



Nitrogen removal in domestic wastewater. Effect of nitrate recycling and COD/N ratio



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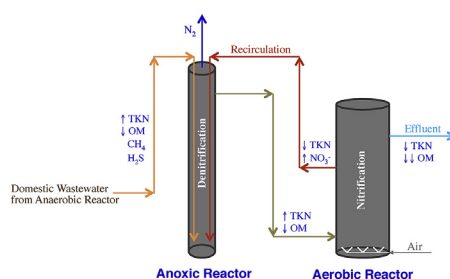
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HIGHLIGHTS

- Denitrification/nitrification of domestic wastewater after anaerobic treatment.
- The HRT was 1 h for the anoxic bioreactor and 2 h for the aerobic one.
- Evaluation of the influence of COD/N and NO_3^- recycling ratios in nitrogen removal.
- The system achieved 85% and 96% removal of nitrogen and organic matter respectively.

GRAPHICAL ABSTRACT



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ABSTRACT

A denitrification/nitrification pilot plant was designed, built and put into operation, treating the effluent of an anaerobic reactor. The operation of the plant examined the effect of the nitrate recycling and the COD/N ratio on the nitrogen and the remaining organic matter removal at 18 °C. The system consisted of a two-stage treatment process: anoxic and aerobic. The hydraulic retention time (HRT) of the system was 1 h for the anoxic bioreactor and 2 h for the aerobic one. The increase in the nitrate recycling ratio did not cause a significant improvement in the nitrogen removal due to the insufficient carbon source. The wastewater to be treated had a C/N ratio of 1.1 showing a lack of organic carbon. The addition of methanol was a key point in the denitrification process used as a model for the traditional wastewater by-pass in the WWTP. The maximum nitrogen and organic matter removal (87.1% and 96%, respectively) was achieved with a nitrate recycling ratio of 600% and a C/N of 8.25, adjusted by methanol addition.

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1. Introduction

Wastewater treatment plants (WWTPs) are currently programmed to remove particulate and dissolved organic fractions

and, in more sensitive areas, nitrogen and phosphorus compounds. The most conventional well-known intensive system for treating domestic wastewater is the activated sludge process (Yang et al., 2014). However, the anaerobic treatment of wastewater has become the most commonly used method for processing effluents because of its advantages over conventional activated sludge treatment. Its energy consumption is low, while causing little

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wastage of biological solids, and transforming the organic matter into valuable biogas (Gouveia et al., 2015). Among the disadvantages of the anaerobic treatment, post treatments are necessary in order to achieve discharge standards.

According to the Official Bulletin of Spain (BOE), the characteristic parameters of the activity, its emission limit values and reference measurement methods for discharges from wastewater treatment plants derive from Directive 91/271/CEE, transposed by RDL 11/1995, RD 509/1996 and RD 2116/1998. The requirements for discharges from WWTP are 125 mg O₂/L for the chemical oxygen demand (COD) or a minimum reduction percentage of 75% (reduction in relation to the influent load), and 15 mg N/L for the total nitrogen (TN) or a minimum reduction percentage of between 70 and 80% (Manual para la gestión de vertidos, 2007, Real Decreto 509/1996, BOE 77 1996).

In the last decade, increasingly stringent environmental requirements have been imposed on nutrient discharge into receiving waters, because an excess of nutrients in the water is considered to be the primary cause of eutrophication (Cherchi et al., 2009). Most of the efforts of recent studies have been focused on the development of new technologies capable of obtaining better effluent quality, with special attention to nitrogen removal and the reduction of treatment costs (Ruscalleda Beylier et al., 2011). To control eutrophication in receiving water bodies, biological nutrient removal (BNR) of nitrogen has been widely used in wastewater treatment practice, both for the upgrade of existing wastewater treatment facilities and the design of new facilities (Hu et al., 2011). BNR constitutes the most economical and sustainable technique for meeting increasingly rigorous discharge requirements (Alzate Marin et al., 2016; Xu et al., 2013).

BNR is achieved through two processes: nitrification and denitrification. In the nitrification process, under aerobic conditions, ammonium (NH₄⁺) is converted to nitrite (NO₂⁻) by the ammonium oxidizing bacteria (AOB). Then, nitrite is oxidized to nitrate (NO₃⁻) by the nitrite oxidizing bacteria (Hatamoto et al., 2016). Denitrification is an anoxic process in which nitrate is reduced into nitrite and then into molecular nitrogen gas (N₂). This process is performed by a functional group of heterotrophs that use nitrite and/or nitrate as the electron acceptor in respiration. The denitrification process requires electron donors like organic carbon sources for the heterotrophs (Alzate Marin et al., 2016; Kumar et al., 2012; Tallec et al., 2006; Waki et al., 2008; Xu et al., 2013). Although conventional denitrification uses organic matter as electron donor for the denitrifying process, the possibility of using alternative electron donors, such as methane and sulfide, have been experimentally applied to wastewaters for denitrification (Deng et al., 2009; Pelaz et al., 2017; Waki et al., 2008).

There are different denitrification terms, such as pre-denitrification and post-denitrification depending on the order of nitrification and denitrification. In a post-denitrification configuration, wastewater is fed into a nitrification system prior to denitrification. This configuration usually leads to a total consumption of the COD before the start of the denitrification process; and therefore an exogenous carbon source should be supplied to carry out the post-anoxic denitrification (Alzate Marin et al., 2016; Kim et al., 2015). In contrast, in most BNR systems, the anoxic stage is located upstream of the aerobic zone. Wastewater is fed directly into the denitrification system, supplying organic carbon to remove nitrite and nitrate that are recycled from the nitrification system. High denitrification rates can be achieved with the pre-anoxic regime, given the supply of readily biodegradable carbon. However, this system is accompanied by some disadvantages, such as the higher energy costs derived from mixed liquor recycle flows, dissolved oxygen (DO) return from the aerobic stage, and the dilution of influent carbon (Kim et al., 2015; Xu et al., 2013).

After anaerobic treatment, a nitrogen removal plant receives an influent containing mainly the residual soluble fraction of organic carbon present in domestic wastewater, and a large fraction of the nitrogen. Therefore, the influent presents a low COD/N ratio, which is favorable to the nitrification stage but may be an obstacle for the denitrification step (Yang et al., 2014).

The denitrification potential of wastewater is mainly governed by the availability of biodegradable organic carbon, commonly expressed as the C/N ratio (Ruscalleda Beylier et al., 2011). Therefore, the C/N ratio of the influent is one of the most critical parameters that can directly affect the biological nitrogen removal efficiency. This occurs because different microorganism populations compete for the substrate causing a fluctuation in the effectiveness of organic and nitrogen removal (Fu et al., 2009; Kumar et al., 2012). Theoretically, the stoichiometric requirement of organic substrate for denitrification is 2.86 g COD/g N, considering the electron transmitting balance between organic substrate and NO₃⁻. But some studies have demonstrated that C/N values of approximately 6–11 g COD/g N could allow proper nitrogen removal to occur (Ruscalleda Beylier et al., 2011). In the case of Kim et al. (2015), with a C/N = 8 ratio, an average denitrification efficiency of around 72% was obtained. In another example, Fu et al. (2009), achieved a nitrogen removal efficiency of 90.6% when the C/N ratio was 9.3.

The amount of biodegradable organic carbon in domestic wastewater after anaerobic treatment is limited, and nitrogen removal is limited by the lack of bioavailable electron donors for heterotrophic denitrification (Cherchi et al., 2009; Santos et al., 2016). Therefore, the addition of external carbon sources often becomes necessary in order to achieving high-efficiency BNR, especially for facilities with a weak influent biochemical oxygen demand (BOD) and/or those facing strict effluent limits (Cherchi et al., 2009; Shi et al., 2015). Methanol is the most commonly used electron donor, as a result of its higher denitrification efficiency, indicated by the relatively low methanol-to-nitrate ratio, lower cost, and broad availability in the market. The main disadvantage of using methanol lies in the safety issues associated with its transportation, handling, and storage. It has been estimated that an additional 25–31% of the capital construction cost for methanol storage, pumping, and delivery systems is required in order to meet the safety standards regarding the use of a non-flammable, non-hazardous product (Cherchi et al., 2009).

Among the technologies available, biofiltration has been widely deployed in urban wastewater treatment plants. Biofiltration technology combines both physical and biological treatment by using an immersed filter material. During biofiltration treatment, the wastewater is simply passed through a fixed bed of media, which acts both as a filter and as a support for the growth of nutrient consuming bacteria. The advantages of these immersed biological systems reside in their compactness (small footprint) and low residence time (Rocher et al., 2015).

This work focuses on the study of the integration of denitrification/nitrification process treating domestic wastewater after anaerobic treatment. The specific aim of the study was to evaluate the influence of the COD/N ratio and the nitrate recycling ratio in nitrogen removal. To do so, a denitrification/nitrification pilot plant was designed, built and operated at different conditions.

2. Materials and methods

2.1. Experimental setup

The pilot plant consists of two fixed bed bioreactors able to carry out the denitrification and nitrification in wastewater. Both reactors were designed as vertical cylinders. The height and diameter of the

anoxic cylinder used for denitrification was 2.78 m and 0.15 m, respectively, with a working volume of 20 L. The height and diameter of the nitrification cylinder was 1.86 m and 0.30 m, respectively, with 40 L of working volume. The anoxic bioreactor was filled with corrugated PVC rings (17 mm in length, inner diameter of 12 mm and outer diameter of 16 mm), while the aerobic one used Filtralite® (3–6 mm of size particle) as the filter medium. A diagram of the pilot plant is shown in Fig. 1. The plant was placed in a lab provided with an air-conditioning system, in order to maintain the temperature of the bioreactor at 18 °C during both winter and summer time. The denitrifying biofilter was equipped with measurement systems for pressure, gas flow and oxidation-reduction potential (ORP), while the nitrifying biofilter included a probe to measure the dissolved oxygen and temperature. The incoming flow was set to 20 L/h. The denitrification reactor was operated with a HRT of 1 h while the nitrification one was set at 2 h. The aeration rate was controlled through a flow meter, maintaining the dissolved oxygen (DO) concentration between 2.0 and 2.5 mg O₂/L. Four aerators were fixed on the bottom to ensure that the bubbles were distributed uniformly.

The plant was fed with the reject water of an anaerobic membrane bioreactor (AnMBR) that treated domestic wastewater under psychrophilic conditions (18 °C). AnMBR is an Upflow Anaerobic Sludge Blanket (UASB) reactor with a submerged ultrafiltration membrane placed on top of the same reactor. The AnMBR pilot plant has been explained in detail in a previous work (Gouveia et al., 2015). The wastewater with a high concentration of NH₄⁺ and low level of organic matter was pumped into the anoxic reactor. In this first step, NH₄⁺ did not change, and passed through the aerobic reactor. In the second step, the NH₄⁺ was oxidized to NO₃⁻ in the presence of oxygen. This stream was then recycled using a peristaltic pump proceeding from the aerobic bioreactor and feeding into the anoxic reactor, where the denitrifying bacteria were able to use the COD from the feedstream.

Due to the high DO concentration in the recycled water proceeding from the aerobic bioreactor, the organic carbon available in the feed water from anaerobic treatment tended to be oxidized instead of being used for denitrification. As a consequence, denitrification efficiencies would fall. To avoid this effect as far as possible, a degassing tank was placed in the recycling line to prevent dissolved oxygen entering the anoxic tank.

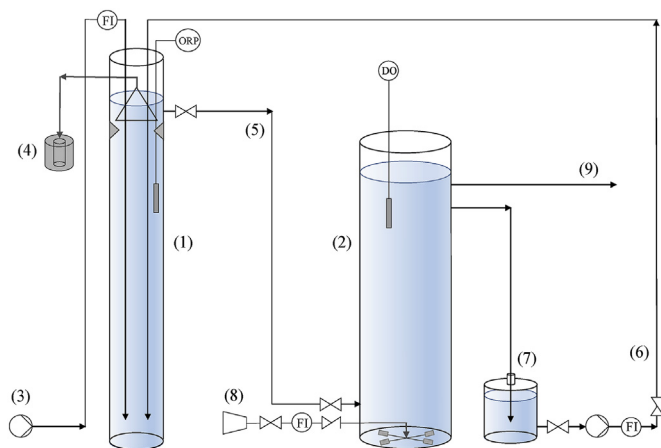


Fig. 1. Pilot plant flow scheme. (1) Denitrification reactor, (2) nitrification reactor, (3) filling pump, which supplies the wastewater from the anaerobic treatment to the denitrifying reactor, (4) gas flow meter, (5) effluent from anoxic to aerobic reactor, (6) nitrate recycling from aerobic to anoxic reactor, (7) degassing tank, (8) compressor, responsible for supplying the air, (9) final effluent. ORP: oxidation-reduction potential probe; DO: dissolved oxygen; FI: flow-rate indicator; PI: pressure indicator.

2.2. Inoculum and feed wastewater

The inoculum of the denitrifying bioreactor was a mix of anoxic sludge and anaerobic digested sludge, taken from the wastewater treatment plant of Valladolid (Spain). The inoculum of the nitrifying bioreactor was secondary aerobic sludge from the same WWTP.

The plant being studied was fed with the effluent from an AnMBR fed with raw municipal wastewater from the city of Valladolid (Spain). The average concentration of the main parameters of wastewater after anaerobic treatment are given in Table 1. It can be seen that the concentration of NH₄⁺-N dominated the TN, leading to a COD/N ratio as low as 1.04. When the AnMBR effluent was sampled, the sulfide contained in the wastewater was oxidized to sulfate, and by chromatographic techniques this compound could be determined. In the inlet stream (from the anaerobic treatment), sulfur is represented by the corresponding amount of sulfide oxidation without quantifying the supersaturation, so the real value for sulfide was expected to be higher than showed.

2.3. Analytical methods

Samples of wastewater were collected periodically before and after the denitrification reactor, and after the aerobic reactor, with this stream being the effluent of the process. Nitrate nitrogen (NO₃⁻-N), nitrite nitrogen (NO₂⁻-N), sulfate (SO₄²⁻), soluble phosphorus (P-PO₄³⁻) and Chloride (Cl⁻) concentrations were analyzed by HPLC-IC using a Waters 515 HPLC pump (Waters, Milford, USA) coupled with an ion conductivity detector (Waters 432, Milford, USA) and equipped with an IC-Pak Anion Guard-Pak column (Waters, Milford, USA) and an IC-Pak Anion HC (150 mm × 4.6 mm) column (Waters, Milford, USA). Ammonium concentration was determined using an ammonia-selective electrode: Orion, model 9512HPBNWP. The analyses of Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen (TKN) as well as total and volatile suspended solids (TSS, VSS) were determined according to the *Standard methods for the examination of water and wastewater* manual APHA-AWWA-WPCF (APHA et al., 2005). The measurement of dissolved oxygen concentration was determined with an oximeter WTW, model oxi 330/SET and a dissolved oxygen probe CeliOx 325. Gas production from the anoxic bioreactor was measured volumetrically by water displacement, and its composition in terms of methane, carbon dioxide, nitrogen, oxygen, hydrogen sulfide and hydrogen was determined by gas chromatography (Varian CP-3800, Palo Alto, CA, USA) coupled with a thermal conductivity detector and equipped with a CP-Molsieve 5A (15 m × 0.53 mm × 15 μm) and a CP-Pora BOND Q (25 m × 0.53 mm × 15 μm) columns. The injector, oven and detector temperatures were 150 °C, 40 °C and 175 °C, respectively. Helium was used as the carrier gas at 13.7 mL/min. Pressure, temperature and oxidation reduction potential (ORP) were measured by using sensors and probes.

2.4. Operation strategy

The denitrification/nitrification experiments were run for more than five consecutive months. Eight different scenarios were studied until reaching the optimum C/N ratio and nitrate recycling ratio (R). Each case was analyzed for around 20 days at a steady state. Table 2 depicts the recycling ratio of nitrate (R), the COD, whether or not the addition of an external carbon source occurred and the C/N ratio established for each case studied.

In cases 1 and 2, R was modified. The same parameter was changed in cases 4, 5 and 6 but with other C/N ratios in contrast with cases 1 and 2. All these cases are analyzed in section 3.1.

In cases 2 and 3, R was maintained but the C/N ratio was increased by the addition of methanol. A different R was kept in

Table 1Feed composition of the process studied. (Mean \pm standard deviation of the mean. < QL: Lower than quantification limit).

sCOD (mg O ₂ /L)	TKN (mg N/L)	NH ₄ ⁺ (mg N/L)	NO ₂ ⁻ (mg N/L)	NO ₃ ⁻ (mg N/L)	SO ₄ ²⁻ (mg S/L)	sol P (mg P/L)
122.4 \pm 3.4	118.0 \pm 3.5	109.3 \pm 3.3	< QL	< QL	8.7 \pm 0.2	10.7 \pm 0.3

Table 2

Characteristics of the cases implied in the operation strategy.

Case	R	Methanol	Soluble COD (mg O ₂ /L)	C/N ratio
1	Q	No	106.0 \pm 2.1	1.09
2	2Q	No	105.7 \pm 1.0	1.12
3	2Q	Yes	286.5 \pm 3.6	2.59
4	3Q	Yes	454.2 \pm 4.3	3.74
5	4Q	Yes	448.7 \pm 1.5	3.94
6	5Q	Yes	476.2 \pm 5.4	4.87
7	6Q	Yes	574.0 \pm 3.2	5.37
8	6Q	Yes	848.2 \pm 1.7	8.25

cases 7 and 8, but with a higher C/N ratio than that of cases 2 and 3. These cases are discussed in section 3.2.

3. Results and discussion

3.1. The effect of the recycling ratio of nitrate

It was studied the removal efficiency of organic matter and nitrogen in the denitrification-nitrification system while changing the nitrate recycling ratio. COD concentration in the influent was maintained constant, and the recycling R from the aerobic bioreactor effluent into the anoxic one was increased in order to study its effect. An increase in the recycling rate from the aerobic to the anoxic column provides more nitrates to the denitrification reactor, and can thus improve the overall nitrogen removal and minimize the TN concentration in the effluent.

Table 3

COD concentration in the inlet, after the denitrification reactor and at the end of the process for the different conditions evaluated. (1: wastewater influent from AnMBR; 2: Stream from the denitrification reactor to nitrification; 3: Nitrification effluent and the outlet of the plant). (Mean \pm standard deviation of the mean).

Case	Soluble COD (mg O ₂ /L)		
	1	2	3
1	106.0 \pm 2.1	44.4 \pm 1.6	13.1 \pm 1.3
2	105.7 \pm 1.0	68.5 \pm 0.4	27.1 \pm 0.2
3	286.5 \pm 3.6	76.5 \pm 0.7	22.3 \pm 0.9
4	454.2 \pm 4.3	81.9 \pm 2.4	31.9 \pm 2.8
5	448.7 \pm 1.5	72.5 \pm 2.6	20.0 \pm 3.7
6	476.2 \pm 5.4	66.7 \pm 2.1	27.7 \pm 3.3
7	574.0 \pm 3.2	68.2 \pm 1.0	21.5 \pm 0.6
8	848.2 \pm 1.7	107.0 \pm 0.6	33.8 \pm 0.2

Table 4

Evolution of nitrogen compounds during the denitrification/nitrification process. (1: wastewater influent from AnMBR; 2: Stream from the denitrification reactor to nitrification; 3: Nitrification effluent and the outlet of the plant). (Mean \pm standard deviation of the mean. < QL: Lower than quantification limit).

Case	TKN (mg N/L)			NH ₄ ⁺ (mg N/L)			NO ₂ ⁻ (mg N/L)			NO ₃ ⁻ (mg N/L)		
	1	2	3	1	2	3	1	2	3	1	2	3
1	96.9 \pm 1.0	33.6 \pm 0.6	13.4 \pm 0.3	93.0 \pm 1.0	32.3 \pm 0.6	12.9 \pm 0.3	< QL	< QL	< QL	< QL	32.5 \pm 0.3	61.2 \pm 0.6
2	94.2 \pm 1.9	68.1 \pm 1.2	28.4 \pm 0.5	94.0 \pm 1.9	57.6 \pm 1.2	24.8 \pm 0.5	< QL	< QL	< QL	< QL	28.6 \pm 0.6	49.3 \pm 1.0
3	110.5 \pm 0.6	52.8 \pm 1.2	20.6 \pm 0.4	107.3 \pm 0.6	48.7 \pm 1.2	17.8 \pm 0.4	< QL	2.3 \pm 0.1	< QL	< QL	24.8 \pm 0.3	48.8 \pm 0.2
4	121.4 \pm 1.4	42.9 \pm 1.5	17.9 \pm 1.0	119.0 \pm 1.3	38.1 \pm 1.3	15.2 \pm 0.9	< QL	1.1 \pm 0.1	< QL	< QL	25.0 \pm 0.5	34.0 \pm 0.8
5	114.0 \pm 0.5	49.8 \pm 0.4	22.5 \pm 0.3	111.0 \pm 0.5	44.9 \pm 0.5	20.8 \pm 0.4	< QL	0.6 \pm 0.1	< QL	< QL	10.2 \pm 1.1	13.2 \pm 1.3
6	97.7 \pm 1.0	37.7 \pm 0.7	8.6 \pm 0.8	85.8 \pm 1.5	21.0 \pm 1.4	5.4 \pm 0.9	< QL	0.6 \pm 0.1	< QL	< QL	32.8 \pm 1.0	43.8 \pm 1.5
7	106.9 \pm 0.04	18.1 \pm 0.9	0.0 \pm 0.0	102.5 \pm 0.1	12.0 \pm 0.4	< QL	< QL	< QL	< QL	< QL	28.9 \pm 0.2	32.5 \pm 0.1
8	102.8 \pm 1.7	33.5 \pm 0.2	7.2 \pm 0.01	98.9 \pm 0.3	29.8 \pm 0.2	5.7 \pm 0.5	< QL	< QL	< QL	< QL	3.9 \pm 0.05	6.1 \pm 0.04

On the one hand, during the first part of the work, case 1 and 2 were tested, analyzing the recycling effect from R = Q (Q: incoming flow) to R = 2Q, being the COD/N ratio of 1.09 and 1.12 for each condition.

Tables 3 and 4 summarize the average concentration of the COD and the nitrogen compounds at different nitrate recycling ratios. The NH₄⁺ concentration decreased significantly in the anoxic reactor due to the dilution of the nitrate recycling stream. The average ammonium removal efficiency of the overall process was 86.1% in case 1, and 73.6% for case 2 (Table 4). The removal profile of NH₄⁺-N was analogous to that of COD, indicating that the utilization of organic matter and the degradation of NH₄⁺ occurred simultaneously. Fig. 2 shows the percentage of COD and TN removed. With the same COD influent of 106 mg O₂/L, the average COD removal efficiency was 87.6% and 74.4% for each situation, indicating a good ability to remove the organic matter. On the contrary, the total nitrogen removal efficiency was poor, with values of about 20%. The C/N ratio in the system was very low, and this became a limiting factor for the denitrification process, which was not able to remove the nitrogen compounds. NO₃⁻ was the prominent compound of TN in the effluent and this residual nitrogen was mainly due to the exhaustion of the carbon source of heterotrophs.

On the other hand, cases 4, 5 and 6 were analyzed by pumping methanol into the system. It is interesting to make note of cases 4, 5 and 6, where the COD concentration in the feed was approximately constant (460 mg O₂/L). In these situations, the C/N ratio was adjusted to around 4.1 by the addition of methanol. This adjustment was done to increase the available organic matter in the feed for the denitrification process. In those cases, the nitrate recycling ratio was changed as follows: R = 3Q, 4Q and 5Q. For this reason, the results showed a higher percentage of TN removal than in cases 1 and 2, with a TN removal of 57.3%, 59.7% and 56.2% for cases 4, 5 and 6, respectively, as can be seen in Fig. 2.

Contrary to expectations, as regards TN and COD, there was no appreciable improvement in the removal efficiencies corresponding to the increase in the nitrate recycling rate in cases 4, 5 and 6 (Fig. 2). In the cases compared in this part of the study, the same amount of organic matter was available for denitrifying. By increasing the recycling ratio of nitrate, the nitrate load supplied to the anoxic reactor was increased. There were more electron acceptors for the same amount of electron donors. Therefore, increasing R in the system did not provoke an enhancement in the yield of the process, because of the lack of organic matter in the feed. For the wastewater studied, with a low C/N, a higher nitrate

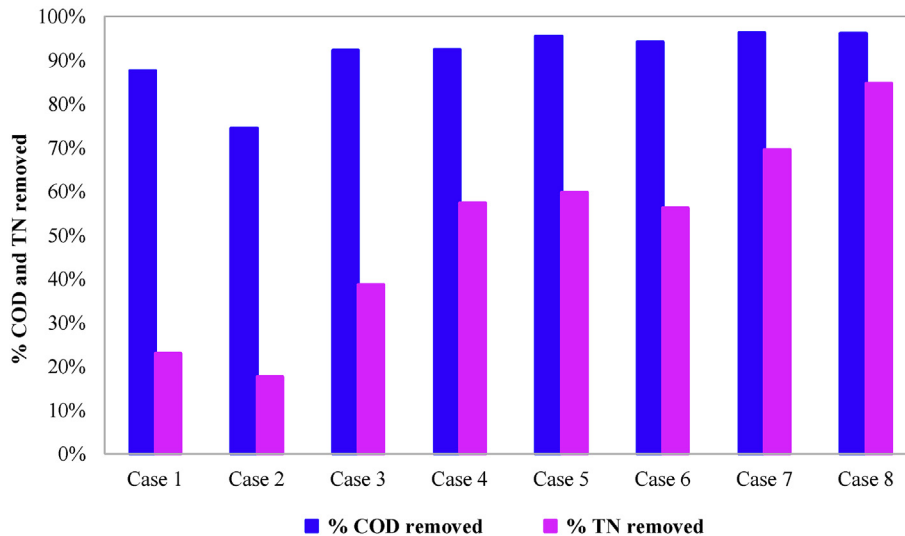


Fig. 2. COD and TN removal percentages after the denitrification/nitrification process.

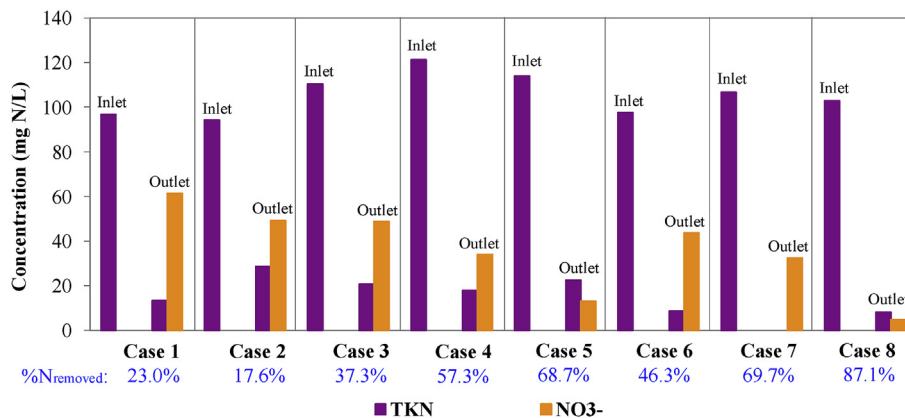


Fig. 3. Comparison of nitrogen compound concentrations in the different cases, before and after denitrification/nitrification treatment. In blue, the efficiency of nitrogen removal in each case. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

recycling ratio was not beneficial for nitrogen removal, and could be economically non-profitable. The enhancement in the TN removal efficiencies in cases 4, 5 and 6 versus cases 1–2, was due to the addition of methanol, which provided organic matter that was to be used by denitrifying bacteria. The results obtained are in agreement with the results reported by Fongsatitkul et al. (2011), showing no improvement on the COD removal with respect to the influence of R. On the other hand, this author observed a modest improvement of 4–5% TKN removal when R doubled from Q to 2Q, but no further increase at a recycling ratio of 4Q. In the case of Chen et al. (2015), at the low COD/N ratio of 3.0, the N removal efficiency decreased when R increased, due to the limited carbon sources in anoxic zones. Only at the high COD/N ratio of 5.5 did the N removal efficiency steadily increase with R.

Fig. 3 depicts the evolution of TKN and NO₃⁻-N concentration in the different situations studied in this work. The left column represents the feed, and the right one represents the effluent after the denitrification/nitrification process. A clear decrease in the TKN effluent can be observed, when compared to the inlet concentration in all the analyzed cases, indicating a good nitrification yield. Case 2, the most unfavorable case in terms of operating conditions, shows the worst yield of nitrification and TN removal. The graphs of the cases 4, 5 and 6, show no considerable differences between

them.

Sometimes incomplete denitrification can produce N₂O, which is an intermediary product in denitrification processes. This can be problematic as N₂O is a potent greenhouse gas and contributes to increasing the earth's temperature and destroying the ozone layer (Liu et al., 2015). Gas samples taken from the bioreactor showed concentrations lower than 9 mg/L of N₂O gas in its headspace, corresponding to less than 10% of the N removed.

3.2. The effect of increasing the COD/N ratio

The removal efficiency of nutrients and organic carbon in the denitrification–nitrification system with different COD/N ratios was also studied. In a WWTP, part of the stream that feeds the anaerobic reactor is directed through a bypass, to the stream that feeds the denitrification reactor. In this way, it is possible to increase the soluble COD available in the liquid stream that feeds the anoxic reactor, without adding an external carbon source. In order to simulate this behavior, the addition of methanol was employed as an extra carbon source. The use of methanol on a commercial scale entails extra costs, and thus the process may not be viable from an economic point of view. Methanol addition was carried out in order to simulate the increment of the denitrification potential by

increasing the concentration of organic matter available in the system.

The C/N ratio of the wastewater after anaerobic treatment was around 1.1 (cases 1 and 2), showing a lack of the carbon source needed to promote the denitrification process. On the one hand, comparing cases 2 and 3, methanol was added in order to enhance the denitrification step by increasing the C/N ratio from 1.1 to 2.6, while the nitrate recycling ratio was maintained at 200% ($R = 2Q$). The corresponding removal efficiencies of TN were doubled from 17.6% to 38.7% as can be seen in Fig. 2. As regards the organic matter, the removal efficiency of COD was enhanced, rising from 74.4% to 92.2%, with a concentration effluent of 27.1 mg O₂/L in case 2, and 22.3 mg O₂/L in case 3 (Table 3). As depicted in Fig. 3, the concentration of NO₃⁻-N in the effluent of the denitrification/nitrification process remained almost the same. Looking at TKN, the effluent concentration in case 3 was around 25% lower than in case 2, despite the fact that in case 3 the feeding concentration was almost 15% higher than in case 2.

On the other hand, looking at cases 7 and 8, the nitrate recycling ratio from the aerated bioreactor was maintained at 600% ($R = 6Q$) and methanol was added to increase the amount of COD in the feed. In case 7, the value of COD was 574.0 mg O₂/L and the C/N ratio was 5.37, as indicated in Table 2. More methanol was added in case 8, where the inlet COD value was 848.2 mg O₂/L, changing the COD/TN ratio from 5.37 to 8.25. In comparison with case 7, due to this raise in the concentration of COD in the feed, the nitrogen removal efficiency showed a substantial improvement, rising from 69.6% to 87.1% (Fig. 2), obtaining effluents with 32.5 mg N/L and 13.3 mg N/L of TN in cases 7 and 8, respectively (Table 4). Both situations achieved a high COD removal of around 96.1%. Fig. 3 shows the high decrease in the NO₃⁻-N concentration column after the denitrification/nitrification process.

In light of the results achieved, the greater the ratio of the influent C/N, the better the values for TN removal. Similar observations were made by Han et al. (2015), Wang et al. (2009) and Kumar et al. (2012). Therefore, based on the results, the denitrification capacity of the system was affected by the availability of COD in the influent; and the addition of COD was a very important step in the nitrogen removal.

Fu et al. found removal efficiencies of 96.2% for COD and 83% for TN, with rather longer HRT values than those achieved in this work: 1.5 days versus 3 h. The process they developed was a modified membrane bioreactor with two parts for the anoxic and aerobic compartments that treated synthetic wastewater with a C/N ratio of 9.3 (Fu et al., 2009).

Azhdarpoor et al. (2016) obtained 92% and 86% of COD and TN removal respectively, with a SBR configuration, however they used synthetic wastewater with a C/N ratio much higher than that tested in this work (C/N = 19 versus C/N = 8.3) and 8 h of HRT (versus 3 h in this study).

Analyzing all the cases studied in this work, the largest increases in the TN removal efficiency took place in cases 7 and 8. More specifically, the removal efficiency of TN increased by 40.7% between cases 1 and 3; a TN removal efficiency increase of 35.1% was observed when comparing cases 3 and 5; and there was an increase of 29.5% in the TN removal efficiency between the cases 5 and 8.

There was no significant difference in the phosphorus concentration between the influent and effluent in any case. The wastewater would require one specific treatment for its elimination.

Thus the denitrification-nitrification system could achieve long-term stability for the removal of nitrogen with the addition of methanol, obtaining an effluent that most likely complies with the legislative requirements for discharge into waters, as regards organic matter and nitrogen (Real Decreto 509/1996, BOE 77, 1996).

The results obtained in this work show a big improvement over

the processes already developed by other authors and described in literature. Similar values of COD and TN removal were achieved, compared with those developed in literature, but using shorter residence time and lower COD concentration, which implies, as a consequence, a process intensification. For a certain volume to treat, the required reactors are smaller than using the conventional denitrification process and also is needed a lower addition of chemicals. That is translated into lower operational and capital costs. Also, this means a good opportunity for the developing economies because the reactors are more compact making it possible to build small plants to supply small areas. Thus, enabling the development of small WWTP to solve problems in small and medium-sized towns that are isolated.

As future study is proposed, in order to evaluate the effect of increasing the carbon ratio on the nitrogen elimination potential, using a mixture of the anaerobic reactor effluent and the raw feed.

4. Conclusions

The process developed here is an interesting alternative for eliminating the nitrogen and organic matter present in the wastewater from an anaerobic reactor, with very low C/N ratios. The proposed system was a denitrification/nitrification integrated process, with a short HRT of 1 h for the anoxic bioreactor and 2 h for the aerobic one.

The success of the system in removing COD and TN from domestic wastewater after anaerobic treatment was achieved mainly due to the addition of methanol, that increased the molar ratio of C/N. On the other hand, nitrate recycling did not contribute to a significant improvement in the process due to the insufficient carbon source, it only improved the homogeneous distribution of microbial communities in the reactors.

The optimal nitrogen and organic matter removal values were 87.1% and 96%, respectively. The optimized process was performed using a nitrate recycling ratio of six times the feeding flow (600%) and the addition of methanol until an inlet C/N ratio of 8.25 and a COD concentration of almost 850 mg O₂/L was obtained. As result of these combined effects, it was possible to obtain an effluent that met the requirements of wastewater discharge, in terms of both organic matter and nitrogen content.

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