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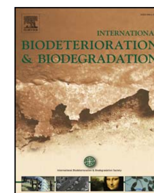

Anaerobic co-digestion of commercial laundry wastewater and domestic sewage in a pilot-scale EGSB reactor: The influence of surfactant concentration on microbial diversity

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ABSTRACT

Different molecular tools (PCR-DGGE, 16S rRNA high-throughput sequencing and sequencing of the *bamA* gene) were used to assess and compare the microbial diversity in a pilot-scale expanded granular sludge bed (EGSB) reactor used for the anaerobic co-digestion of commercial laundry wastewater and domestic sewage and subjected to increasing concentrations of linear alkylbenzene sulfonate (LAS). Using PCR-DGGE, a microbial stratification along the sludge bed of the reactor was observed. When analyzed using 16S rRNA gene sequencing, the samples exhibited high microbial diversity and richness, with the lowest Shannon index obtained for the highest concentration of surfactant. For the Bacteria domain, the genera *Bellilinea*, *Syntrophus*, *Syntrophobacter*, *Cytophaga*, *Bacteroides* and *Synergistes* were the most abundant, whereas for the Archaea domain, the genera *Methanosaeta* and *Methanolinea* were predominant. The *Pseudomonas* genus was the only genus that was predicted to be involved in all steps of surfactant degradation. Additionally, *bamA* gene sequencing indicated the presence of the species *Syntrophorhabdus aromaticivorans*, *Desulfosarcina cetonica* and *Syntrophus aciditrophicus*, which have genetic potential for the aromatic ring cleavage under anaerobic conditions. Therefore, despite the high toxicity of LAS under anaerobic conditions, the use of different molecular tools revealed the great diversity and richness of the microbial community from the granular biomass of the EGSB pilot reactor, indicating that a microbial consortium is necessary for complete LAS degradation. Additionally, the sequencing analysis of the *bamA* gene represents a step forward in the understanding of the core microbial community involved in aromatic ring cleavage for anaerobic digestion of real laundry wastewater, which may guide future studies.

1. Introduction

Surfactants are substances that have both polar (hydrophilic) and apolar (hydrophobic) regions in their chemical structures. This characteristic has led to their use in a wide variety of products, mainly in the field of detergents (soaps, soap powders, detergents). In this context, an anionic surfactant, namely, linear alkylbenzene sulfonate (LAS), has received attention. With a worldwide production of millions of tons per year, LAS is the most widely used anionic surfactant in the world (García et al., 2005). Commercially, LAS is sold as a mixture of homologs (depending on the size of the alkyl chain) and position isomers (depending on the position of the aromatic ring). LAS is structurally composed of an alkyl chain with different numbers of carbon atoms (from 10 to 14), while the hydrophilic part corresponds to the sulfonate group. The sulfonate group may be attached to any carbon atom except for the terminal carbons of the alkyl chain.

Inadequate treatment wastewater containing LAS can lead to the formation of foams, which inhibit the natural self-purification processes of rivers and lagoons as well as the aerobic and/or anaerobic processes in sewage treatment plants. Approximately 0.02–1.0 mg LAS L^{-1} in aquatic environments may cause gill damage and excessive mucus secretion in fish, decreased respiration in *Pomatoschistus microps*, and damaged movement in mussels (Venhuis and Mehrvar, 2004); while 40–60 mg LAS g^{-1} in soil interferes with the reproduction and growth

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of invertebrates (Holmstrup et al., 2001).

Due to the high consumption of surfactants, especially LAS, and due to the effects of the inadequate treatment of surfactants, there is a need to develop technologies to treat this macro-pollutant. In this context, the biological removal of LAS has been sought through the use of different configurations of anaerobic reactors. Commercial laundry wastewater is characterized by the presence of high concentrations of LAS (12–1024 mg LAS L^{-1}) and organic matter (620–4800 mg DQO L^{-1}; Braga and Varesche, 2014). Depending on the products used during washing (stabilizers and neutralizers), the sulfate concentration in commercial laundry wastewater can be as high as 372 ± 223 mg L^{-1} (Delforno et al., 2014). In the literature, few studies deal with the biological treatment of wastewater from commercial laundry; physicochemical treatments, such as advanced oxidative process and reverse osmosis, are the most commonly used treatment methods for commercial laundry wastewater.

Microbiology is a valuable tool for the elucidation of the microbial consortium that participates in the degradation of the LAS molecule. According to Almendariz et al. (2001), acidogenic bacteria are responsible for the degradation of this complex molecule, while methanogenic archaea are more sensitive to LAS and do not participate directly in the degradation process. In this regard, populations belonging to the Bacteria domain are significantly affected by LAS because they play a central role in the degradation of this compound. Higher surfactant concentrations and longer exposure times have a toxic effect on Bacteria, inhibiting or decreasing bacterial activity. On the other hand, low LAS concentrations (< 3 mg L^{-1}) can have a positive effect on bacterial populations (Mosche and Meyer, 2002).

The degradation of LAS is initiated by Ω-oxidation, which oxidizes the methyl end group, thereby enabling further β-oxidation. The second stage of LAS degradation begins by desulfonation of the sulfate group, followed by the opening of the aromatic ring, yielding carbonic gas, water, sulfate and biomass (Scott and Jones, 2000; Kuntze et al., 2008; Mungray and Kumar, 2009; Lara-Martin et al., 2010). Kuntze et al. (2008) proposed the use of the *bamA* gene as a molecular marker for the identification of microorganisms that have the coenzyme 6-oxocyclohex-1-ene-1-carbonyl, which is related to the last step of aromatic ring cleavage.

Therefore, the present study aimed to evaluate the influence of LAS concentration on the composition and dynamics of microbial communities during the anaerobic co-digestion of commercial laundry wastewater and domestic sewage in an expanded granular sludge bed (EGSB) pilot-scale reactor by using a combination of DGGE fingerprinting, 16S rRNA gene sequencing and bamA marker-gene amplification.

2. Material and methods

2.1. EGSB reactor and experimental setup

The EGSB pilot-scale reactor consisted of an acrylic and polyvinyl chloride (PVC) apparatus with a volume of 62 L, a height of 4.1 m and a diameter of 0.150 m (Fig. 1). At the top end of the reactor, there was a device to separate the solid, liquid and gas phases, and at the base of the reactor, there was a flow distributor. Twelve sampling points were installed in the reactor. The reactor was operated for 232 days at room temperature with a hydraulic retention time (HRT) of 40 h and an upflow velocity of 4 m h^{-1} with effluent recirculation. The reactor was inoculated with a granular sludge obtained from a full-scale UASB plant that treats effluent from a poultry slaughterhouse.

The EGSB reactor was operated in three stages (Table 1). Stage I consisted of the reactivation of microorganisms. Thus, the reactor was fed only with domestic sewage for 39 days. Real domestic sewage was provided by a wastewater interceptor from the neighborhood near Campus 2 of the University of São Paulo (São Carlos, Brazil). In stages II and III, commercial laundry wastewater was added, and the proportion of domestic sewage and laundry wastewater was maintained as a

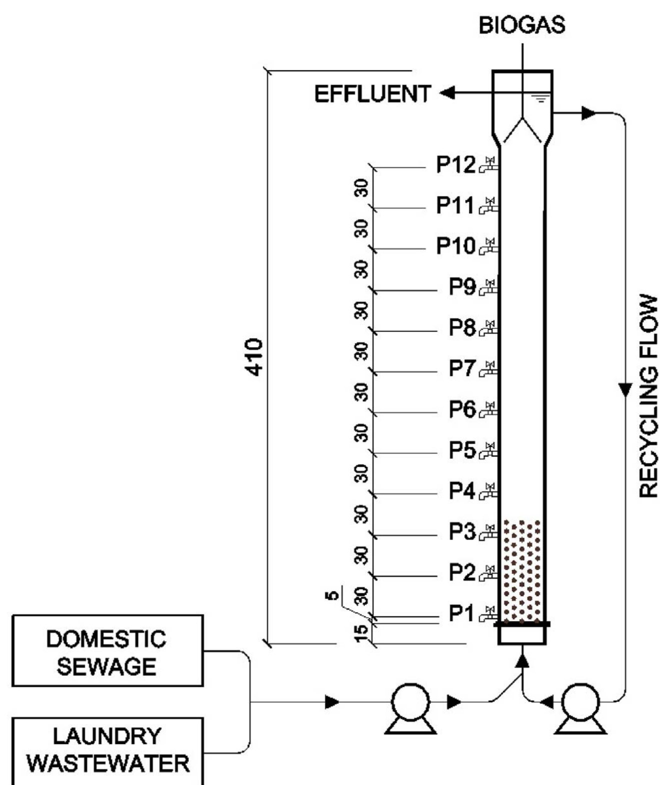

Fig. 1. EGSB pilot scale reactor layout (values shown are in centimeters).

function of the anionic surfactant (LAS) concentration for each stage (Stage II: 4.0 mg LAS each stage (Stage II: 4.0 and Stage III: 16.0 mg LAS each stage (Stage II: 4.0). The commercial laundry wastewater was collected from a commercial laundry located in São Carlos, Brazil. Samples were collected from the first rinse without softener in 20-L, high-density, polyurethane flasks and stored at 4 °C until DNA extraction.

2.2. DNA extraction

The PowerSoil® DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, USA) was used for DNA extraction according to the manufacturer's instructions. DNA quality and concentration were assessed using gel electrophoresis (1% agarose) and an ND-2000 spectrophotometer (NanoDrop Inc., Wilmington, DE), based on a ratio of 260/280 nm > 1.8.

2.3. Microbial community composition

2.3.1. Sampling, 16S rRNA Illumina MiSeq sequencing and data analyses

Three samples, namely, 16S_0 (0 mg L^{-1} LAS), 16S_4 (4 mg L^{-1} LAS) and 16S_16 (16 mg L^{-1} LAS) (Table 1), were sequenced for in-depth analysis of microbial composition. Briefly, at the end of each operational stage of the reactor, approximately 20 mL of a composite sample of the sludge bed was taken. The homogenized sample was composed of approximately 5 mL of biomass from each of the first four sampling points (P1, P2, P3, and P4; Fig. 1) mixed in a 50 mL Falcon tube, washed successively with phosphate-buffered saline (PBS 1X), and centrifuged (6000 rpm for 10 min). The pellets were then stored at −20 °C until DNA extraction.

Library preparation and 16S rRNA amplicon sequencing were performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA). Amplification of the 16S rRNA gene V4 variable region was performed using the primer pair 515F (5′ GTGYCAGCMGCCGCGGTAA 3′) and 806R (5′ GGACTACNVGGGTWTCTAAT 3′) with a barcode on the

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