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## Titration biosensors for the estimation of the biochemical nitrate demand of municipal and industrial wastes

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**Abstract** An anoxic titrimetric test was investigated for measuring denitrification potential of different wastewaters, both municipal and industrial, and to quantify the denitrifying activity in an activated sludge system. The method measures the amount of acid that is required to maintain the pH set-point value in a batch denitrification experiment, and it was performed using a DENICON (denitrification controller) biosensor. The amount of acid is proportional to the nitrate used to oxidise the biodegradable chemical oxygen demand present in the wastewater, while the acid consumption rate is used to derive the denitrifying activity. The wastewaters tested were a municipal wastewater (MW), an industrial-municipal wastewater (MIW; 70% and 30%, respectively), and four industrial wastewaters drawn from an ice-cream factory (IW1), a beet-sugar factory (IW2), a brewery (IW3), and a tuna cannery industry (IW4). Good correlation between titration data and analyses was found.

**Keywords** Denitrification potential · Industrial wastewaters · Titrimetric biosensor

### Introduction

Denitrification is a biological process in which nitrate is reduced, acting as the terminal electron acceptor during the oxidation of organic substrates in the absence of oxygen. Temperature and pH are among the factors affecting this process, but the denitrification rate is also influenced by the concentration and nature of the carbon source, with the highest rates being obtained with the most easily degradable forms [3, 4].

To obtain a high nitrogen removal efficiency in biological nitrogen removal (BNR) systems, not only must the chemical oxygen demand (COD)/N ratio lie within the range of 5–10 g COD/g N [2] but a high denitrification rate must also be guaranteed. The utilisation rate of the internal carbon source in the wastewater used by the denitrifying biomass to reduce nitrate is often quite low. In these cases the controlled addition of external organics can improve process stability and flexibility.

To limit operating costs, the availability and compatibility of organic residues from other processes (industrial wastewater) should be investigated. This may allow denitrification to be enhanced, while at the same time reducing the amount of industrial waste requiring treatment [7]. Monteith et al. [6] tested several industrial wastes as organic carbon sources; they found that some organic wastes, such as formaldehyde and dextrose wastes, were less efficiently degraded than distillery oils or brewery wort. Tsonis [9] investigated the possibility of using an olive oil mill wastewater as a non-nitrogenous external carbon source in the second anoxic stage modified Bardenpho system for nutrient removal; he found that the addition of this waste is acceptable only up to a certain amount due to additional colour problems in the treated effluents. To identify organic wastes for use as possible external carbon sources to enhance the denitrification process, an accurate characterisation study of those wastes needs to be undertaken.

In this experimental study an anoxic titrimetric biosensor was used to characterise industrial wastewaters with respect to their denitrification potential—expressed as biochemical nitrate demand (BND)—as well as the estimation of the denitrifying activity of activated sludges under different conditions. This device can measure the amount of nitrate used to oxidise the biodegradable COD present in the wastewater from the amount of acid added to neutralise alkalinity produced in the denitrification reaction (approximately 1 mol OH<sup>-</sup> for every mole of nitrate removed); nitrate concentration is in this case maintained in excess over requirement. At the same time the denitrifying activity can be derived from the

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acid consumption rate. Details of DENICON's operation (DENI-trification CONTroller, Austep, Milan, Italy) and of data processing can be found in Rozzi et al. [8] and Massone et al. [5]. The BNDs of the wastewaters tested obtained with the titrimetric method were compared with those obtained by analysis of nitrate reduced during the tests.

## Materials and methods

### Wastewaters and sludge: origin and preparation

Titrimetric measurements for assessment of denitrification potential were conducted both on domestic sewage and industrial effluents, including dairy (ice-cream), beet-sugar, brewery, and tuna cannery wastewaters exhibiting a wide spectrum of organic matter content and structure, reflected by COD concentrations varying from 1,250 mg/L for brewery waste to 8,000 mg/L for beet-sugar wastes. The wastewater samples drawn at the different facilities were analysed for pH, COD, total nitrogen (TN), suspended solids (SS) and total phosphate (TP) according to standard methods [1], and stored at 4°C until use. Table 1 outlines the results of the conventional characterisation.

It should be noted that the samples were prepared to represent the expected quality of biological treatment influent. Therefore primary effluents were collected for domestic and dairy samples; chemical settling and lagoon-neutralised effluents were collected for tuna cannery and beet-sugar samples, respectively, because they are routinely subjected to this type of pre-treatment before biological processes. The brewery sample was collected in the equalisation tank at the factory. In our experiments, the samples were also adjusted to a pH of 7–8, a range suitable for biological activity. The experimental tests (batch tests) were carried out utilising denitrifying biomass samples (hereafter referred to as AS-1) drawn at the wastewater treatment plant of Cagliari located in the south of Sardinia, Italy, which is a conventional activated sludge system, and from a laboratory-scale SBR initiated for this research, which was fed with a mixture of domestic and beet-sugar wastewater (AS-2). The samples of sludge drawn at the full-scale plant were analysed for total suspended solids

(TSS) and volatile suspended solids (VSS) and stored at 4°C for a period of not longer than 15 days. Reagent grade chemicals were used throughout.

### Experimental procedure

Tests were carried out using DENICON, an automated titration device developed by Massone et al. [5] that allows production of alkalinity following nitrate reduction to be determined. The anoxic titrimetric method records the cumulative amount of 0.05 M HCl added during a batch denitrification test in a 1 L thermostated, stirred vessel in order to maintain a constant pH ( $pH_{eq}$ ). To determine the  $pH_{eq}$ , samples of 0.5–1 L activated sludge were transferred into the pH-stat reactor, where they were stirred, thermostated (20–25°C), and bubbled with a mixture of N<sub>2</sub> and CO<sub>2</sub> (at a ratio of 100:1). The pH was measured until its value did not change more than 0.02 units over a period of 10–20 min. The  $pH_{eq}$  is set as the working pH of the pH-stat titration unit; KNO<sub>3</sub> is then added to the denitrifying sludge to give an initial concentration of 15–40 mg NO<sub>3</sub>-N/L. Once a stable endogenous respiration rate is obtained, indicated by constant acid addition, the organic waste (industrial wastewater) to be tested, is added to give a range of F/M ratios from 0.03 to 0.07 g COD/g VSS. Acid addition is monitored until the organic substrate has been consumed, and a steady endogenous rate is again observed.

During determination of  $pH_{eq}$  and the denitrification test, sparging the mixed liquor at a constant rate with a mixture of N<sub>2</sub> and CO<sub>2</sub> allows the CO<sub>2</sub> concentration to be kept constant, thus avoiding possible interference caused by additional CO<sub>2</sub> production (e.g. by facultative heterotrophic microorganisms), which could shift the carbonate equilibrium and so modify the system pH.

The characterisation tests with beet-sugar waste were carried out using both sludges AS-1 and AS-2; the latter is a biomass acclimatised to this wastewater so those tests give information on the impact of the acclimatisation on the denitrification rate and denitrification potential; all other tests were conducted only with AS-1.

Samples of mixed liquor were taken during the batch experiments, filtered through 0.45 µm membranes and analysed to determine nitrate-N/nitrite-N concentrations.

**Table 1** Conventional characterisation of selected domestic and industrial wastewaters. COD Chemical oxygen demand, SS suspended solids, TN total nitrogen, TP total phosphate

Waste	pH	COD (mg/L)	SS (mg/L)	TN (mg/L)	TP (mg/L)
Domestic	7.5	160	110	26.3	3.9
Ice-cream	4.4	2,870	508	27.5	5.5
Beet-sugar	1.1	8,000	–	230	6.2
Brewery	8.2	1,250	–	15	–
Tuna cannery	6.5	1,400	150	170	–

### Calculation of BND of the wastes and denitrification activity of the biomass

An example of the titration curve obtained with the dairy wastewater is shown in Fig. 1, where significant parameters for data evaluation are also represented.

The shape of the curve reflects the complex composition of the wastewaters. In fact, when a pure compound (e.g. acetate) is added as a carbon source in an anoxic titrimetric test, the response is acid dosage at a constant rate until all the substrate is used by the

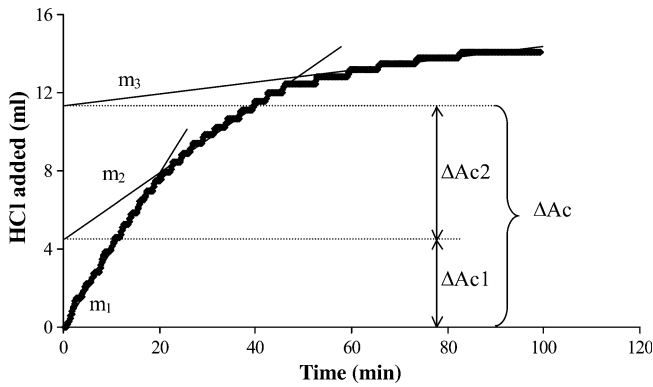


Fig. 1 Example of wastewater titration curve

biomass to reduce nitrate, whereas when a real wastewater is added to the pH-controlled reactor, the rate at which the organic carbon of the waste is utilised by the denitrifying biomass, and thus the alkalinity production and neutralisation rate, depends strongly on the amount and the kind of COD (readily or slowly biodegradable) available. It is then possible to determine the different denitrification rates on the different COD fractions.

The maximum denitrification rate (in  $\text{mgN gVSS}^{-1} \text{h}^{-1}$ ) was determined from the first slope of the titration curve ( $m_1$  in Fig. 1) after the addition of the wastewater sample using following equation:

$$k_{D\max} = \frac{m_1 \cdot MW_N \cdot C_{Ac} \cdot 60}{X_V \cdot V} \quad (1)$$

where  $MW_N$  is the molecular weight of nitrogen,  $C_{Ac}$  the acid concentration in mol,  $X_V$  the mixed liquor suspended solids (MLSS) concentration in g/L, and  $V$  is the total volume of the batch. Using Eq. 1 and substituting  $m_1$  with the difference between  $m_1$  and  $m_3$ , or the difference between  $m_2$  and  $m_3$  (also in Fig. 1), it is possible to calculate the denitrification rates on the readily and slowly biodegradable COD fractions, respectively.

The denitrification potential of the wastewater expressed as the amount of nitrates, in  $\text{mg NO}_3\text{-N/L}$ , that can be denitrified on the COD of the wastewater was determined from the titration curve using the expression:

$$\text{BND} = \frac{\Delta \text{Ac} \cdot MW_N \cdot C_{Ac}}{V_{\text{WW}}} \quad (2)$$

where  $\Delta \text{Ac}$  is defined in Fig. 1 and  $V_{\text{WW}}$  is the volume in litres of wastewater added to the reactor vessel. Even in this case it is possible to distinguish the contribution to the BND of the different fractions.

## Results and discussion

The results of the anoxic tests for the assessment of the denitrification potential in selected industrial effluents and in domestic sewage are outlined in Table 2.

The results obtained with the domestic sewage and the ice-cream wastewaters showed very little variation:

Table 2 Results of anoxic tests. BND Biochemical nitrate demand

Waste	$k_{D\max}$ ( $\text{mgN gVSS}^{-1} \text{h}^{-1}$ )		BND ( $\text{mgN/L}$ wastewater)	
	Mean	95% confidence interval	Mean	95% confidence interval
Domestic	4.05	$\pm 0.98$	9.05	$\pm 1.52$
Ice-cream	6.02	$\pm 0.75$	272	$\pm 30.2$
Beet-sugar	5.84	$\pm 2.3$	1,020	$\pm 430$
Brewery	7.23	$\pm 2.0$	117	$\pm 18.1$
Tuna cannery	3.53	$\pm 0.8$	61	$\pm 40$

the denitrification potential of the sewage is very low, probably due to the amount of readily biodegradable compounds in the wastewater available for denitrification; a previous study using a respirometric technique found the readily biodegradable fraction of this sewage to be 13% of total COD. In contrast, the ice-cream waste has quite a high denitrification potential and could be considered as an alternative carbon source in denitrification. In this case a problem that could arise is the variation in quality and quantity due to the production cycle, which determines peak of flow rate and organic loading during defined periods of the year. Thus, before selecting this kind of wastewater, a study of the annual variation should be carried out, or pre-treatment of the waste to equalise loading should be considered.

The wide range obtained for beet-sugar waste is due to the different results obtained with non-acclimatised and acclimatised sludges. In the latter case, denitrification rates were considerably higher, and a second slope that still had a high rate (i.e.  $2.38 \text{ mgN gVSS}^{-1} \text{h}^{-1}$ ) was observed. Furthermore, it was noticed that accumulation of nitrite took place when using non-acclimatised sludge, leading to lower production of alkalinity and thus slower acid addition. In fact, during denitrification it is the second step, i.e. the reduction of nitrite to nitrogen gas that influences the pH in the reactor. The different denitrification rates observed can thus be

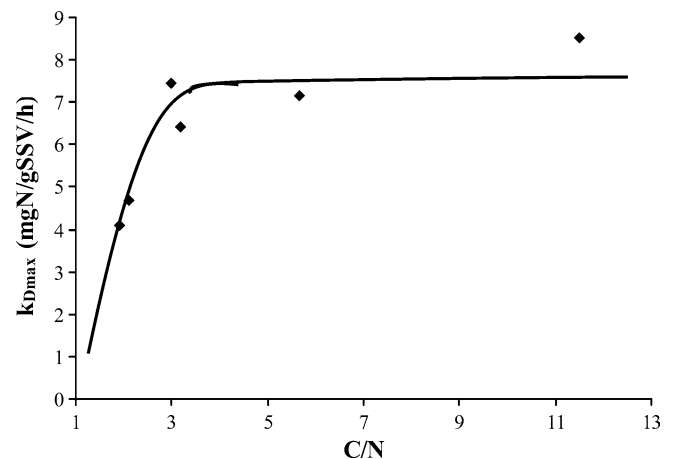


Fig. 2 Denitrification rates of brewery waste at different C/N ratio

explained by the presence in the acclimatised biomass of microorganisms and enzymes able to reduce nitrites at higher rate and to more easily utilise the different organic substances in the wastewater.

The range obtained for the maximum denitrification rate with the brewery waste could be explained by considering the operating conditions of the tests, particularly when different COD/N ratios were applied. Figure 2 shows the relationship between the COD/N ratio applied and the maximum denitrification rates observed in the tests for this waste.

The tests with the tuna cannery waste were performed over a period of 1 week. It was observed that even if the maximum denitrification rate did not change significantly, the denitrification potential of the waste decreased from the 120 mg N/L wastewater obtained the 1st day to 20 mgN/L in the test performed after 1 week. This might mean that wastewater storage influenced its composition; in this case it would be necessary to perform the test as soon as possible after sampling to obtain reliable results.

The nitrates reduced during the tests were deducted from the total amount of acid added by the titration unit, and correlated to those measured with analytical methods. Figure 3 outlines the correlation found.

The graph in Fig. 3 shows a very good correlation, indicating that the titrimetric method is a useful tool with which to determine nitrate concentration in wastewaters or in biological reactors (in this case with an excess of carbon), which can be integrated, in its on-line version, into an automatic control system, especially in alternated or intermittent aeration processes, in order to verify the end of nitrification and denitrification phases and so switch aeration off or on.

Similar correlations were found for the single wastewaters, both for the nitrate reduced and the denitrification potential. Table 3 shows the correlation

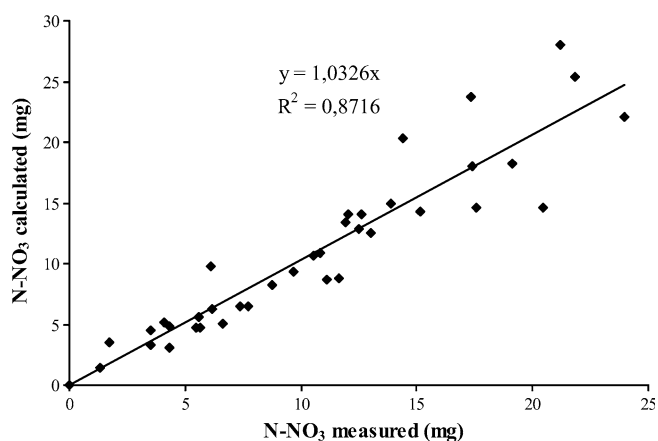


Fig. 3 Correlation between measured nitrate and that calculated from the acid added during the tests

Table 3 Correlation between analytical results and biosensor data

Waste	$BND_{analysis} = a \times BND_{biosensor}$	
	a	$R^2$
Domestic	1.018	0.909
Ice-cream	0.9442	0.887
Beet-sugar	1.0916	0.914
Brewery	1.0873	0.973
Tuna cannery	0.9653	0.943

obtained for all the different wastes with respect to BND.

The data show that values of denitrification potential were determined with a relatively error smaller ( $< 10\%$ ).

## Conclusions

The main advantage of using the biosensor for denitrification monitoring during anoxic tests consists in the possibility of continuous and instantaneous observation of the process. Therefore, it allows the different slopes of the titration curve to be identified and thus better kinetic evaluation; moreover a cost saving derives from reagents required for the analyses.

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