAS degraders count in DR drastically decreased. At 8-h HRT the LAS degraders in LR were twice than in DR. When microalgae and bacteria are in symbiotic association, the microalgal activities would enhance or stimulate bacterial activity. The presence of SPCs in the effluent suggests the primary oxidation of LAS. The toxicity of the LAS compound was reduced after the primary biodegradation step which would have assisted the growth of LAS tolerant/degrading bacteria in LR.

3.3. Caffeine

The daily variation and segment-wise distribution during the reactor operation of LR and DR in removing caffeine are shown in Fig. 6. The distribution of caffeine along the depth of the reactor is shown in Fig. 6(b). The influent caffeine concentration of 0.05 ± 0.02 mg/L, was reduced to 0.002 ± 0.001 mg/L at 12-h HRT in both reactors. After decreasing HRT to 8-h the caffeine concentration in effluent was 0.007 ± 0.004 mg/L and 0.011 ± 0.005 mg/L in LR and DR respectively. The caffeine removal in LR at 12- and 8-h HRT was 96.3 ± 1.9% and 86.2 ± 7.1%. The DR showed 96 ± 1.7% removal at 12-h HRT and 78.6 ± 10.8% removal at 8-h HRT. The caffeine concentration below the top segment reduced >70% in both reactors although there was a decrease in caffeine degraders with decreasing the HRT to 8-h.

Caffeine which is neutral at the studied pH (pKa = 14) could be removed from the reactors by either sorption or biodegradation. A preliminary batch test was conducted to observe the adsorption of LAS and caffeine onto the sponge media for 7 days (data not shown). No significant change in the LAS and caffeine concentrations were observed indicating the adsorption onto the sponge was negligible. The bacterial degradation of caffeine initiates with the parallel conversion of caffeine to paraxanthine and theobromine parallelly by demethylases. The oxidation of xanthine, mono, and dimethylxanthine was followed by the purine catabolism pathway (Asano et al., 1993; Blecher and Lingens, 1977). The bacterial strains *Pseudomonas*, *Alcaligenes*, *Aspergillus*, *Serratia*, *Penicillium*, *Klebsiella*, *Stemphylium*, *Rhizopus*, *Rhodococcus*, and *Phanerochaete* have been reported to have the ability to degrade caffeine (Asano et al., 1993; Madyastha et al., 1999). These bacterial species were able to utilize caffeine as a sole source of carbon, nitrogen, and energy for growth (Chi et al., 2009; Mohanty et al., 2012). It was reported that algae can increase the caffeine removal efficiency of the reactor by either releasing exudates, which aid the biodegradation processes (Subashchandrabose et al., 2011),
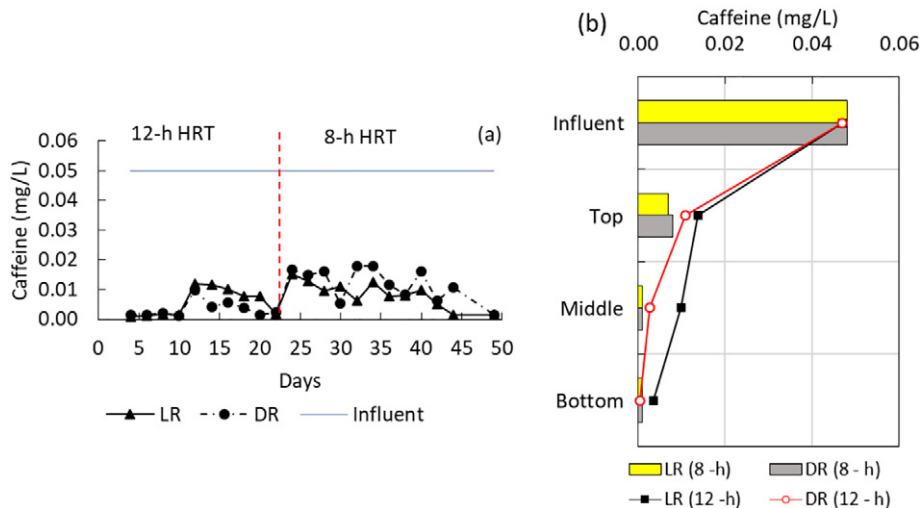

Fig. 6. Caffeine profile in LR and DR at 8 and 12-h HRTs (a) daily effluent values (b) segment wise distribution.
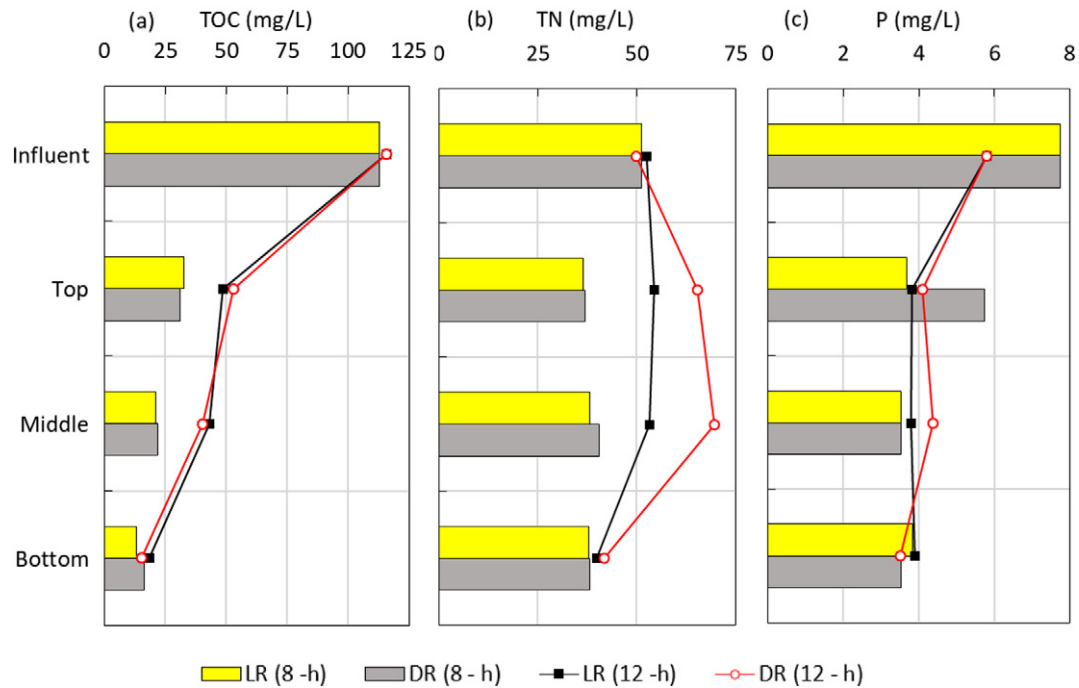
Fig. 7. The effluent quality profile along the reactor depth in LR and DR at 8 and 12-h HRTs (a) TOC, (b) TN, and (c) P.

or by microalgal uptake. Nevertheless, due to the fact that algae fed with nutrients were not capable of removing caffeine (Matamoros et al., 2016), it can be hypothesized that the main removal process of this compound is through bacterial biodegradation and the microalgae uptake is unlikely. On the other hand, no apparent difference was observed in LR and DR during caffeine removal in both HRTs (p-value is >0.05). Whereas the higher HRT (12-h) had shown better performance in removing caffeine in both LR and DR (p ≤ 0.05).

3.4. Performance of reactors

The overall performance of the two reactors with respect to TOC, TN and P removal are presented in Table 3. The initial wastewater concentration of TOC reduced from 113.6 ± 4.5 mg/L to 16.5 ± 2.1 mg/L at 12-h HRT and to 10.8 ± 1.5 mg/L at 8-h HRT in LR. The TOC in DR effluent water was 13.9 ± 1.5 mg/L in 12-h HRT and 18.1 ± 4.3 mg/L in 8-h HRT. The TN concentration reduced from 50.4 ± 2.2 mg/L to 42.9 ± 2.1 and 38.3 ± 1.8 mg/L for 12 and 8-h HRT in LR, and to 41.5 ± 3.6 and 40.9 ± 3.4 mg/L in DR respectively. The P content reduced from 7.5 ± 2.3 mg/L to 3.8 ± 0.2 and 4.7 ± 0.1 mg/L for 12 and 8-h HRT in LR, and to 4.3 ± 0.4 and 4.7 ± 0.1 mg/L in DR. The LR showed a significant difference (p ≤ 0.05) in TOC, LAS, and P removal compared to DR. The LR showed higher TOC and TN removal at 8-h and higher P removal at 12-h HRT than the DR (p ≤ 0.05). The P removal in LR (49%) was slightly higher than DR (43%) at 12-h HRT whereas at 8-h HRT both reactors showed almost the same performance. In LR, the TN and TOC removal was positively affected by decreasing HRT whereas the P removal decreased from 48.9 to 36.9%. In DR, no difference was observed in TOC and TN removal in 12- and 8-h HRTs whereas the P removal decreased from 43.1 to 37.3%. The decrease in HRT had shown a negative effect on the P removal in both reactors. The effluent quality profile along the reactor depth is shown in Fig. 7. It suggests that >50% of the TOC removal occurred in the first segment of the two reactors in both HRTs.

4. Conclusion

In this study, a trickling filter-type photoreactor packed with the semi-transparent porous sponge was operated for removing LAS and caffeine from wastewater under light and dark conditions. The trickling filter has a relatively low power requirement just for pumping the water to the top of the filter. The LR exhibited better performance than DR in removing the organic carbon and LAS. The porous substrate (sponge) helped in the formation of dense biofilm culture. Longer retention time (12 h) is required for preserving the algae-bacteria consortium (especially for LAS- and caffeine-degrading bacteria) in the reactors. At 12-h HRT, LR showed rapid ω-oxidation of LAS and β-oxidation of SPCs than DR. The SPC metabolite profile indicated the benefits of free radicals either produced by algae during photosynthesis or through photodegradation. Further studies are needed for elucidating the photosynthesis-enhanced mechanism for the removal of micropollutants. This trickling filter type algal-bacterial photobioreactor is a promising technology that can remove both macro and micropollutants in the same system with a lesser footprint.

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