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Effect of salinity in heterotrophic nitrification/aerobic denitrification performed by acclimated microbiota from oil-produced water biological treatment system

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ABSTRACT

Mixed cultures salt acclimated showed high efficiency in heterotrophic nitrification/aerobic denitrification process in hypersaline conditions. They were able to remove 80% of ammonium in Heterotrophic Nitrification Medium (HNM) with 12 and 14% of salt. Above these salinity, the process still had 40% ammonium removal up to 20% of salt. Chromatography analysis validated the occurrence of the heterotrophic nitrification/aerobic denitrification process in all studied salinities (6%–20% of NaCl). However, with increasing salinity, the N_2 production was smaller and took longer than the unsalted control. Microbial diversity analysis of mixed cultures showed that different groups of nitrifying microorganisms were involved in ammonium removal, including heterotrophic nitrifying/aerobic denitrifying genera such as *Pseudomonas*, *Paracoccus*, *Bacillus*, *Halomonas*, *Acinetobacter* and *Klebsiella*. In addition, this analysis also revealed that the acclimation process allowed the adaptation of the microorganisms to high saline conditions and ammonium removal up to 20% of salt. This work showed that heterotrophic nitrification/aerobic denitrification process could occur in high salinity after microbiota acclimation step, and these mixed acclimated cultures have a potential for application in hypersaline effluent treatment.

1. Introduction

Effluents generated by oil industry have high amounts of ammonium, so they should be treated before being discarded to not be harmful to the environment (Abdel-Raouf et al., 2012; Sarioglu et al., 2012; Munirasu et al., 2016). Biological processes for pollutants removal are the most used in effluent treatment plants, since they combine high removal efficiency with low operational cost (Da Motta et al., 2003). The conventional biological process for the ammonium removal consists in two stages: aerobic autotrophic nitrification, followed by anaerobic denitrification. The nitrification process is the oxidation of the ammonium to nitrite, followed by the oxidation of the nitrite to nitrate, realized by autotrophic microorganism and aerobic conditions, where the first stage is carried out by bacteria and archaeas, and the second only by bacteria, and the denitrification is the reduction of nitrate to gaseous nitrogen by heterotrophic bacteria under anaerobic conditions (Madigan et al., 1997). This conventional biologic ammonium removal requires a longer time, since the microorganisms are slow growing, and increased the plant operational complexity, because the two steps take place under different conditions (Chen and Ni, 2012).

Heterotrophic nitrification/aerobic denitrification (HN/AD) is another nitrogen removal process that has gained attention in effluent treatment plants, which a single heterotrophic microorganism is able to perform the two steps, nitrification and denitrification, in aerobiosis

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(Robertson and Kuenen, 1983). Due to their ability to convert ammonium into gaseous nitrogen and its growth to be faster than the autotrophic nitrifying, these microorganisms present several advantages for application in effluent treatment plants, such as: removal of organic matter in addition to nitrogen removal; simplicity in the operational process, since nitrification and denitrification occur in aerobiosis; and discards the need to add alkalinizers, since the denitrification process alkalinizes the medium that has been acidified by nitrification (Third et al., 2005; Padhi et al., 2013; Lei et al., 2016). These advantages may allow a better removal efficiency of ammonium and carbon in a single step in an aerobic reactor.

However, like most biological processes, the HN/AD can be affected by high salinity (Quartaroli et al., 2017). Salt directly interferes with the oxidation rate of ammonium by reducing the transport of chemical compounds between the medium and the cell, altering the microbial metabolism, causing dehydration and cellular lysis (Measures, 1975). At the effluent treatment plants, salt is considered an instability factor. Effluents with high saline concentrations can affect the microbiota in the sludge, and compromise the pollutant removal processes. According to literature, the saline concentration of the production water can vary from 3.5%, salinity of the sea water, up to approximately 20%, depending on the place of oil exploration. With the exploration of oil in the pre-salt layers in Brazilian coast, this salinity can be higher, reaching 30% of salt, creating a challenge for the biological treatment of this effluent (Neff et al., 2011; PETROBRAS, 2012). Several works in the literature show that an effective strategy used to minimize the effects of salt in biological processes is the acclimation process, which consists in submit the microbiota to a gradual increase of salt (Marchetto et al., 2003; Bassin et al., 2012; Quartaroli et al., 2016). During the acclimation process, microorganisms can develop mechanisms of adaptation to the new salt conditions (Oren, 2002).

Some studies have already shown that salt can interfere directly in the process of HN/AD, but performed by bacterial isolates (Guo et al., 2013; Lei et al., 2016). However, no study was done to evaluate the effect of salt on the process performed by NH/DA populations present in a mixed culture from activated sludge. A acclimated mixed culture capable of performing NH/DA under hypersaline conditions can optimize the removal of ammonia in salt water treatment plants.

Due to the need for ammonium removal in hypersaline effluent, the importance of heterotrophic nitrification knowledge in Wastewater Treatment Plant (WWTP), and the absence of studies of this process in high salinities, the aim of the study was to evaluate the occurrence of the heterotrophic nitrification/aerobic denitrification process under hypersaline conditions after NaCl microbiota acclimation, as well as to analyze the microbial populations involved in the ammonium removal along the increase of the salt, focusing in those nitrifying heterotrophic/aerobic denitrifying microorganisms, which according to literature belong to genera more tolerant to salt than autotrophic nitrifying.

2. Materials and methods

2.1. Sampling

Two liters of activated sludge from produced water treatment system of Marine Terminal Almirante Barroso/TEBAR (São Sebastião/SP) were collected. The sample was stored in gallon plastic at room temperature and transported to the Cellulose and Paper Laboratory of the Federal University of Viçosa - Minas Gerais/Brazil. The sample was aliquoted in 15 mL Falcon tubes and frozen for future analysis.

2.2. Activated sludge acclimation

The activated sludge acclimation to high salinity was performed in two different preselected culture media: R_2A (Difco) (Reasoner and Geldreich, 1985), and MOD (Moderate) (Rohban et al., 2009) (Fig. S1). The selection of two media was done through analysis of bacterial growth in 5 different media, where the highest richness was observed in the MOD and R_2A media. In 250 mL Erlenmeyer flasks, 5 mL of bioreactor sample were inoculated in 50 ml of MOD or R_2A media. Every 6 days a 10% (v/v) aliquot of acclimated microbiota was transferred to a new flask containing 50 mL of MOD or R_2A medium with 2% more NaCl than the previous saline concentration. The flasks were kept under agitation of 150 rpm at 30 °C. Every six days, there was a new peal with an increase of 2% NaCl, until a final concentration of 20%. After each salt addition (6, 8, 10, 12, 14, 16, 18 and 20%), a culture aliquot was preserved in 20% glycerol and frozen at −80 °C. In total, 16 mixed cultures were obtained from acclimation, 8 from R_2A and 8 from MOD medium.

2.3. Treatment assembly

For treatment assembly, 500 µL of each mixed culture was reactivated in 5 mL of R_2A or MOD culture medium with addition of 6–20% NaCl. After reactivation step, 8 mixed cultures were obtained, each containing an aliquot of 5.0 mL of inoculum from R_2A medium and 5.0 mL of inoculum from MOD medium, both with the same salt concentration (6–20% NaCl). Five milliliters of each mixed culture were inoculated into 250 mL erlenmeyers containing 50 mL of culture medium for nitrifying heterotrophic bacteria (HNM) (Zhang et al., 2012) and were subjected to two treatments: **treatment I)** HNM medium without NaCl and **treatment II)** HNN medium with 6–20% NaCl. The amount of salt added to each mixed culture was the same in which the samples were acclimated (Fig. S1 and Table 1). Bioreactor sample, without acclimatization, able to remove ammonium in 5% salt concentration was used as positive control. The HNM medium without inoculum was used as negative control. The treatments were incubated at 30 °C under 150 rpm for 10 days.

2.4. Ammonium removal evaluation

The ammonium removal was evaluated by colorimetry according to the methodology of Chaney and Marbach (1962). The analyzes were performed in 96-well microplates containing in each well: 5 µL of each mixed culture grown in HNM medium, 100 µL of phenol reagent (50 g of solid phenol, 0.25 g of sodium nitroprusside, complete to 1.000 mL with distilled water) and 100 µL of hypochlorite reagent (25 g NaOH, 16.9 mL of hypochlorite with 4–6% chlorine, complete to 1.000 mL with distilled water). The microplates were incubated for 20 min at 39 °C, and then the absorbance were read at a wavelength of 630 nm in the Multiskan GO spectrophotometer (Thermo Scientific). As a positive control was inoculated 5 µL of bioreactor sample grown in HNM medium, and as negative, was added 5 µL of HNM medium without inoculum. The ammonium removal analyzes were conducted over 10 days, when the rate remained constant. Analyses were performed in duplicate.

Table 1
NaCl concentration in acclimation process and in treatments.

Mixed cultures	NaCl concentration in acclimation process	Treatment I (HNM without salt)	Treatment II (HNM with salt*)
1	6%	TI.1	TII.1 (6%)
2	8%	TI.2	TII.2 (8%)
3	10%	TI.3	TII.3 (10%)
4	12%	TI.4	TII.4 (12%)
5	14%	TI.5	TII.5 (14%)
6	16%	TI.6	TII.6 (16%)
7	18%	TI.7	TII.7 (18%)
8	20%	TI.8	TII.8 (20%)

*NaCl concentration added to HNM culture medium.

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