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## Revealing the bacterial profile of an anoxic-aerobic moving-bed biofilm reactor system treating a chemical industry wastewater



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### ABSTRACT

This study aimed at the characterization of the microbial community diversity of a moving bed-biofilm reactor (MBBR) system by pyrosequencing the 16S rRNA gene. The MBBR process comprised two stages (anoxic and aerobic) for organic matter and nitrogen removal from a pesticide-producing industry wastewater. Over 15,000 sequence reads from each reactor were analysed after 400 days of operation. Despite similar values of bacterial richness, the aerobic reactor exhibited a more equitable distribution of the bacterial groups, leading to higher values of diversity. Proteobacteria was observed to be the most dominant classified phylum in both reactors, accounting for more than 80% of the reads, while Alphaproteobacteria was preponderant at the class level. As observed for both phylum and class levels, the genus diversity was less pronounced in the anoxic reactor, which was highly dominated by very few genera. Among them, *Hyphomicrobium* (21%) was the most frequent one. These organisms possibly played an important role in denitrification, as COD abatement in the non-aerated reactor was mainly coupled to nitrite/nitrate reduction. The aerobic tank was dominated by the genus *Methylobacillus* (13%). *Thiobacillus* corresponded to the second most relevant genus in both reactors. As expected, no nitrifying bacteria were detected in the anoxic biofilm. In the aerated tank, however, ammonium oxidizers were represented by the *Nitrosomonas* genus, accounting for 2.5% of the entire bacterial population. *Nitrospira*- and *Nitrobacter*-like nitrite oxidizers coexisted in the aerobic reactor. The former was almost 10 times more frequent than the latter, despite the occurrence of salt shock loading events.

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## 1. Introduction

Many industries generate wastewaters with complex and variable composition, representing important sources of pollution (Friha et al., 2014). Among them, pesticides-producing factories occupy an important role in the modern society, given the wide applicability of the products processed in such industrial facilities. Insecticides, fungicides, acaricides and herbicides, commonly known classes of pesticides, are often employed in many agricultural applications (Campos et al., 2014). Some of the components used in the formulation of pesticides may be hazardous and toxic to many classes of organisms. Hence, to both environmental and human protection, these constituents should be properly handled and

reduced to acceptable levels before being discharged into natural receiving waters (Gilliom, 2007).

Two of the most important classes of pollutants are organic matter and nitrogen. When organic compounds are released into an aquatic environment, heterotrophic bacteria use them as carbon source in their metabolism, resulting in oxygen consumption. In some circumstances, dissolved oxygen in water may become depleted, and, consequently, a massive death of aquatic organisms is expected. This is actually one of the most drastic effects of the pollution caused by organic matter. Thus, oxygen demand-based methods (e.g., chemical and biochemical oxygen demand – COD and BOD, respectively) are often employed to assess the potential environmental impact derived from the presence of organic compounds. The presence of excess nitrogen is associated with the occurrence of eutrophication in aquatic ecosystems. Thus, elimination of nitrogen compounds from wastewaters is crucial for environmental preservation.

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Biological processes are widely implemented for the removal of organic matter and nitrogen from wastewaters. Biodegradable organic compounds are oxidized by ordinary heterotrophs, which transform them into CO<sub>2</sub> and bacterial biomass (Ekama and Wentzel, 2008). On the other hand, nitrogen removal is conventionally accomplished by a two-step process: nitrification and denitrification. The first step involves the oxidation of ammonium to nitrite by ammonium-oxidizing bacteria (AOB) and then to nitrate by nitrite-oxidizing bacteria (NOB), both chemolithoautotrophic organisms. In the second step, nitrate is reduced to nitrogen gas by organic-dependent denitrifying bacteria (Henze et al., 1997).

Biological treatment is often conducted in engineered bioreactors where the microorganisms grow in suspension. Within this category, the activated sludge (AS) process stands out, playing an important role in wastewater treatment all over the world (Yan et al., 2015). However, depending on the operating conditions to which the reactor is subjected, suspended biomass-based reactors present certain disadvantages. The possibility of occurring poor settling sludge is well documented (Nielsen et al., 2009), making the separation between the biosolids and the treated effluent in secondary clarifiers more challenging. Moreover, the biomass concentration in AS-related processes is relatively low, implying the need for large areas for the installation of the treatment facility (Van Haandel and Van der Lubbe, 2012).

Recently, advances in the wastewater treatment sector have culminated in the development of new processes showing high treatment performance and stability to cope with strict environmental legislations worldwide. Essentially, most of the new technologies are based on the growth of bacteria adhered to a solid surface, which can be fixed or mobile. An example of a growing technology is the moving-bed biofilm reactor, known by the acronym MBBR. In this process, free-moving elements, usually made of plastic, serve as support for the establishment of bacterial colonies, which grow in the form of biofilms. As the majority of the biomass is attached to the carriers, the retention of microorganisms within the system is favoured and consequently the volumetric treatment capacity is enhanced. Moreover, effluent clarification operation becomes much easier (Rusten et al., 1998), which substantially facilitates the overall treatment process.

Given the key features shown by the MBBR technology, it has been applied for the treatment of many kinds of waste streams, from domestic sewage to complex industrial wastewaters (Kermani et al., 2008; Bassin et al., 2011; Dong et al., 2011; Hassani et al., 2014). Nevertheless, the treatment of wastewaters generated by pesticide-producing industries in MBBRs is scarcely reported in the literature (Chen et al., 2007). In a previous study, we investigated the operation of a two-stage anoxic-aerobic MBBR system designed to achieve simultaneous removal of organic matter and nitrogen from a wastewater produced in a chemical industry where a great variety of products are manufactured, including pesticides used in agriculture (Cao et al., 2016).

As an emerging technology, the MBBR needs to be better understood. The characterization of the microbial community associated with the biological system is a critical step that needs to be done, since bacteria plays a major role in the bioreactor functioning (Muszyński et al., 2015). The bacterial community screening is the basis to obtain a deeper insight into the relation between main biological conversions taking place in the biofilm and their corresponding microbial functional groups. Therefore, we decided to characterize the bacterial community of the biofilms cultivated in both anoxic and aerobic environments. To address this goal, biomass samples were collected from each stage (anoxic and aerobic) of the MBBR system and next generation sequencing was used to reveal the identity of the microorganisms involved in the

treatment of the industrial wastewater under study.

## 2. Materials and methods

### 2.1. Wastewater source

The wastewater used in this study was generated by a chemical industry located in the city of Belford Roxo, Rio de Janeiro, Brazil. The industrial plant is responsible for the production of pesticides (e.g., insecticides, fungicides, acaricides, and herbicides), animal health products and raw materials for polyurethane manufacturing. An illustration of the main production units composing the industrial facility is shown in Fig. S1 (Supplementary Material). The biological process currently employed to treat the industrial wastewater consists of an activated sludge reactor, preceded by an equalization tank. Given the operational problems encountered in the running treatment facility, a biofilm-based process was investigated as an option for the removal of pollutants present in the referred wastewater. As many products are manufactured over time, the physicochemical composition of the wastewater significantly changes. Hence, the characterization of specific compounds becomes a difficult task. The average values of some wastewater parameters are shown in Table S1.

### 2.2. Operation and control of the two-stage moving-bed bioreactor system

A lab-scale MBBR system composed of two reactors in series (R1 and R2) was continuously researched for over 400 days as described in a previous study (Cao et al., 2016). To ensure both organic matter and total nitrogen removal from the chemical industry wastewater previously described, the biofilm-based system was operated in pre-denitrification mode, as illustrated in Fig. 1. R1 (1 L capacity) was maintained under non-aerated (anoxic) conditions by bubbling nitrogen gas through the reactor. On the other hand, R2 (2 L volume) was intended to accomplish nitrification and was connected to a central compressed air system which provided aeration and circulation of the plastic media. Dissolved oxygen (DO) concentrations in R2 was kept within 6.5–7.0 mg/L. Nitrogen gas and air were provided at a rate of 4 L/min by means of porous rock diffusers placed at the bottom of R1 and R2, respectively. The internal mixed-liquor recycle ratio from the aerobic to the anoxic tank was gradually increased from 2 to 4 to bring more nitrate/nitrite to be denitrified in R1 and therefore enable a higher nitrogen removal to be obtained. The operating conditions of the biological treatment step are summarized in Table 1. The hydraulic retention time (HRT) of R1 and R2 corresponded to 12 and 24 h respectively. Hence, the overall HRT of the system was 36 h pH was controlled close to neutrality (6.5–7.5). Kaldnes® K1 with volume-specific surface area of 500 m<sup>2</sup>/m<sup>3</sup> was the media type used to promote attached biomass growth. The media filling ratio ( $V_{\text{media}}/V_{\text{reactor}}$ ) was 50% in both reactors. The seed sludge was obtained from a municipal sewage treatment plant in the city of Rio de Janeiro, Brazil (CEDAE Alegria). A small volume of inoculum (around 50 mL) was regularly added to the MBBR system during the first 15 days of operation to enhance biofilm development in the carriers.

### 2.3. Bacterial community analysis: polymerase chain reaction (PCR) amplification, sequence identification and bioinformatics analysis

At the last experimental condition (run 3, Table 1), the highest organic matter and nitrogen removal efficiencies were observed. Furthermore, in that period, the MBBR system had already been running for a long time, and the microorganisms were well adapted to the environmental conditions prevailing in the bioreactor.

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