Linear alkybenzene sulfonate and alkyl sulfate biodegradation in laboratory and field activated sludge systems

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Abstract

The fate and biodegradation of two xenobiotic compounds, linear alkylbenzene sulfonate (LAS) and alkyl sulfate (AS), were investigated in laboratory and field activated sludge systems. Two 1-L porous pot (65 μ stainless steel mesh) reactors were fed synthetic wastewater (200 mg COD/L including 2 mg/L LAS and 1 mg/L alkyl sulfate) to evaluate the influence of specific operating conditions (i.e. hydraulic retention time, HRT, and solids retention time, SRT) on the measured rate of biodegradation. The reactors were operated in parallel under a constant SRT of 10 d, and HRTs of 2, 4, 6, and 12 h. Subsequently, the reactors were operated under a constant HRT of 6 h, and SRTs of 3, 6, 10, and 15 d. The extant kinetic parameters obtained from respirometric experiments suggest that the HRT had little impact on the measured kinetic parameters ($\hat{\mu}$ = 0.14 ± 0.06 h^{-1}, K$_S$ = 0.4 ± 0.3 mg COD/L, Y = 0.67 ± 0.02 mg biomass COD formed/mg LAS COD utilized and $\hat{\mu}$ = 0.0.25 ± 0.01 h^{-1}, K$_S$ = 0.35 ± 0.07 mg COD/L, and yield = 065 ± 0.02 mg biomass COD formed/mg AS COD utilized) at a constant SRT of 10 d. The SRT had a more noticeable effect on the measured biodegradation kinetics (e.g., Y increased from 0.50 ± 0.08 to 0.66 ± 0.05 mg/mg and 0.49 ± 0.07 to 0.61 ± 0.07 when the SRT increased from 3 to 10 d at a constant HRT of 6 h for LAS and AS, respectively). Extant kinetics for LAS biodegradation were measured in the field at three activated sludge wastewater treatment plants operated at different conditions. The field results were similar to the results from laboratory systems operated to simulate the field conditions. Using the COD fraction to calculate the competent biomass concentration, the field measured extant kinetic parameters were used to accurately predict effluent concentrations within 2% on average at one plant and within 4 μg/L at two other plants. Day to day predictions were not as accurate, possibly due to the non-steady-state nature of the field systems.
Keywords: linear alkylbenzene sulfonate, alkyl sulphate, biodegradation, kinetics, competent biomass, respirometry, activated sludge.

1   Introduction

Surface active agents, or surfactants, are components of widely used household laundry detergents, household cleaners, shampoos, cosmetics, antistatic agents, textile aids, and textile softeners. Linear alkyl benzene sulfonate (LAS) was introduced in 1965 as a biodegradable alternative to non-biodegradable branch-chained alkyl benzene sulfonates (ABS) and since has become the common anionic surfactant in commercial detergent formulations. For example, worldwide use of LAS in 1997 exceeded 2 million metric tons and AS use was approximately half that of AS [1]. Trehy et al. [2] reported environmental LAS concentrations in the influent (3.0 – 7.7 mg/L) and effluent (0.003 – 0.086 mg/L) of the activated sludge process from 10 U.S. wastewater treatment plants. Influent concentrations in European WWTPs are estimated at 13.2 and 4.7 mg/L for LAS and AS, respectively [3]. Kaiser *et al.* [4] reported LAS concentrations in the influent to domestic wastewater treatment plants typically ranges from 1 mg/L to 5 mg/L. Krueger *et al.* [5] observed that LAS was readily biodegraded in activated sludge systems and the LAS had a half-life of 1-2 days. Others [2, 6–8] have reported on the complexity in the measurement of LAS biodegradation intermediates which caused difficulties in evaluating biodegradation. van Ginkel [9] demonstrated the importance of SRT for maintaining sufficient surfactant-degrading microorganisms in wastewater treatment plants, while Temmink and Klapwijk [3] suggest that the influent concentration and adsorption to biomass were important in describing LAS removal. In a previous study [4] using porous pots to simulate activated sludge, effluent concentrations of LAS were found to be a function of influent concentrations when the HRT was less than 10 h. Federle et al. [7] determined that primary and complete biodegradation of LAS were best described by a first-order approach with the first-order rate constants of 0.50-0.53h^{-1}. The kinetics and removal of LAS in municipal activated sludge treatment systems have been previously studied [4]using laboratory continuous-flow activated sludge systems and radio-labeled LAS. First-order rate constants of LAS removal were determined during this study, but the methods to determine the kinetic constants were labor-intensive and time consuming. Zhang *et al.* [10] found that the biodegradation rate of anionic surfactants over a range of concentrations (i.e., sub-and supra-critical micelle concentrations) followed Monod kinetics.

   The main objective of the present study was to determine the efficacy of extant respirometry to provide accurate measurements of the biodegradation kinetic parameters of surfactants in laboratory and field activated sludge systems. The goal was to be able to use the resulting kinetics to describe, and eventually to predict, the fate of LAS in activated sludge systems. The extant kinetic parameters of LAS were measured over a range of operating conditions at several activated sludge wastewater treatment plants and in laboratory porous pot reactors simulating activated sludge.

2   Materials and methods

The laboratory apparatus consisted of two porous pot reactors (stainless steel mesh pore size of 65 μ) with a working volume of 1-L. They were operated in parallel as continuous stirred tank reactors (CSTRs) for a period of one year. Sludge wastage was performed automatically three times per day using a timer or once per day manually to maintain the desired SRT. Surfactant LAS was a mixture of LAS homologs with 96.8% C_{12} LAS, dodecyl benzene sulfonate-sodium salt, in liquid form with 51.5% active fraction. For all kinetic measurements, an injection concentration of 2 mg/L as COD was used. 1 mg/L LAS was found to be equivalent to 2.7 mg/L as COD by the standard COD test. The fraction of the biomass involved in the biodegradation of LAS was assumed to be equal to the fraction of energy (COD) supplied to the culture by LAS [11–13]. Consequently, a competent biomass fraction of 2.7% was used for the laboratory experiments. A similar competent biomass fraction (2.9%) was determined for the field experiments. Phosphate buffer was added to the biomass sample to maintain the pH at approximately 6.8 to 7.0 during the respirometer tests [14].

Respirometric tests were used as a surrogate measurement of LAS and AS disappearance in batch kinetic tests as described previously [15]. Samples were placed in 250 mL sealed respirometric vessels with constant temperature water jackets (Tudor Glass Co., Belvedere, SC). The dissolved oxygen (DO) concentration was measured by a DO meter (Model 3550, Yellow Springs, CO), and the DO probes were fitted with high sensitive membranes. The temperature was controlled by a water bath at 25 $\pm$ 0.1°C. The initial dissolved oxygen concentration was elevated to approximately 16-18 mg/L using pure oxygen to prevent oxygen from becoming limiting during the entire respirometric response. The biomass was continuously stirred using a magnetic stir bar. When a linear decrease in DO was observed, a low concentration (2 mg/L as COD) injection of LAS or AS was made to the respirometric vessel, resulting in a low initial substrate to biomass concentration ratio (S_0: X_0) ensuring that the test was a measure of the extant kinetics of the biomass [16]. The DO response data was collected continuously at a sampling frequency of 10 Hz through a data acquisition board installed in a personal computer. The resulting data file was averaged to provide a DO measurement every 2 to 4 seconds and normalized to subtract the background endogenous oxygen uptake rate. The biodegradation response was modeled in an Excel™ spreadsheet, and the kinetic parameters were estimated by nonlinear regression using a fourth-order Runge-Kutta approximation of the Monod equation .

Preliminary experiments to ascertain the reliability and reproducibility of the extant technique for measuring surfactant kinetics included experiments to determine the effect of the initial substrate concentration, the effect of the initial DO concentration, and the effect of re-injecting LAS to the same biomass sample. The tests were conducted with biomass from the Boone Water Pollution Control Plant (WPCP), Boone, Iowa. Grab samples of the influent to Boone

plant were collected and measured by liquid chromatography/mass spectroscopy (LC/MS) to determine the LAS and AS influent concentrations.

To evaluate the validity of using synthetic wastewater for the laboratory studies, a side-by-side comparison of porous pot reactors fed synthetic and raw wastewater was performed. Extant tests were run to determine whether the kinetic parameters in the two reactors were comparable. The raw wastewater was collected from the Boone WPCP and diluted to 200 mg/L as COD.   The composition of the synthetic wastewater is shown in Table 1. Both wastewaters were fed at a concentration of 200 mg COD/L, and 2 mg/L LAS (5.4 mg/L as COD) and 1 mg/L AS (2.2 mg/L as COD) were added to the synthetic wastewater. It was assumed that the actual wastewater contained a similar amount of LAS and AS (which was confirmed by subsequent analysis).   The reactors were operated at room temperature $22 \pm 2°C$.   To maintain a diverse microbial consortium, 5 ml of mixed liquor from a local municipal wastewater treatment plant was added to the reactors every other day.   This addition represented less than 2% of the feed COD to the reactors and less than 2% of the porous pot mixed liquor concentration at all operating conditions.

Table 1:   Composition of synthetic wastewater fed to porous pot reactors.

Macronutrients	Conc., mg/L	Micronutrients	Conc., µg/L
Urea	37.6	$NiSO_4 \cdot 6H_2O$	100
Nutrient broth	60.0	$ZnCl_2$	62
Lauric acid	7.0	$CuCl_2 \cdot 2H_2O$	159
Potato starch	6.0	$CoCl_2 \cdot 6H_2O$	18
Non-fat dried	60.0	$MnSO_4 \cdot H_2O$	22
milk	10.0	EDTA	74
Dietary fiber	20.0	K_2MoO_4	5
Sodium acetate	10.0		
$Na(HCO_3)$	3.0		
$K_3PO_4 \cdot H_2O$	0.5		
$FeSO_4 \cdot 7H_2O$	2.0		
LAS	1.0		
AS			

After it was determined that synthetic wastewater was an acceptable substitute for real wastewater, two porous pot reactors were fed synthetic wastewater to evaluate the influence of HRT and SRT on the biodegradation responses. The reactors were operated in parallel under a constant SRT of 10 d, and HRTs of 2, 4, 6, and 12 h. Subsequently, the reactors were operated under a constant of HRT of 6 h, and SRTs of 3, 6, 10, and 15 d. An additional porous pot operating condition was run at 18 h HRT and 30 d SRT to simulate the operating conditions at the Boone WPCP, an extended aeration activated sludge plant with an HRT of approximately 18 h and SRT in the range of 24 - 30 d. Additional field tests were conducted at the Iowa City Wastewater Treatment

Plant (WWTP), a conventional activated sludge plant with an HRT of approximately 4 - 6 h and SRT of approximately 6 d.

3 Results and discussion

LAS and AS concentrations were measured in the influent to the Boone WPCP, and the results indicated a range of LAS and AS concentrations from 1- 5 mg/L as shown in Table 2. These findings are similar to the results reported by Kaiser *et al.*, 1997. From this table it can be seen that C_{12} LAS accounted for about 20% of the total LAS concentration. The C_{12} homolog was used as the representative test compound for this study. To estimate of the competent fraction of LAS degrading biomass, an average LAS concentration of 2.86 mg/L (7.72 mg COD/L) and influent COD concentration of 265 mg/L were used to calculate a COD fraction of 2.9%. Therefore, since the biomass obtained 2.9% of its energy from LAS, it was assumed that 2.9% of the biomass had the capacity to degrade LAS [12,13]. Similarly, the competent fraction of degrading biomass in the porous pot reactors was estimated as 2.7%, since LAS contributed 2.7% of the feed COD.

Table 2:   LAS and AS concentrations in the influent to the Boone WPCP.

Sample Week (day)	C_{10} LAS mg/L	C_{11} LAS mg/L	C_{12} LAS mg/L	Total LAS $C_{10\text{-}14}$ mg/L	C_{12} AS mg/L	C_{13} AS mg/L	C_{14} AS mg/L	Total AS $C_{12\text{-}15}$ mg/L
1	0.74	0.54	0.18	1.46	0.22	0.16	<0.05	0.38
2	0.66	0.36	0.1	1.12	0.16	0.06	<0.05	0.22
3	1.1	0.82	0.44	2.36	0.42	0.16	0.12	0.7
4 (1)	1.08	0.94	0.56	2.58	0.50	0.22	0.16	0.88
4 (2)	1.76	1.88	0.9	4.72	2.22	1.04	0.70	4.06
4 (3)	1.86	1.68	1.16	4.92	1.70	0.86	0.68	3.48
avg ± s.d.	1.2 ± 0.5	1.0 ± 0.6	0.6 ± 0.4	2.9 ± 1.6	0.9 ± 0.9	0.4 ± 0.4	0.3 ± 0.3	1.6 ± 1.7

Experiments to test the influence of a range of HRTs (2 h, 4 h, 6 h and 12 h) with a constant SRT of 10 d on the biodegradation kinetics of LAS were performed. Subsequently, the experiments to test the influence of a range of SRTs (3 d, 6 d, 10 d, 15 d) with a constant HRT of 6 h were conducted. The results of the extant biodegradation tests are shown in Table 3.

From Table 3 it can be seen that the measured kinetic parameters were fairly constant when the SRT was maintained. There was some variability at the short HRT conditions, possibly due to the difficulty in maintaining steady-state

conditions (e.g., plugging of the stainless steel screen and subsequent cleaning may caused the true SRT/HRT to deviate slightly from short circuiting and./or loss of solids). Similar results were observed by Kaiser *et al.* [4] where effluent concentration of LAS remained constant and relatively independent of influent concentrations at higher HRTs, but varied at lower HRTs. At the long SRT conditions, there was a slight decrease in the $\hat{\mu}$ and K_S values for LAS, possibly indicative of low velocity, high affinity enzyme systems at these conditions. The evidence for this, however, was not strong. In pure culture studies, Sokol [17–19] and Sokol and Migiro [20] observed a much more pronounced decrease in $\hat{\mu}$ values for cultures grown at increasingly long SRTs. A similar decrease was not observed for the measured kinetic parameters for AS.

Table 3:   Extant kinetic parameters in porous pot reactors at various HRT and SRT conditions.

HRT (SRT)	LAS			AS			VSS, mg/L
	$\hat{\mu}$, h^{-1}	K_s, mg COD/L	Y, mg/mg as COD	$\hat{\mu}$, h^{-1}	K_s, mg COD/L	Y, mg/mg as COD	
2(10)	0.18±0.07	0.8±0.9	0.68±0.10	0.26±0.01	0.2±0.1	0.68±0.04	3574
4(10)	0.20±0.05	0.5±0.3	0.68±0.07	0.23±0.09	0.3±0.2	0.69±0.07	2233
6(10)	0.08±0.03	0.2±0.1	0.66±0.05	0.25±0.07	0.4±0.3	0.61±0.06	1283
12(10)	0.09±0.02	0.2±.02	0.65±0.05	0.26±0.01	0.5±0.0	0.61±0.07	794
6(3)	0.05±0.01	0.2±0.1	0.50±0.08	0.17±0.02	0.2±0.2	0.49±0.07	260
6(6)	0.08±0.02	0.1±0.1	0.65±0.04	0.20±0.06	0.2±0.1	0.52±0.07	461
6(10)	0.08±0.03	0.2±0.1	0.66±0.06	0.25±0.07	0.4±0.3	0.61±0.07	1283
6(15)	0.06±0.01	0.1±0.1	0.69±0.03	*	*	0.49±0.09	1550
18(15)	0.02±0.01	0.1±0.0	0.68±0.10	0.21±0.12	0.6±0.1	0.47±0.03	980
6(30)	0.07±0.02	0.1±0.1	0.64±0.11	0.29±0.06	1.3±0.3	0.52±0.08	2760
18(30)	0.03±0.01	0.1±0.1	0.48±0.13	*	*	0.43±0.15	2250

*Unique estimates were not obtainable for these tests due to parameter identifiability difficulties. However, the pseudo-first order rate coefficient was determined and was found to be 0.36±0.09 and 0.10±0.02 for the 6(15) and 18(30) conditions, respectively.

To evaluate the ability of extant kinetic parameters to predict effluent concentrations in full-scale wastewater treatment plants, field tests were conducted whereby the influent and effluent concentrations of LAS were measured as were the extant kinetic parameters. The method of Ellis and Eliosov [13] was used to estimate the competent biomass concentration by using a calibration period whereby the steady-state mass balance equation [23] and measured kinetic parameters are used to estimate the percent of competent biomass that would result in the measured effluent concentration of LAS. As can be seen from Table 4, the influent COD fraction provided a reasonable estimate of the competent biomass fraction. Consequently, the COD fraction was used for subsequent predictions of effluent concentrations.

Table 5 shows the results of the predictions of effluent LAS concentrations using measured extant kinetic parameters. Since the competent fraction measured during the calibration period was relatively close to the influent COD fraction (Table 4), the influent COD fraction was used to calculate the biomass involved in LAS degradation.

Table 4:   Comparison of influent COD to estimated competent biomass fraction.

Week (Day)	Influent COD fraction, %	Effl. conc., µg COD/L	K_S, mg COD/L	$\hat{\mu}$ X, mg COD/(LXhr)	$\hat{\mu}$, h⁻¹	Comp. biomass, mg COD/L[1]	MLVSS mg COD/L	Comp. biomass fraction, %
1	0.32	16	0.42	6.7	0.41	16.3	2272	0.72
2	0.30	46	0.62	7.2	0.21	34.3	2499	1.37
3	0.58	7	1.02	6.7	0.43	15.6	2200	0.71
4	1.57	84	1.00	6.3	0.19	34.0	1988	1.71
5(1)	2.66	57	1.34	7.5	0.36	20.8	3237	0.64
5(3)	1.60	24	0.78	11.3	0.50	22.6	3209	0.70
5(4)	1.38	19	1.19	12.2	0.48	25.4	3152	0.81
Av±s.d.	1.13±0.8	36±27	0.91±0.32	8.2±2.4	0.4±0.1	24±7	2650±535	0.95±0.42

[1] A measured k_d value of 0.11 d⁻¹ was used.

Table 5:   Comparison of predicted and measured effluent LAS concentrations at three wastewater treatment plants.

Week (Day)	Effluent concentration, µg COD/L	
	Measured	Predicted[2]
Cedar Rapids WPCF		
1	16	98
2	46	143
3	7	17
4	84	15
5(1)	57	32
5(3)	24	9
5(4)	19	15
Ave. ± s.d.	43±16	41±47
Boone WPCP		
1	3.6	9.9
2	2.9	2.5
3(1)	2.3	7.6
3(3)	3.9	5.8
Ave.±s.d.	2.9±0.7	6.5±3.1
Iowa City WWTP		
1[1]	5.9	13.2
2	4.4	9.5
3	4.0	8.8
4	3.9	6.5
Ave.±s.d.	4.5±0.7	9.5±2.8

[1] Calculation was based on the first order rate coefficient[2] k_d values of 0.11, 0.2, and 0.2 d⁻¹ were used for the Cedar Rapids, Boone, and Iowa City plants, respectively.

4   Conclusion

The efficacy of the extant respirometric technique to measure the extant kinetics of LAS biodegradation by field and laboratory biomass was demonstrated in this

study. LAS biodegradation kinetics in laboratory porous pot reactors were independent of whether the reactor was fed synthetic or real wastewater. The influence of HRT on the measured biodegradation kinetics was minimal with the possible exception that the biokinetic parameters were more variable at the low HRT conditions (i.e., when HRT < 6 h). The SRT had more noticeable effect on the measured biodegradation kinetics (e.g., the Y value for increased from 0.50 ± 0.08 to 0.66 ± 0.05 mg/mg and 0.49 ± 0.07 to 0.61 ± 0.07 mg/mg when the SRT increased from 3 to 10 d at a constant HRT of 6 h for LAS and AS, respectively).

The similarity in kinetic parameters between laboratory and field activated sludge systems was apparent from the study. The COD fraction proved to be a reliable indicator of the competent biomass concentration as validated by the calibration of the steady-state model. Extant kinetic parameters predicted average effluent LAS concentrations within 2% at one plant and within 4 μg/L at two other plants. Day to day predictions were not as close probably due to the non-steady state nature of the full-scale wastewater treatment plants and the limitations of the steady-state model.

Acknowledgements

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