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Autotrophic denitrification with sulfide as electron donor: Effect of zeolite, organic matter and temperature in batch and continuous UASB reactors

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ABSTRACT

The effect of zeolite and temperature on the autotrophic denitrification process for hydrogen sulfide removal in the presence of organic matter was evaluated. Four batch reactors (1 l) at 35 °C were used to study the effect of zeolite on denitrification, without the presence of organic matter. Two continuous UASB reactors (6 l) were used for the autotrophic denitrification with addition of natural zeolites at mesophilic controlled temperature (35 °C) and room temperature (13–20 °C). In continuous assays, COD/N ratios between 1.4 and 10 were used. It was found that zeolites have a positive effect on the autotrophic denitrification startup process (batch and continuous modes), decreasing by up to 50% the time required to achieve stabilization conditions of the process. Autotrophic denitrification with zeolite as amendment in a continuous UASB in the presence of organic matter could be carried out at uncontrolled room temperatures (13 °C – 20 °C), although processes at a controlled mesophilic temperature of 35 °C were more efficient than the room temperature process. The proposed system allows the simultaneous heterotrophic-autotrophic denitrification, with removal percentages better than 95% for nitrogen and COD, while sulfide had removal percentages better than 75%.

1. Introduction

The potential and actual environmental problems caused by dumping liquid wastes with high concentrations of nitrogen compounds into watercourses are well known (Dupas et al., 2015). For example, according to the environmental regulations of Chile, the maximum total nitrogen (Kjeldahl + nitrites + nitrates) concentration allowed in discharged wastewater is 10 mg l^{-1}.

Nitrates can be found at high levels in various industrial wastewaters (Fernandez-Nava et al., 2008; Sahinkaya et al., 2015), and in a number of activated sludge systems the effluent has nitrate concentrations that exceed those allowed for discharge into the environment, so it is necessary to decrease that concentration prior to final disposal of the effluent (Amand and Carlsson, 2012; Sahinkaya et al., 2015).

Autotrophic denitrification is carried out by a consortium of sulfur oxidizing bacteria (SOB), among which *Thiobacillus denitrificans* stands out (Oh et al., 2000). Autotrophic denitrification is a combination of nitrogen and sulfur cycles to reduce nitrates to nitrogen gas, while the sulfur compounds, mainly sulfides (S^{2-}), are oxidized to sulfate (SO_4^{2-}), which is environmentally friendly (Vaiopoulou et al., 2005).

Autotrophic denitrification is an attractive process for wastewater treatment because several studies have reported nutrient removal efficiencies above 90% (Ramanathan et al., 2014; Zou et al., 2014). This process can also be considered suitable for removing sulfides from wastewater and groundwater (Sierra-Alvarez et al., 2007; Tanaka et al., 2007) or even to remove H_2S from biogas generated during anaerobic digestion of effluents containing sulfate (canneries, petrochemical industries, tanneries, among other) or flue gas (Baspinar et al., 2011; Qian et al., 2015). However, these effluents generally also contain organic matter, which may decrease the removal rate of sulfide because the nitrates may be used by heterotrophic denitrifying biomass. It has been established that autotrophic and heterotrophic denitrifiers compete fiercely when the nitrate supply becomes limited (Chen et al., 2008). The

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simultaneous removal of nitrate, sulfide and organic matter has been called the *denitrifying sulfide removal* (DSR) process, and research initiatives dealing with this process have only been reported in the last decade (Show et al., 2013). Most of the research on DSR involves the use of completely stirred tank reactors (CSTR) (Reyes-Avila et al., 2004; Wang et al., 2005), and expanded granular sludge bed (EGSB) (Chen et al., 2008), downflow-anaerobic filter, fixed-bed (Show et al., 2013), and sulfur-packed reactors (Kim et al., 2004) have been documented. However, an upflow anaerobic sludge blanket (UASB) has not been used for autotrophic denitrification or the DSR process. UASB reactors have many operational advantages (Choeisai et al., 2014) and have been widely evaluated and checked, leading to high chemical oxygen demand (COD) removal efficiencies operating with low hydraulic retention times (θ) (Basu and Gupta, 2010; Zhang et al., 2015). Therefore, the use of UASB for autotrophic denitrification in the presence of organic matter was one of the goals of this work.

On the other hand, autotrophic denitrifying bacteria have a relatively low growth rate, which is one of the main limitations of the process (Fajardo et al., 2014). However, if a suitable support medium is used to favor bacterial growth, this limitation can be significantly reduced. Several studies state that natural zeolites have properties useful for immobilizing microorganisms, among them acting as a suitable surface to enable the colonization of microorganisms, being relatively inert to most substances and compounds, being resistant, having a relatively large surface area, and being inexpensive (Fernandez et al., 2007; Montalvo et al., 2012). In the literature there are some reports on the use of UASB reactors with zeolite for heterotrophic denitrification with satisfactory results (Guerrero et al., 2013; Montalvo et al., 2014). Operation in the mesophilic range (35 °C) turns out to be an additional cost of the process and this is the reason to study temperature in autotrophic denitrification.

The present paper is focused on the study of autotrophic denitrification in UASB reactors in the presence of organic matter and operating with natural zeolites. The influence of temperature on the process will also be discussed.

2. Materials and methods

The experimental work was carried out in three series or assays: In the first (Assay 1) the potential of zeolite for accelerating the startup phase of the process was evaluated in batch reactors; in the second (Assay 2) the effect of zeolite on autotrophic denitrification carried out in a UASB reactor was studied in order to establish a startup protocol; and in the third (Assay 3) the effect of zeolite on the behavior of autotrophic denitrification operating under various conditions was evaluated.

In Assay 1 four batch reactors of 1 L each were operated at a controlled temperature of 35 °C. Each reactor operated at 20% by volume of inoculum (from a heterotrophic denitrification system wastewater). The use of this inoculum was in order to see if this kind of inoculum could be used for autotrophic denitrification. Table 1 shows the main characteristics of Chilean natural zeolite used in the three assays, obtained from the Maule Region, Chile. Assay 1 was performed in duplicate, leaving two reactors without zeolites (R1 and R2) and two reactors with zeolites (R3 and R4), each containing 20 g of zeolite/g of volatile suspended solids (VSS) inoculum at a concentration of 10 g l^{-1}. Wastewater under autotrophic denitrification was prepared according to Fajardo et al. (2012) using two solutions (A and B) in the same proportions by volume (see Table 2). The S/N ratio was greater than stoichiometric (0.68 mol S mol^{-1} N) in order to remove all the nitrogen compounds. The assay was performed by 20 days.

In Assays 2 and 3, UASB reactors of 6.4 L each were operated. The reactors operated with zeolites, initially loaded with 20 g of zeolite/g SSV of heterotrophic denitrifying inoculum. The VSS inoculum concentration was 10 g l^{-1}. Wastewater undergoing treatment had the same composition as that used in Assay 1, but waste activated sludge (WAS) from a sewage treatment plant was added, diluted to obtain a (COD) of 100–200 mg O_2 l^{-1}. This organic matter addition is performed to simulate real full scale wastewater, where the effluent from aerobic processes has this concentration.

Table 1
Chemical and mineral composition of the zeolites used in all the assays.[a]

Chemical composition (%)		Mineral composition (%)	
SiO_2	64.19	Clinoptilolite	35
TiO_2	0.51	Mordenite	15
Al_2O_2	11.65	Montmorillonite	30
Fe_2O_3	2.53	Other minerals	20
MnO_2	0.03		
MgO_2	0.66		
CaO	3.42		
Na_2O	0.75		
K_2O_2	1.60		
P_2O_5	0.03		
Ignition residue	14.64		

[a] Particle diameter: 0.6–1.4 mm.

In Assay 2, UASB reactors (duplicate) were operated at COD/N = 5 and COD/N = 1.4 ratios and hydraulic detention times (θ) of 4 and 2 h. The denitrification processes were carried out at 35 °C by 30 days.

In Assay 3 the reactors (duplicated) operated at COD/N = 10 and COD/N = 4 ratios, and θ of 2 and 1.3 h, upward velocities of 0.25, 0.5 and 0.75 m h^{-1}, and operating temperatures of 35 °C for R1 and R2, and room temperature (13 °C – 20 °C), respectively. The assay was performed by 45 days.

Solids, COD, nitrate and nitrite analyses were carried out according to the recommendations of the APHA's Standard Methods for the Examination of Waters and Wastewaters (APHA et al., 2012). Nitrate, nitrite and pH were determined with selective electrodes. Sulfide was determined by potentiometric titration using Titro-Line® 7000 of SI Analytics.

Minitab 17 software was used for the statistical processing and analysis of the data. An ANOVA analysis was carried out and a comparison of confidence intervals for mean values was made with a confidence of 95%.

3. Results and discussion

3.1. Effect of zeolite on batch autotrophic denitrification (Assay 1)

Fig. 1A shows pH evolution in batch reactors. One characteristic of denitrification is that in the conversion of nitrate to nitrogen gas, H^+ are consumed as shown in Equation (1.1), which leads to increasing pH during the process:

$$5S^{2-} + 8NO_3^- + 8H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O \quad (1.1)$$

In Fig. 1A it is seen that pH increased, showing that the denitrification process was carried out according to Equation (1.1). It is also seen that pH increases faster in the reactors with zeolites, giving values around 7.3 on the fourth day. In reactors without zeolite the pH increased slowly, reaching the value of 7.3 at the end of the assay (day 20). With respect to the range obtained, studies have shown that pH between 7 and 7.5 improves autotrophic denitrification (Mahmood et al., 2008), so the reactors operated in the proper range.

Fig. 1B shows the evolution of nitrite in batch reactors, where at