Bull Environ Contam Toxicol (2010) 85:269–273
DOI 10.1007/s00128-010-0096-8

Anaerobic Biodegradability and Inhibitory Effects of Some Anionic and Cationic Surfactants

Beatriz Pérez-Armendáriz · Yésica Mayett Moreno · Oscar Monroy-Hermosillo · Jean Pierre Guyot · Rosa O. González

Received: 16 July 2010 / Accepted: 27 July 2010 / Published online: 5 August 2010

Abstract The anaerobic biodegradability and inhibitory effects on the methane production of three different surfactants, two anionic: sodium lauryl sulfate (SLS) and sodium dodecylbenzene sulfonate (SDBS), and a cationic surfactant: trialkyl-methylammonium chloride (TMAC), were evaluated with two different anaerobic sludges, granular and flocculent. Five different concentrations of the surfactants, 5, 50, 100, 250 and 500 mg/L, were tested. SLS was biodegraded at concentrations of 5, 50 and 100 mg/L with flocculent sludge and at 100 and 250 mg/L with granular sludge. However an inhibitory effect on methane production was observed in both sludges at 500 mg/L. The results indicate that SDBS was not biodegradable under anoxic conditions. TMAC was slightly degraded 50 and 100 mg/L with the flocculent sludge, and from 100 to 500 mg/L with the granular sludge.

Keywords Anaerobic degradation · Anaerobic sludge · Anionic surfactants · Cationic surfactant · UASB

B. Pérez-Armendáriz (✉) · Y. M. Moreno
Universidad Popular Autónoma del Estado de Puebla. Centro Interdisciplinario de Posgrados Investigación y Consultoría, 21 Sur 1103 Col, 72160 Santiago, Puebla, México
e-mail: beatriz.perez@upaep.mx

O. Monroy-Hermosillo · J. P. Guyot
Departamento de Biotecnología, Universidad Autónoma Metropolitana-I, Calle Rafael Atlixco No. 186, Col, Vicentina, México City, México

R. O. González
Departamento de Matemáticas, Universidad Autónoma Metropolitana-I, Calle Rafael Atlixco No. 186, Col, Vicentina, México City, México

Anionic and cationic are the most commonly used surfactants in the world and both their toxicity and occurrence in natural environments are well documented (Fuchedzhieva et al. 2008; Mungray and Kumar 2008; Sibila et al. 2008).

Surfactants are common constituents of municipal waste waters and the corresponding receiving freshwater and saltwater (Sharvelle et al. 2008). Surfactants can generate problems by foaming in the aeration tanks of activated sludge plants and reducing the settling properties of sludge flocks. Thus, their aerobic degradability, and the factors that affect it, remain very active areas of research (Hosseini et al. 2007).

In contrast, the study of the removal of surfactants using anaerobic digestion has not received the same attention, and several aspects of these processes have not yet been completely elucidated. Few works are dedicated to explaining the mechanisms of surfactant biodegradation under anaerobic conditions, a fact that can be explained because anaerobic digesters are generally used to treat industrial wastewaters, and, with a few exceptions, anaerobic reactors such as up flow anaerobic sludge blanket (UASB) reactors, have been used to treat low strength wastewaters e.g. municipal effluents which contain surfactants.

Nevertheless, anaerobic treatment of domestic wastewaters could be a good technical and economical option in tropical areas, where the ambient temperature would allow anaerobic digestion to proceed at acceptable rates. Furthermore, the incidence of detergents in industrial anaerobic digesters has not been precisely determined. Notwithstanding, based on the high efficiency of numerous existing anaerobic treatment plants, it can be said that detergents used to wash the equipment that reach industrial wastewaters would not affect the anaerobic process. Anaerobic degradation of nonionic surfactants (i.e., linear alcohol ethoxylate, fatty alcohol polyglycol ethers) has been reported (Liwarska-Bizukojc et al. 2008). Wagener and

Schink (1987) also showed the anaerobic biodegradability of anionic surfactants, using one type of anaerobic sludge in enrichment cultures and for seeding a fixed-bed reactor.

The inhibitory effect of the anionic surfactant sodium dodecylbenzene sulfonate (SDBS) on mesophilic fermentation in a lab-scale, continuously stirred digester, was reported by Khalil et al. (1988). The same authors (1989) showed that the growth and methanogenesis of *Methanosarcina barkerii* are completely inhibited by SDBS, at concentrations between 15 and 20 mg/L.

In this work, we studied the anaerobic biodegradability and inhibitory effects of two anionic surfactants, sodium dodecylbenzene sulfonate and sodium lauryl sulfate, and one cationic surfactant, trialkyl-methylammonium chloride, on two different types of sludge: a granular sludge from an industrial UASB reactor treating wastewaters from the paper industry, and a flocculent sludge from a pilot scale UASB reactor treating domestic wastewater.

Materials and Methods

Two different sludges were used in this study, see Table 1. One was a granular sludge (GS) obtained from an industrial up-flow anaerobic sludge blanket (UASB) reactor treating wastewaters from a paper manufacturer. The other was a flocculent sludge (FS) obtained from a pilot scale UASB reactor ($50\ m^3$) treating domestic wastewaters. The sludges were selected because of their very different characteristics, as shown in Table 1.

Batch reactors and medium. All experiments were performed in serum bottles (60 mL) closed with butyl rubber stoppers, under a nitrogen atmosphere (1 atm). Four mL of sludge were anaerobically added to 16 mL of anaerobic medium in the serum bottles. The medium was reduced by first adding 0.2 mL of a 2.5% anaerobic solution of sodium sulfide, followed by 0.4 mL of a different anaerobic stock solution of detergent. Finally, the serum bottles were incubated at 36°C, in the dark, on a reciprocal shaker with gentle mixing.

Controls without substrates were designed to determine the endogenous production of methane by the sludge. Parallel sterilized controls were run to determinate the abiotic methane.

Table 1 Characteristics of the sludge from the industrial UASB reactor (granular sludge) and from the pilot scale UASB reactor (flocculent sludge)

Characteristics	Granular sludge	Flocculent sludge
Total suspended solids (mg/L)	123.8	39.5
Volatile suspended solids (mg/L)	104	24
% VSS	80.5	60.8
Specific activity from acetate mmol CH_4/g VSS-h	0.48	0.09

The medium was prepared according to strict anaerobic procedures as described by Balch et al. (1979). The RAMM medium (g/L) 0.27 K_2HPO_4, 0.35 KH_2PO_4, 0.53 NH_4Cl, 0.075 $CaCl\cdot 2H_2O$, 0.1 $MgCl\cdot 6H_2O$, 0.02 $FeCl_2\cdot 4H_2O$ supplemented with trace elements (g/L) 0.5 $MnCl_4\cdot H_2O$, 0.05 $ZnCl_2$, 0.05 H_3BO_3, 0.03 $CuCl_2$, 0.01 $NiCl_26H_2O$, 0.05 Na_2SeO_3, 0.05 $CoCl_2\cdot 6H_2O$ (Shelton and Tiedje 1984) and a vitamin solution containing (in mg/L): 0.02 biotin, 0.02 folic acid, 0.1 pirydoxine, 0.05 thiamine, 0.05 riboflavin, 0.05 pantothenic acid, 0.001 and 0.05 vitamin B12, final pH = 7.0.

Anionic surfactants, sodium lauryl sulfate (SLS), $CH_3–(CH_2)_{11}–OSO_3–Na$, sodium dodecylbenzene sulfonate (SDBS) $CH_3–(CH_2)_{10}–CH_3–C_6H_6–SO_3–Na$, and the cationic detergent trialkyl-methylammonium chloride (TMAC) $((CH_3–CH_2)_n)_3–NH_3–Cl$ (n = 8–10 alkyl groups), and tri-hydrated acetate sodium were purchased from Sigma-Aldrich (Mexico).

Analytical procedures, volatile and total suspended solids were determined according to Standard Methods (APHA 1989). Methane production was determined by gas chromatography in batch reactors, as previously described (Guyot et al. 1990). Sampling was done at regular intervals by taking 0.5 µL of headspace gas.

Biodegradation kinetics was adjusted, after trying different models, to a square root model through time, using curvilinear regression. The slopes (biodegradation rate) were compared employing the *t*-student test. All experiments were done in triplicate. In order to determine significant differences between experiments and controls without surfactants, data were statistically analyzed using SPSS Inc. (Chicago, USA) software.

The percentages of surfactants biodegradation on methane production for both sludges were calculated according to the following expression:

$$\%\ \text{Biodegradation} = 100 \times [(P_i - P_{control}]/P_{theoretical})$$

where P_i = net CH_4 produced by each reactor, µmol CH_4/mL headspace gas, $P_{control}$ (biodegradability test) = net CH_4 produced by sample without surfactant and without acetate, µmol CH_4/mL headspace gas, $P_{theoretical}$ (biodegradability test) = was calculated considering the total conversion of surfactant according to the stoichiometry described by Tervin and Buswel (1932), µmol CH_4/mL gas.

Results and Discussion

In the flocculent sludge, SLS at concentrations of 5, 50 and 100 mg/L (100% biodegradation) produced no significant differences (Table 2). However at 250 mg/L SLS was

Table 2 Biodegradation test for three surfactants at different concentrations

Surfactant concentration (mg/L)	Final value of CH_4 less control for each assay [μmol CH_4/mL] (n = 3)	Biodegradation (%)	Adjusted model	Significant Difference (*)	R^2
Flocculent sludge (SLS)					
5	0.76	100	$CH_{4(5)} = 0.56 + 4.07\sqrt{t}$	b	0.96
50	3.16	100	$CH_{4(50)} = 0.86 + 4.53\sqrt{t}$	b	0.98
100	5.07	100	$CH_{4(100)} = -0.29 + 5.38\sqrt{t}$	b	0.98
250	5.92	68	$CH_{4(250)} = -1.42 + 5.23\sqrt{t}$	b	0.95
500	−10	0	$CH_{4(500)} = 0.13 + 2.19\sqrt{t}$	a	0.97
Granular sludge (SLS)					
5	0	0	$CH_{4(5)} = -0.68 + 6.48\sqrt{t}$	b	0.98
50	0	0	$CH_{4(50)} = -0.71 + 6.25\sqrt{t}$	b	0.98
100	3.50	100	$CH_{4(100)} = -0.92 + 9.06\sqrt{t}$	c	0.97
250	5.92	68	$CH_{4(250)} = -0.91 + 8.1\sqrt{t}$	c	0.96
500	−4.5	0	$CH_{4(500)} = -0.51 + 3.94\sqrt{t}$	a	0.97
Flocculent sludge (SDBS)					
5	1.5	0	$CH_{4(5)} = 0.70 + 3.29\sqrt{t}$	c	0.92
50	0.8	47.1	$CH_{4(50)} = 0.46 + 3.42\sqrt{t}$	c	0.97
100	1.61	47.4	$CH_{4(100)} = 0.53 + 1.36\sqrt{t}$	b	0.95
250	−6.07	0	$CH_{4(250)} = 0.48 + 1.5\sqrt{t}$	b	0.92
500	−10.24	0	$CH_{4(500)} = 0.84 + 0.15\sqrt{t}$	a	0.74
Granular sludge (SDBS)					
5	0	0	$CH_{4(5)} = 2.81 + 10.11\sqrt{t}$	c	0.81
50	0.43	25.35	$CH_{4(50)} = 3.3 + 10.09\sqrt{t}$	c	0.84
100	1.08	31.83	$CH_{4(100)} = 3.6 + 10.01\sqrt{t}$	b	0.79
250	3.05	36	$CH_{4(250)} = 2.62 + 11.13\sqrt{t}$	b	0.77
500	−3.6	0	$CH_{4(500)} = 3.58 + 6.48\sqrt{t}$	a	0.74
Flocculent sludge (TMAC)					
5	0	0	$CH_{4(5)} = -0.97 + 4.09\sqrt{t}$	a	0.93
50	0.78	15	$CH_{4(50)} = -0.92 + 4.07\sqrt{t}$	a	0.92
100	0.22	7.7	$CH_{4(100)} = -0.83 + 4.35\sqrt{t}$	a	0.95
250	0.12	0	$CH_{4(250)} = -0.87 + 3.93\sqrt{t}$	a	0.90
500	0.23	0	$CH_{4(500)} = -0.72 + 4.40\sqrt{t}$	a	0.92
Granular sludge (TMAC)					
5	0	0	$CH_{4(5)} = 2.00 + 2.79\sqrt{t}$	a	0.92
50	0.02	1.42	$CH_{4(50)} = 2.18 + 2.71\sqrt{t}$	a	0.92
100	2.94	100	$CH_{4(100)} = 2.43 + 3.19\sqrt{t}$	b	0.89
250	5.71	80.76	$CH_{4(250)} = 3.43 + 3.25\sqrt{t}$	b	0.89
500	8.57	60.60	$CH_{4(500)} = 2.23 + 4.07\sqrt{t}$	c	0.91

* Same letters did not show significant differences between slopes ($p < 0.05$)

partially degraded (68%) and at a significantly slower rate. At 500 mg/L, SLS inhibited methane production, and had the slowest rate.

With the granular sludge, SLS was totally degraded (100%), at concentrations of 100 mg/L. At 250 mg/L the final biodegradation percentage was 68%, in agreement with the theoretical prediction (Table 2). At 5 and 50 mg/L there were no significant differences between slopes ($\alpha = 0.01$) and the control. At 500 mg/L SLS inhibited methane production.

Biodegradation of linear alquilbenzene sulfonate (LAS) surfactants has already been reported; Abboud et al. (2007) reported biodegradation of LAS by a consortium of the mixed facultative anaerobes *Acinetobacter calcoaceticus* and *Pantoea agglomerans* up to 300 mg/L of LAS, however higher concentrations of LAS showed a toxicity to the mixer co-culture. In our experiment, we also observed a toxic effect up of 500 mg/L with both sludges. Abboud et al. (2007) indicate that high surfactant concentration affects Acetoclastic bacteria, specifically the structural bacteria of anaerobic sludge. This was demonstrated by the observation of an increase of the filamentous form released from aggregate structure, depending on the time of exposure and surfactant concentrations (Costa et al. 2007).

Regarding to biodegradation of sodium dodecylbenzene sulfonate with flocculent sludge at concentrations of 50 and 100 mg/L, SDBS was slightly biodegradable (47.1% and 47.4% respectively) (Table 2), and was strongly inhibitory at 250 and 500 mg/L. With granular sludge, SDBS was slightly biodegradable at 50 mg/L (25.35%), 100 mg/L (31.8%) and 250 mg/L (36%) and became inhibitory at 500 mg/L. Toxic effect on methanogenic bacteria at 500 mg/L was found for both sludges (Table 2).

Under conditions studied in this work, the anionic surfactants, SLS and SDBS, were wholly or partially biodegraded except at 500 mg/L, which was toxic to methanogenic bacteria. Biodegradation results of anionic surfactants for both sludges were different, due to their intrinsic differences. Recent studies indicate the importance of the type of sludge used to degrade toxic substances. Two factors were identified in anaerobic biodegradation: the occurrence of adapted microorganisms and the structure of bacterial communities [can be granular or flocculent sludge (Lozada et al. 2006)]. Similarly, McHugh et al. (2004) demonstrated a high diversity of anaerobic microflora with different capacities to remove polluting agents, such as anionic surfactants. Other authors suggest that biodegradation of LAS is due to the possible use of alkylbenzenesulphonate as a source of sulphur by anaerobic bacteria under anoxic conditions (Denger and Cook 1999).

The metabolic pathways for anaerobic degradation of substituted monobenzenes have not been clearly elucidated, except for benzoate (Macarie et al. 1991). In addition, a relatively limited range of mononuclear aromatics are known to be anaerobically mineralized to methane and CO_2. Our results appear to partially contradict those of Wagener and Schink (1987). These authors found that both alkylsulfonates and alkylbenzenesulfonates were not anaerobically degraded at concentrations higher than 10 mg/L, and that SLS was partly degraded at concentrations lower than 100 mg/L; however, they used only one type of anaerobic sludge. The differences between our results and the data of Wagener and Schink (1987) can be explained by the behavioral variability of sludges from different origins.

TMAC was only slightly degraded at 50 mg/L (15%) and 100 mg/L (7.7%), but with no significance difference to the control. At 250 and 500, TMAC was not degraded by flocculent sludge, even after 22 days of incubation. The granular sludge did not degrade TMAC at concentrations of 5 and 50 mg/l. From 100 to 500 mg/l TMAC was degraded, but the percentage of biodegradation decreased from 100% to 60.6% (Table 2). The 100, 250 and 500 mg/L slopes were not significantly different, except at 500 mg/L which was the highest of all. In this case, a concentration threshold was observed; below 100 mg/L of TMAC the rate of biodegradation was slower.

The difference between sludges and their response to surfactant biodegradation was most marked with the cationic detergent (TMAC); in this case, the granular sludge (of industrial origin) was observed to have an increased rate of methane production compared with the flocculent sludge. Similar results were reported by Federle and Schwab (1992) who tested the biodegradability of stearyltrimethylammonium chloride (a cationic surfactant) under anaerobic conditions, using a well-adapted microorganism. The sample obtained was taken from a Laundromat wastewater pond which had been exposed to surfactants for over 25 years. As mentioned earlier, it is important to consider the distinct biodegradation capabilities of anaerobic sludges in terms of different aspects, such as the adaptation of microorganisms.

References

Abboud MM, Khleifat KM, Batarseh M, Tarawneh K, Al-Mustafa A, Al-Madadhah M (2007) Different optimization conditions required for enhancing the biodegradation of linear alkylbenzosulfonate and sodium dodecyl sulfate surfactants by novel consortium of *Acinetobacter calcoaceticus* and *Pantoea agglomerans*. Enzyme Microb Tech 41:432–439

APHA—AWWA—WPCF (1989) Standard methods for the examination for water and wastewater. In: Clesceri LS, Greenberg AE, Trussell RR (eds) 17 th ed, American Public Health Association, Washington DC

Balch W, Fox G, Magrum C, Woese C, Wolfe R (1979) Methanogens: reevaluation of a unique biological group. Microbiol Rev 43:260–296

Costa JC, Abreu AA, Ferreira EC, Alves MM (2007) Quantitative image analysis as a diagnostic tool for monitoring structural changes of anaerobic granular sludge during detergent shock loads. Biotechnol Bioeng 98:60–68

Denger K, Cook AM (1999) Note: linear alkylbenzenesulphonate (LAS) bioavailability to anaerobic bacteria as a source of sulphur. J Appl Microbiol 86:165–168

Federle TW, Schwab BS (1992) Mineralization of surfactants in anaerobic sediments of a laundromat wastewater pond. Water Res 26:123–127

Fuchedzhieva N, Karakashev D, Angelidaki I (2008) Anaerobic biodegradation of fluoranthene under methanogenic conditions in presence of surface-active compounds. J Hazard Mater 153:123–127

Guyot JP, Noyola A, Monroy O (1990) Evolution of microbial activities and population in granular sludge from an UASB reactor. Biotechnol Lett 12:155–160

Hosseini F, Malekzadeh F, Amirniiozafari N, Ghaemi N (2007) Biodegradation of anionic surfactants by isolated bacteria from activated sludge. Int J Environ Sci Tech 4:127–132

Khalil EF, Whitmore TN, Gamel-El-Din H, El-Bassel A, Lloyd D (1988) The effects of detergents on anaerobic digestion. Appl Microbiol Biot 29:517–522

Khalil EF, Whitmore TN, Gamel-El-Din H, El-Bassel A, Lloyd D (1989) The effect of detergent on methanogenesis by *Methanosarcina barkeri*. FEMS Microbiol Lett 57:313–316

Liwarska-Bizukojc E, Scheumann R, Drews A, Bracklow U, Kraume M (2008) Effect of anionic and nonionic surfactants on the kinetics of the aerobic heterotrophic biodegradation of organic matter in industrial wastewater. Water Re 42:923–930

Lozada M, Figuerola E, Itria R, Erijman L (2006) Replicability of dominant bacterial populations after long. Environ Microbiol 8:625–638

Macarie H, Noyola A, Guyot JP (1992) Anaerobic treatment of a petrochemical wastewater from a terephthalic acid plant. Water Sci Technol 25:223–235

McHugh S, Cartoon M, Collins G, O'Flaherty V (2004) Reactor performance and microbial community dynamics during biological treatment of wastewaters at 16–37°C. FEMS Microbiol Ecol 48:369–378

Mungray AK, Kumar P (2008) Anionic surfactants in treated sewage and sludges: Risk assessment to aquatic and terrestrial environments. Bioresource Technol 99:2919–2929

Sharvelle S, Garland J, Banks MK (2008) Biodegradation of polyalcohol ethoxylate by a wastewater microbial consortium. Biodegradation 19:215–221

Shelton DR, Tiedje JM (1984) General method for determining anaerobic biodegradation potential. Appl Environ Microb 47:850–857

Sibila MA, Garrido MC, Perales JA, Quiroga JM (2008) Ecotoxicity and biodegradability of an alkyl ethoxysulphate surfactant in coastal waters. Sci Total Environ 394:265–274

Tervin D, Buswel AM (1932) The methane fermentation of organic acids and carbohydrates. J Am Chem Soc 56:1751–1755

Wagener S, Schink B (1987) Anaerobic degradation of nonionic and anionic surfactants in enrichment cultures and fixed-bed reactors. Water Res 21:615–622