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Metagenomic analysis of the microbiome in three different bioreactor configurations applied to commercial laundry wastewater treatment

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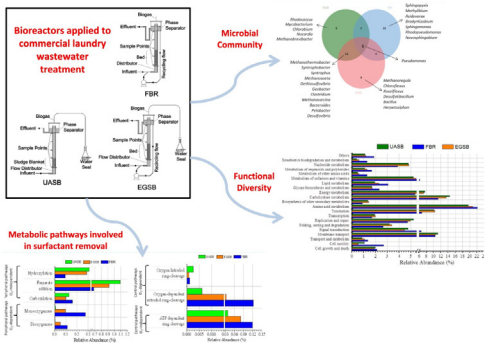
HIGHLIGHTS

- Taxonomic and functional diversity of distinct bioreactors were compared.
- Metabolic pathways involved in aromatic compounds removal were evaluated.
- Biological data showed EGSB and UASB were more closely related than the FBR.
- Anaerobic aromatic degradation pathways were enriched in UASB and EGSB.
- In contrast, FBR showed enrichment of aerobic microbiota and pathways.

GRAPHICAL ABSTRACT



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ABSTRACT

The taxonomic and functional diversity of three different biological reactors (fluidized bed reactor, FBR; up-flow anaerobic sludge blanket reactor, UASB; and expanded granular sludge bed reactor, EGSB) used for commercial laundry wastewater treatment was investigated using metagenome shotgun sequencing. Metagenomes were sequenced on the Illumina Hiseq platform and were analyzed using MG-RAST, STAMP and PAST software. The EGSB and UASB reactors were more closely related based on taxonomic and functional profiles, likely due to similar granular sludge and procedures adopted to ensure anaerobic conditions. The EGSB and UASB reactors showed a predominance of methanogens and genes related to methanogenesis, with a prevalence of the acetoclastic pathway, in addition to the peripheral and central O_2-independent pathways for aromatic compound degradation. By contrast, FBR showed a dominance of aerobic microbiota and pathways for O_2-dependent aromatic compound degradation. Therefore, although the reactors showed similar surfactant removal levels, the microbial composition, functional diversity and aromatic compound degradation pathways were significantly distinct.

1. Introduction

As a result of their chemical versatility, the global market of anionic surfactants reached approximately $7.5 billion in 2012, and Brazil accounted for 10% of the total amount (Frost, 2013 apud Bain and

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Company and GasEnergy, 2014). These numbers suggest an annual production of anionic surfactants of approximately 293,000 tons/year in Brazil. In this context, linear alkylbenzene sulfonate (LAS), an anionic surfactant, has high levels of global production. Its chemical structure consists of an alkyl chain of 10–14 carbon atoms that are bonded to a sulfonated aromatic ring.

Because of its intense use and recalcitrance in anaerobic and aerobic wastewater systems, LAS can be found in domestic sewage (1–18 mg L^{-1}) and laundry wastewater (17–1024 mg L^{-1}; Braga and Varesche, 2014). Therefore, previous studies focused on LAS removal using physico-chemical treatment (Sostar-Turk et al., 2005) and anaerobic reactors. The biological reactors studied are fluidized bed reactors (FBR) (Braga and Varesche, 2014; Macedo et al., 2015; Oliveira et al., 2009), expanded granular sludge bed reactors (EGSB) (Delforno et al., 2014, 2012) and up-flow anaerobic sludge blanket reactors (UASB) (Okada et al., 2014; Okada et al., 2013).

According to Buffière et al. (1995), several factors contribute to the treatment efficiency in FBR, such as the maximum contact between liquid/support material, preferential channels avoided, operation with a wide range of organic concentrations, dilution provided by recirculation and other factors. EGSB reactors show similar features; however, the up flow velocity is lower than FBR because granular sludge presents low density and, consequently, lower energy costs (Kato et al., 1994). Additionally, granular sludge can work as a collective defense against toxic compounds, thus optimizing the survival of microbial populations (McHugh et al., 2003). The UASB reactor is simple in construction and operation, with lower energy costs, tolerant of a high organic load and low hydraulic retention time (HRT). However, contact between the biomass and wastewater is not maximized, and preferential channels are common (Chernicharo et al., 2015).

In combination with studies of specific parameters of biological reactors (HRT, co-substrates, bioavailability, stability and production of volatile acids, temperature conditions, support material and applied LAS load), molecular biology methods are used to understand the taxonomies and pathways involved in anionic surfactant removal, focusing on new strategies for biodegradation in biological reactors. The main molecular approaches used in association with the operation of biological reactors include fluorescence in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (TRFLP) and the sequencing of 16S rRNA gene amplicons.

Using FISH, Lobner et al. (2005) observed the migration of archaea populations into granules when exposed to surfactant, whereas Delforno et al. (2015) used massive 16S rRNA gene sequencing to evaluate the microbial core related to the biodegradation of LAS. However, the use of 16S rRNA gene amplicon sequencing to infer functions in biological reactors has many limitations, such as several artifacts due to PCR amplification and sequencing errors (Quince et al., 2009). To avoid inconsistencies attributed to 16S rRNA gene amplicon sequencing, the whole genome shotgun metagenomic (WGSM) sequencing approach is used to assess the taxonomic and functional profiles directly. Therefore, WGSM sequencing may provide more detailed information about the genetic potential and structure of the microbial community and thus connect biological information with physicochemical data obtained from biological reactors. Moreover, the decreasing cost and increasing sequencing depth of high throughput sequencing technologies enable the high-resolution analysis of complex samples, such as biological reactors used for wastewater treatment.

Knowledge about the aerobic and anaerobic pathways involved in the biodegradation of LAS is scarce. According to Shorberl (1989), the aerobic pathways consist of ω-oxidation (conversion of one of the two methyl groups of the alkyl chain into a carboxyl group), β-oxidation (oxidative shortening of the alkyl chain by two carbon units) and oxidative ring cleavage following the desulfonation. Under anaerobic conditions, fumarate is added to the alkyl chain and the LAS molecule is converted in sulfophenyl carboxylic acid (SPC). Then, subsequent β-oxidation reactions occur, resulting in ring cleavage and the desulfonation process (Lara-Martin et al., 2010).

According to current knowledge, the degradation of aromatic compounds by microorganisms involves peripheral and central pathways. The main function of peripheral pathways is to convert aromatic compounds into key central intermediates. These intermediate compounds are used in central pathways for the generation of carbon and energy for the microorganism (Fuchs et al., 2011). In general, the strategies used by microorganisms are dependent or independent of oxygen for peripheral and central pathways. Normally, O_2-independent peripheral pathways include carboxylation, the addition of fumarate and hydroxylation, whereas O_2-dependent peripheral pathways include the action of dioxygenases and monooxygenases. O_2-independent central pathways include ATP-dependent ring cleavage. O_2-dependent central pathways may act in two different ways: oxygen intradiol ring-cleavage and oxygen extradiol ring-cleavage. In general, anaerobic strategies are considered less efficient than aerobic strategies due to low energy yields.

Therefore, this study characterized and compared three distinct biological reactors used for laundry wastewater treatment using whole genome shotgun metagenomics. The microbial community structures, functional profiles and metabolic pathways were revealed, providing deep insight into the microbiota of bioreactors used for the wastewater treatment of recalcitrant compounds.

2. Materials and methods

2.1. Sampling and description of reactors

Three samples were collected from three different configurations of biological reactors used for commercial laundry wastewater treatment (Fig. 1). The biological reactor configurations were as follows: i) a fluidized bed reactor (FBR) (Macedo et al., 2015), ii) an up-flow anaerobic sludge blanket reactor (UASB) (Okada et al., 2014), and iii) an expanded granular sludge bed reactor (EGSB) (Delforno et al., 2015). The physicochemical parameters of each reactor are summarized in Table 1.

The EGSB and UASB reactors consisted of an acrylic apparatus with a total volume ranging from 0.65 L to 1.40 L, inoculated with granular sludge, which was derived from a UASB reactor applied for poultry slaughterhouse wastewater treatment and operated under a mesophilic condition (30 °C) in a climatic chamber. These reactors showed a hydraulic retention time (HRT) of approximately 35 h. Moreover, the following two procedures were used to ensure anaerobic conditions in both systems: the use of a water seal and the use of a siphon at the outlet of the system, which prevented the entry of oxygen via the effluent hose.

The FBR reactor consisted of an acrylic apparatus with sand as the support material (1.4–1.7 mm diameter), inoculated with the sludge of a full-scale UASB reactor used for the treatment of swine manure and operated under a mesophilic condition (30 °C) in a climatic chamber. In contrast to EGSB and UASB reactors, the HRT of the FBR reactor was approximately 18 h and no procedure was used to prevent reoxygenation. In the FBR and EGSB, the up-flow velocity with effluent recirculation was 106 L h^{-1} and 5 L h^{-1}, respectively, whereas the UASB was operated without effluent recirculation.

The oxygen concentration was not measured directly. However, the redox potential was analyzed before the sampling procedure using resazurin 0.1% (Aldrich), an oxidation-reduction indicator that changes color to indicate partial (pink) or full (colorless) anaerobic conditions.

The reactors were fed only with laundry wastewater diluted with a public water supply to obtain an influent LAS concentration of approximately 15 mg L^{-1} and 400 mg L^{-1} sodium bicarbonate. The wastewater was collected from a commercial laundry located in São Carlos, SP, Brazil.

For in-depth microbiome characterization, one homogeneous sample of the sludge blanket from the UASB and from the EGSB was taken at the end of the reactor operation. For the sampling of the FBR reactor,

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