Study of the efficiency of moving bed biofilm reactor (MBBR) in LAS Anionic Detergent removal from hospital wastewater: determination of removing model according to response surface methodology (RSM)

Reza Shokoohi, Zahra Torkshavand, Hassan Zolghadnasab, Mohammad Yousef Alikhani and Meisam Sedighi Hemmat

ABSTRACT

Detergents are considered one of the important pollutants in hospital wastewater. Achieving efficient and bio-friendly methods for the removal of these pollutants is considered as a concern for environmental researchers. This study aims at studying the efficiency of a moving bed biofilm reactor (MBBR) system for removing linear alkyl benzene sulfonate (LAS) from hospital wastewater with utilization of response surface methodology (RSM). The present study was carried out on a reactor with continuous hydraulic flow using media k_1 at pilot scale to remove detergent from hospital wastewater. The effect of independent variables including contact time, percentage of media filling and mixed liquor suspended solids (MLSS) concentration of 1000-3000 mg/l on the system efficiency were assessed. Methylene blue active substances (MBAS) and chemical oxygen demand (COD) 750-850 mg/l were used by closed laboratory method in order to measure the concentration of LAS. The results revealed that the removal efficiency of LAS detergent and COD using media k_1, retention time of 24 hours, and MLSS concentration of around 3,000 mg/l were 92.3 and 95.8%, respectively. The results showed that the MBBR system as a bio-friendly compatible method has high efficiency in removing detergents from hospital wastewater and can achieve standard output effluent in acceptable time.

Key words | hospital wastewater treatment, LAS Anionic Detergent, MBBR, modelling

Reza Shokoohi
Zahra Torkshavand
Hassan Zolghadnasab
Meisam Sedighi Hemmat (corresponding author)
Department of Environmental Health Engineering,
School of Public Health,
Hamadan University of Medical Sciences,
Hamadan,
Iran
E-mail: *meisam2005_r@yahoo.com*

Mohammad Yousef Alikhani
Department of Microbiology,
Hamadan University of Medical Sciences,
Hamadan,
Iran

INTRODUCTION

Linear alkyl benzene sulfonate (LAS) is one of the most important anionic surfactants with high global production, mainly found in hospital wastewater, which has a significant effect on the performance of microorganisms in wastewater biological treatment. Structurally, it consists of a hydrophobic region, ranging from 10 to 14 carbon atoms, and a hydrophilic region corresponding to a sulfonated aromatic ring (Garcia *et al.* 2006; Delforno *et al.* 2014; Razavi *et al.* 2016). Once used in detergents, vegetables and fruits, LAS can reach the environment as a component of the effluents of sewage treatment plants (Sanz *et al.* 2003). LAS is one of the largest anionic surfactants with biological degradation capacity of around 97–99%, which can replace the alkyl benzene in non- linear sulphonates (Gupta *et al.* 2003; Petrovic *et al.* 2007). The moving bed biofilm reactor (MBBR) system was developed in Norway about 30 years ago for the treatment of wastewater. This reactor, with moving suspended beds, provides a surface for the growth of microorganisms (Zhang *et al.* 2014; Mazioti *et al.* 2015). The main advantage of the biofilm reactor with moving bed is the capability of accumulating suspended biomass and biofilm in a single reactor. This property and advantage provides the possibility of the presence of a large number of different types of microorganisms in the system (Dvořák *et al.* 2014). The use of new hybrid systems for the treatment of different kinds of wastewater has become a necessity for the purpose of achieving high quality output wastewater to maintain surface water (Park *et al.* 2011; Leyva-Díaz *et al.* 2016), reuse of wastewater, and considering the limited land available in the majority of hospitals in the country (9). To this end, the efficiency and performance of new hybrid systems in the removal of different kinds of pollutants

doi: 10.2166/wst.2018.014

have been proved and also numerous investigations have been carried out on these systems (Leyva-Díaz *et al.* 2016). Thus, the present study aims at studying the efficiency of a biofilm reactor system with moving bed (MBBR) for the purpose of removing LAS Anionic Detergent from hospital wastewater.

METHODS AND MATERIALS

Pilot-scale MBBR

Real influent wastewater was supplied from Beasat Hospital in the city of Hamadan, Iran. In this study, an MBBR reactor with a volume of 100 liters at pilot scale with up-flow and continuous hydraulic flow was used. The reactor was made from Plexiglas in cylindrical shape with an inner diameter of 30 cm, total height of 150 cm and effective volume of 90 liters. The air required to supply the dissolved oxygen and rotate the substrate material in reactor volume is entered into the system using an air compressor and through hoses embedded in the bottom of the reactor. Kaldnes media (k_1) (Pakan Ghatreh, Iran) was used as the bed to study the efficiency of MBBR system in removing LAS Anionic Detergents from hospital wastewater. This media was made from polyethylene (PE) with special weight of 0.96 gr/cm^3 and surface area of 500 m^2/m^3 for the growth of biofilm. The general scheme of the pilot is shown in Figure 1. Raw hospital wastewater was fed into the reactors using a peristaltic pump with flow rates ranging from 0.001 to 0.003 L/s in up flow mode for the desired hydraulic retention time (HRT).

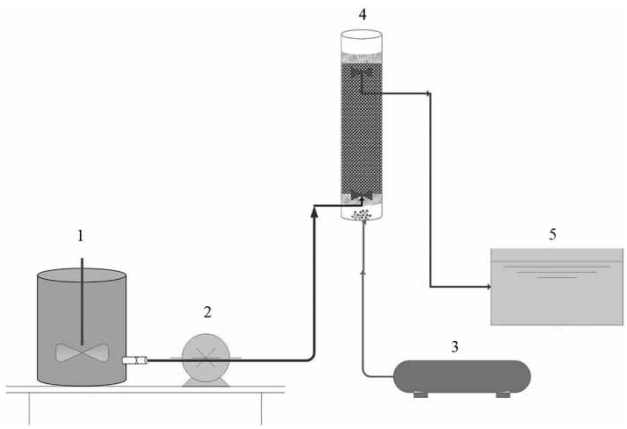

Figure 1 | The general scheme of the pilot used in this research (1-Wastewater injection tank; 2- Injection pump; 3- Air compressor; 4- Bioreactor; 5- Secondary settling tank).

Reactor setup

In the beginning, 30 percent of the reactor volume was filled with Kaldnes media for initial setup of the reactor. Then, more than a third of the volume of the reactor was filled with return sludge from the secondary sedimentation basin of the hospital treatment plant and the rest of the volume of reactor was filled with wastewater so that the reactor is used discontinuously. The amount of dissolved oxygen in this study was around 2 to 3.5 mg/l. An automatic aquarium heater with temperature set between 22 to 27 °C was used for the optimal activity of microorganisms and temperature maintenance. It should be mentioned that the pH of hospital wastewater ranged between 7.5 and 8.5. In this study, effective factors in microorganism's activity including temperature, dissolved oxygen, pH and other parameters were continuously assessed. Ten weeks after the reactor setup, significant biofilm growth was observed on the Kaldnes media and after this stage, the reactor entered into continuous mode in order to be used for studying the amount of detergent removal from hospital wastewater in different conditions. In this study, the experiments associated with anionic detergent concentration (LAS) were assessed using methods mentioned in the standard method book (554 c) (APHA/AWWA/WEF 2005).

Experimental design

The effect of three main parameters, namely packing rate, HRT, and mixed liquor suspended solids (MLSS) concentration (Table 1) were surveyed for the efficiency of the system in terms of LAS Anionic Detergent removal (%). In the present study, to collect the maximum information with fewer number of experiments, a Central Composite Design (CCD) (one of RSM family designs) was applied for modeling and analysis of the experiments using Design- Expert 7.0 software. Table 2 shows the experimental conditions according to the factorial design.

Table 1 | Independent variables and their levels used in the response surface design

		Coded level		
Factors	**Symbol**	**−1**	**0**	**1**
MLSS concentration	A	1,000	2,000	3,000
HRT	B	8	16	24
Packing rate	C	30	50	70

Table 2 | Response surface CCD design and results

Run	A: MLSS (mg/L)	B: HRT (hr)	C: Packing rate (%)	Removal efficiency
1	2,000	16	50	48.3
2	2,000	16	70	72.2
3	3,000	16	50	61.2
4	2,000	16	50	48.3
5	3,000	8	30	31.4
6	3,000	24	70	92.3
7	2,000	24	50	57.3
8	2,000	16	30	30.1
9	1,000	8	70	62
10	2,000	16	50	48.3
11	1,000	24	70	73
12	2,000	8	50	41.8
13	2,000	16	50	48.3
14	1,000	16	50	44.3
15	2,000	16	50	48.3
16	1,000	24	30	31
17	3,000	8	70	77.7
18	1,000	8	30	17.6
19	2,000	16	50	48.3
20	3,000	24	30	52

Isolation and identification of bacteria

The microbial carriers were separated from the reactor and placed in a phosphate buffer solution of 0.1 mM. Then the microbial layer was removed from the carriers and homogenized for 10 minutes. The isolated bacteria from microbial layers were identified using biochemical standard methods such as Gram and spore staining, catalase, oxidase, Motility test medium (SIM), Methyl Red and Voges Proskauer (MR-VP) Broth, Triple Sugar Iron (TSI), Kligler Iron Agar (KIA), Simon citrate, urease, oxidative-fermentative (OF), Phenylalanine deaminase and gelatin hydrolysis tests. Materials required for initial isolation and identification of isolated bacteria were purchased from Merck and Sigma Aldrich Companies.

DISCUSSION AND FINDINGS

Determining the effect of MLSS concentration on process efficiency

The results of this stage of the experiment are shown in Figure 2. In this stage, the effects of MLSS concentration (1,000, 2,000 and 3,000 mg/l) on the anionic detergent removal efficiency at retention times of 8, 16 and 24 hours were studied. As shown in Figure 2, with increasing retention time and filling percentage of the system, the efficiency of the process increase in terms of the anionic detergent removal (LAS). Results reveal that the proper designing of the system consists of aeration, retention time, choosing appropriate bed and filling rate of the tank are very effective in achieving the desired result (Lee *et al.* 2006). According to the results obtained at higher concentration of MLSS and longer retention times, the efficiency rate of the system increases. The higher efficiency of the system could be associated with the growth of biofilm and growth in the number of microorganisms at higher retention time (Delnavaz *et al.* 2008).

Study of the bacteria identified and detached from biofilm

The bacteria that were identified in this study consisted of *Escherichia coli*, *Enterobacter* spp., *Acinetobacter* spp. and *Pseudomonas* spp. In the study conducted by Wagner on the population of bacteria available in the wastewater treatment systems, more than 750 species of bacteria were analyzed and these species of bacteria play an important part in suspended growth and biofilm systems (Wagner & Loy 2002). Also, in other studies carried out on the dominant bacteria in the biofilm formed in connected growth systems (biofilm), the existence of a variety of bacteria has been proved. In a study conducted by Satu and colleagues on the active microbial population biomembrane reactor system, the frequency percentage for Alpha – Beta – Gamma bacteria and Epsilon proteo-bacteria, Espingo-bacterium were 9.3, 13.6, 39.4, 5.6,9.3 and 14, respectively (Sato *et al.* 2015). In a study conducted by Yang and colleagues on physicochemical characteristics and microbial community evolution of biofilms during the start-up period in a moving bed biofilm reactor, Gram-negative bacteria mainly including *Sphaerotilus*, *Zoogloea* and *Haliscomenobacter* were predominant in the initial stage (Zhu *et al.* 2015).

Determining the amount of COD removal

The results of this stage of the experiment have been illustrated in Figure 3. It was observed that under 30 percent filling of the reactor with media, the removal efficiency of chemical oxygen demand (COD) at hydraulic retention times of 8, 12 and 24 hours were 62.6, 78 and 86.9%, at

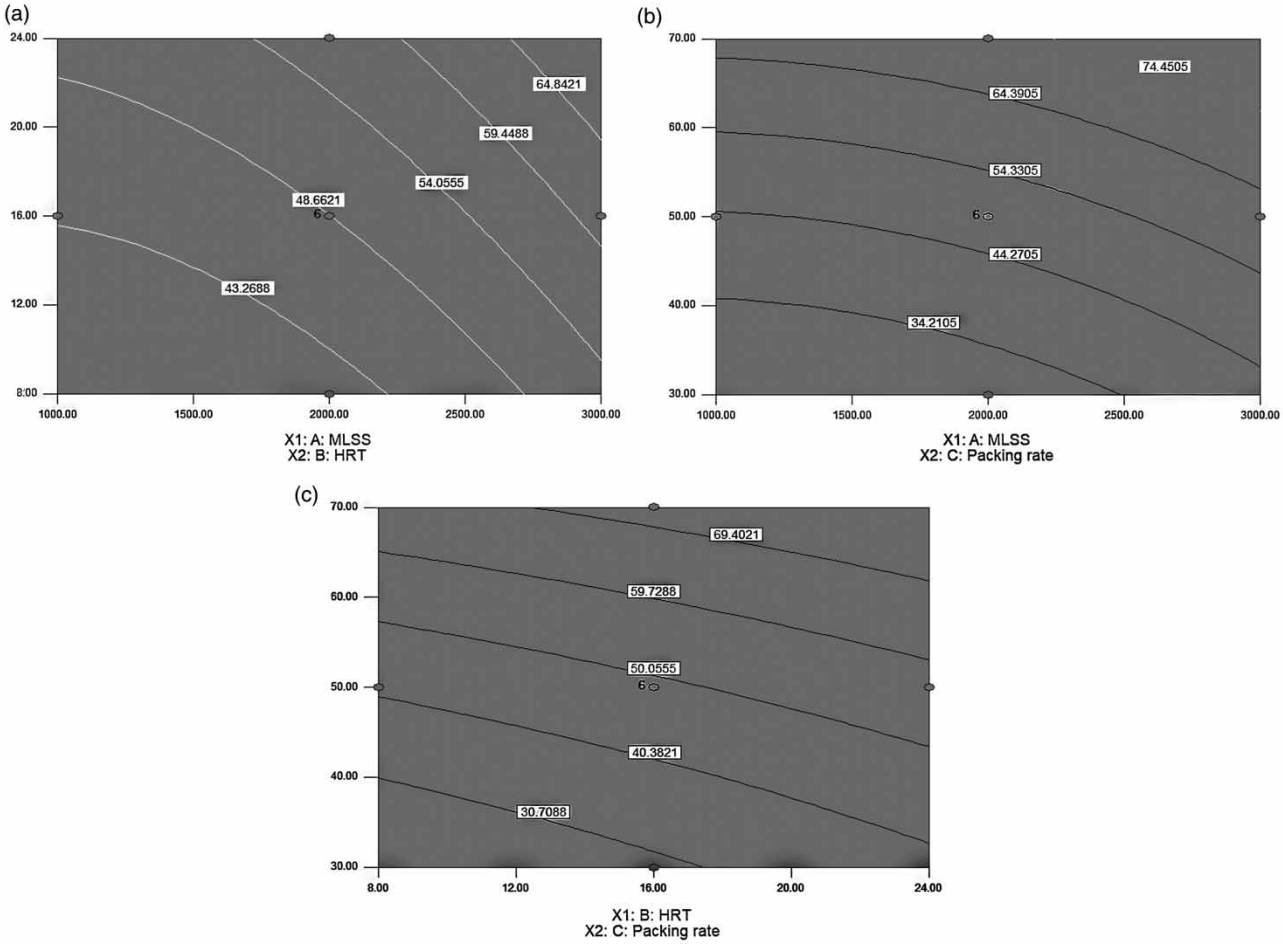

Figure 2 | Determining the effect of MLSS concentration on the efficiency of Anionic Detergent (LAS) removal.

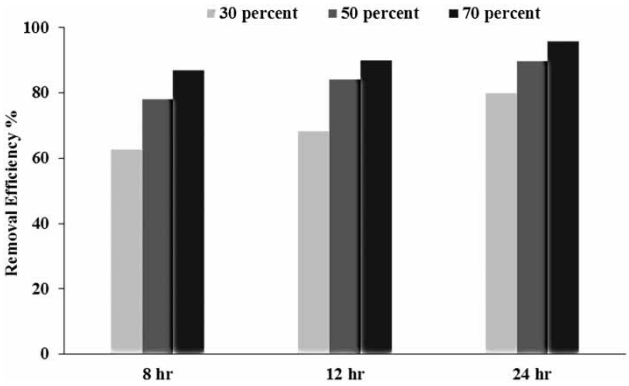

Figure 3 | COD removal efficiency in 30, 50, and 70 percent reactor filling with k_1 media (MLSS = 3,000 mg L^{-1}).

50 percent filling, the removal efficiencies of COD were 68.3, 54.2 and 89.9% and for 70 percent reactor filling, the removal efficiencies of COD were 80, 89.8 and 95.8%, respectively. In biofilm systems, treatment efficiency depends on the access level for biofilm growth and this action is associated with the characteristics and rate of reactor filling with media. Hence, it could have significant effect on the biological activity of the microbes in the bioreactor (Feng *et al.* 2012). Thus, increasing the percentage of filling raises the surface for the growth of biofilm. In the study carried out by Feng and colleagues on the removal of organic compounds and nitrogen using MBBR system, similar results were obtained (Feng *et al.* 2012). In a study conducted by Delforno and colleagues on the removal of anionic surfactant in an expanded granular sludge bed reactor, it was observed that surfactant addition and change of HRT did not affect the COD removal (Delforno *et al.* 2012).

Statistical analysis and the model fitting

In this study, the CCD model was used to evaluate the interactive effect of parameters for optimizing the removal of

LAS Anionic Detergent from hospital wastewater. There were a total of 20 runs for optimizing the variables in the CCD statistical design. Design-Expert was used for analysis of variance (ANOVA) software analysis of the experimental data obtained earlier. The model was found to be adequate for predictions made within the range of experimental variables. The model expressed by Equation (1), where the variables take their coded values, represents the efficiency of LAS removal (Y) as a function of MLSS concentration (A), HRT (B) and packing rate (C).

$$Y = +48.62 + 8.67*A + 7.51*B + 21.51*C + 1.35*A*B + 0.025*A*C - 1.05*B*C + 3.64*A^2 + 0.44*B^2 + 2.04C^2 \quad (1)$$

The statistical significance of the factors and their interactions at various levels of probability are shown in Table 3. The value of determination coefficient (R^2) of 0.9993 was obtained for removal efficiency of LAS Anionic Detergent. This indicates that more than 95% of the total variability could be explained by the quadratic model. The adjusted regression values for removal of LAS was more than 0.9986, which suggested that very good correlations between the response and variables. Note that P value $<$ 0.05 implies that the model terms are significant at confidence level of 95% or more, while values greater than 0.10 indicate that the model terms are not significant. A relatively low value of coefficient of variance of the model for LAS removal was obtained 1.3%, implying precision and reliability of the experiments. The lack-of-fit F-test describes the variation of the data around the fitted model. According to Table 3, the lack of P values of >0.05 obtained statistically significant for LAS removal, implying a significant model correlation between the variables and LAS removal.

Residuals plots, which define the difference between the observed values of a response and its predicted value, are considered to be important for addressing the adequacy of the model. Normal test plots are graphical tools for demonstrating residuals' departure from a straight line. The normal probability plot of LAS removal (Figure 4(a)) show that almost all data points are normally scattered close to the straight line and no gross distribution can be seen around the line. The adequacy of the model was also checked by the plot of residuals versus the predicted responses. As shown in Figure 4(b), random scatter of the residuals around zero indicates that the models are well behaved and the constant variance assumptions are satisfied. Furthermore, in a well-designed model, the residuals should be independent of time or any other parameter(s).

CONCLUSION

This study was aimed at investigating the efficiency of the MBBR in removing LAS Anionic Detergent from hospital wastewater. The experimental data demonstrated that the

Table 3 | ANOVA for response surface quadratic model analysis of variance table

Source	Sum of squares	Df	Mean square	F value	p-value prob$>$ F	
Model	6113.59	9	679.29	1485.02	<0.0001	Significant
A-MLSS	751.69	1	751.69	1643.30	<0.0001	
B-HRT	564.00	1	564.00	1232.99	<0.0001	
C-Packing rate	4626.80	1	4626.80	10114.83	<0.0001	
AB	14.58	1	14.58	31.87	0.0002	
AC	5.000E-003	1	5.000E-003	0.011	0.9188	
BC	8.82	1	8.82	19.28	0.0014	
A^2	36.45	1	36.45	79.69	<0.0001	
B^2	0.53	1	0.53	1.17	0.3050	
C^2	11.45	1	11.45	25.04	0.0005	
Residual	4.57	10	0.46			
Lack of fit	4.57	5	0.91			
Pure error	0.000	5	0.000			
Cor total	6118.17	19				

R-squared: 0.9993, Adj R-squared: 0.9986, Pred R-squared: 0.9913, C.V. %: 1.3.

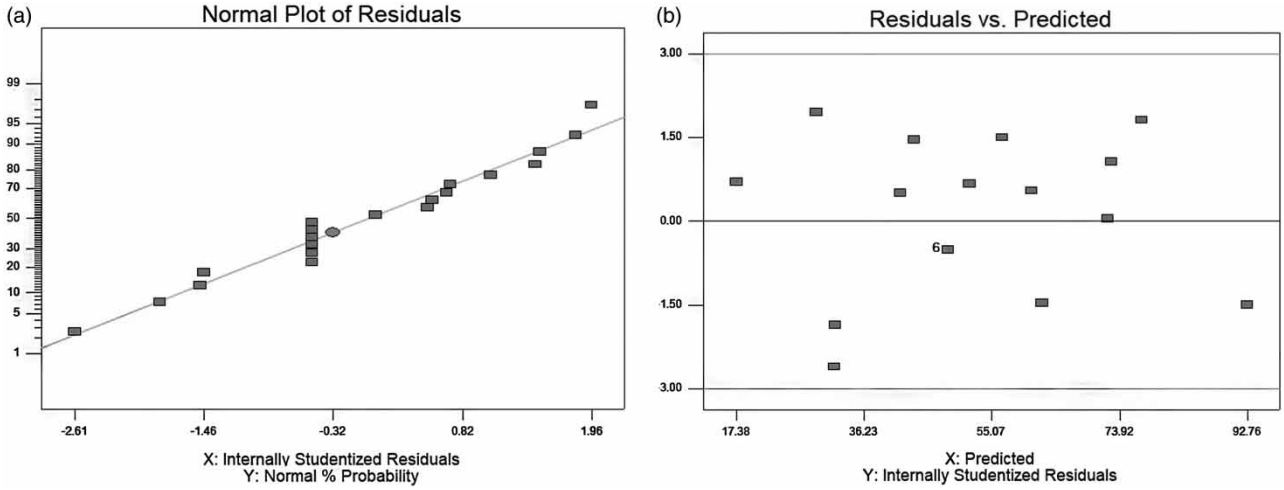

Figure 4 | (a) Normal probability distribution of residuals, (b) residuals versus predicted.

MBBR system have the potential for highly efficient removal of LAS from hospital wastewater. The CCD was found to be a valuable tool in establishing optimal conditions through a response surface study. Three variables, namely packing rate, HRT, and MLSS concentration, were regarded as factors in the optimization study. The results revealed that the removal efficiency of LAS detergent and COD using media k1, retention time of 24 hours, and MLSS concentration of around 3,000 mg/l were 92.3 and 95.8%, respectively. The findings in this study strongly suggested that the MBBR system proved to be a promising technique for the treatment of wastewater and aqueous solution containing LAS Anionic Detergent.

ACKNOWLEDGEMENTS

The research was supported by Hamadan University of Medical Sciences, Hamadan, Iran (Grants No. 9503111151, 9403121122).

REFERENCES

APHA/AWWA/WEF 2005 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, DC, USA.

Delforno, T. P., Okada, D. Y., Polizel, J., Sakamoto, I. K. & Varesche, M. B. A. 2012 Microbial characterization and removal of anionic surfactant in an expanded granular sludge bed reactor. *Bioresource Technology* **107**, 103–109.

Delforno, T., Moura, A., Okada, D. & Varesche, M. 2014 Effect of biomass adaptation to the degradation of anionic surfactants in laundry wastewater using EGSB reactors. *Bioresource Technology* **154**, 114–121.

Delnavaz, M., Ayati, B. & Ganjidoust, H. 2008 *Biodegradation of Aromatic Amine Compounds Using Moving Bed Biofilm Reactors*.

Dvořák, L., Lederer, T., Jirků, V., Masák, J. & Novák, L. 2014 Removal of aniline, cyanides and diphenylguanidine from industrial wastewater using a full-scale moving bed biofilm reactor. *Process Biochemistry* **49**, 102–109.

Feng, Q., Wang, Y., Wang, T., Zheng, H., Chu, L., Zhang, C., Chen, H., Kong, X. & Xing, X.-H. 2012 Effects of packing rates of cubic-shaped polyurethane foam carriers on the microbial community and the removal of organics and nitrogen in moving bed biofilm reactors. *Bioresource Technology* **117**, 201–207.

Garcia, M. T., Campos, E., Dalmau, M., Illán, P. & Sanchez-Leal, J. 2006 Inhibition of biogas production by alkyl benzene sulfonates (LAS) in a screening test for anaerobic biodegradability. *Biodegradation* **17**, 39–46.

Gupta, S., Pal, A., Ghosh, P. K. & Bandyopadhyay, M. 2003 Performance of waste activated carbon as a low-cost adsorbent for the removal of anionic surfactant from aquatic environment. *Journal of Environmental Science and Health, Part A* **38**, 381–397.

Lee, W.-N., Kang, I.-J. & Lee, C.-H. 2006 Factors affecting filtration characteristics in membrane-coupled moving bed biofilm reactor. *Water Research* **40**, 1827–1835.

Leyva-Díaz, J., Muñío, M., González-López, J. & Poyatos, J. 2016 Anaerobic/anoxic/oxic configuration in hybrid moving bed biofilm reactor-membrane bioreactor for nutrient removal from municipal wastewater. *Ecological Engineering* **91**, 449–458.

Mazioti, A. A., Stasinakis, A. S., Pantazi, Y. & Andersen, H. R. 2015 Biodegradation of benzotriazoles and hydroxy-benzothiazole in wastewater by activated sludge and moving bed biofilm reactor systems. *Bioresource Technology* **192**, 627–635.

Park, H. O., Oh, S., Bade, R. & Shin, W. S. 2011 Application of fungal moving-bed biofilm reactors (MBBRs) and chemical coagulation for dyeing wastewater treatment. *KSCE Journal of Civil Engineering* **15**, 453–461.

Petrovic, M., Gehringer, P., Eschweiler, H. & Barceló, D. 2007 Radiolytic decomposition of multi-class surfactants and their biotransformation products in sewage treatment plant effluents. *Chemosphere* **66**, 114–122.

Razavi, T., Fadaei, A., Sadeghi, M. & Shahsavan Markadeh, S. 2016 Study of the impact of combination of ZnO nanoparticles with ultraviolet radiation (photocatalytic process) on the removal of anionic surfactant linear alkyl benzene sulfonate (LAS) from aqueous solutions using Taguchi statistical method. *Desalination and Water Treatment* **57**, 28755–28761.

Sanz, J. L., Culubret, E., De Ferrer, J., Moreno, A. & Berna, J. L. 2003 Anaerobic biodegradation of linear alkylbenzene sulfonate (LAS) in upflow anaerobic sludge blanket (UASB) reactors. *Biodegradation* **14**, 57–64.

Sato, Y., Hori, T., Ronald, N. R., Habe, H. & Ogata, A. 2015 Effect of a microbiota activator on accumulated ammonium and microbial community structure in a pilot-scale membrane bioreactor. *The Journal of General and Applied Microbiology* **61**, 132–138.

Wagner, M. & Loy, A. 2002 Bacterial community composition and function in sewage treatment systems. *Current Opinion in Biotechnology* **13**, 218–227.

Zhang, S., Wang, Y., He, W., Wu, M., Xing, M., Yang, J., Gao, N. & Pan, M. 2014 Impacts of temperature and nitrifying community on nitrification kinetics in a moving-bed biofilm reactor treating polluted raw water. *Chemical Engineering Journal* **236**, 242–250.

Zhu, Y., Zhang, Y., Ren, H.-Q., Geng, J.-J., Xu, K., Huang, H. & Ding, L.-L. 2015 Physicochemical characteristics and microbial community evolution of biofilms during the start-up period in a moving bed biofilm reactor. *Bioresource Technology* **180**, 345–351.

First received 12 July 2017; accepted in revised form 23 December 2017. Available online 12 January 2018