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Denitrification of high nitrate concentration wastewater using alternative carbon sources

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ABSTRACT

The use of different organic carbon sources in the denitrification of wastewater containing 2500 mg nitrates/L in a SBR was studied. Three alternative sources of carbon were tested: wastewater from a sweet factory, a residue from a soft drinks factory and a residue from a dairy plant. The first two are sugar-rich, whereas the third presents a high content in lactic acid. Maximum specific denitrification rates of between 42 and 48 mg NO₃-N/g VSS h were obtained. The effluents were nitrate-free and very low COD concentrations were obtained in 4–6 h reaction time, especially with the sugar-rich carbon sources. The values of the denitrific net yield coefficient were higher than when using methanol (0.93–1.75 g VSS_{formed}/g NO_x-N_{reduced}). The lowest value was obtained using the lactic acid-rich residue. The optimum COD/N ratios varied between 4.6 for the lactic acid-rich carbon source and 5.5–6.5 for the sugar-rich carbon sources.

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1. Introduction

One of the surface treatment industries that has taken on increased importance in recent times is that of stainless steel manufacturing owing to the greater consumption of products of this type in the chemical, petrochemical, building and food industries. Wastewaters containing high concentrations of metals, nitrates and fluorides are generated in the stainless steel manufacturing process. These wastewaters are treated at the plant itself, undergoing a precipitation process (generally with Ca(OH)₂) to remove fluorides and metals in the form of sludge, thus obtaining treated wastewater which still contains high nitrate concentrations (between 500 and 1000 NO₃-N mg/L) as well as dissolved calcium as a consequence of the aforementioned treatment [1].

Nitrogen compounds discharged into the environment can cause serious problems such as the eutrophication of rivers and deterioration of water sources, as well as hazards to human health. Furthermore, nitrates can also form nitrosamines and nitrosamides, potentially carcinogenic compounds [2–4].

Biological denitrification is a reliable method for removing nitrogen from wastewater. Denitrification is an anoxic process in which the nitrate is reduced to nitrite and subsequently to nitrogen gas by means of the action of *heterotrophic* denitrifying bacteria in accordance with the following sequence:

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

The presence of an organic carbon source is needed in heterotrophic denitrification. When not enough COD is present in the wastewater being treated for denitrification to occur, for example in wastewaters with a low COD/N ratio, or because of high COD consumption in previous steps such as nitrification, additional COD needs to be added to the system [5–7]. This is the case of rinse waters from the stainless steel pickling process, which contain hardly any organic matter [1].

The characteristics of the added carbon source have been found to have major effects on important parameters of the denitrification process such as the denitrification rate, COD demand, the biomass yield and biomass composition [6,8]. Several factors have been highlighted which have to be considered when choosing a carbon source: costs, sludge production, denitrification rate, kinetics, degree of utilization, handling and storage safety, the content of unfavourable/toxic compounds and the potential for complete denitrification without the need for adaptation of the microflora [5,8].

As regards costs, carbon source and waste management costs together are responsible for more than 50% of the total costs of treated wastewater [9]. It is thus very important to find an economical carbon source.

Methanol is the most commonly employed external carbon source due to being easily assimilated by denitrifying bacteria and its low cost [10-14]. Ethanol and acetic acid constitute other equiv-

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alent commercial sources [5,15,16]. Although the results obtained with these carbon sources are very satisfying, the essential problem when dealing with a high nitrate concentration may be the accumulation of nitrites produced during denitrification [16], thus causing inhibition of bacterial development.

Several authors have considered waste products as possible carbon sources from an economic and environmental perspective, such as industrial wastes or municipal and agricultural effluents [6,17–23]. Among the waste compounds most widely used as alternative sources of carbon are to be found those which contain volatile fatty acids, with specific denitrifying rates of between 0.46 and 20.25 mg NO₃-N/g VSS h being achieved, depending on the study [17,20,21].

Cappai et al. [18] used two industrial wastewaters originating from an ice-cream production factory and a beet-sugar factory, obtaining a mean specific denitrification rate of 3.28 mg NO_3 -N/g VSS h and 2.72 mg NO_3 -N/g VSS h, respectively.

Rodríguez et al. [24] used agro-food wastewater, obtaining a maximum denitrification rate of 4.1 mg NO_3 -N/g VSS h when potato processing wastewater was used.

The aim of this research work was to study the denitrification of high nitrate concentration wastewater using three alternative carbon sources from different industrial processes: Carbon Source 1 (CS1) is the wastewater produced in the cleaning of the reactors used in the production of sweets; Carbon Source 2 (CS2) is a saccharose-rich residue from the production of soft drinks; and Carbon Source 3 (CS3) is a lactic acid-rich residue from a dairy plant. Different COD-N ratios were investigated for each of the carbon source tested and reaction kinetics was determined.

2. Materials and methods

2.1. System configuration and operation

The 3-L volume closed glass reactors used for the laboratory experiments were equipped with mechanical stirrers IKA/WERKE (Eurostar digital model) to improve contact between the microorganisms and the synthetic wastewater. At the end of the denitrification reaction period, the stirrer was turned off and settling of the biomass commenced. When both phases (biomass and supernatant) were completely separate, the supernatant was unloaded by pumping (Watson-Marlow SCIQ 323). The Sequencing Batch Reactor system was operated in the following sequential phases: loading period (40 min), anoxic reaction period (6-22 h), depending on the operational conditions being tested, settling period (30 min), and unloading period (40 min). Sludge from a landfill leachate treatment plant was used as inoculum [1]. The leachate treatment consists of a pressurized nitrification-denitrification process followed by ultrafiltration to separate the sludge (Biomembrat process).

Prior to the commencement of experiments, the sludge underwent a three-week acclimation period (15 operating cycles with an anoxic reaction period of 24 h), introducing 0.75 L of inoculum (sludge) and 2 L of synthetic wastewater into the reactor. During start-up, the reactor was fed with synthetic wastewater diluted 50% with drinking water with the aim of progressively acclimating the biomass to the high nitrate concentration of the wastewater to be treated [25,26]. The COD/NO₃-N ratio initially employed was 5, using sodium acetate as carbon source [16]. Phosphorus was also added as a nutrient in the form of Na₂HPO₄ at a N/P ratio of 10 [6,17,27,28].

After this acclimation period, acetate was replaced for the different carbon sources. During the study carried out with each carbon source, the reactor containing the inoculum (0.5 L) was loaded with 2 L of the same synthetic wastewater. The COD/N/P ratios were varied on the basis of experimental results. Five consecutive cycles

Table 1

Average composition of the industrial carbon sources used in the experiments.

Parameter	Unit	CS1	CS2	CS3
рН		3.3	6.8	4.2
TS	g/L	140	750	285
VS/TS	%	98	100	67
COD	g/L	155	850	370
TOC	g/L	77	-	198
TKN	mg/L	21	< 0.05	254
NH4 ⁺ -N	mg/L	8.0	< 0.05	720
PO4 ³⁻ -P	mg/L	4.2	<0.1	3350

were needed to achieve stable operating conditions for each of the studied ratios and carbon sources. Another five cycles were then maintained, after which measurements were taken to obtain the reaction kinetics.

Biomass was purged periodically from the reactor keeping the concentration as constant as possible (usually between 5.0 and 6.0 mg VSS/L). All the processes were performed at room temperature (20 ± 1 °C) in an anoxic environment. Experiments were carried out at pH values of between 7.5 and 8.

2.2. Characteristics of the wastewater and the alternative carbon sources

Wastewater from the stainless steel industry was characterised over a period of one month, during which two samples were collected each week. Substantial variation in its characteristics was observed, possibly due to variation in the industrial process as well as in the pre-treatment of the wastewater with lime [1]. For the experiments, synthetic wastewater was used (pH: 8.5, fluoride: 5 mg/L, nitrate-N: 700 mg/L, sulphate: 200 mg/L, calcium: 150 mg/L, chloride: 177 mg/L). These concentrations were employed due to their being the most common values in the samples analyzed during the characterisation of the industrial wastewater. No metal ions were added, since the presence of metals is practically inappreciable after pre-treatment with lime [1]. The synthetic wastewater contained oligoelements, as it was prepared using drinking water.

The characteristics of the carbon sources (CS) employed are given in Table 1. CS1 is the wastewater originating from the cleaning of the reactors used in the production of sweets. It contains 14% total solids (in weight) and is very rich in sugars (53.6–57% glucose and 32.1–46.4% saccharose, both % weight on a dry basis) and may contain some lactose and whey in smaller amounts (6.4–8.6% and 3.6–5.0%, respectively, also expressed on a dry basis). It has a COD of 155 g/L.

CS2 is a saccharose-rich residue (98-99% saccharose on dry basis) from a soft drinks factory and presents high organic matter content (850 g COD/L) and a water content of 48%. CS3 is a lactic acid-rich residue from a dairy plant, with water content of 71.5%. It contains 16.1-16.8% lactose and 2.8-4.2% lactic acid, as well as fats and proteins in small amounts (2.5-2.8%) (percentages are expressed on a dry basis). It presents an organic matter content of 370 g COD/L, as well as containing ammonium nitrogen and phosphorus. Despite the high nutrient content of CS3, it should be stressed that the amount of waste added to the reactor in the different cycles (between 7.8 and 8.9 mL, depending on the COD/N ratio employed) meant that the concentrations of nitrogen and phosphorus in the reaction medium were negligible. The ammonium nitrogen levels were kept around 2 mg NH₄⁻⁻N/L in all the trials. As regards phosphorus, the addition of CS3 allowed N/P ratios of between 24 and 27 to be obtained, depending on the COD ratio employed, external addition of phosphorus being needed to achieve the ratio employed in all the trials (N/P = 10).

2.3. Sampling and analysis

Performance of the bioreactors was monitored by measuring the nitrate-N, COD, biomass concentrations, pH and dissolved oxygen in the reactors at the beginning and the end of each operating cycle. All measurements in this research study were performed in triplicate to provide greater reliability of the results. A kinetic study was conducted when the steady state was reached in the reactor, after 10 operating cycles (stable biomass concentration and high nitrate removal percentages). Samples were taken once every hour and immediately filtered and analyzed for N-nitrate, N-nitrite and COD concentrations. In these studies, three profiles were elaborated for each carbon source and experimental COD/N ratio investigated.

Nitrate concentration was monitored spectrophotometrically at 420 nm using the sodium salicylate method [29]. Nitrite detection was determined at 585 nm using the ferrous sulphate method (HACH manual, adapted from McAlpine and Soule [30]). COD (colorimetric method with closed reflux), fluoride (potentiometry), total (TSS) and volatile (VSS) suspended solids (gravimetry) were measured according to Standard Methods [31]. The spectrophotometer. The concentration of fluoride was determined using an ORION 96-09 fluoride-selective electrode. The concentration of dissolved oxygen (DO) and pH were measured using a YSI 55/25 FT oximeter and a CRISON pH 25 pH-meter.

3. Results and discussion

3.1. Start-up period

Sodium acetate was used as the carbon source to acclimate the biomass. Based on data from the bibliography, a COD/N ratio of 4 was used. After two weeks (10 operating cycles with an anoxic reaction period of 24 h), complete denitrification had not been achieved. To avoid possible organic carbon limitations, the COD/N ratio was increased up to 5, thereby obtaining a nitrate-free effluent after one more week of acclimation (five operating cycles). In these experiments, the biomass concentration was kept between 3 and 3.5 g VSS/L with a VSS/TSS-ratio of 70–78%.

After this acclimation period, acetate was replaced by the different carbon sources. The evolution of NO_3^- -N, COD and VSS levels during the acclimation period is shown in Fig. 1. No accumulation of nitrites was observed during this period.

3.2. Denitrification with alternative carbon sources

The kinetic data for the alternative carbon sources were taken after 10 acclimation cycles for each of the studied ratios and carbon sources. The variation in NO_X -N concentration (the nitrogen provided by nitrates and nitrites) with reaction time when using wastewater from a sweet factory (CS1) as carbon source is shown in Fig. 2. This variation does not give a perfect fit to a straight line, as occurs when a simple carbon source (methanol, acetate, for example) is used. According to the results of other researchers [23,32,33], three linear phases of nitrate reduction occur simultaneously during the process of denitrification employing activated sludge from denitrifying systems acting upon complex carbon sources such as industrial effluents. The highest denitrification rate is provided by the most readily biodegradable COD; a slower rate is provided by the more slowly biodegradable COD, which needs to be hydrolysed prior to denitrification; while the lowest denitrification rate is provided by endogenous carbon. The same behaviour can be observed in our case. The maximum denitrification rates are obtained in the first 2h of reaction, with values ranging between 30.4 and 41.6 mg NO_x -N/g VSS h, depending on the COD/N ratio employed (Table 2). The greatest consumption of



Fig. 1. Evolution of NO₃-N and COD in the effluent during acclimation period (operating cycles with an anoxic reaction period of 24 h).

organic matter also takes place in this period, with values ranging between 5.6 and 7.2 mg $COD_{consumed}/mg NO_x-N_{removed}$, depending on the COD/N ratios employed. The organic matter consumed in this period would correspond to the easily biodegraded fraction contained in the wastewater. At lower COD levels, the denitrification process became incomplete, resulting in increased COD and nitrate concentrations in the effluent. In those experiments (COD/N ratios of 5 and 5.5), accumulation of nitrites was observed (with a maximum nitrite concentration in the final effluent of 7 mg NO₂-N/L), whereas this was not the case in trials with higher COD levels.

With respect to residual COD in the effluent, values lower than 90 mg COD/L were obtained in the treated effluent after 6 h of reaction (Fig. 5).

In view of the results obtained, the optimum COD/N ratio for this waste carbon source is 6.5. The maximum denitrification rate is obtained with this ratio (41.6 mg NO_X -N/g VSS h), which, after 6 h of reaction with a biomass concentration of 4.4 g/L, affords a treated effluent that is nitrate-free and which has an organic matter content of 90 mg/L.

The use of a residue from a soft drinks factory (CS2) as carbon source led to a faster reduction of the nitrates present, achieving complete denitrification after 4 h of reaction for some of the COD/N ratios employed. Observing the kinetic



Fig. 2. NO_x-N profiles for different COD/N ratios using a carbon source from a sweet factory (Y error bar < 10%).

behaviour of the process, three linear phases can also be distinguished during the nitrogen-reducing process (Fig. 3). The difference with respect to the previous case is that the maximum denitrification rate is achieved in 1.5 h, with values ranging between 31.8 and 48.1 mg NO_x-N/g VSS h and organic matter consumptions of between 3.6 and 5.2 mg COD_{consumed}/NO_x-N removed, depending on the COD/N ratios employed (Table 2). No accumulation of nitrites was observed during the process.

The values of the COD in the effluent were higher than those obtained when using CS1 (Fig. 5 and Table 2). The optimum COD/N ratio was 5.5, since, although the highest denitrification rate is not achieved with this ratio, it does afford a nitrate-free treated effluent with the lowest content in organic matter (163 mg COD/L) after 4 h of reaction, with a biomass concentration of 5.8 g/L.

When using a residue from a dairy products factory (CS3) as carbon source, a residual nitrogen concentration in the effluent \leq 0.4 mg NO_x-N/L in 6 h for COD/N ratios of 4.6 and 4.7 (Table 2). Three linear phases were also observed in this case in the overall removal process (Fig. 4), achieving maximum denitrification

rates in the first 2 h of reaction of between 36.2 and 44.1 mg NO_x -N/g VSS h, with organic matter consumptions of between 3 and 3.5 mg COD_{consumed}/NO_x-N _{removed} depending on the COD/N ratios employed. When using this carbon source, an accumulation of nitrites was also observed at the lowest COD/N ratio assayed (COD/N of 4.3), although the concentration of nitrites in the final effluent was never higher than 10 mg NO₂-N/L. Therefore, the accumulation of nitrite, which is temporarily transported outside the cell by some types of bacteria during the denitrification process, but is not taken back up to be used as electron acceptor when not enough COD is present [16], will have probably led to the inhibition of the denitrification process.

The organic matter consumption in the process of denitrification was slower than when using the other two carbon sources, obtaining COD values of around 250–450 mg/L in the effluent (Fig. 5 and Table 2).

On the basis of the results obtained with this carbon source, the optimum COD/N ratio is 4.6. The highest denitrification rate is achieved using this ratio, as well as an effluent that is practically free of nitrates after 6 h of treatment.

Table 2

Denitrification rates, COD consumption and composition of the effluent using different carbon sources.

COD/N	Denitrificatio	$n rate(mg NO_x - N)$	√gVSS·h)	COD _{consumed} /NO _x -N _{removed} (mg/mg)		ng/mg)	NO ₃ -N _{eff.} (NO ₂ -N _{eff.})	COD _{eff.}	Reaction
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3	(mg/L)	(mg/L)	time (h)
Organic waste from a sweet factory (CS1)									
5.0	30.4	7.4	2.6	5.6	3.3	5.3	164(5.5)	75	6
5.5	39.8	11.6	4.5	7.3	2.2	0.5	120(7.0)	77	6
6.0	38.2	20.1	4.6	7.8	1.4	1.4	9.5 (<0.05)	64	6
6.5	41.6	15.4	6.3	7.2	2.0	1.2	<0.05 (<0.05)	90	6
Organic waste from a soft drinks factory (CS2)									
4.8	31.8	10.3	3.2	5.2	0.1	-	15.5 (<0.05)	362	5
5.0	48.1	5.2	0	4.1	0.1	-	0.5	286	4
5.5	46.8	12.9	1.2	4.1	5.7	5.5	<0.05 (<0.05)	163	4
6.5	48.0	25.0	0.8	3.6	5.6	12.0	<0.05 (<0.05)	755	4
Organic waste from a dairy products factory (CS3)									
4.3	36.2	5.1	1.0	3.5	1.2	0	32.7 (<0.05)	402	6
4.5	38.7	6.0	0.3	3.0	1.4	11.0	3.8 (9.5)	445	6
4.6	44.1	2.1	0.1	3.2	4.8	32.5	0.4 (<0.05)	257	6
4.7	39.5	5.3	0	3.2	3.9	-	<0.05 (<0.05)	435	4



Fig. 3. NO_x-N profiles for different COD/N ratios using a carbon source from a soft drinks factory (Y error bar < 10%).

3.3. Comparison of the results

The denitrification rates obtained when using the three carbon sources tested were found to be higher than those obtained in previous studies using methanol as a carbon source [1] and those obtained by other researchers using different carbon sources (Table 3). The values varied with the C/N ratio employed. At the optimum C/N ratios (where both N-NO₃ and COD concentrations reached a minimum in the effluent) the denitrification rates were higher than 41 mg NO_x-N/g VSS h for the three carbon sources, ranging between 41.6 and 46.8 mg NO_x-N/g VSS h.

An important factor to be taken into account is the yield coefficient (Y_D) , which represents the influence of nitrates and nitrites on the microbial growth rate. Y_D is defined according to the following expression:

$$Y_D = \frac{\text{mg VSS formed}}{\text{mg NO}_x - \text{N reduced}}$$

The biomass growth varies significantly per carbon source. Alternative carbon sources usually lead to higher yields than methanol [11,34]. Of the alternative carbon sources tested in this study, the use of CS3, which is rich in lactic acid, produced less



Fig. 4. NO_x-N profiles for different COD/N ratios using a carbon source from a dairy products factory (Y error bar < 10%).



Fig. 5. COD profiles for different COD/N ratios using different carbon sources (a) CS1: carbon source from a sweet factory; (b) carbon source from a soft drinks factory; (c) carbon source from a dairy products factory (Y error bar < 10%).

sludge (Y_D = 0.93 kg VSS_{formed}/kg NO_x-N_{reduced}). This value was lower than those obtained with the other two carbon sources under study and lower than that found by Hwang et al. [34] for isopropanol. The yield coefficients for sugar-rich carbon sources were 1.18 for CS1 and 1.75 for and CS2.

The difference in biomass growth can also be observed in the mean sludge retention time (SRT), with values of 5.9, 2.0 and 7.8 d for CS1, CS2 and CS3, respectively. High SRT is desirable in bioprocesses, as this will also allow for the retention of slow growing bacteria and will lead to a better acclimated biomass in the reactor [35–37].

According to theory, based on electron balances, the total consumption of COD per gram of nitrate-nitrogen converted to nitrogen gas is 2.86 g. In practice, however, more COD will be consumed, as COD is not only used for respiration, but also for cell growth and maintenance [15]. Therefore, the consumption rates found for CS3 at the optimum COD/N ratio ($3.2 \text{ gCOD}_{\text{formed}}/\text{gNO}_{x}$ -N_{reduced}) are surprisingly low, whereas the COD consumption with CS1 was much higher compared to the other carbon sources (7.2 gCOD_{formed}/gNO_x-N_{reduced} at the optimum COD/N ratio).

Table 3

Summary of denitrification rates achieved with various organic carbon source.

Carbon source	Maximum specific denitrification rate (mg NO3-N/g VSS h)	рН	T(°C)	Reference
Methanol	5.42 ^a	-	23 ± 3	Bilanovic et al. [17]
Acetate	19.79 ^a			
Effluent from	20.25			
anaerobic digestion	20120			
Paunch liquor	68	72-77	20	Bickers and
raunen nquoi	0.8	7.2-7.7	20	van Oostrom [32]
Rendering stickwater	10.5			
Ice-cream factory wastewater	3.28	-	20	Cappai et al. [18]
Beet-sugar factory wastewater	2.72			
Acetic acid	1.46	6.5	30	Elefsionitis and Li [21]
Propionic acid	1.21			
Mixed VFAs	1 75			
Mined VIIIb	1.75			
Acetate	4.7	7.3	20	Rodriguez et al. [24]
Urban sewage	4.3			
Potato processing	4.1			
Milk bottling	3.8			
Cheese industries	2.8			
Tomato processing	2.7			
Reet_sugar processing	2.5			
Winory	2.5			
whitery	2.0			
Methanol	30.4 ^b	9	20 ± 1	Fernández- Nava et al. [1]
Sweet factory	41.6 ^b	7.5–8	20 ± 1	The present study
Soft drinks factory	46.8 ^b			
Dairy plant	44.1 ^b			
J F				

^a Alternating anoxic conditions.

^b At optimum COD/N ratio.

These low COD consumption values are difficult to explain, more so seeing that COD consumption during the first hour was always higher than for the second hour, whereas nitrate removal was similar throughout the 2 h. This fact may be attributed to intercellular storage [38,39].

Normally, a reverse relationship between COD consumption and the denitrification rate is found [8,15,19,21]. As can be seen (Table 2), this relation was not found in this study. Carbon sources with a high biomass yield showed higher denitrification rates. Activity of non-denitrifying bacteria under anoxic conditions has been reported, the metabolic routes and activity of which depend on the type of carbon source [8,21,27]. Therefore, as regards COD consumption, the activity of the total bacterial community should be taken into account, rather than just that of the denitrifying bacteria. Furthermore, competition between different types of bacteria does not necessarily have to result in lower denitrification rates.

4. Conclusions

Three linear phases of nitrate reduction occur simultaneously during the denitrification of high nitrate concentration wastewater when using residual organic carbon sources. The highest denitrification rates were reached within the first 2 h and varied with the COD/N ratio employed. Maximum values of between 42 and 47 mg NO_x -N/g VSS·h were obtained for the carbon sources tested here. The optimum COD/N ratios were higher for the sugar-rich carbon sources (6.5 and 5.5 for CS1 and CS2, respectively) than for the lactic acid-rich carbon source (CS3: 4.6).

The use of residual carbon sources may constitute an economical alternative for the denitrification of wastewater containing high nitrate concentrations. The use of CS1 and CS2 afforded a nitrate-free effluent with a very low content in organic matter in relatively short reaction time: 90 mg COD/L in 6 h and 163 mg COD/L in 4 h, respectively. The main drawback of employing sugar-rich carbon sources in terms of industrial implementation is the high values of the yield coefficient, which mean that the sludge must be purged from the reactor more often, thus increasing the management costs of this sludge. When using CS3, the residue rich in lactic acid, the yield coefficient was lower and the final effluent was also free of nitrates, but the COD values in the effluent were slightly higher than those obtained using sugar-rich carbon sources (257 mg/L).

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