Future of Anaerobic Biodegradation Criteria for Ecolabel: The Challenge Posed by Linear Alkylbenzene Sulfonate (LAS) Anaerobic Biodegradation in Bio-reactors

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The EU Flower and Nordic Swan Ecolabel programs

- **Include a requirement for anaerobic biodegradability of surfactants in cleaning products.**
- **This criterion has been criticized:**
  - **European Commission's SCHEER (Scientific Committee on Health, Environment and Emerging Risks) expert advisory committee (2005, confirmed 2008):**

    "A poor biodegradability under anaerobic conditions is not expected to produce substantial modifications in the risk for freshwater ecosystems as the surfactant removal in the STPs seems to be regulated by its aerobic biodegradability."
  - **HERA Project, a joint AISE + Cefic effort (2013):**

    "The requirement of ultimate biodegradability under anaerobic conditions cannot be considered an effective measure for environmental protection."

Essential Background:

- **LAS does not meet ecolabel criterion for anaerobic biodegradability**
  - **based on failure to observe 60% biodegradation (mineralization) in the ECETOC 28, ISO 11734, OECD 311 or equivalent tests measuring headspace gas production.**
  - **This requirement applies to all surfactants classified as aquatic Acute Category 1 (H400) or Chronic Category 3 (H412).**
- **These programs do not consider recent studies demonstrating LAS anaerobic biodegradation in relevant environmental compartments:**
  - **Anaerobic marine sediments (C. Corada-Fernandez et al., Cadiz University)**
    - **Following paper in this session**
  - **Wastewater treatment vessels known as bio-reactors**
    - **This presentation**
    - **Poster "LAS Biodegradation in Anaerobic Bio-Reactors is Well Documented"**

Bio-reactor Studies – Quality Requirements

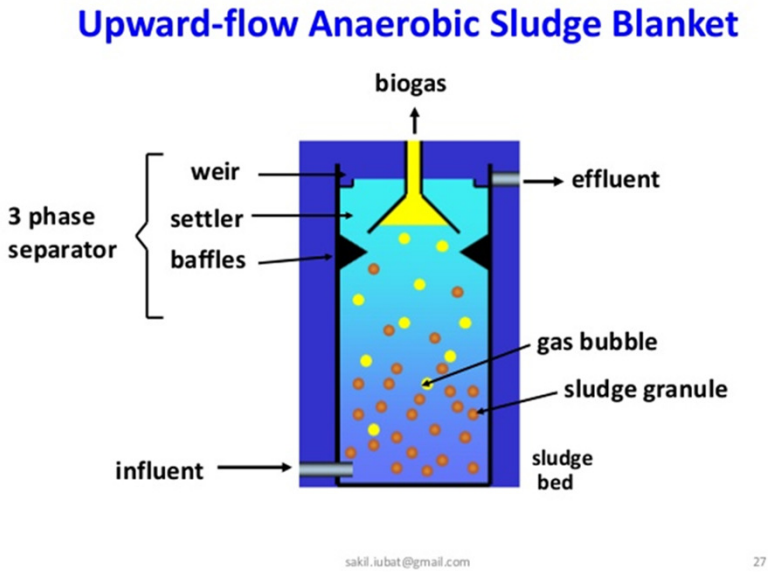
- Published studies
- Anaerobic conditions verified
  - Use of resazurin (oxidation/reduction indicator dye), or
  - Other measurement method.
- Concentrations measured using analytical methods specific for LAS, typically: HPLC with UV detection.
- Mass balance removals measured, to account for any LAS bound to solids/sludge.

Overview of Bio-Reactors

Types

- **Batch (sealed vessel)**
- **Flow-through Reactors**
  - **Granular/immobilized sludge**
    - **Upflow (schematic)**
    - **Sequencing (cyclic operation)**
    - **Expanded Granular (co-substrates in feed for sludge growth)**
    - **Horizontal (support bed for sludge)**
  - **Non-immobilized sludge**
    - **Continuous stirred**

Schematic



Bio-reactor Studies Meeting Quality Criteria

Batch Reactors

- **3 Positive studies, demonstrating LAS anaerobic biodegradation**
- **3 Negative studies, showing no/minimal LAS anaerobic biodegradation**

Flow-through Reactors

***Granular/Immobilized Sludge* – All 15 studies positive**

- **Upflow – 4 Studies**
- **Sequencing – 3 Studies**
- **Expanded Granular – 5 Studies**
- **Horizontal – 3 Studies**

Non-immobilized Sludge

- **Continuous Stirred – 2 Positive studies + 1 Negative study**

Batch Reactors – 3 Positive Studies

- **25 tests showed LAS anaerobic biodegradation**
  - **Mean value = 43%, but wide range = 5.4-93%**
- **Wide range of test conditions:**
  - **Co-substrates (yeast extract plus carbon source, such as sucrose or acetate, propionate, and butyrate)**
  - **Microbial inoculums (digester sludge or sludge from bio-reactor)**
  - **LAS sources (pure LAS or digester sludge or laundry wastewater containing LAS),**
  - **LAS concentrations (12 – 350 mg/L) and**
  - **Incubation periods (1 to 250 days).**
- **No obvious correlation of test conditions with extent of biodegradation**

Batch Reactors – 3 Negative Studies (19 Tests)

- **Comparing positive and negative tests identifies experimental conditions essential for LAS anaerobic biodegradation:**
  - **Analytical method specific for LAS**
    - **Gas production not sensitive enough even in tests with 93% LAS primary biodegradation (Prats et al. 2000).**
  - **Co-substrates – either from direct addition in feed or use of unwashed sludge (co-substrates present in sludge)**
    - **Studies with <u>washed</u> sludge and <u>no</u> co-substrates found no/minimal biodegradation, <u>even</u> with HPLC/UV detection.**

New Test Developed to Predict Ultimate Biodegradability in Anaerobic Digesters

- **AnBUSDiC (Anaerobic Biodegradation under Sludge Digester Conditions) Test (Bendt & Willing, 2012).**
  - **A batch test with two-step addition of test substance; biodegradation is measured after the second addition.**
- **LAS produces negligible gas production (Eadsforth et al. 2013).**
- **These results are consistent with other batch test results which indicate LAS anaerobic biodegradation cannot be detected by gas production.**

Conclusions on Batch Reactors

- **LAS anaerobic biodegradation has been observed in numerous tests using batch reactors.**
- **Detection requires measurement of parental material (primary biodegradation).**
  - **Gas production (mineralization) not observed.**
- **Measurable biodegradation requires co-substrates, either added directly or present in sludge.**
  - **Batch tests using washed sludge and no co-substrate (to optimize measurement of gas production) are unlikely to detect LAS anaerobic biodegradation, even primary biodegradation.**

Flow-through Reactors using Granular/Immobilized Sludge

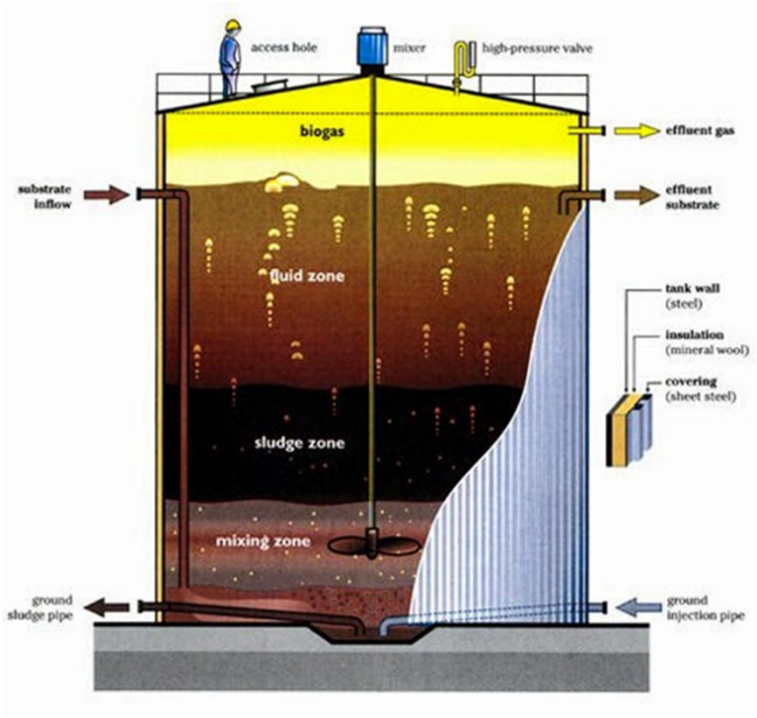
- **LAS anaerobic biodegradation demonstrated in all four reactor types**
  - **15 published studies, 27 tests, mean value = 53%, range = 24-87%.**
- **Experimental conditions:**
  - **Observed with no co-substrate (where compared, better without).**
  - **Similar removals at thermophilic (55°C) as mesophilic (30-37°C) temperatures (nearly all use mesophilic).**
- **LAS anaerobic biodegradation observed with range of:**
  - **Microbial inoculums (digester sludge, sludge from bioreactors, or river sediments).**
  - **LAS sources (pure LAS, or laundry detergent, laundry wastewater or sewage containing LAS).**
  - **LAS concentrations (4 - 32 mg/L).**
  - **Hydraulic resident times (12-48 hours).**
  - **Operational periods (100 hours to 11.5 months).**

Key Findings – Flow-through Reactors using Granular/Immobilized Sludge

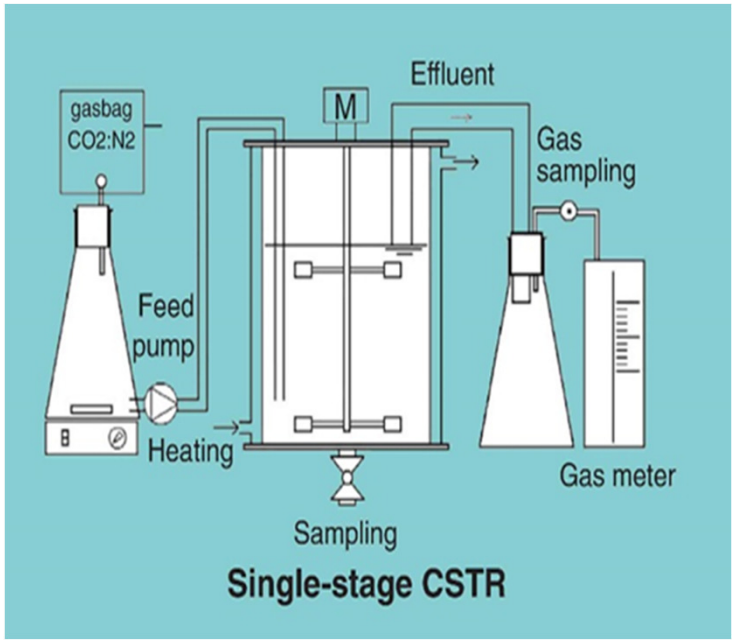
- LAS mineralization observed with ^{14}C-LAS yielding $^{14}CO_2$ and ^{14}C-methane (Angelidaki et al. 2004).
  - Extent of mineralization 5.6% vs. 40% primary biodegradation.
- Predominant microbes present in bioreactors treating LAS/laundry wastewater identified (Delforno et al. 2018):
  - Same biodegradation pathway (based on metabolic activity) as in marine sediments (Lora-Martin et al. 2010).
  - Pathway is for complete biodegradation (mineralization).

Continuous Stirred Reactors – Most like WWTP Anaerobic Digesters

WWTP Anaerobic Digester



Continuous Stirred Reactor



Study Results with Continuous Stirred Reactors

- **LAS anaerobic biodegradation observed in two studies**
  - **6 tests, mean value = 22%, range = 14-28%**
- **No LAS anaerobic biodegradation observed in third study**
  - **2 tests**
- **All studies conducted in same laboratory (Denmark Technical University) using very similar experimental procedures**
  - **Procedures also similar to other flow-through studies, except HRT = 15 days, similar to anaerobic digesters.**

Conclusions on Continuous Stirred (CS) Reactors

- **Key difference among studies: Use of pre-adapted sludge inoculum in all positive tests vs. not pre-adapted in all negative tests.**
- **During pre-adaption, the sludge is starved for organic compounds (none in feed) and microbes digest anaerobically biodegradable substances in sludge.**
- **Hypothesis: competition from more readily anaerobically biodegradable substances in sludge explains the lack of LAS biodegradation in sludge that has not been preadapted.**
- **Hypothesis may explain the lack of LAS biodegradation in sludge digesters (due to presence of more readily anaerobically biodegradable substances)**

Conclusions on LAS

- **LAS anaerobic biodegradation demonstrated in batch and flow-through bio-reactors (20 studies).**
- **LAS mineralization demonstrated in one study.**
- **Even primary biodegradation, as observed in all studies, improves LAS aquatic safety**
  - **LAS biodegradation intermediates (sulfophenyl carboxylates, SPCs) have much lower (~20 fold) aquatic toxicity than LAS (Kimerle and Swisher, 1977).**

Conclusions on Ecolabel Programs

- **LAS anaerobic biodegradation is well documented in relevant environmental compartments:**
  - **Wastewater treatment bio-reactors (this study)**
  - **Marine sediments (Corada-Fernández, this session).**
- **Such observations support the conclusions of the HERA Project (2013):**

  "The requirement of ultimate biodegradability under anaerobic conditions cannot be considered an effective measure for environmental protection."
- **Ecolabel criteria requiring anaerobic biodegradability should be reviewed taking into consideration these recent studies.**