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SIMULTANEOUS DENITRIFICATION AND METHANOGENESIS IN AN ANAEROBIC EXPANDED BED REACTOR

DESNITRIFICACIÓN Y METANOGÉNESIS SIMULTÁNEA EN UN REACTOR ANAEROBIO DE LECHO EXPANDIDO

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Abstract
Wastewaters from some industries contain high levels of nitrate but an insufficient amount of electron donor to sustain biological denitrification. An option of treatment is combining anaerobic digestion and denitrification process in a single unit. During this research, an anaerobic expanded bed reactor was operated at different C/N ratios (10, 7, 4, 3, 2 and 1) in order to study nitrogen and organic matter removal. A 3 L reactor was used with a flow feed of 3 L/d, upflow velocity of 7 m/h and temperature between 30-35°C. Organic matter removal remained above 90% throughout the 177 days of experimentation. Nitrogen removal was over 90%, when C/N ratios were greater than the stoichiometric (C/N>1), however, with a ratio C/N=1, nitrogen removal was 60%, causing the accumulation of nitrite. The high removal of nitrate and organic matter reached in this study demonstrate the viability to use this type of reactors for the treatment of such effluents, in spite of the fact that the increase of nitrate concentration changed the biomass distribution, which caused a decrease in the size of anaerobic granules.
Keywords: denitrification, C/N ratio, methanogenesis, expanded bed reactor, nitrate.

Resumen
Aguas residuales de diversas industrias contienen altos niveles de nitratos, pero una insuficiente cantidad de donadores de electrones para llevar a cabo la desnitrificación biológica. Una alternativa de tratamiento es combinar la digestión anaerobia y la desnitrificación en un solo reactor. Durante esta investigación, un reactor anaerobio de lecho expandido fue operado con diferentes relaciones C/N (10, 7, 4, 3, 2 y 1) con el fin de estudiar la eliminación de nitrógeno y materia orgánica. Se utilizó un reactor de 3 L con flujo de alimentación de 3 L/d, velocidad ascensional de 7 m/h y temperatura de 30-35°C. La remoción de materia orgánica se mantuvo por arriba del 90 %, durante los 177 días del experimento, y la remoción de nitrógeno fue superior al 90 %, cuando las relaciones C/N fueron superiores a la estequiométrica (C/N>1). En la relación C/N=1, la eliminación de nitrato fue del 60 %, causando la acumulación de nitrito. Las altas remociones de nitrato y materia orgánica obtenidas demuestran la factibilidad de utilizar este tipo de reactores para el tratamiento de dichos efluentes, a pesar de que el incremento de la concentración de nitrato provocó una disminución en el tamaño de los gránulos anaerobios.
Palabras clave: desnitrificación, relación C/N, metanogénesis, reactor de lecho expandido, nitrato.

1 Introduction

Organic matter and nitrogen compounds represent some of the most important pollutants in water. Nitrogen compounds, principally ammonia, nitrates and nitrites, are released by various industrial and agricultural activities. The discharge of nitrogen compounds on the environment can cause serious problems, for example, eutrophication of rivers and deterioration of water sources, as well as to be dangerous for human health, due to nitrates can form nitrosamines and nitrosamides, which are

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potentially carcinogenic compounds (Andalib *et al*., 2011; Arredondo *et al*., 2007).

There are many physicochemical and biological methods for disposal of this kind of pollutants. Even if physicochemical treatments have many advantages, including plant simplicity, insensitivity to temperature changes and adaptability in a wide pollutant concentration range, their benefits are counteracted by disadvantages such as high operational costs, energy consumption and post-treatment costs for precipitation sludge or concentrated liquid disposal (Halling and Jörgensen, 1993). Anaerobic biological processes are the most effective, sustainable and economically feasible way of removing organic matter and nitrogen as long as the necessary electron donor is available (Cervantes *et al*., 2011).

Effluents of industries such as food processing, explosive manufacturing, fertilizers, cellophane, metal production, and nuclear weapon production contain high levels of nitrate but an insufficient amount of electron donor to sustain biological denitrification (Tugtas *et al*., 2009). For this case, an alternative is to combine anaerobic digestion and nitrate reduction process in a single unit, which may resolve the deficient electron donor problem in wastewater and allow partial conversion of organic matter to energy. In a simultaneous process, the organic substrate or hydrogen is oxidized and one electron acceptor, other than oxygen, such as nitrate, nitrite, sulphur, ferric iron and carbon dioxide, is reduced (Chowdhury *et al*., 2010). This process is determined by the following reaction:

$$5C_6H_{12}O_6+24NO_3^-+24H^+ \rightarrow 12N_2+30CO_2+42H_2O$$

The main factors that influence the development of a simultaneous process are: carbon-nitrogen (C/N) ratio, type of carbon source, alkalinity, pH, temperature, and organic loading rate. C/N ratio is an important parameter for optimal operation of denitrification systems, from a technical and economic standpoint. Simultaneous denitrification and anaerobic digestion are possible when the carbon source is easily assimilated and when there is a C/N ratio appropriate to prevent methanogenic inhibitions (Ruiz *et al*., 2006).

Investigations have shown that was possible to maintain both processes in a single reactor at low organic loading rates, such as Mosquera *et al*. (2001), who reported 100 % denitrification and 80 % methanogenic activity in an upflow biofilter treating fish cannery wastewater. Ruiz *et al*. (2006) investigated the removal of nitrate and carbon in upflow sludge blanket (UASB) reactor with nitrate and peptone as nitrogen source and acetate as the carbon source. The system resulted in 98 % denitrification and 96 % COD simultaneously under different COD/N ratios. Ahan *et al*. (2007) worked in an anaerobic upflow bed filter combined with a membrane-aerated bioreactor to investigate simultaneous organic and nitrogen removal in the anaerobic system. Where 99 % organic removal and 46 % nitrogen removal was obtained, and a significant increase in membrane fouling was reported. In addition, Zhang (2003) studied the integration of methanogenesis and denitrification in an expanded granular sludge bed (EGSB) which is considered a high organic loading rate reactor (up to 40 kgCOD/m^3·d) (Seghezzo *et al*., 1998). The system reached a complete denitrification of 97-100 % with anaerobic COD reduction of 92-97 %. However, the effect of wastewater composition in this kind of reactors has not been investigated in depth. For that reason, the main goal of this work was to study the simultaneous denitrification and methanogenic process in an anaerobic expanded bed reactor at different C/N ratios.

2 Materials and methods

2.1 Experimental set-up

Experiments were conducted in an acrylic column of 1.2 m of height and an inside column diameter of 4.6 cm. An operating volume of 3 L, hydraulic retention time (HRT) of one day and upflow velocity of 7 m/h. The reactor was inoculated with 900 mL of anaerobic biomass with 55 gTS/mL and 40 gVS/mL from a Wastewater Treatment Plant of the brewing industry and added calcium (100 mg/L) as granulating agent. The temperatures in the reactor were maintained in the range of 30-35 °C. Fig. 1, shows a schematic representation of reactor set-up. The reactor was fed with synthetic wastewater consisting of (mg/L): KCl (270), KH_2PO_4 (169), $MgCl_2·6H_2O$ (150), $CaCl_2$ (277), yeast extract (18), trace element solution (1 mL/L) consisting of (mg/L): $FeCl_2·4H_2O$ (2000), $MnCl_2$ (500), EDTA (500), Na_2SeO_3 (100), H_3BO_3 (50), $ZnCl_2$ (50), $(NH_4)_6Mo_7O_{24}·4H_2O$ (50), $AlCl_3$ (50), $CoCl_2·2H_2O$ (50), $CuCl_2·2H_2O$ (50), HCl (1 mL/L) and the addition of $C_6H_{12}O_6$ (1880 mg/L) and $NaNO_3$ (45-453 mg/L) to obtain carbon/nitrogen (C/N) ratios of 10, 7, 4, 3, 2 and 1, assuming that all substrates are soluble.

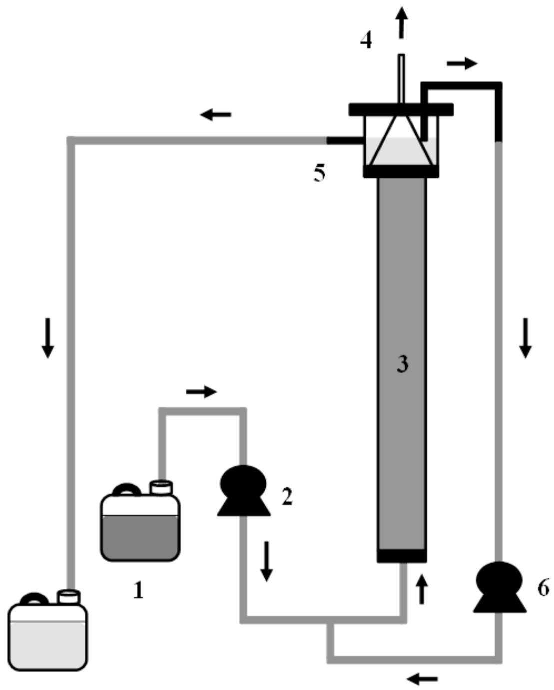

Fig. 1. Experimental set-up of the anaerobic expanded bed reactor. (1) Feed tank, (2) feed pump, (3) reactor, (4) biogas outlet, (5) effluent outlet, and (6) effluent recycling pump.

2.2 Analytical methods

In this system, nitrate (NO_3^--N) was analyzed by the cadmium reduction method with Nitra-Ver high range kit from Hach® and nitrite (NO_2^--N) by the sulfanilic acid reaction with Nitri-Ver low range kit from Hach®. Biomass was determined by volatile solids concentration, and consumption of organic matter (COD) was measured by closed reflux dichromate oxidation, both based on standard methods (Standard Methods for the Examination of Water and Wastewater, 1995). Granulometry was obtained by sieving (Laguna *et al*., 1999) and the pH was determined using an Accumet XL60 pH meter.

2.3 Reactor operation

In order to activate the biomass, a start-up procedure of 64 days was performed before conducting experiments, increasing in steps the applied organic loading rate up to 2 gCOD/L·d, reaching a stable operation with high COD removal under these conditions. No nitrate was added to the influent during this period. The acclimatization operating conditions are summarized in Table 1. Once start-up was finished, studies on simultaneous methanogenesis and denitrification processes were conducted. In this period of operation, the C/N ratio was varied by increasing periodically the concentration of nitrate, resulting in six stages of treatment (10, 7, 4, 3, 2 and 1 C/N ratios) and using glucose as the carbon source. Consumptions of substrates (organic matter and nitrate) by denitrification and methanogenesis were calculated based in mass balances, assuming only soluble organic matter.

2.4 Experimental data processing

An anaerobic expanded bed reactor can be modeled as a complete stir tank reactor due to the high rate of recirculation and gas generation in the sludge bed, assuming that it allows a complete mixing of the substrate and reaction product (Chávez, 2010).

The mass balance can be expressed as:

$$V\frac{dS}{dt} = F(S_0 - S) - \gamma V \tag{1}$$

Hydraulic retention time is given by HRT = V/F and in a steady state, there is no accumulation (VdS/dt = 0), Eq. (1) can be written as:

$$\gamma = \frac{(S_0 - S)}{HRT} \tag{2}$$

Replacing Monod or Michaelis-Menten expression for γ in Eq. (2):

$$S = V_{max} \cdot HRT \frac{S}{(S_0 - S)} - K_s \tag{3}$$

Table 1. Acclimatization operating conditions

Time (days)	pH	Alcalinity	Biogas (L/d)	Influent COD (g/L)	% COD removal
1-27	7.56±0.44	0.74±0.09	0.38±0.18	1±0.1	91.52
28-64	7.36±0.39	0.74±0.12	1.5±0.46	2±0.1	92.50

Table 2. Operating conditions of the reactor in the simultaneous denitrification and methanogenesis process

C/N ratio	Operation time (days)	Influent COD (g/L)	COD removal (%)	Influent nitrate (g/L)	Nitrate removal (%)
10	65-77	2±0.14	94.51	0.33±0.11	98.16
7	78-91	2±0.12	97.83	0.47±0.13	92.59
4	92-120	2±0.10	91.38	0.83±0.10	90.85
3	121-140	2±0.15	96.73	1.10±0.11	94.29
2	141-160	2±0.13	92.44	1.65±0.13	93.58
1	161-177	2±0.12	90.33	3.30±0.12	60.76

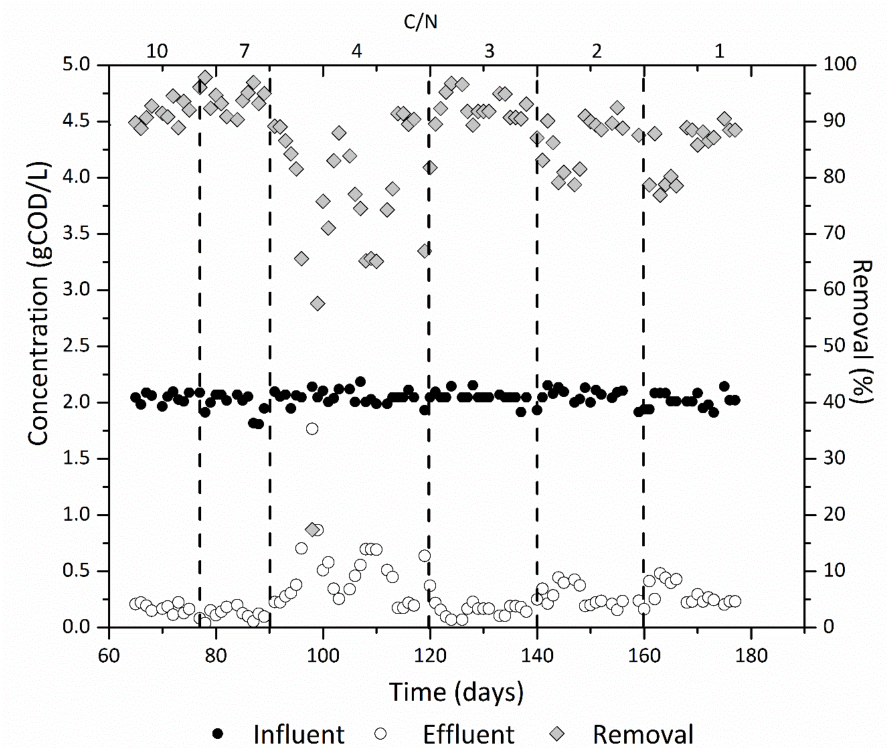

Fig. 2. Organic matter removal in the simultaneous denitrification and methanogenesis process.

A plot, called Lineweaver-Burk, of S versus HRT $S/(S_0 - S)$ gives a straight-line with an intercept of K_s and a slope of V_{max}.

3 Results and discussion

3.1 Nitrogen and organic matter removal

The study of simultaneous denitrification and methanogenesis process starts from day 65. Operating conditions of each stage, as well as COD and nitrogen removal efficiencies, are shown in Table 2.

Fig. 2 shows the evolution of the organic matter consumption at different C/N ratios (10, 7, 4, 3, 2 and 1). The C/N ratio of 10 showed a stable operation period, with a removal efficiency of organic matter above 90% in 77 days of operation, after increasing nitrate concentration up to 0.475 g/L to obtain C/N ratio of 7, no significant changes were observed in COD removal (97 % average). In a period where, C/N ratio was 4 (from day 90); the reactor started to be unstable, and a disintegration of anaerobic granules was observed, this was evident due to the presence of biomass suspension inside the reactor, which caused a removal efficiency of 50 %; due to this, it was necessary to reduce the upflow velocity from 7 to 3 m/h to avoid the reactor washing out. Once the efficiency was stabilized, the influent C/N ratio began to decrease gradually, until obtain the lowest ratio of 1.

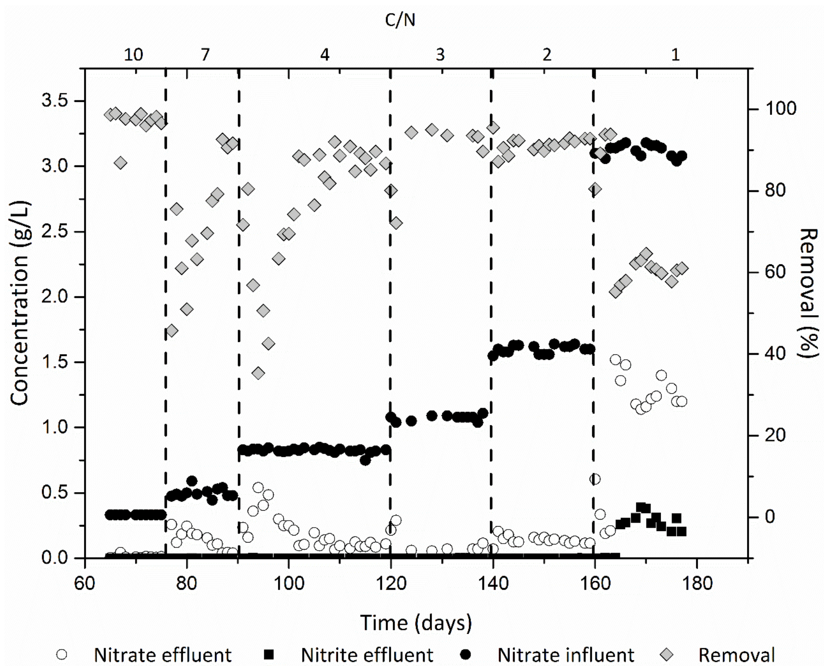

Fig. 3. Nitrogen removal in the reactor at different C/N ratios during the experiment.

A COD removal efficiency superior to 90% was obtained in the next experiments, using a C/N ratio of 3 (day 120) with 20 days of operation, which not present significantly alterations during nitrogen load variations, this is because of denitrifying bacteria contribution to the organic matter removal, and possibly to the methanogenic microorganisms' acclimatization at nitrate presence. The same behavior was observed during the period C/N ratio of 2 (day 141-160), as well as the 177 day by the addition of 3.3 g of nitrate (C/N ratio of 1).

Removal of organic matter was similar at the obtained by Vidal *et al.* (1997), and Gharsallah *et al.* (2002), treating effluents of fish processing industry, which were around 90%.

Fig. 3 shows the behavior of nitrogen compounds at different operating conditions of the C/N ratio. Besides when nitrate was added to the reactor feeding to obtain a C/N ratio of 10 during 18 days of operation, the nitrate removal was 97%, however, in the C/N ratio of 7 and 4; the nitrate removal dropped 50%, until reaching levels up to 90 % at the end of period. The reactor operation maintained a removal higher than 90%, using the C/N ratio of 3 and 2, but in the C/N ratio of 1, even when the organic matter maintained an efficiency of 90%, nitrate removal was 60%, due to the insufficient carbon source needed to carry on the denitrification, allowing the accumulation of nitrites (0.3 g/L).

The results obtained shows the importance of establishing a balanced C/N ratio and demonstrate that the use of C/N relations closer to the stoichiometric (1.4, including biomass formation) allow higher efficiencies of denitrification without the accumulation of intermediaries, meanwhile the use of high ratios causes a significant methanogenic activity allowing to have both processes in the same reactor (Andalib *et al.*, 2011).

In case of nitrogenous compounds, nitrates removal in C/N ratio from10 to 2 turned out to be 97% at the end of each stage, which is comparable to that obtained by Chen and Lin (1993) who studied the effect of nitrate loading rate in a continuous stirred tank reactor (CSTR) co-immobilized with methanol as the carbon source, and concluded that a high concentration of nitrate of more than 774 mg/L in the influent and a C/N ratio above 1.3, nitrate removal was higher than 99% with removal efficiencies of the carbon source of 98% and bigger. Furthermore, nitrite accumulation as an intermediary was observed when an insufficient carbon source was provided in a simultaneous denitrification and methanogenesis system, with a C/N ratio lower than 1.5, causing inhibition to methanogenic activity, which is similar to the situation occurring in this study.

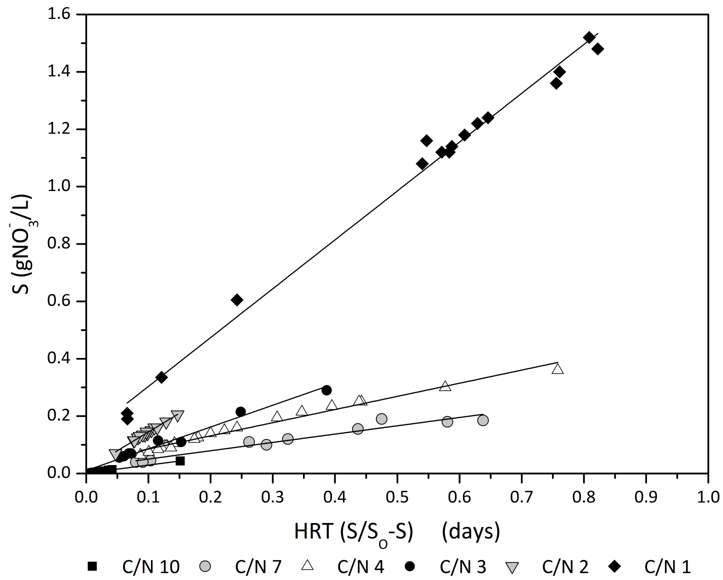

Fig. 4. Schematic representation of the mass balance in the reactor for each relation C/N.

Table 3. Mass balance parameters in the anaerobic expanded bed reactor

C/N ratio	Substrate (gNO_3^-/L)	V_{max} (gNO_3^-/L·d)	Ks (gNO_3^-/L)	R^2
10	0.33±0.11	0.28	0.009	0.99
7	0.47±0.13	0.29	0.021	0.94
4	0.83±0.10	0.46	0.039	0.98
3	1.10±0.11	0.76	0.010	0.98
2	1.65±0.13	1.30	0.013	0.99
1	3.30±0.12	1.70	0.133	0.99

Similarly, the experiment done by Oh and Silverstein (1999), in sequenced batch reactors, using acetate as reducing agent, it was found that a C/N ratio of 1 showed a nitrite accumulation of 30%. However, when the C/N ratio was in 2 and 3, the nitrate was consumed without nitrite accumulation. Martínez (2003) working in an upflow anaerobic sludge bed reactor (UASB) with toluene as the carbon source and a C/N ratio of 1.4, obtaining carbon and nitrogen consumptions above 95 and 87% respectively, determined that the starting point for the C/N relation study, lies in its stoichiometric value. This allowed a denitrifying process without significant accumulation of nitrogenous and organic compounds. Additionally, Plascencia (2010), using an expanded granular sludge bed reactor (EGSB) coupled with an aerobic reactor to treat effluents from crab processing with C/N ratios near stoichiometric, managed to remove 81% of carbon and 74% of nitrogen compounds.

3.2 Nitrate mass balance in the reactor

Mass balance allows to describe the behavior observed in the biological process of simultaneous methanogenesis and denitrification. Fig. 4 presents the graphical results for nitrate balance in a stationary state according to Eq. (3). For each line on this figure, the slope corresponds to the maximum consumption rate of nitrate, and the interception corresponds to the affinity constant.

It is observed that when the substrate concentration increases, the maximum consumption rate of nitrate increases as well, and their values are presented in Table 3. The rates values obtained in this study are higher than those reported by Zhang (2003) and Rustrian (1997) who worked with C/N ratios of 4.9 to 61 and obtained consumption rates from 0.03 to 1 gNO_3/L·d, while Ruiz *et al*. (2006) mentioned that after a strategy of acclimatization in gradual increases in nitrate concentrations, with loading rates from 0.075

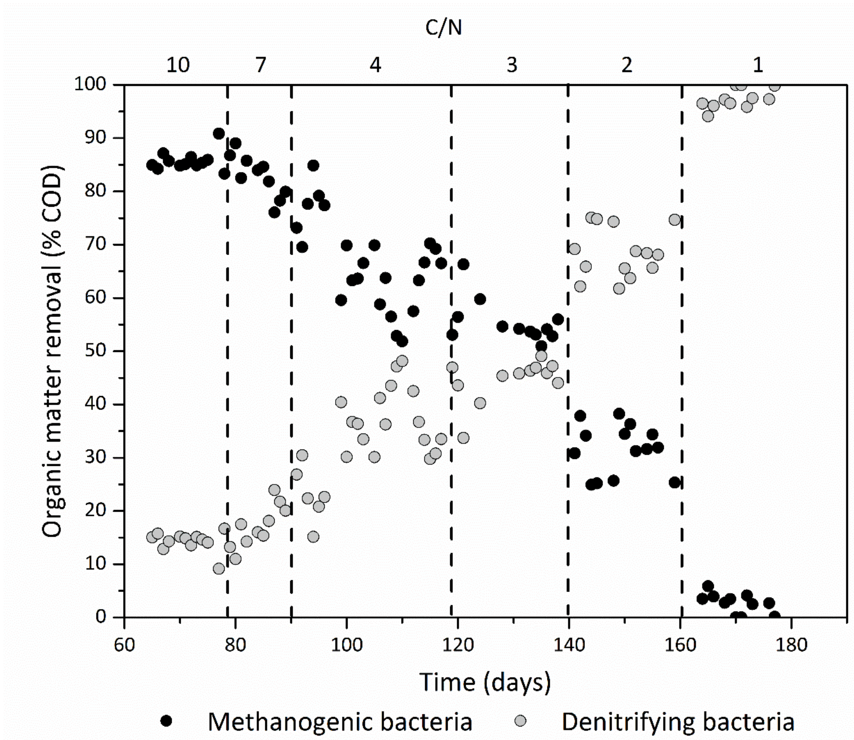

Fig. 5. Competition between methanogenic and denitrifying bacteria in the system.

to 7.5 gNO_3/L·d and C/N ratios from 1.7 to 170, higher removals are achieved (more than 80%) in an UASB reactor, in addition, it has been observed that the type of reactor influences the consumption rates of nitrate as Chen *et al*. (1997) in a CSTR fixed reactor obtained velocities from 0.58 to 4.7 gNO_3/L·d with C/N ratios from 1.7 to 5.8.

3.3 Competition between methanogenic and denitrifying bacteria

Denitrifying bacteria compete between methanogenic bacteria for organic substrates during anaerobic treatment of water containing high levels of nitrate. The result from this competition is important because is possible to determine the performance of the finished products like nitrogen and methane (Andalib *et al*., 2011). Fig. 5 shows that when the amount of nitrates are raised, denitrifying activity also increases, while the methanogenic activity decreases. Initially, methanogenic activity was widely dominant, since the day 90 (C/N ratio from10 to 7), 85% of the COD was consumed by methanogenic bacteria. In C/N ratio 4 (day 110) methanogenic activity decreased 20%, and denitrifying activity increased, consuming 35% of organic matter. In C/N ratio of 3 (day 130) the COD was removed 50% by both consortia, due to they can grow in different regions of the granule.

In relation to this effect, Chen and Lin (1993), studied a mixed culture immobilized on gel beads at pH 7, fed with 800 mg/L methanol and 100 mg/L NO_3-N, demonstrating that methanogenic and denitrifying bacteria could grow simultaneously occupying different regions of the gel beads. Denitrifying bacteria grow mainly in the peripheral surface while methanogenic bacteria grow on the internal part of the gel beads. Zhang (2003) working in an EGSB reactor, also noted a change in color in the reactor sludge, revealing that a community of bacteria has changed, because the denitrifying bacteria was outside the granule, and the methanogenic was inside.

Finally, denitrifying activity dominated the process during the day 160 (C/N ratio of 1), where 97% of the COD was consumed by denitrifying bacteria. This behavior was observed by Klüber and Conrad, (1998) who found that the addition of nitrate affects not only the activity, also changes the composition of the methanogenic community, and reported that nitrate caused inhibition in methanogenic microorganisms.

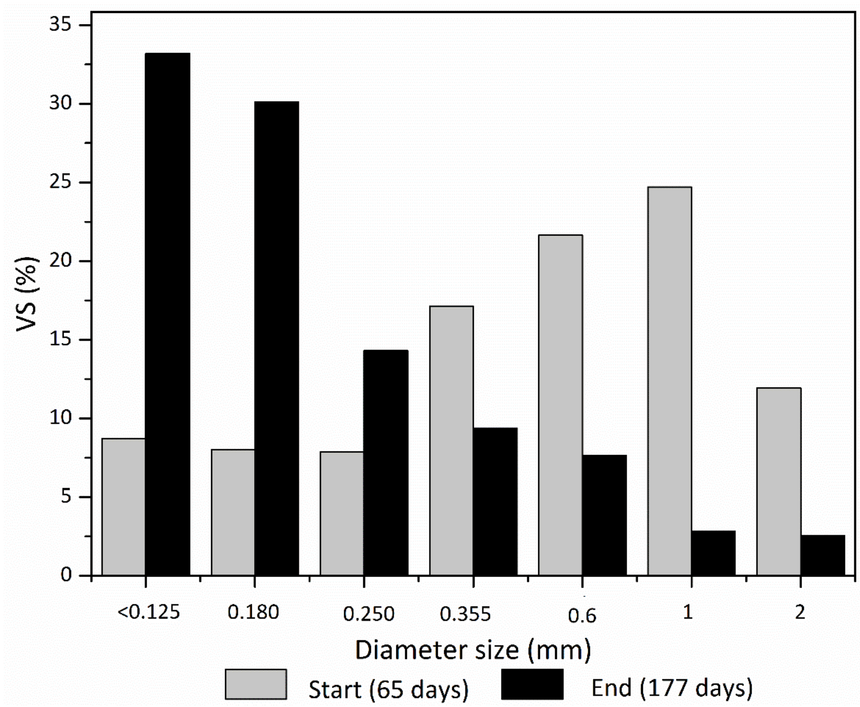

Fig. 6. Granulometry made at the beginning and end of simultaneous denitrification and methanogenesis process.

3.4 Effect of C/N ratio on granule size

The increase of nitrate concentration affected the size of the granules, as can be seen in Fig. 6, at the beginning of the C/N ratio of 10 (day 65), 58.3% of the volatile solids (VS) were distributed in bigger sized granules (0.6 <D<2). Meanwhile, when the study was concluded with a C/N ratio of 1 (day 177) a change of color was observed (from black to brown) in the reactor biomass, and the granules size for this period of C/N ratio was reduced by 13%. This due to the bacteria consortium establishment, which do not present the physiological characteristics to form granules by themselves, besides, there are not rupture and abrasion forces in the anaerobic expanded bed reactor linked to the ascendant velocity, which causes granule's disintegration (Seghezzo *et al*., 1998).

Conclusions

C/N ratio has a strong influence on biomass activity and consequently, on the nitrogen and organic matter removal of the reactor. The organic matter removal remained above 90% throughout the experimentation. This removal was carried out by the participation of both consortia, where it was observed that at high C/N ratios, methanogenic activity was predominant, while at low ratios, the degradation was carried out mainly by denitrifying bacteria, and in intermediate ratios (C/N of 3) both consortia participated in 50 %. Nitrogen removal was over 90%, when C/N ratios were greater than the stoichiometric (C/N>1), however, in C/N ratio of 1, nitrogen removal was 60%, causing the accumulation of nitrite.

A decrease in C/N ratio also effects the biomass granular structure, because this cannot stay in the anaerobic expanded bed reactor with C/N ratio of 4 and lower, where the granule is replaced by a flocculent sludge, making the reactor operation difficult, by the increase of sludge washout.

The results obtained during this study shown the feasibility of using expanded bed reactors for the treatment of industrial wastewater with C/N ratios above the stoichiometry, which is beneficial because a large amount of effluents with these characteristics can be treated in smaller spaces than the conventional treatment systems.

Nomenclature

COD (g/L)	chemical oxygen demand
HRT (d)	hydraulic retention time

OLR (g COD/L-d)	organic loading rate
TS (g/L)	total solids
VS (g/L)	volatile solids
C/N	carbon/nitrogen ratio
COD/N	chemical oxygen demand/nitrogen ratio
NO_3^--N	nitrate-nitrogen
NO_2^--N	nitrite-nitrogen
EDTA	ethylenediaminetetraacetic acid
EGSB	expanded granular sludge bed
UASB	upflow anaerobic sludge bed
CSTR	continuous stirred tank reactor
D	diameter
So (g/L)	inlet substrate
S (g/L)	outlet substrate
F (L/d)	flux
V (L)	volume
t (d)	time
γ (g/L·d)	consumption rate: Monod equation
Vmax (g/L·d)	maximum consumption rate
Ks (g/L)	affinity constant

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