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Research article

Using natural biomass microorganisms for drinking water denitrification

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ABSTRACT

Among the methods that are studied to eliminate nitrate from drinking water, biological denitrification is an attractive strategy. Although several studies report the use of denitrifying bacteria for nitrate removal, they usually involve the use of sewage sludge as biomass to obtain the microbiota. In the present study, denitrifying bacteria was isolated from bamboo, and variable parameters were controlled focusing on optimal bacterial performance followed by physicochemical analysis of water adequacy. In this way, bamboo was used as a source of denitrifying microorganisms, using either Immobilized Microorganisms (IM) or Suspended Microorganisms (SM) for nitrate removal. Denitrification parameters optimization was carried out by analysis of denitrification at different pH values, temperature, nitrate concentrations, carbon sources as well as different C/N ratios. In addition, operational stability and denitrification kinetics were evaluated. Microorganisms present in the biomass responsible for denitrification were identified as *Proteus mirabilis*. The denitrified water was submitted to physicochemical treatment such as coagulation and flocculation to adjust to the parameters of color and turbidity to drinking water standards. Denitrification using IM occurred with 73% efficiency in the absence of an external carbon source. The use of SM provided superior denitrification efficiency using ethanol (96.46%), glucose (98.58%) or glycerol (98.5%) as carbon source. The evaluation of the operational stability allowed 12 cycles of biomass reuse using the IM and 9 cycles using the SM. After physical-chemical treatment, only SM denitrified water remained within drinking water standards parameters of color and turbidity.

1. Introduction

The increased contamination of water by nitrogen containing compounds requires particular attention since is a serious global concern, elicited from diverse and several causes. Nitrate, due to its high-water solubility, is likely the most widespread contaminant in ground waters, representing a serious risk to the proper supply of drinking water. Nitrates can quickly reach groundwater and watercourses, causing human health harm due to the consumption of contaminated water (Bartucca et al., 2016; Bhatnagar and Sillanpää, 2011; Ganesan et al., 2013).

As an example, the excess of nitrates in drinking water can cause methemoglobinemia in newborns, also called the “blue baby syndrome.” In addition, the reduction of nitrate to nitrites may induce the formation of nitrosamines *in vivo*, which may lead to cancer (Bucco et al., 2014; Ergas and Rheinheimer, 2004; McAdam and Judd, 2007; Salem et al., 2007; Wang and Chu, 2016). The highest permissible nitrate concentration (as nitrogen-nitrate: $N\text{-}NO_3^-$), established by the United States Environmental Protection Agency

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in drinking water is 10 mg/L, and 50 mg/L as nitrate (NO_3^-) by the World Health Organization (WHO, 2011).

There are different sources of nitrate groundwater contamination. The intensive use of nitrogen-based fertilizers as well as the use of improper sanitation systems are the main causes of nitrate ground water contamination (Emamjomeh et al., 2017; Espejo-Herrera et al., 2015; Liu et al., 2012; Ravnjak et al., 2013; Wick et al., 2012). The use of septic tanks is widespread in Brazil mainly because of its low cost, simple operation and compactness. However, in such cases, sewage compounds can infiltrate the soil, reaching the water table, which is often used as the main source of water supply (Esteller et al., 2009).

The rapid increase of surface and groundwater contamination by nitrogen containing substances worldwide requires the development of improved decontamination strategies based on both physicochemical as well as biological processes. Among the different methods available, the most commonly used are ion exchange, membrane technology, adsorption and biological treatments (Huang et al., 2011; Liu et al., 2012; Loganathan et al., 2013; Sanaeepur et al., 2012; Shrimali and Singh, 2001; Zhang and Angelidaki, 2013). However, these techniques present severe limitations such as high cost and the need of further residue disposal that is generated during the process. Thus, studies focused on nitrate removal are essential for the development feasible and cost-effective strategies (Liu et al., 2012; Ravnjak et al., 2013).

Through biological removal, nitrate is transformed into gaseous nitrogen by a wide range of facultative anaerobic organisms, eliminating the formation of nitrogenous residues as in the case of physical-chemical treatments. After oxygen consumption, these organisms rely only on nitrate to generate energy through cellular respiration. The denitrification process involves the formation of several intermediate products, culminating in the formation of nitrogen gas (Salem et al., 2007), such shown in Equation (1).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad (1)$$

The bacteria responsible for denitrification are mostly heterotrophic, therefore, is necessary the presence of external organic carbon source for bacterial growth as well as to generate energy for the conversion of nitrate into gaseous nitrogen. (Mohseni-Bandpi et al., 2013; Salem et al., 2007; Vasiliadou et al., 2009). Although several studies report the use of denitrifying bacteria for nitrate removal, most of the studies involve the use of sewage sludge as biomass to obtain the microbiota.

Alternatively, bamboo is highly resistant to a broad range of climatic conditions, presenting fast growing rates in tropical and subtropical areas (Mognon, 2017). Studies have shown that different species of bamboo are associated with many different microorganisms (Tu et al., 2014), which may present potential for use in biological denitrification processes. There is only one study focused on the use of bamboo as a source of denitrifying microorganisms (Bucco et al., 2014) and therefore there is a vast field to be exploited due to its great diversity.

The objective of this work was the use of natural biomass from bamboo as a source of denitrifying bacteria, controlling the process variables with a focus on the evaluation of bacterial performance.

2. Materials and methods

2.1. Source of denitrifying microorganisms

This work was carried out using the bamboo specie *Phyllostachys aurea* Carrière ex Rivière & C. Rivière, collected from the campus of the Agroveterinary Sciences Center of the State University of Santa Catarina (CAV/UDESC). Microorganisms utilized in this study were either Immobilized in Bamboo Biomass (IM) or Suspended Microorganisms in Solution (SM).

2.2. Identification of the presence of denitrifying microorganisms in the biomass

Microorganisms were isolated from bamboo biomass as following: Bamboo pieces were briefly washed with running water and mixed with nitrate contaminated distillated water (25 mg N-NO_3^-/L) in a 30% ratio (m/v) and incubated under anaerobic conditions until complete denitrification. Soon after complete nitrate removal, the bamboo biomass was removed from the solution, and the denitrified water was again contaminated with nitrate ions (25 mg N-NO_3^-/L) and a source of carbon. After incubation during 48 h, stirring at room temperature, solution was centrifuged for 5 min at 5000 rpm. Supernatant containing denitrified water was discarded and bacterial pellet was inoculated into a fresh 250 mL flask containing nitrate contaminated distillated water and a source of carbon and incubated under anaerobic conditions until complete denitrification.

This last cycle was repeated three consecutive times to perform selective pressure for the growth of denitrifying microorganisms. After the last denitrification cycle, the bacterial pellet was seeded into Blood Agar plates (0.5% of ovine blood) by platelet exhaustion technique and incubated at 37 °C for 24 h. Bacterial identification was performed by The Center of Microbial Diagnostic (CEDIMA) from the Center of Agroveterinay Sciences and was based on morphological and biochemical tests as previously described (Silva et al., 2017).

2.3. Denitrification using immobilized microorganisms

The bamboo samples were collected from the field, stems were removed and biomass was weighted, washed with running water and mixed to nitrogen contaminated water (25 mg N-NO_3^-/L) in a 30% ratio (m/v) in 250 mL Erlenmeyer flasks. The solution was kept under orbital shaking in Shaker EC 720 incubator (Cienlab) for specific amounts of time at 25 °C. Control experiments were performed, using sterilized biomass, where the bamboo pieces were previously autoclaved using a CS (Prismatec) autoclave at 121 °C for 15 min before mixing to the nitrate contaminated water. Also, control experiments were performed in the absence of external carbon source.

2.4. Suspended Microorganisms

Bamboo pieces were briefly washed with running water and mixed with nitrate contaminated distillated water (25 mg N-NO_3^-/L) in a 30% ratio (m/v) and incubated under anaerobic conditions until water had been completely denitrified. Soon after the complete nitrate removal, the biomass was removed, and the denitrified water was again contaminated with nitrate ions (25 mg N-NO_3^-/L) and external carbon source was added. After complete denitrification, cells were centrifuged using Eppendorf 5804 AG Centrifuge for 5 min at 5000 rpm. Supernatant was discarded and bacterial pellet was inoculated into a fresh 250 mL flask containing nitrate contaminated distillated water and a source of carbon and incubated under anaerobic conditions until removal of all the nitrates from solution.

Control experiments for the SM studies were performed where autoclaved bamboo biomass was utilized as microorganism source.

2.5. Optimization of the operational parameters of biological denitrification

The main operational parameters, reported in the literature, that