Future of Anaerobic Biodegradation Criterion for Ecolabel: Linear Alkylbenzene Sulfonate (LAS) Biodegradation in Anaerobic Bio-Reactors is Well Documented

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Abstract

The requirement for anaerobic biodegradation of the surfactants used in laundry and cleaning products is a criterion for ecolabel programs in Europe. The relevance to environmental safety of this requirement has been questioned for surfactants such as Linear Alkylbenzene Sulfonate (LAS) that undergo rapid and complete biodegradation under aerobic conditions. Since this requirement was adopted, some studies have demonstrated that LAS can undergo anaerobic biodegradation. This poster reviews the available data on LAS anaerobic biodegradation in bio-reactors, vessels designed to facilitate treatment of wastewater by microorganisms. The review concludes LAS anaerobic biodegradation in bio-reactors is well documented (18 studies to date).

Introduction

- Previous studies have demonstrated that LAS undergoes anaerobic biodegradation under sulfate-limited conditions (Denger and Cook, J. Appl. Microbiol. 86: 165-168, 1999) and in marine sediments (latest study: Corada-Fernández et al., J. Hazard. Mat. 360: 24-31, 2018, and see platform presentation by C. Corada Fernández).
- Ecolabel programs in Europe (Nordic Swan, EU Flower) include a requirement for anaerobic biodegradation of H400 and/or H412 classified surfactants used in laundry and cleaning products.
- The relevance of environmental safety to this requirement has been questioned for surfactants such as LAS that undergo rapid and complete biodegradation under aerobic conditions.
- Studies demonstrating LAS anaerobic biodegradation add further weight to this question.
- This poster is an update of a 2017 paper (J.E. Heinze, *CLER Review*, vol. 15, pp. 20-59, available at www.CLER.com/review) which can be consulted for more detailed information.

Methods

- A search of the available scientific literature was conducted in two stages:
  - An initial review of studies on LAS anaerobic biodegradation in the CLER in-house library, and
  - Systematic monitoring (last 10 years) of Chemical Abstracts and Medline databases using the SciFinder® tool (American Chemistry Society, CAS® Division) with "LAS" or "linear alkylbenzene" as the search terms.
- Search results are current through February 2019.

- **Quality criteria for Including bio-reactor studies in this review:**
  - Published studies
  - Anaerobic conditions verified via:
    - Use of oxidation/reduction indicator dye (resazurin)
    - Direct measure of anaerobic processes, such as nitrate reduction
    - Confirmation that predominate microorganisms are anaerobes, and/or
    - Other measurement method.
  - LAS biodegradation measured using analytical methods specific for LAS:
    - Typically, HPLC with UV detection (primary biodegradation).
    - Use of LAS radio-isomers uniformly ring labeled with carbon-14 (^{14}C). Production of ^{14}C-CO_2 and ^{14}C-methane gas is a measure of ultimate biodegradation (mineralization).
  - Anaerobic biodegradation determined from mass balance removals, which accounts for any LAS bound to solids/sludge.

- In this poster, studies demonstrating LAS anaerobic biodegradation are grouped by bio-reactor type in the following tables. A graphic image for each type of bio-reactor is provided with the corresponding table of study results.

Table 1. LAS Anaerobic Biodegradation in Batch (Sealed Vessel) Reactors (Figure 1)

Study	Experimental Conditions: • Essential Salts Medium • Mesophilic temperatures (30-36°C) • Unwashed sludge and/or co-substrates (yeast extract + carbon sources) used in all studies*	LAS Biodegradation (Mass balance removal measured with analytical method specific for LAS)
Sanz et al. *Riv. Ital. Sostanze Grasse* 76, 307-11, 1999; *CLER Rev.* 6, 26-30, 2000	Test 1 (14 days) 9 LAS concentrations, 12.5 – 350 mg/L Test 2 24 hrs., LAS @ 90 mg/L 72 hrs., LAS @ 40 mg/L Test 3 24 hrs., LAS @ 150 mg/L 72 hrs., LAS @ 50 mg/L	**5.4-43.6%** (no trend with concentrations) **55%** **50%** **70%** **64%**
Pratts et al. *CLER Rev.* 6, 22-5, 2000	LAS @ 60 mg/L: Test 1 (250 days) Alicante sludge (4 tests) Madrid sludge (4 tests) Test 2 (Madrid sludge) 14 days 92 days	 **30-93%** **36-84%** **37%** **47%**
Motteran et al. *J. Int. Biodeterior. Biodegrad.* 96, 198-204, 2014; *J.Environ. Sci. Health, Part A 51, 1286-1302*, 2016	LAS in laundry wastewater (28 days): LAS @ 16 mg/L LAS @ 75 mg/L	 **22%** **63%**

* Two studies using washed sludge and no co-substrate (Garcia et al. 2005, 2006) showed no LAS biodegradation; a third study (Garcia et al. *Biodegrad.* 17: 39-46, 2006) showed LAS biodegradation ranging from no significant detection to 22%.

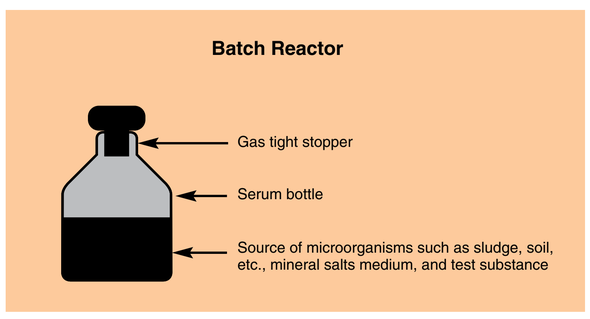

Figure 1. Batch (sealed vessel) bio-reactor for anaerobic biodegradation testing.

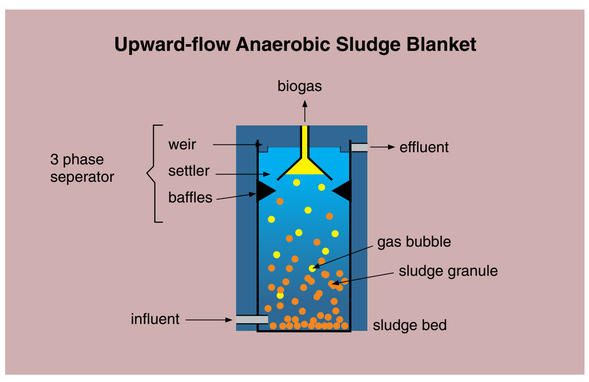

Figure 2. Flow-through Bio-reactors Using Granular/Immobilized Sludge: Upward-flow Anaerobic Sludge Blanket (UASB) Reactor.

Table 2. LAS Anaerobic Biodegradation in Upward-flow Anaerobic Sludge Blanket (UASB) Bio-Reactors (Fig. 2), Flow-through Vessels using Granular/Immobilized Sludge

Study	Experimental Conditions: • Feed: Essential Salts Medium (ESM) • Sludge from bio-reactor or anaerobic digester • Mesophilic temperatures • Hydraulic residence time = 24-48 hrs.	LAS Biodegradation (Mass balance removal measured with analytical method specific for LAS)
Sanz et al. *Biodegrad.* 14, 57-64, 2003	LAS, operated 3 months Feed: ESM + carbon sources Feed: ESM only	 **64%** **85%**
Angelikadi et al. *Water Sci. Technol.* 49(10), 115-122, 2004	C12-LAS, operated 87 days ^{14}C-LAS, operated 5 days	**40%** **5.6% converted to $^{14}CO_2$** (mineralization)
Løbner et al. *Biotech. Bioeng.* 89, 759-65, 2005	LAS, operated 8 months	**51%** (similar daily removal at 30°and 55°C)
Delforno et al. *Sci. Total Environ.* 649: 482-94, 2018	Operated 100 hours LAS in ESM + yeast extract + carbon sources LAS in commercial laundry wastewater	**45%*** **47%**

* The biodegradation pathway in bio-reactors is the same as in marine sediments, based on identifying bacteria with the metabolic capability for executing each step of the LAS anaerobic biodegradation pathway observed in marine sediments.

Table 3. LAS Anaerobic Biodegradation in Horizontal Anaerobic Immobilized Bed (HAIB) Bio-Reactors (Fig. 3), Flow-through Vessels Using Granular/Immobilized Sludge

Study	Experimental Conditions: • Feed: Essential Salts Medium (ESM) • Support: Polyurethane (PU) foam • Waste water treatment plant sludge • Mesophilic temperatures • Hydraulic residence time = 12 hrs. • Operated 9.5 months • LAS: 7-14 mg/L	LAS Biodegradation (Mass balance removal measured with analytical method specific for LAS)
Duarte et al. *J. Braz. Chem. Soc.* 17, 1360-7, 2006; *Biodegradation* 19, 375-85, 2008	Feed: ESM + yeast extract + sucrose	**35%**
Oliveira et al. *J. Environ. Manage.* 90, 1261-68, 2009	Support: charcoal Support: PU foam + clay beads	**28%** **27%**
Duarte et al. 2010B	Feed: ESM +/- co-substrates (meat extract, starch, sucrose)	**34%**

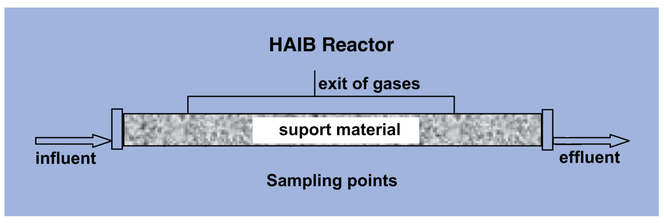

Figure 3. Flow-through Bio-reactors Using Granular/Immobilized Sludge: Horizontal Anaerobic Immobilized Bed (HAIB) Reactor.

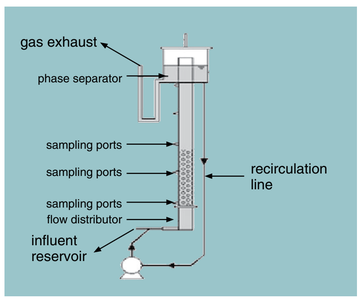

Figure 5. Expanded Granular Sludge Bed (EGSB) reactor

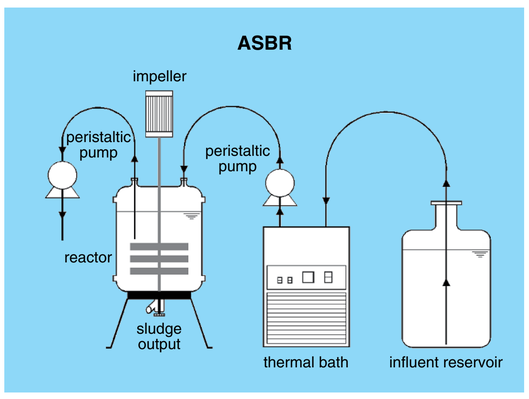

Figure 4. Anaerobic Sequencing Batch Reactor (ASBR).

Table 4. LAS Anaerobic Biodegradation in Anaerobic Sequencing Batch Reactors (ASBR, Fig. 4), Flow-through Vessels Using Granular/Immobilized Sludge

Study	Experimental Conditions: • Feed: Essential Salts Medium (ESM) • Solids: Waste water treatment plant sludge • Mesophilic temperature • Hydraulic residence time = 23-24 hrs. • Operated 14-67 days per test • LAS: from liquid laundry detergent	LAS Biodegradation (Mass balance removal measured with analytical method specific for LAS)
Duarte et al. *Internat. Biodeter. Biodegrad.* 64, 129-134, 2010	LAS = 22 mg/L Feed: ESM + yeast extract + starch + sucrose Feed: Same as above except 10x yeast extract Feed: ESM only	 **37%** **24%** **53%**
Duarte et al. *Rev. Biol. Trop.* 63, 295-302, 2015	Solids: river sediment with high levels of denitrifying bacteria: LAS = 15 mg/L, feed: ESM + KNO_3 + yeast extract + starch + sucrose LAS = 15 mg/L, same feed as above except yeast extract + starch + sucrose at ½ level) LAS = 30 mg/L, same feed as first	 **60%** **55%** **47%**
Duarte et al. *Braz. Arch. Biol. Technol.* 58, 326-332, 2015	LAS = 22 mg/L, feed: ESM + KNO_3 (170 mg/L) + yeast extract + sucrose	**87%**

Table 5. LAS Anaerobic Biodegradation in Expanded Granular Sludge Bed (EGSB) Reactors (Fig. 5), Flow-through Vessels Using Granular/Immobilized Sludge

Study	Experimental Conditions: • Sludge from UASB reactor Mesophilic temperature • Operated 173-237 days	LAS Biodegradation Mass balance removal measured with analytical method specific for LAS)
Delforno et al. *Bioresource Technol.* 107, 103-9, 2012	Feed: ESM + ethanol + methanol, HRT = 26 or 32 hrs., LAS = 13-14 mg/L	**57%**
Delforno et al. *Bioresource Technol.* 154, 114-21, 2014	Feed: ESM + ethanol + methanol + yeast extract, HRT = 38 hours: LAS at 13 mg/L LAS at 11 mg/L from laundry wastewater LAS at 12 mg/L from laundry wastewater	 **56%** **73%** **78%**
Delforno et al. *Bioresource Technol.* 192, 37-45, 2015	Feed: ESM + ethanol + methanol + yeast extract, HRT = 39 hours, LAS at 29 mg/L from laundry wastewater	**52%**
Delforno et al. *J. Environ. Manage.* 183, 687-93, 2016	Feed: Same as Delforno et al. 2014, except 7.3 mM Fe(III) added, LAS at 13 mg/L	**71%**
Granatto et al. 2019, *Int. Biodeter. Biodegr.* 138, 23-32, 2019	Pilot scale reactor (69 L) Feed: primary treated (settled) sewage from waste water treatment plant, HRT = 36 hours, LAS in sewage at ~6.2 mg/L, operated 314 days	**50%**

Table 6. LAS Anaerobic Biodegradation in Continuous Stirred Tank Reactors (CSTR) (Fig. 6), Flow-through Vessels Not Using Granular/Immobilized Sludge

Study	Experimental Conditions: • Feed: anaerobic digester sludge spiked with 2-phenyl C-12 LAS; LAS bioavailability as measured in binding studies. • Sludge pre-adapted by operating for several months with feed but no LAS* • Mesophilic temperature • Hydraulic residence time = 15 days • Each phase operated 35-72 days	LAS Biodegradation (Mass balance removal measured with analytical method specific for LAS)
Haggensen et al. *Wat. Sci. Technol.* 46(10), 159-65, 2002	Two reactors (R1, R2), each with two operational phases (A, B) Phase A, LAS = ~104 mg/L: R1, bioavailable LAS = 16% R2, bioavailable LAS = 18% Phase B: R1, LAS = 268 mg/L, bioavailable LAS = 50%) R2, LAS = 108 mg/L, bioavailable LAS = 24%)	 **14%** **20%** **23%** **25%**
Mogensen et al. *Environ. Toxicol. Chem.* 22, 706-11, 2003	LAS ~100 mg/L: Phase A, bioavailable LAS = 18% Phase B, bioavailable LAS = 24%	 **20%** **28%**

* No LAS biodegradation observed in study (Angelidaki et al. 2004) with no pre-adaption of sludge.

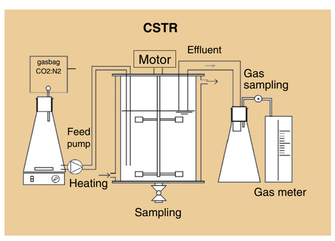

Figure 6. Continuous Stirred Tank Reactor (CSTR).

Conclusions

- LAS anaerobic biodegradation in batch and flow-through bio-reactors is documented in 18 studies in which oxygen-free (anoxic) conditions were verified and biodegradation measured using analytical methods specific for LAS.
- Biodegradation observed was primary biodegradation (removal of parent material (LAS)) but mineralization (ultimate biodegradation) was documented in one study using radiolabeled LAS.
- The biodegradation pathway in bio-reactors appears to be the same as in marine sediments, based on identifying bacteria (in a bio-reactor study) with the metabolic capability for executing each step of the LAS anaerobic biodegradation pathway observed in marine sediments.
- These data further challenge the environmental relevance of anaerobic biodegradation requirements that are part of ecolabel programs in Europe.
- Ecolabel criteria requiring anaerobic biodegradability should be reviewed taking into consideration these recent studies.
- Findings will be further discussed in the Safety & Regulatory Affairs Session 3, 17:30 Tuesday.