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Original Article

Study of Aerobic Biodegradation of Surfactants and Fluorescent Whitening Agents in Detergents of a Few Selected Asian Countries (India, Indonesia, Japan, and Thailand)

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ABSTRACT

Consumption of detergents containing linear alkyl benzene sulfonate (LAS) and fluorescent whitening agents (FWAs) is increasing rapidly in Asian countries. A respirometric oxygen uptake method was conducted to examine the aerobic biodegradability of some of the commercially available laundry and dishwashing detergents of India, Japan, Indonesia, and Thailand. Indian detergents and Japanese detergents showed 95–100% of LAS removal. However, the three-dimensional fluorescent spectra results indicated the presence of residual metabolites such as sulfophenyl carbonate. Indonesian detergents and Thai detergents showed less LAS removal, but the progress of benzene ring cleavage of LAS in these detergents was observed from the test results. The FWAs were observed mainly in laundry detergents. The removal of FWAs during biodegradation test was observed to be between 12.4 and 78.8%. The complex changes in oxygen uptake curve during the test period suggested the presence of various organic compounds in the detergents. The tested detergents were classified into three clusters based on the relationship between the total organic carbon and the fluorescence (220/290 nm) removals. The diverse biodegradation results among the tested detergents from different Asian countries suggested the effects of various ingredients present in the detergents.

Keywords: biodegradation, detergents, fluorescent whitening agents, LAS, respirometer

INTRODUCTION

Detergents are the most extensively used products in our day-to-day life in every aspect of cleaning. The per capita consumption of detergents is increasing significantly every year. In Asian countries, detergent consumption is approximately 3 kg/capita/year, which is comparatively lower than the consumption in Western countries [1]. The individual per capita consumptions of India, Japan, Indonesia, and Thailand are 2.7, 8.9, 2.0, 3.4 kg/capita/year, respectively, which show that the consumption of detergents in Japan is 2–3 times greater than in India, Indonesia, and Thailand [1]. This also indicates that the production and consumption of detergents are more in developed countries. Although these detergents vary from country to country based on their local washing habits, practices, and consumer demands, all detergents consist mainly of surfactants, water softeners, bleaching agents, brighteners, and fragrance. The loading of these detergents on wastewater treatment plants is directly proportional to the consumption rate. The treatment efficiency of these detergent-rich domestic wastewaters mainly depends on local treatment processes. The main environmental effects due to the addition of these detergent compounds are dropping of the surface tension of the water, decrease in the breeding ability of aquatic organisms, destruction of the external mucus layer and gills of fish, and algal blooms that subsequently discharge toxins and reduce oxygen in water bodies through their degradation [2]. The toxicity of these detergents is

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mainly due to the non-biodegradable surfactants and fluorescent whitening agents (FWA).

Linear alkyl benzene sulfonate (LAS) is the most commonly used anionic surfactant in laundry detergents and is also used in other cleaning products such as dishwashing detergents and shampoos. It has a complex structure with alkyl chains ranging from 10 to 14 carbon units. The toxicity of LAS mainly depends on the position of the benzene ring: the central position is less toxic compared to the terminal position, and toxicity increases with chain length. Under aerobic conditions LAS is easily biodegraded but is less degradable under anaerobic conditions. Long chain LASs have higher adsorption and are insoluble when bound to calcium [3], which suggests that the biodegradation of LAS depends on the hardness of water/ wastewater.

Fluorescent whitening agents (FWAs), also known as optical brighteners, are present in detergents. They are used for brightening clothes. Approximately 20 − 95% of these compounds get stuck to fabrics during washing [4]. These compounds absorb ultraviolet (UV) light, usually between 340 − 370 nm, and re-emit it as blue light, usually between 420 − 470 nm. These FWAs are classified by their structure and property. All the compounds are highly substituted ring (aromatic) structures and contain many double bonds that are activated by UV light [5]. Approximately 80% of these compounds are stilbene derivatives. The most widely used FWAs in the detergent industry are benzenesulfonic acid, 2,2 ([1,1-biphenyl]-4,4-diyldi-2,1-ethenediyl) bis-disodium salt or Tinopal® CBS-X, and disodium 4,4-bis [(4–anilino-6-morpholion-1,3,5- trizin-2-yl) amino] stilbene-2,2-disulfonate or CXT [6].

Detergents are almost completely degraded in aerobic conditions without inhibition. The aquatic bacteria of Otamiri River, Nigeria degraded detergents [2]. There was initially a decrease in the microbial count, and then a slight increase in the degradation process, indicating the inability of the isolates to utilize the detergents as a nutrient source. Phosphate and carbon sources play an important role in the degradation of detergents. During the oxidation process, the phosphate position in detergent from the enzyme site plays a key role in detergent degradation [2]. Six types of detergents, of 0.01% (w/v), spiked in wastewater samples showed a greater reduction in approximately 30 days [7]. Other detergents showed degradation by the microbial community isolated from the New Calabar River located in Nigeria in the Niger River Delta [8].

Although these detergents are aerobically biodegradable, the anionic surfactant, linear alkyl benzenes (LABs) were detected in water and sediment samples of Cambodia, India, Indonesia, Malaysia, Philippines, Thailand, Japan, and Vietnam [9]. The FWAs were detected in surface water and sewage effluent samples of Taiwan and Tokyo Bay [10–12]. This shows that the treatment of this detergent-rich wastewater is the most persistent problem in many Asian countries. Although consumption of detergent with LAS and FWAs is increasing rapidly in Asian countries, little is known on their biodegradability. The main objective of this study was to compare the biodegradability of different Asian detergents as sole substrates and to determine the removal of LAS and FWAs present in these detergents in aerobic processes.

MATERIALS AND METHODS

Collection of detergents

A few popular commercial detergents were selected for the study, but their commercial names have been withheld. The detergents have been marked as shown in **Table 1**.

Aerobic seed

The aerobic seed (activated sludge) was collected from an aerobic master culture reactor which was introduced from the Chemical Evaluation and Research Institute (CERI), Japan, and operated in a draw and fill mode, then fed with substrate (Glucose) at 25°C in Ritsumeikan University (Biwako-Kusatsu Campus) located in Shiga Prefecture, Japan.

Biodegradation test

The biodegradability tests were conducted in duplicate using an OxiTop® (WTW, Weilheim, Germany) respirometer under dark condition. The test was performed for 10 days with detergent concentrations ranging from 50 mg/L to 250 mg/L. The 50 mg/L concentration was used for Indian detergents (I2 − I6), and the 65 mg/L for I1, all Indonesia detergents, and all Thai detergents. The detergent concentration of 250 mg/L was selected for Japanese detergents, which were expected to have high biodegradability. To each test bottle, 3 mL of aerobic seed sludge and 6 mL of nutrients were added. The test was performed by following the protocol given in the Organization for Economic Co-operation and Development 301F [13].

Analytical methods

The initial and final samples were filtered through 0.25 μm filter paper and then analyzed for LAS, FWA, and TOC. For the filtered samples LAS was directly analyzed using high-performance liquid chromatography (HPLC) (pump

Table 1 Summarized description of detergents used in this study.

Country of origin	Marker	Type/use	LAS Concentration (mg/g)	LAS Distribution (%)	TOC (mg/g)
India	I1	Laundry (Powder)	165	C_{11} (36) > C_{10} (26) > C_{12} (25) > C_{13} (14)	150
	I2		68.4	C_{11} (32) > C_{12} (30) > C_{13} (20) > C_{10} (17)	105
	I3		140	C_{12} (34) > C_{11} (31) > C_{13} (18) > C_{10} (18)	146
	I4		101	C_{12} (31) > C_{11} (30) > C_{10} (23) > C_{13} (16)	122
	I5		27.4	C_{12} (32) > C_{11} (30) > C_{10} (21) > C_{13} (18)	78.7
	I6	Dish-washing (Powder)	68.4	C_{12} (31) > C_{11} (31) > C_{10} (22) > C_{13} (16)	78.0
Indonesia	IN1	Laundry and dish-washing (Gel)	66.9	C_{11} (30) > C_{13} (30) > C_{12} (29) > C_{10} (10)	89.0
	IN2		141	C_{11} (38) > C_{12} (26) > C_{13} (24) > C_{10} (12)	116
	IN3		84.4	C_{11} (30) > C_{13} (30) > C_{12} (28) > C_{10} (12)	91.2
	IN4	Laundry and dish-washing (Liquid)	75.0	C_{13} (34) > C_{12} (29) > C_{11} (27) > C_{10} (11)	118
	IN5		98.1	C_{13} (30) > C_{12} (29) > C_{11} (28) > C_{10} (13)	112
Japan	J1	Laundry (Powder)	82.2	C_{11} (39) > C_{13} (30) > C_{12} (19) > C_{10} (12)	111
	J2		15.2	C_{13} (36) > C_{12} (32) > C_{11} (27) > C_{10} (6)	116
	J3		167	C_{11} (33) > C_{12} (33) > C_{13} (26) > C_{10} (8)	116
Thailand	T1	Laundry (Powder)	64.8	C_{12} (33) > C_{11} (31) > C_{13} (22) > C_{10} (14)	103
	T2		74.2	C_{12} (33) > C_{11} (30) > C_{13} (28) > C_{10} (10)	111
	T3	Dish-washing (Liquid)	60.8	C_{11} (32) > C_{12} (30) > C_{13} (24) > C_{10} (14)	166

L-2130, autosampler L-2200, fluorescence detector L-7485 (Hitachi, Tokyo, Japan) with ODS-80TM column (Tosoh, Tokyo, Japan)). The eluent was prepared using 1.5 mM ammonium acetate in an 80:20 (v/v) mixture of methanol and water with a 0.5 mL/min flow rate. Three-dimensional excitation-emission matrices (EEMs) were measured using a fluorescence spectrophotometer F-7000 (Hitachi, Tokyo, Japan). The spectra gave a group of successive emission spectra over a range of excitation wavelengths, which can be used to identify the fluorescent whitening agents (FWAs) present in the detergents. In this study, the excitation and emission wavelengths were increased from 200 to 600 nm at 10 nm intervals. The x-axis represents the emission spectra from 200 nm to 600 nm, while the y-axis indicates the excitation wavelength from 200 nm to 600 nm, and the third dimension, i.e., the contour line, is shown to express the fluorescence intensity. The total organic carbon (TOC) was measured using TOC-V (Shimadzu, Kyoto, Japan).

Statistical analysis

The results obtained from the fluorescence spectroscopy (220/290 nm) and TOC removal were subjected to hierarchical cluster analysis (HCA) by Ward's method, with the Euclidean distance as a measure of the proximity between two variables using SPSS Statistics 23 (IBM, Chicago, USA). The dendrogram was obtained from SPSS, with a 0 ± 25 scale, which was the rescaled linkage distance.

RESULTS AND DISCUSSION

Characterization of detergents

The detergents were characterized based on the concentrations of anionic surfactant (LAS) and organic carbon, given in **Table 1**. The highest concentration of LAS was observed in J3 and I1 with 167 and 165 mg/g concentrations, respectively, whereas the lowest was J2 with 15.2 mg/g concentration. Overall, a wide range of difference in concentration of LAS was observed in the selected detergents ranging between 1.5 − 16.7% (w/w). In general, anionic surfactants constitute

5 − 15% of the detergents by weight [14]. The varied concentrations of LAS showed the composition diversity of each detergent from different countries. However, in this study, no noticeable difference was observed in the concentration of LAS for laundry and dishwashing detergents. All the detergents showed C_{10} to C_{13}-LAS homologue chains. The percentage distribution of the homologues in LAS is given in **Table 1**. The C_{11}–C_{12} homologues are used to give wetting and foaming property whereas, C_{13}–C_{14} is used to give detergency (ability to remove dirt) [15]. The distribution and structure of LAS are some of the important factors for the biodegradation of LAS. Higher concentrations of C_{13}-LAS were observed in one Japanese (J2) and two Indonesian (IN4 and IN5) detergents. Detergents such as I1, I2, IN1, IN2, IN3, J1, J3, and T3 had higher concentrations of C_{11}-LAS. On the other hand, detergents such as I3, I4, I5, I6, T1, and T2 had higher concentrations of C_{12}-LAS.

The TOC content of the detergents varied from 7.8 − 16.6%. The highest concentration of TOC was observed in the Thai dishwashing detergent (T3) with 166 mg/g, whereas lowest in the Indian dishwashing detergent (I6) with 78 mg/g. In laundry detergents, highest and lowest concentrations were observed for I1 (150 mg/g) and I5 (79 mg/g). All the Japanese detergents had approximately showed a similar concentration of TOC (111 − 116 mg/g). Although the LAS concentration in J2 is the least, it showed a similar concentration of TOC which indicates the presence of other organic substances in the formulation.

Removal of LAS

The concentrations of LAS were analyzed using HPLC. The removal of LAS during the biodegradation test is given in **Table 2**. In general, LAS is regarded as a biodegradable surfactant, and 97 − 99% of removal has been observed in some wastewater treatment plants using aerobic processes [16, 17]. During aerobic biodegradation, the initial cleavage of LAS occurs at the terminal methyl group of the alkyl chain by ω-oxidation to carboxylic acids, which undergo β-oxidation, and the metabolites enter the tricarboxylic acid cycle as acetyl-coA. The next stage of LAS degradation is desulfonation, and the final stage is ring cleavage [18]. The biodegradation kinetics of LAS, in combination with other organic matter in activated sludge treatment, was found to be affected by LAS concentrations greater than 20 mg/L [19]. The aerobic biodegradation of LAS mainly depends on the concentrations of the LAS; presence of microorganisms adapted to LAS; composition of LAS (homologue/isomer); temperature; the bioavailability of the compound and nutrients; and the chain structure (linear or branched) [14, 20].

In this study, three Indian detergents (I3, I4, and I6) and all Japanese detergents (J1, J2, and J3) showed 95–100% LAS removal, which is similar to that reported in the literature [16, 17]. The LAS profile of Indian detergent (I4) before and after biodegradation test is shown in **Fig. 1**. Out of 17 detergents tested, only 6 detergents showed the LAS removal percentage greater than 95%, and two Indian detergents, I1 and I2 showed 92.6 and 90.3% LAS removal, respectively. The detergents, especially the Indonesian and the Thai detergents, showed relatively low (53.9 − 78.9%) LAS removal and some LAS remained after the test, for example, IN5 as shown in **Fig. 1**. The order of LAS removal from high to low is as follows: J2 > I6 > I3 > J1 > I4 > J3 > I1 > I2 > IN2 > I5 > IN5 > IN3 > T1 > IN1 > T2 > IN4 > T3. The highest concentration of LAS is 42 mg/L in J3, which showed 95.2% removal, whereas the lowest LAS concentration of 1.4 mg/L in I5 showed only 74.5% removal. This suggests that the initial concentration of LAS in detergents has shown no effect on the removal efficiency. According to the literature, the longest alkyl chain homologues show highest biodegradation rates [18]. However, the Thai and Indonesian detergents showed less removal of C_{13} and C_{12} homologues. The ratio of carbon chains before and after the biodegradation test also showed that more than 50% of the C_{13} homologue remained in solution in case of the detergents IN1, IN4, T1, T2, and T3. This observation suggests that the initial cleavage of LAS, especially in longer alkyl chain homologues, was possibly inhibited by some unknown constituents of detergents.

The EEMs have been extensively used to characterize dissolved organic matter in water, wastewater, and soil samples. The three-dimensional EEMs for detergents (IN4 and T2) before and after the biodegradation test are shown in **Fig. 2**. The EEM of the detergents mainly showed two peaks. The first main peak (peak L) located at the Ex/Em (excitation/emission) wavelength of 220/290 nm seemed to include the peak of LAS, or its metabolites, produced after biodegradation such as sulfophenyl carbonate (SPC) [21]. The removal percentage of fluorescence intensity at peak L is given in **Table 3**. The intensity of peak L in some Indian (I1) and Japanese detergents (J1, J2, and J3) showed a small decrease or a slight increase after the biodegradation test, in spite of a large removal of LAS (**Table 2**). This suggested the presence of high concentrations of SPC. In contrast, the intensity of peak L in the Thai and Indonesian detergents decreased. This decrease in the intensity is slightly smaller than the removal of LAS. The result indicates the progression of benzene ring cleavage of LAS in these detergents during the test. Five

Table 2 Change of LAS concentration and removal during biodegradation test.

Sample name	Type	Before test (mg/L)						After test (mg/L)						C_{10A}/C_{10B}	C_{11A}/C_{11B}	C_{12A}/C_{12B}	C_{13A}/C_{13B}	LAS removal (%)
		C_{10}	C_{11}	C_{12}	C_{13}	LAS	Remarks	C_{10}	C_{11}	C_{12}	C_{13}	LAS	Remarks					
I1	L	2.8	3.9	2.6	1.5	10	$C_{11} > C_{10} > C_{12} > C_{13}$	0.37	0.16	0.03	0.23	0.79	$C_{10} > C_{13} > C_{11} > C_{12}$	0.13	0.04	0.01	0.16	92.6
I2	L	0.59	1.1	1.0	0.70	3.4	$C_{11} > C_{12} > C_{13} > C_{10}$	0.20	N.D.	N.D.	0.13	0.33	$C_{10} > C_{13}$	0.34	0.00	0.00	0.19	90.4
I3	L	1.2	2.2	2.4	1.3	7.0	$C_{12} > C_{11} > C_{13} > C_{10}$	0.02	N.D.	N.D.	0.09	0.11	$C_{13} > C_{10}$	0.02	0.00	0.00	0.07	98.4
I4	L	1.2	1.5	1.6	0.82	5.0	$C_{12} > C_{11} > C_{10} > C_{13}$	0.08	N.D.	N.D.	0.10	0.18	$C_{13} > C_{10}$	0.07	0.00	0.00	0.12	96.4
I5	L	0.29	0.41	0.43	0.25	1.4	$C_{12} > C_{11} > C_{10} > C_{13}$	0.24	N.D.	0.03	0.09	0.35	$C_{10} > C_{13} > C_{12}$	0.83	0.00	0.07	0.36	74.5
I6	D	0.70	0.97	0.99	0.50	3.2	$C_{12} > C_{11} > C_{10} > C_{13}$	0.01	N.D.	N.D.	N.D.	0.01	C_{10}	0.01	0.00	0.00	0.00	99.7
IN1	B	0.45	1.3	1.3	1.3	4.4	$C_{11} > C_{13} > C_{12} > C_{10}$	0.12	0.22	0.54	0.77	1.7	$C_{13} > C_{12} > C_{11} > C_{10}$	0.27	0.17	0.43	0.58	61.8
IN2	B	1.1	3.5	2.4	2.2	9.2	$C_{11} > C_{12} > C_{13} > C_{10}$	0.15	0.28	0.69	0.83	1.9	$C_{13} > C_{12} > C_{11} > C_{10}$	0.14	0.08	0.29	0.38	78.9
IN3	B	0.66	1.7	1.6	1.6	5.5	$C_{11} > C_{12} > C_{13} > C_{10}$	0.12	0.31	0.64	0.79	1.8	$C_{13} > C_{12} > C_{11} > C_{10}$	0.18	0.19	0.41	0.49	67.0
IN4	B	0.51	1.3	1.4	1.6	4.9	$C_{13} > C_{12} > C_{11} > C_{10}$	0.08	0.30	0.75	0.84	1.9	$C_{13} > C_{12} > C_{11} > C_{10}$	0.16	0.23	0.54	0.51	59.4
IN5	B	0.85	1.8	1.9	1.9	6.4	$C_{13} > C_{12} > C_{11} > C_{10}$	0.17	0.28	0.63	0.76	1.8	$C_{13} > C_{12} > C_{11} > C_{10}$	0.20	0.16	0.34	0.40	71.3
J1	L	2.7	10	9.9	9.3	31	$C_{11} > C_{13} > C_{12} > C_{10}$	0.24	0.37	N.D.	N.D.	0.61	$C_{11} > C_{10}$	0.09	0.04	0.00	0.00	98.0
J2	L	0.24	1.0	1.2	1.4	3.8	$C_{13} > C_{12} > C_{11} > C_{10}$	N.D.	N.D.	N.D.	N.D.	0.00	-	0.00	0.00	0.00	0.00	100
J3	L	3.4	14	14	11	42	$C_{11} > C_{12} > C_{13} > C_{10}$	0.47	1.32	0.18	N.D.	2.0	$C_{11} > C_{10} > C_{12}$	0.14	0.10	0.01	0.00	95.2
T1	L	0.57	1.3	1.4	0.94	4.2	$C_{12} > C_{11} > C_{13} > C_{10}$	0.24	0.17	0.42	0.76	1.6	$C_{13} > C_{12} > C_{10} > C_{11}$	0.42	0.13	0.30	0.81	62.2
T2	L	0.48	1.4	1.6	1.3	4.8	$C_{12} > C_{11} > C_{13} > C_{10}$	0.25	0.24	0.55	0.81	1.9	$C_{13} > C_{12} > C_{10} > C_{11}$	0.52	0.17	0.35	0.61	61.6
T3	D	0.57	1.3	1.2	0.93	4.0	$C_{11} > C_{12} > C_{13} > C_{10}$	0.21	0.23	0.55	0.83	1.8	$C_{13} > C_{12} > C_{11} > C_{10}$	0.37	0.18	0.47	0.89	53.9

N.D.: not detected; L: laundry detergent; D: dishwashing detergents; B: both L and D (Can be used as both laundry and dishwashing detergent).

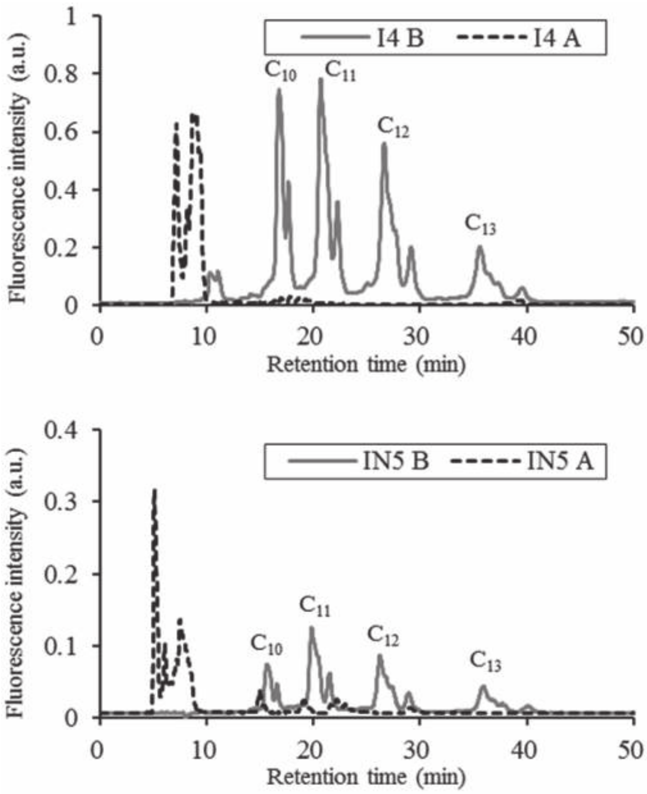

Fig. 1 Examples of HPLC chromatogram change during biodegradation test.
(B: before the test; A: after the test). a.u.: arbitrary unit.

Indian detergents also showed partial benzene ring cleavage. Such differences of LAS biodegradability seemed to be caused by the different ingredients present in the detergents, although the details were not clear.

Removal of FWAs

In this study, FWAs in the detergents were measured using fluorescence spectroscopy (EEM). The second peak (peak F) was observed at an Ex/Em of 340/430 nm, as shown in **Fig. 2**. The second peak seemed to be mainly due to the brightening agents used in preparing the detergents according to the literature [22]. These optical brighteners (OBs in the chemical industry), or fluorescent whitening agents (FWAs in the detergent industry), are compounds that are excited (activated) by wavelengths of light in the near-ultraviolet (UV) range (360 to 365 nm) and then emit light in the blue range (400 to 440 nm) [22, 23]. These FWAs were seen mainly in laundry detergents, but their presence was completely absent in dishwashing detergents (D and B types). The fluorescence intensity of peak F before and after the biodegradation test and the removal percentage of FWAs are also given in **Table 3**. The FWAs intensities had a wide range. The highest intensity (I3) was nearly nine times larger than the lowest intensity (J3). The removal of FWAs from the eight laundry detergents during the biodegradation test was observed to be between 12.4 and 78.8%.

Very few studies have been done on the biodegradation of FWAs. The FWAs exhibit greater affinity to sewage sludge which leads to partial removal (55 − 98%) in sewage treatment plants [4]. The concentration of CXT in detergents was observed to be between 0.02% and 0.10% with greater than 70% photodegradation in 28 days [24]. On the other hand, the concentration of CBS-X in household detergents varies from 0.05 − 0.15%, with >50% photodegradation in 12 months [25]. Due to the abundant use of these FWAs, detergents are potential sources of ecosystem pollution. In domestic wastewater, laundry detergents are the primary sources of whitening agents. However, in contrast with anionic surfactants such as LAS, FWAs show little or no biodegradation and tend to accumulate in the sewage sludge [4]. These pollutants remain in wastewater for long periods of time and negatively affect water quality, and animal and plant life.

Biodegradation study

Aerobic biodegradation study was done on the detergents using an OxiTop® respirometer. The BOD curve for the detergents is shown in **Fig. 3**. The pH was higher than 8 in some test reactors at the beginning of the study, which is due to the basic nature of detergents. After the test, it was between 7 and 8. It was reported by Perez Garcia *et al*. [19] that LAS can decrease the pH during the aerobic degradation of organic matter and pH neutralization is essential. However, there was no such significant decrease in pH in this study. There was no lag phase observed in the curve, which suggests that some of the organic materials contained in these detergents can be readily used by the aerobic microbes, and no acclimation period is required. The BOD curves increased step-wise during the test period. The nature of the curves indicates the presence of different constituents in detergents which have varied degradation pattern.

The biodegradation test results for TOC removal are given in **Table 4**. The TOC removal showed a very wide range, from 10.3 − 76.3%. The TOC removal in Indonesian detergent (IN3) is higher but lower in Indian detergent (I1). The higher TOC removal in the detergents can be due to adsorption phenomena. The hydrophobic nature of surfactants and FWAs exhibits higher tendency for adsorption. The main factor that affects adsorption is the type of homologue. Longer alkyl chains have greater hydrophobicity, thus increasing

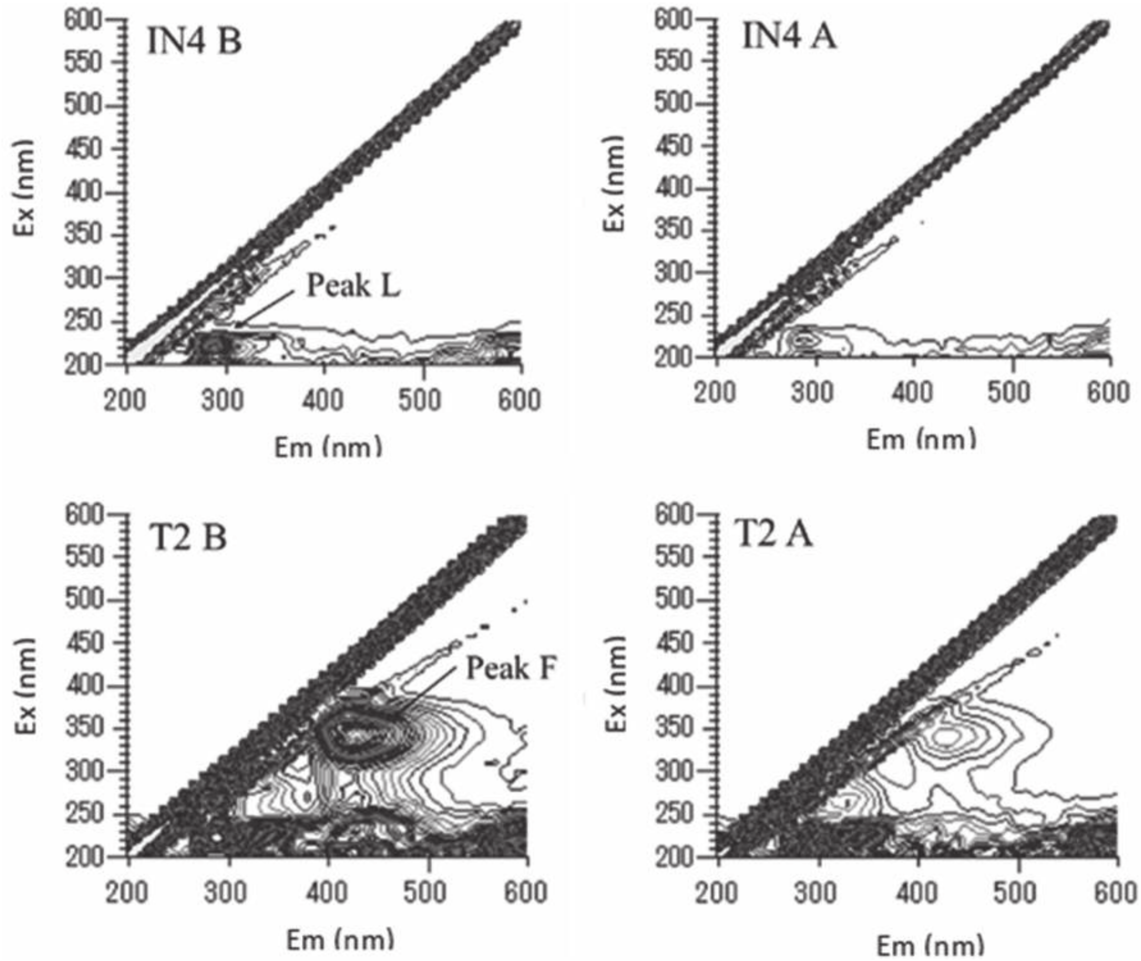

Fig. 2 Examples of EEM change during biodegradation test.
(B: before the test; A: after the test)

the adsorptive tendency [26]. The FWAs show little or no biodegradation and tend to accumulate in the sewage sludge. Lower BOD can be due to the antimicrobial properties of the detergents that can inhibit the activity of microorganisms. The antimicrobial agents most commonly used in laundry and dishwashing detergents are quaternary ammonium chlorides and alcohols. Some of them include triclosan (TCS), triclocarban, benzalkonium chloride, chlorhexidine, chloroxylenol and many more. These antimicrobial agents can either kill or inhibit the growth of bacterial cells [27]. During wastewater treatment, less than half fraction of TCS undergoes biodegradation or lost to other mechanism, while the remaining gets adsorb in sewage sludge [28]. The TCS concentration of 20 mg/L reduces the oxygen consumption by 50%. On the other hand, TCS can inhibit nitrification at a concentration as low as 0.5 mg/L under aerobic condition [29]. The half maximum effective concentration (EC_{50}) values for benzalkonium chloride were in the range of 0.12 and 3.6 mg/L. Didecyl dimethyl ammonium chloride affected the performance of a rotating biological contactor at a concentration above 20 mg/L, and the biofilm was completely eliminated at 160 mg/L [30].

From the cluster analysis, the fluorescence (220/290 nm) removal and TOC removal are presented in the form of a dendrogram in **Fig. 4**. The dendrogram revealed two distinct groups separated at a linkage distance lower than 25. In the next step, at the rescaled distance lower than 5, three statistically significant clusters were grouped which are shown in **Fig. 5**. The relationship between the fluorescence (220/290 nm) removal and TOC removal is shown in **Fig. 5**. Although there is no linear relationship between them, TOC removal increased with an increase in fluorescence removal. Some detergent plots are along the line (y = x) as shown in the figure. The cluster 1 includes five detergents

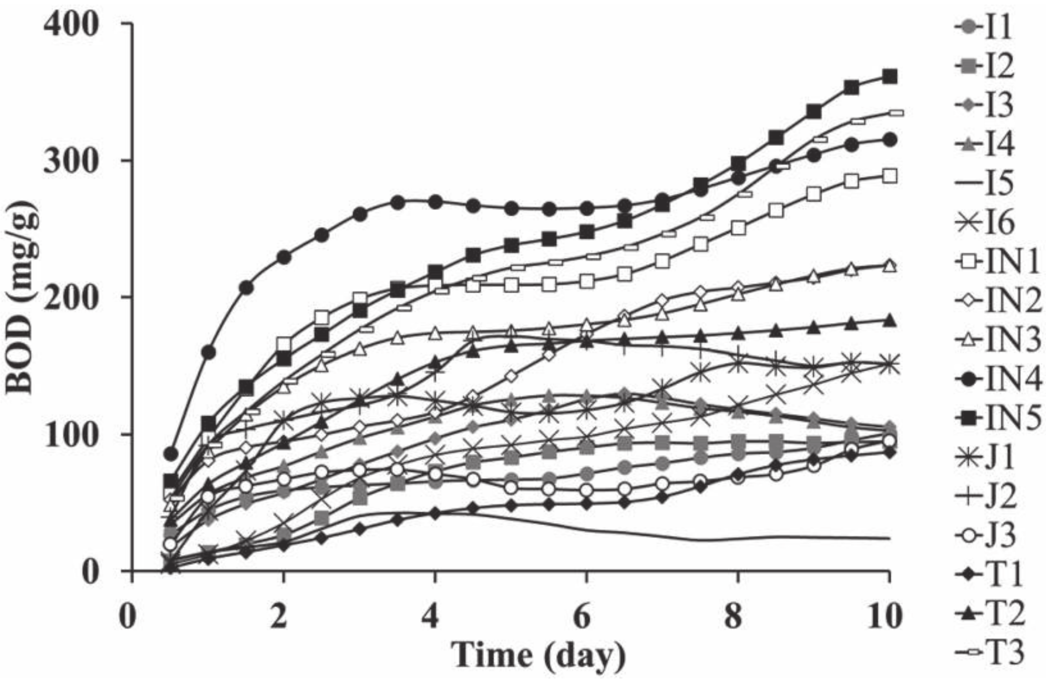

Fig. 3 BOD curve (cumulative oxygen consumption) for 10 days.

Table 3 Change of fluorescence EEM intensity and removal during biodegradation test.

Detergent	Type	Peak L (Ex/Em = 220/290)			Peak F (Ex/Em = 340/430)		
		Intensity-before (10^3)	Intensity-after (10^3)	Removal (%)	Intensity-before (10^3)	Intensity-after (10^3)	Removal (%)
I1	L	588	666	< 0.0	30.6	26.8	12.4
I2	L	397	115	71.0	N.D.	N.D.	
I3	L	671	257	61.7	49.6	39.5	20.4
I4	L	440	240	45.4	10.3	4.44	56.9
I5	L	137	62	54.5	19.2	4.26	77.9
I6	D	406	101	75.2	N.D.	N.D.	
IN1	B	418	112	73.2	N.D.	N.D.	
IN2	B	602	322	46.5	N.D.	N.D.	
IN3	B	331	160	51.8	N.D.	N.D.	
IN4	B	1103	139	87.4	N.D.	N.D.	
IN5	B	448	184	58.9	N.D.	N.D.	
J1	L	302	265	12.2	64.3	32.9	48.8
J2	L	87.9	76.9	12.6	79.0	59.2	25.1
J3	L	389	353	9.4	8.88	6.39	28.0
T1	L	392	192	50.9	N.D.	N.D.	
T2	L	358	228	36.4	41.8	8.87	78.8
T3	D	313	231	26.3	N.D.	N.D.	

N.D.: not detected; L: laundry detergent; D: dishwashing detergents; B: both L and D.

Table 4 Change of TOC concentration and removal during biodegradation test.

Sample name	Type	TOC_B (mg/L)	TOC_A (mg/L)	TOC removal (%)
I1	L	9.75	8.71	10.3
I2	L	5.22	1.92	63.5
I3	L	7.29	2.92	60.3
I4	L	6.09	3.95	36.1
I5	L	3.94	1.86	51.3
I6	D	3.90	2.28	41.0
IN1	B	5.78	1.93	67.2
IN2	B	7.51	2.50	66.7
IN3	B	5.93	1.41	76.3
IN4	B	7.68	2.31	70.1
IN5	B	7.25	2.47	65.3
J1	L	36.7	23.6	35.7
J2	L	29.1	12.1	58.4
J3	L	28.9	18.3	36.7
T1	L	6.70	2.40	64.2
T2	L	7.24	2.53	65.3
T3	D	10.8	2.71	75.0

L: laundry detergent; D: dishwashing detergents; B: both L and D; TOC_B: TOC before biodegradation test; TOC_A: TOC after biodegradation test.

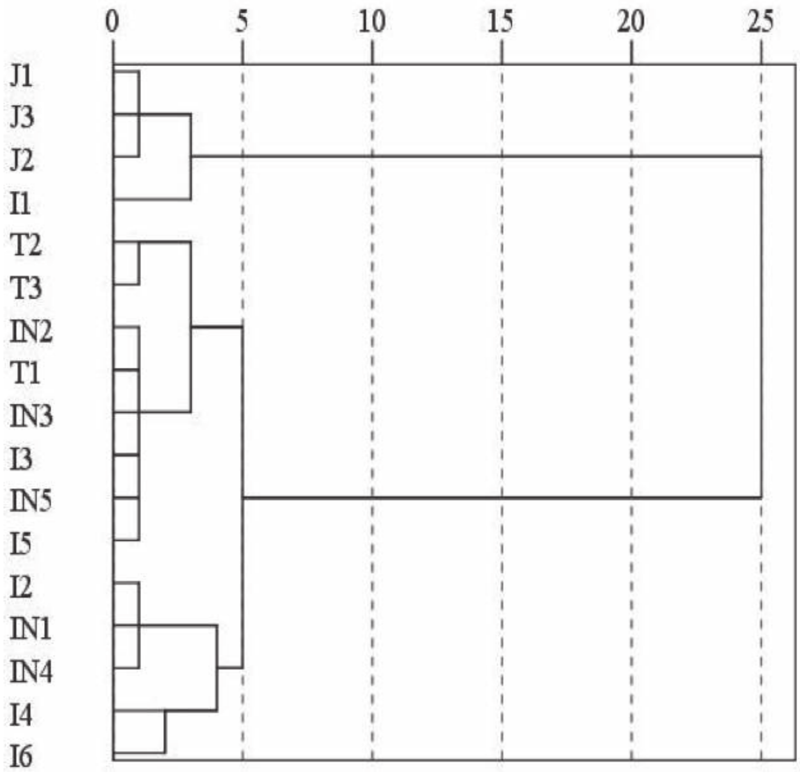

Fig. 4 Dendrogram of the hierarchical cluster analysis using Ward's method and Euclidean distance.

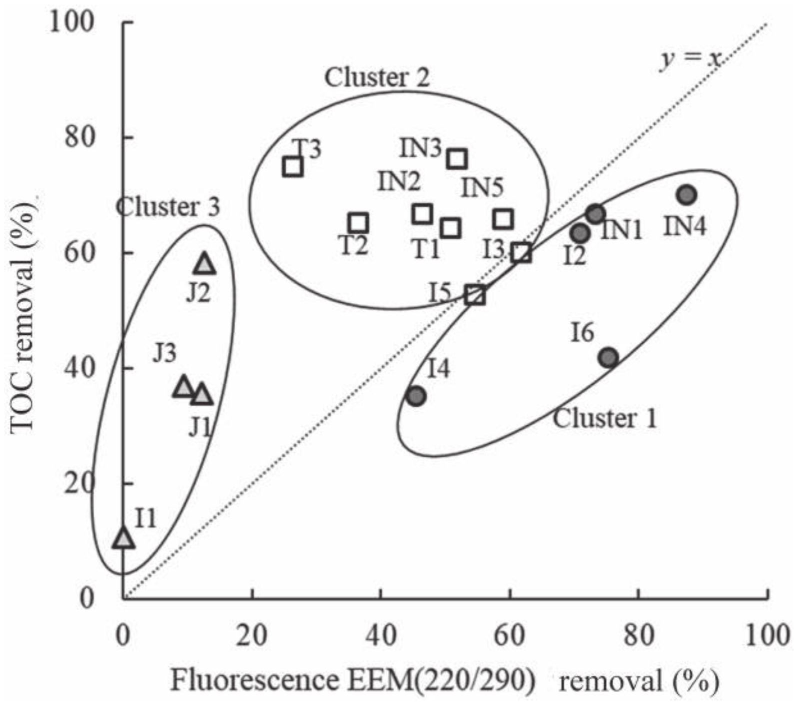

Fig. 5 Relationship between fluorescence EEM (220/290 nm) removal and TOC removal.

of which three are Indian (I2, I4, and I6) and two Indonesian detergents (IN1 and IN4). The largest cluster, cluster 2 includes eight detergents, with two Indian (I3 and I5), three Indonesian (IN2, IN3, and IN5), and all Thailand (T1, T2, and T3) detergents. The cluster 3 includes one Indian (I1) and all Japanese detergents (J1, J2, and J3). The detergents in the cluster 1 suggest that TOC decreased due to benzene ring cleavage of LAS. The cluster 2 indicates an additional removal of organic matter which do not contain benzene rings. The cluster 3 shows the detergents with a lot less removal of TOC but with more than 92% LAS removal as given in **Table 2**. The result indicates that the LAS of these detergents had undergone the primary step of biodegradation, but not the complete mineralization, and the metabolites were still not degraded. This explains the cause of less removal of their peak L in EEM, as shown in **Table 3**, and supports the suggestion that high concentrations of SPC would remain in the mixed liquor after biodegradation. In contrast, the TOC removals of Indonesian detergents and Thai detergents were almost same as that of their corresponding LAS removals as given in **Table 2**. This result suggests that approximately 60 − 70% of LAS in these detergents were completely mineralized. This agrees with the results of the removals of peak L in EEM, showing the progression of benzene ring cleavage.

Significant variability in biodegradability was noticed among the tested detergents which indicate the presence of a wide variety of ingredients in the detergents. Detergents are mainly composed of surfactants, water softeners, bleaching agents, brighteners, antimicrobial agents, and fragrance. In this study, we observed the complexity of biodegradability of brightening agents and surfactants. Under aerobic conditions, LAS showed greater removal efficiencies, but metabolites were still present after the primary break down of carbon chains. It was suggested that biodegradation of detergents depends not only on surfactants alone but also on the presence of other ingredients. For example, in real conditions, these anionic surfactants can react with cationic surfactants and form a complex compound that is difficult to degrade [31].

CONCLUSIONS

Biodegradation of a few popular commercial detergents from four Asian countries was studied. The pattern and the extent of biodegradation varied significantly among the detergents. This is possibly due to their different chemical composition. Some Indian detergents and Japanese detergents showed a high removal of LAS during aerobic biodegradation test. However, a high concentration of SPC remained in the mixed liquor. In contrast, some Indonesian detergents and Thai detergents showed less LAS removal but could be linearly related to TOC removal. The removal of FWAs during the biodegradation test was less than LAS in all cases. It is understood that the biodegradation of the detergents is complex and is dependent on the presence of other ingredients in the detergent.

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REFERENCES

[1] Tsumadori M: Soap and detergent product trends in Asian and Pacific countries. In: Cahn A (Ed.): Proceedings of the 3rd World Conference on Detergents: Global Perspectives, 3rd September, 1993 Montreux, Suiza (No. L-0256), Switzerland, AOCS Publishing, Urbana, USA, 1994.

[2] Ogbulie TE, Ogbulie JN, Umezuruike I: Biodegradation of detergents by aquatic bacterial flora from Otamiri River, Nigeria. *Afr. J. Biotechnol.*, **7**(6), 824–830, 2008.

[3] Jensen J: Fate and effects of linear alkylbenzene sulphonates (LAS) in the terrestrial environment. *Sci. Total Environ.*, **226**(2-3), 93–111, 1999. PMID:10085562, doi:10.1016/S0048-9697(98)00395-7

[4] Poiger T, Field JA, Field TM, Siegrist H, Giger W: Behavior of fluorescent whitening agents during sewage treatment. *Water Res.*, **32**(6), 1939–1947, 1998. doi:10.1016/S0043-1354(97)00408-9

[5] Neiditch OW: Minor additives in heavy-duty laundry detergents. *J. Amer. Oil Chem. Soc.*, **58**(2), A162–A165, 1981.

[6] Bohnet M: Ullmann's encyclopedia of industrial chemistry, 6th ed. Wiley-VCH, Weinheim, Germany, 2003.

[7] Ojo OA, Oso BA: Biodegradation of synthetic detergents in wastewater. *Afr. J. Biotechnol.*, **8**(6), 1090–1109, 2009.

[8] Okpokwasili GC, Olisa AO: River-water biodegradation of surfactants in liquid detergents and shampoos. *Water Res.*, **25**(11), 1425–1429, 1991. doi:10.1016/0043-1354(91)90121-6

[9] Isobe KO, Zakaria MP, Chiem NH, Minh Y, Prudente M, Boonyatumanond R, Saha M, Sarkar S, Takada H: Distribution of linear alkylbenzenes (LABs) in riverine and coastal environments in South and Southeast Asia. *Water Res.*, **38**(9), 2449–2459, 2004. PMID:15142807, doi:10.1016/j.watres.2004.02.009

[10] Hayashi Y, Managaki S, Takada H: Fluorescent whitening agents in Tokyo Bay and adjacent rivers: their application as anthropogenic molecular markers in coastal environments. *Environ. Sci. Technol.*, **36**(16), 3556–3563, 2002. PMID:12214649, doi:10.1021/es011352o

[11] Managaki S, Takada H: Fluorescent whitening agents in Tokyo Bay sediments: molecular evidence of lateral transport of land-derived particulate matter. *Mar. Chem.*, **95**(1–2), 113–127, 2005. doi:10.1016/j.marchem.2004.08.007

[12] Shu WC, Ding WH: Determination of fluorescent whitening agents in laundry detergents and surface waters by solid-phase extraction and ion-pair high-performance liquid chromatography. *J. Chromatogr. A*, **1088**(1-2), 218–223, 2005. PMID:16130754, doi:10.1016/j.chroma.2005.01.032

[13] OECD: OECD Guidelines for the Testing of Chemicals. Organization for Economic Co-operation and Development, Paris, France, 1994.

[14] de Oude NT (ed.): Detergents. In: Hutzinger O (ed.): The Handbook of Environmental Chemistry. Springer, Berlin/Heidelberg, Germany, Vol. 3 Part F: Anthropogenic Compounds, 1992.

[15] Broze G: Handbook of detergents, Part A: Properties. Marcel Dekker, New York, USA, 1999.

[16] Bevia FR, Prats D, Rico C: Elimination of LAS (linear alkylbenzene sulfonate) during sewage treatment, drying and compostage of sludge and soil amending processes. In: Quaghebeur D, Temmerman I, Angeletti G (eds.): Organic contaminants in waste water, sludge and sediment. Elsevier Applied Science, London, UK, pp. 124–137, 1989.

[17] Brunner PH, Capri S, Marcomini A, Giger W: Occurrence and behaviour of linear alkylbenzenesulphonates, nonylphenol, nonylphenol mono-and nonylphenol diethoxylates in sewage and sewage sludge treatment. *Water Res.*, **22**(12), 1465–1472, 1988. doi:10.1016/0043-1354(88)90157-1

[18] Scott MJ, Jones MN: The biodegradation of surfactants in the environment. *Biochimica et Biophysica Acta*

(BBA)-. Biomembranes, **1508**(1–2), 235–251, 2000. doi:10.1016/S0304-4157(00)00013-7

[19] Perez Garcia M, Romero Garcia LI, Quiroga Alonso JM, Sales MD: Influence of LAS (linear alkylbenzene sulphonates) on biodegradation kinetics. *Chem. Biochem. Eng. Q.*, **10**(2), 75–82, 1996.

[20] León VM, Gómez-Parra A, González-Mazo E: Biodegradation of linear alkylbenzene sulfonates and their degradation intermediates in seawater. *Environ. Sci. Technol.*, **38**(8), 2359–2367, 2004. PMID:15116841, doi:10.1021/es034813+

[21] Managaki S, Kojima S, Harada A, Nakada N, Tanaka H, Takada H: Development of Analytical Method of Linear Alkylbenzenesulfonates and Their Degradation Intermediates by High Performance Liquid Chromatography Equipped with Tandem Mass Spectrometry and Its application to Major Japanese Rivers. *J. Jpn Soc. Water Environ.*, **28**(10), 621–628, 2005. [in Japanese with English Abstract] doi:10.2965/jswe.28.621

[22] Carstea EM, Bridgeman J, Baker A, Reynolds DM: Fluorescence spectroscopy for wastewater monitoring: A review. *Water Res.*, **95**, 205–219, 2016. PMID:26999254, doi:10.1016/j.watres.2016.03.021

[23] Takahashi M, Kawamura K: Simple measurement of 4,4'-bis(2-sulfostyryl)-biphenyl in river water by fluorescence analysis and its application as an indicator of domestic wastewater contamination. *Water Air Soil Pollut.*, **180**(1–4), 39–49, 2006.

[24] HERA: Fluorescent Brightener FWA-1 (CAS 16090-02-1), Human & Environmental Risk Assessment on Ingredients of European Household Cleaning Products, October, 2004. http://www.heraproject.com/files/23-F-04-HERA-FWA1(Version%203_1%20).pdf [accessed in December, 2017].

[25] HERA: Fluorescent Brightener FWA-5 (CAS 27344-41-8), Human & Environmental Risk Assessment on Ingredients of European Household Cleaning Products, November, 2003C. http://www.heraproject.com/files/11-F-04-HERA%20FWA5%20Full%20web%20wd.pdf [accessed in December, 2017].

[26] Hallmann E, Tomczak-Wandzel R, Mędrzycka K: Fate of LAS surfactants in WWTPs based on measured concentrations in wastewater and sludge. Research and Application of New Technologies in Wastewater Treatment and Municipal Solid Waste, Polish-Swedish-Ukrainian Seminar, Krakow, Poland, 2011.

[27] Bhat R, Prajna PS, Menezez VP, Shetty P: Antimicrobial activities of soap and detergents. *Adv. Biores.*, **2**(2), 52–62, 2011.

[28] Heidler J, Halden RU: Mass balance assessment of triclosan removal during conventional sewage treatment. *Chemosphere*, **66**(2), 362–369, 2007. PMID:16766013, doi:10.1016/j.chemosphere.2006.04.066

[29] Stasinakis AS, Petalas AV, Mamais D, Thomaidis NS, Gatidou G, Lekkas TD: Investigation of triclosan fate and toxicity in continuous-flow activated sludge systems. *Chemosphere*, **68**(2), 375–381, 2007. PMID:17337032, doi:10.1016/j.chemosphere.2007.01.047

[30] Zhang C, Tezel U, Li K, Liu D, Ren R, Du J, Pavlostathis SG: Evaluation and modeling of benzalkonium chloride inhibition and biodegradation in activated sludge. *Water Res.*, **45**(3), 1238–1246, 2011. PMID:21030060, doi:10.1016/j.watres.2010.09.037

[31] Utsunomiya A, Mori Y, Hasegawa K: Adsorption of linear alkyl benzenesulfonates and their complexes with cationic surfactants on river sediment, and their biodegradation in river water. *Jpn. J. Toxicol. Environ. Health*, **44**, 264–276, 1998. [in Japanese with English Abstract] doi:10.1248/jhs1956.44.264