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FIXED BIOFILM PROCEDURE FOR EVALUATION OF READILY BIODEGRADABLE SUBSTRATE IN WASTEWATER

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ABSTRACT

In the design and modelling of biological denitrification processes for wastewater treatment, the characterisation of the organic matter is one of the key factors. This work proposes the adaptation of the NUR (nitrogen uptake rate) method to fixed biofilm reactors. The objective is to establish a practical method, based on anoxic respiration, to determine the rapidly biodegradable organic matter available for denitrification in fixed biofilm processes through the measurement of an easily measurable parameter (nitrate). Two procedures are proposed: a batch procedure and a continuous flow procedure. The latter can be run automatically and is more adapted to *in situ* measurement of the production of rapidly biodegradable carbon source. The results obtained demonstrate the viability of the methods and underline the significance of the yield coefficient to the precision of the results.

Key words: Biodegradability, biofilters, characterisation of wastewater, denitrification, organic matter.

INTRODUCTION

The performance of biological denitrification and phosphorus removal processes used for municipal wastewater treatment depends directly on the quality and quantity of the organic matter of the wastewater [1, 2]. The characterisation of the organic matter of the wastewater according to its biodegradability has become a very important stage of biological wastewater treatment design and modelling. The differentiated use of organic matter components available in wastewater by micro-organisms involved in biological wastewater treatment processes, has lead to the division of the wastewater into different compartments, according to their capacity to be biodegraded during treatment. Initially, a very simple division was established which proposed two compartments: readily biodegradable (Ss) and slowly biodegradable (Xs) substrates. Further divisions were proposed later on [3-5] with more detailed subdivision of the organic matter. However, the general trend is to adopt a simple division, due to the difficulties encountered in developing analytical methods to accurately determine all fractions proposed [6]. The more widely used division separates the organic matter of the wastewater into five fractions: readily biodegradable (Ss), slowly biodegradable (Xs), inert soluble (Si), inert particulate (Xi) and active biomass (Xh). This study specifically deals with the readily biodegradable organic matter (Ss) present in the wastewater and available for biological denitrification by fixed biomass processes.

EXISTING ANALYTICAL TECHNIQUES

Analytical techniques to enable the qualitative and quantitative characterisation of the different fractions of the wastewater constitute an area of research still under development. These techniques can be grouped into physical separation tests and biological tests. Physical separation tests use membrane filtration techniques and are based on the hypothesis that the biodegradability of the organic matter is closely linked to the size of the molecule. Small molecules, which are easily transported across the cell membrane, are more readily biodegradable. Large or more complex molecules, which require previous extracellular hydrolysis before assimilation, are considered slowly biodegradable. Dold *et al.* [7] report successful use of ultrafiltration techniques to obtain accurate estimates of influent Ss. The same authors show also that 0.45 mm membrane filtration yield approximate estimates of Ss, with a proportionality factor of 0.8 in relation to the ultrafiltration method. Mamais *et al.* [8] obtained accurate estimates using membrane filtration at 0.45 mm by including a flocculation step before the membrane filtration to remove the colloidal matter which would otherwise pass through the membrane. In practice, the determination of the membrane

pore size which will give representative separation of slowly and readily biodegradable fractions has varied according to the wastewater under study. Torrijos *et al.* [9] obtained good results using 0.1 mm membranes. Furthermore, since both biodegradable and inert organic matter pass through the filter, complementary tests are needed after the filtration step to quantify these two fractions separately.

Biological tests are based on the respirometric response of the biomass when in contact with the wastewater under aerobic, anoxic or anaerobic conditions. Since the division of the organic matter stems from biological response, biological tests have been widely used. Continuous flow systems and batch systems have both been proposed. The continuous flow systems [3, 10] have often been criticised for being expensive and time consuming. The batch systems, although simpler to operate, depend on the availability of biomass adapted to the wastewater being tested. The biomass can be obtained from laboratory scale pilot plants [11-12] or from full scale treatment plants [9-13] when the plants are already in operation. For the anoxic tests [11] and anaerobic tests [14], denitrifying biomass and phosphate removing biomass must be available respectively. The existing biological methods used for the characterisation of municipal wastewater are all based on suspended biomass systems (Tables 1).

Henze [5] suggests the direct measurement of specific compounds such as volatile fatty acids, ethanol and glucose but states that, in the case of domestic wastewater, these measurements account for only 50-70% of the readily biodegradable COD.

FIXED BIOFILM SYSTEMS

Developments in wastewater treatment processes have led to an increasing use of fixed biofilm systems. Since transport phenomena in biofilm systems differ substantially from those in suspended biomass systems [16, 17], specific analytical methods are needed in order to quantify the fractions of organic matter present in the wastewater and available to micro-organisms in a biofilm system.

Table 1. Methods for the measurement of the rapidly biodegradable fraction. Adapted from Henze, [5]. (OUR = Oxygen Uptake Rate, NUR = Nitrogen Uptake Rate, BPR = Biological Phosphorus Release).

Method	Reference
Continuous Dynamic OUR	[10]
Continuous OUR	[3]
Batch NUR	[11]
Batch OUR, NUR	[12]
Specific compounds and molecular weight	[5]
Batch OUR	[15]
Batch OUR	[9]
Batch BPR	[14]
Physico-chemical	[8]

Denitrifying organisms use primarily the readily biodegradable organic matter (Ss) of the wastewater. The kinetics of denitrification are, therefore, influenced by the type of substrate available to the biomass. In the presence of Ss, and considering a fully penetrated biofilm, a zero order intrinsic reaction rate throughout the biofilm will result in an overall zero order surface reaction rate in relation to the concentration of Ss in the bulk liquid [17]. In what concerns the fraction of slowly biodegradable organic matter (Xs), the kinetics of denitrification will be limited by the kinetics of extra cellular degradation (hydrolysis) of Xs into Ss. Two cases are put forward:

1st case - the concentration of Ss in the bulk liquid will be low (limited by the hydrolysis rate), and bulk liquid concentration is such that it results in low diffusion rate and low Ss concentration in the biofilm (Ss << Ks). This leads to first order intrinsic removal rate and first order overall surface removal rate [17].

2nd case - the concentration of Ss in the bulk liquid will be low (limited by the hydrolysis rate), but stays at a level around 3-4 times the value of the half saturation constant, Ks. This leads to a partially penetrated biofilm. The part of the biofilm penetrated by the substrate presents a normal activity and the intrinsic removal rate is zero order. This leads to a half order overall surface removal rate [17].

WASTEWATER CHARACTERISATION

In the microbial respiratory process, the electrons available in the organic matter are transferred to a final electron acceptor (oxygen, for the aerobic processes and nitrate, for the anoxic processes) for the production of energy for bacterial activity and growth. The respirometric tests correlate the amount of electron acceptor consumed (or the kinetics of this consumption) to the concentration of readily biodegradable substrate present in the wastewater. A denitrification (anoxic) batch method using suspended culture was proposed by Ekama *et al.* [11] which determines the concentration of Ss by the nitrogen uptake rate (NUR) observed. The plot of nitrate concentration versus time, for an effluent containing both rapidly and slowly biodegradable organic matter, presents three zones, where the denitrification rates are distinctively different (Figure 1). The first part of the curve is due to denitrification with use of the Ss. During this phase Ss concentration is high and the biofilm is fully penetrated. Under such conditions there is a zero order overall reaction rate in relation to substrate concentration, and nitrate concentration presents a linear rapid decrease during this phase. During the second phase, Xs has to undergo extracellular hydrolysis to Ss before it can be used as carbon source in the denitrification process, the denitrification rate is lower than in the previous phase, limited by the rate of hydrolysis of Xs to Ss.

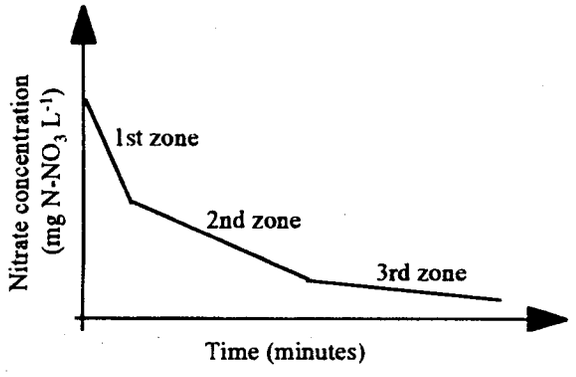

Figure 1. Schematic plot of nitrate concentration versus time in a denitrification batch procedure.

The third phase is due to denitrification using endogenous carbon source (i.e. from decay of biomass). The method establishes that the amount of nitrate (electron acceptor) reduced during the first part of the curve is correlated to the Ss available in the wastewater. The validity of the method was tested for a activated sludge system using acetate and starch as carbon source [13].

The two methods proposed in this work for the characterisation of the readily biodegradable organic matter (Ss) in wastewater, are applicable to fixed biomass processes and specific to denitrifying systems. The procedures are based on the NUR method described above. They consist in evaluating the amount of Ss present in the wastewater by following the kinetics of a denitrifying, fixed biomass system. The first procedure presented uses a batch system and the second procedure, a continuous flow system.

MATERIAL AND METHODS

Biomass development

The biomass used for the experiments presented here was obtained from the anoxic compartment of a laboratory scale nitrification-denitrification high rate submerged biofilter treating domestic wastewater and working under stable operating conditions. The biomass is sampled after filter backwash in order to avoid the presence of excess suspended solids and organic matter which adsorb onto the biofilm during filter cycle.

Biomass concentration and activity

The density and thickness of the biofilm vary according to the type of reactor used, the type of substrate available and the hydraulic conditions at the surface of the biofilm. Jansen [18] uses the thickness of the biofilm as a measure of the amount of biomass. However, this approach implies a biofilm of homogeneous density and thickness. Significant variations are observed for the correlation between biofilm activity, thickness and density when the characteristics of the biofilm are not homogeneous [1]. As this is the case for the biofilms used in this study, we have chosen to use suspended solids (SS) measurement as indicative of the amount of biomass in the system. The measurement of the volatile suspended solids (VSS) is not possible due to the nature of the support media. The standard analytical method used for VSS measurement requires the biomass to be dried at a temperature of 550° C, however the polystyrene beads used as support media do not withstand this temperature. The suspended solids measurement is carried out using an adapted analytical procedure due to the non-existence of an adequate standard procedure. The biomass attached to the polystyrene beads is dried to constant weight, the beads are then washed to eliminate all attached biomass and again dried to constant temperature. The difference between the two weights is considered to be the suspended solids content. In order to compare results of this study to the results presented by other workers and stated in units of volatile suspended solids, we have considered the conversion factor VSS/SS = 0.75, as suggested by Ekama *et al.* [11]. In order to verify initial biomass activity a control test is carried out with a synthetic wastewater having sodium acetate as carbon source. The observed specific denitrification rate is compared to previously observed values of other control tests under the same test conditions.

Batch method

A batch procedure was initially chosen as it enables each parameter to be followed in time and any transient response of the system can be detected and associated to the biochemical mechanisms involved. The reactor used (Figure 2) consists of two parts: a completely closed reactor (A), where the support containing the fixed biomass is placed and an open reactor (B) of the same volume (0.145 l), from which the samples are taken.

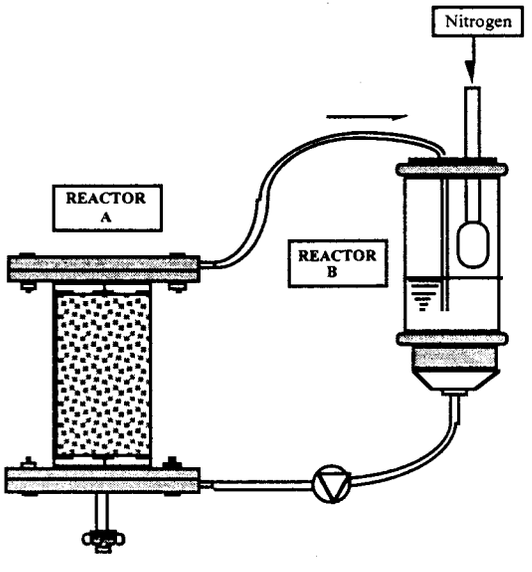

Figure 2. Fixed biomass batch system.

Reactor A is completely filled with the support media so that the filter bed remains immobile. As the biomass is fixed to the support media, no biomass is observed in the liquid phase of the system and therefore, no biomass is present in reactor B. The reactors are Plexiglas cylinders with an internal diameter of 34 mm and a height of 170 mm. A recirculation hydraulic velocity of 34.6 m h^{-1} is used in order to simulate a completely mixed reactor. The characteristics of the support media are given in Table 2.

Continuous flow method

Biofilm process rates are dependent on the diffusion of substances in and out of the biofilm, and this diffusion depends on bulk liquid concentrations as well as on the mixing conditions at the interface of biofilm and bulk liquid. The continuous procedure was set up to avoid possible effects of diffusion limitations which may occur due to the large variations of bulk liquid concentrations which take place during the batch procedure. In addition to this, the constant inlet and outlet concentrations obtained with this procedure lead to more analytical precision in the COD and nitrate measurements. The experimental set up is similar to that of the batch procedure, but, in this case, reactor B is not used (Figure 3).

Table 2. Characteristics of the support media.

Description	Spherical beads
Media material	Polystyrene
Effective diameter	2 - 5 mm
Density	50 kg m^{-3}
Specific surface	1200 m^2 m^{-3}
Porosity	33 %

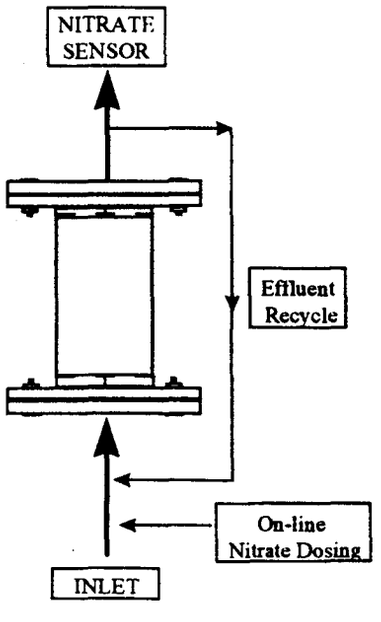

Figure 3. Schematic layout of the continuous feed method.

Reactor A is continuously fed at a hydraulic surface load of 0.7 m h^{-1} and a recirculation hydraulic velocity of 11.0 m h^{-1} is adopted to obtain a completely mixed reactor. Nitrate is added to the feed stream, upstream of the reactor. The outlet flow goes through a filter and is fed to an on-line nitrate sensor. For comparison purposes, at least two of these systems are run in parallel for each experiment.

Residence time in the continuous flow system

In fixed biomass systems, the biomass residence time and the hydraulic residence times are dissociated. The biomass is fixed to the support media and the excess biomass is removed from the system with the retained solids during the filter backwash procedure. The contact time between biomass and wastewater is therefore controlled by the hydraulic residence time. Toettrup [19] showed that hydraulic film diffusion can be neglected for hydraulic velocities above 0.5 m h^{-1} and only biofilm diffusion considered. In the continuous experiments described in this work, the inlet hydraulic surface load adopted was 0.7 m h^{-1}, giving an empty bed retention time of 14.6 minutes. A recirculation hydraulic velocity of 11.0 m h^{-1} was used to obtain a completely mixed system. When the continuous flow method is used to simulate denitrification processes, the retention time may have to be made compatible with the process it is simulating. As a qualitative tool to compare the biodegradability of different wastewaters, a low retention time which enables denitrification to take place is adopted.

Analytical procedures

The analytical methods proposed by the nineteenth edition of the Standard Methods were used to determine the following parameters: COD, nitrate, nitrite, ammonium, phosphates, pH, turbidity. As stated previously, the techniques used for biomass measurement were adapted to the filter support media characteristics.

DATA INTERPRETATION

The concentration of Ss is correlated to the amount of nitrate used as shown by the relationship (i).

$$Ss = \frac{2,86.\Delta N\text{-}NO_3 - 1,71 . \Delta N\text{-}NO_2}{(1 - Yh)} \qquad (i)$$

Ss	=	Rapidly biodegradable substrate (mg O_2 L^{-1})
$\Delta N\text{-}NO_3$	=	Nitrate used during the first phase (mg N- NO_3 l^{-1})
$\Delta N\text{-}NO_2$	=	Nitrite produced during the first phase (mg N- NO_2 l^{-1})
2.86	=	Stoichiometric correlation factor. 1mg N-
NO_3	=	2.86 mg O_2 as electron acceptor to reduce nitrate to nitrogen gas.

1.71	=	Stoichiometric correlation factor. 1mg N-NO_2 = 1.71 mg O_2 as electron acceptor to reduce nitrite to nitrogen gas.
Yh	=	Yield coefficient (0.67 mg DCO/mg DCO)

This expression is based on the bacterial respiration principle by which the electrons obtained from the organic matter used during bacterial activity are either incorporated in new cellular material (synthesis), or transferred to an electron acceptor (energy). In the case of denitrification, the electron acceptor is nitrate. The ratio of the organic matter used for synthesis over the total amount used is defined as the yield coefficient, Yh. As the mass balance is carried out in terms of COD, the amount of electron acceptor used has to be expressed in oxygen equivalents (from stoichiometric correlation we know that 1 mg N-NO_3 is equivalent to 2.86 mg O_2 as electron acceptor). However, although there is no nitrite present in the reactor at time t = 0, if denitrification is not complete, nitrite may accumulate in the reactor. This has to be taken into account in the determination of Ss, as the amount of electrons transferred will not be the same (from stoichiometric correlation we know that 1 mg N-NO_2 is equivalent to 1.71 mg O_2 as electron acceptor).

For the batch experiments, the ΔN-NO_3 necessary for Ss determination is obtained graphically from the plot of nitrate concentration versus time as shown in Figure 4.

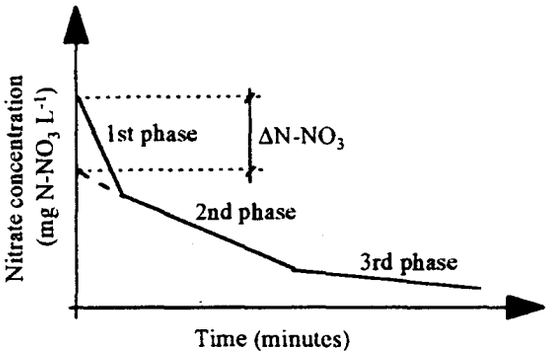

Figure 4. Determination of ΔN- NO_3 from the plot of nitrate concentration versus time for the batch procedure.

The depletion of Ss causes a change in the denitrification rate from a faster rate observed during the first phase to a slower rate, limited by the hydrolysis rate, observed during the second phase. For the continuous procedure, the amount of nitrate reduced is obtained from a mass balance of the reactor, using the inlet and outlet nitrate concentrations and flow rates.

It is considered that during the first phase, denitrification is obtained due to the use of both slowly and rapidly biodegradable carbon sources. Considering that the denitrification using slowly biodegradable carbon source occurs in the first phase at the same rate as in the second phase, then the projection of the denitrification curve of the second phase back to the y-axis enables the determination of the amount of nitrate reduced in the first phase using only the rapidly biodegradable carbon source. The nitrate reduced in the first phase through the use of hydrolysed slowly biodegradable carbon source is in this manner eliminated from the calculations of Ss.

RESULTS FOR THE BATCH EXPERIMENTS

Biomass concentration

The biomass experiments were carried out to verify bacteria activity and density as a function of the biomass concentration and to validate the use of suspended solids measurement as indicator of biomass concentration. The experiments were carried out using different initial biomass concentrations in the range of concentrations observed in submerged biofilters using this type of support medium. The relationship between these concentrations and the denitrification rate obtained was observed. Sodium acetate was used as external carbon source. The initial biomass, COD and nitrate concentrations were as indicated in Table 3.

The linear relationship observed (Figure 5) between the denitrification rate and biomass concentration denotes homogeneous bacteria density and activity in the biofilm and validates the use of suspended solids measurement as indicator of biomass concentration for the concentration range tested.

Table 3. Test conditions for experiments on biomass concentration.

Test	Biomass concentration (g SS m^{-2})	Initial COD concentration (mg O_2 l^{-1})	Initial N-NO_3 concentration (mg N-NO_3 l^{-1})
B1	7.8	799	71.4
B2	8.5	722	64.1
B3	12.5	616	56.9
B4	12.8	844	77.9
B5	17.3	786	68.9
B6	17.7	543	62.5

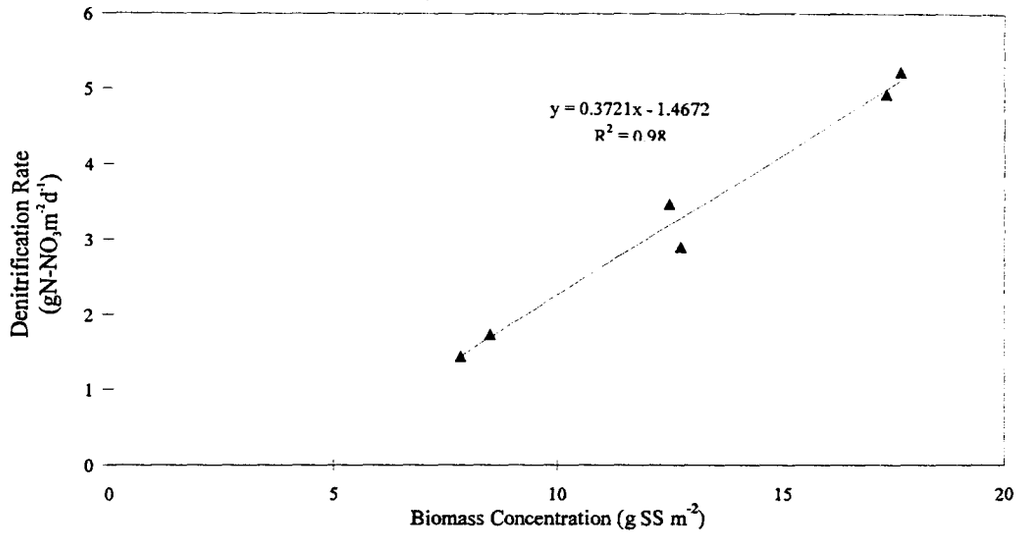

Figure 5. Denitrification rate as a function of the biomass concentration.

The specific denitrification rate (per unity of biomass), given by the slope of the plot on Figure 5, is of 0.37 mg $N\text{-}NO_3$ mg^{-1} SS d^{-1}. This value is slightly lower than the values of 0.577 mg $N\text{-}NO_3$ mg^{-1} SS d^{-1} (which is equivalent to 0.46 mg $N\text{-}NO_3$ mg^{-1} SS d^{-1}, if we consider VSS/SS = 0.75 as suggested by Ekama *et al.* [11] reported by Æesøy and Ødegaard [1] for a laboratory scale, completely mixed rotating disk reactor. However, these specific denitrification rates are significantly superior to the average values found for industrial scale pilot plants operated as secondary or tertiary denitrification units. In these cases, the specific eliminated loads vary from 0.05 to 0.2 mg $N\text{-}NO_3$ mg^{-1} SS d^{-1}, according to the level of treatment and to the operating conditions. These deviations may be due to two factors: the more controlled and constant conditions in which the biomass is grown and to the optimised laboratory test conditions (constant temperature, absence of dissolved oxygen, nitrate in excess).

Influence of the type of carbon source

Three different carbon sources were used to simulate the different urban wastewater compartments: acetate, glucose and starch. Biomass of identical characteristics (Table 4) were used for all tests, in order to avoid the influence of varying biofilm thickness and density on the denitrification kinetics.

Specific denitrification rates of the order of 0.02 to 0.04 mg $N\text{-}NO_3$ mg^{-1} SS d^{-1} were observed for the tests using a slowly biodegradable carbon source (starch) or with no carbon source (endogenous respiration). This is in accordance with data presented by Ekama *et al.* [11] for a denitrifying activated sludge system during endogenous respiration, that is, 0.07 mg $N\text{-}NO_3$ mg^{-1} SS d^{-1} (0.05 mg $N\text{-}NO_3$ mg^{-1} SS d^{-1}, considering VSS/SS = 0.75). The sensitivity of the method, illustrated by these results (Figure 6), was considered sufficient for the purpose of characterising domestic wastewater.

Table 4. Test conditions for experiments using different substrates.

Test	Carbon source	Biomass concentration (g SS m^{-2})	Initial COD concentration (mg O_2 l^{-1})	Initial $N\text{-}NO_3$ concentration (mg $N\text{-}NO_3$ l^{-1})
B7	Sodium Acetate	17.7	543	63.0
B8	Glucose	17.7	632	63.5
B9	Starch	18.7	410	68.2
B10	Endogenous	18.7	42	62.5

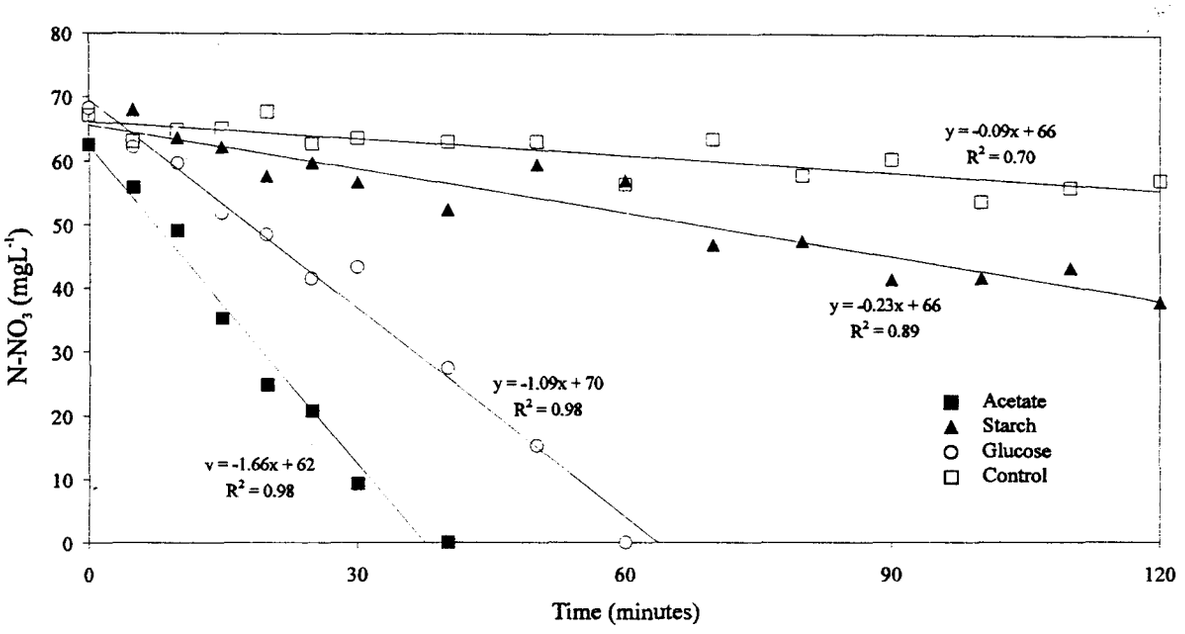

Figure 6. Influence of the type of carbon source on the denitrification rate.

Table 5. Specific denitrification rates for carbon sources of different biodegradability.

Carbon source	Denitrification rate (g $N\text{-}NO_3$ m^{-2} d^{-1})	Specific denitrification rate (g $N\text{-}NO_3$ g^{-1} SS d^{-1})
Sodium acetate	4.8	0.27
Glucose	3.2	0.18
Starch	0.8	0.04
Endogenous	0.3	0.02

Table 6. Test conditions for experiments on carbon source concentration.

Initial COD/N Ratio	Biomass Concentration (g SS m^{-2})	Initial COD concentration (mg O_2 l^{-1})	Initial $N\text{-}NO_3$ concentration (mg $N\text{-}NO_3$ l^{-1})	Temperature (°C)
3.8	16.7	270	71.0	20.0
6.8	16.7	375	55.0	20.0
8.7	16.4	540	62.0	20.0
11.4	16.4	741	65.0	20.0

Influence of the initial carbon Source concentration on the kinetics of the test.

The method was carried out using four different concentrations of rapidly biodegradable carbon source (Table 6) covering the range of COD concentrations normally found for domestic wastewater receiving no or very little influence of industrial effluents.

The denitrification rates obtained for the four tests were the same (5.3 - 5.7 g $N\text{-}NO_3$ m^{-2} d^{-1} or 0.32 - 0.34 g $N\text{-}NO_3$ g^{-1} SS d^{-1}) during the first phase while the rapidly degradable carbon source was still available in the reactor (Figure 7). Those similar rates show that there was no diffusion limitation in the concentration range tested.

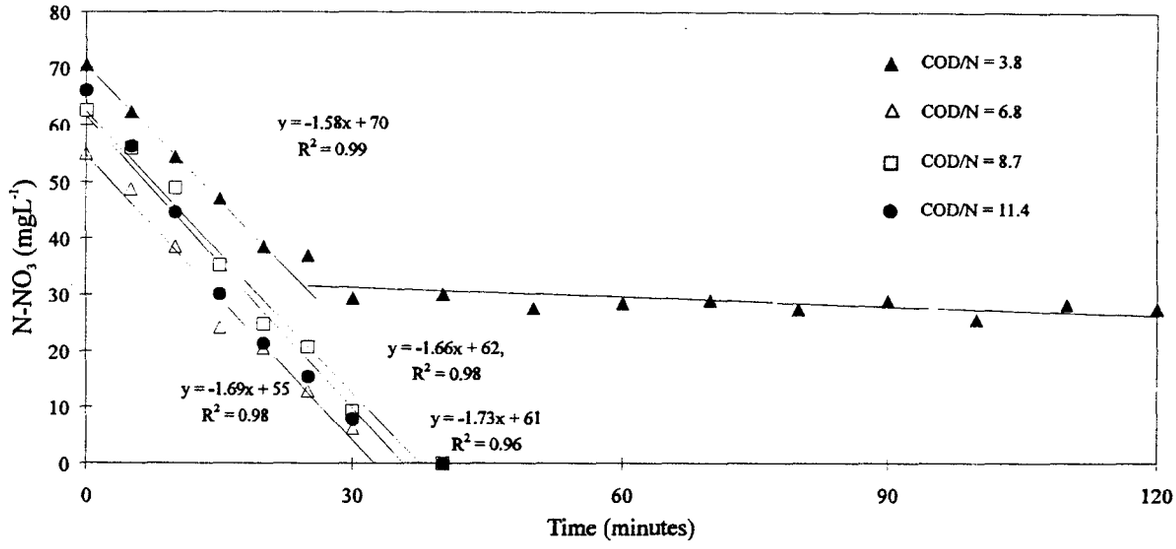

Figure 7. Influence of the initial carbon source concentration.

For the test with a limited concentration of carbon source, the denitrification rate falls, once the carbon source has been totally consumed, to a rate equivalent to the values observed during endogenous respiration. The tests presenting high initial COD/N ratios are nitrate limited and the change in kinetics necessary for the calculations of Ss is not observed. A COD/N ratio of four was established, which enables the necessary changes in kinetics to take place during the test. For the tests carried out with this ratio, the concentration of organic matter falls up till the break point observed on the denitrification versus time curve and then remains stable until the end of the experiment.

Nitrite accumulation

During certain experiments (Table 7), nitrite was observed to accumulate in the reactor. If this accumulation is not taken into account in the determination of Ss, the calculated Ss value will be over estimated. The error incurred by this accumulation will be lower than 10% if the nitrite accumulated is less than 15% of the nitrate reduced. However, depending on the biomass used, this accumulation can reach considerable values, as can be seen in Figure 8.

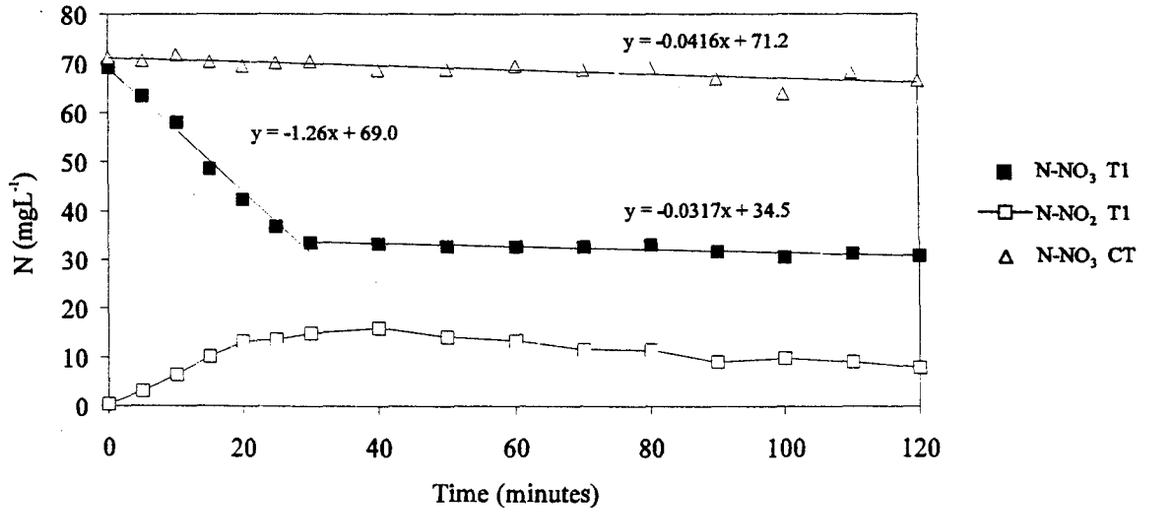

Figure 8. Nitrite accumulation during a denitrification batch experiment. (T1 = Test 1, CT = Control Test without external carbon source.)

Table 7. Test conditions for experiments with nitrite accumulation.

Test	Biomass concentration ($g\ SS\ m^{-2}$)	Initial COD concentration ($mg\ O_2\ l^{-1}$)	Initial N-NO3 concentration ($mg\ N\text{-}NO_3\ l^{-1}$)	Temperature (°C)
Test 1	17.1	420	69.2	20.0
Control test	17.1	65	71.3	20.0

This accumulation was significant for the experiments using a biomass grown on methanol and fed, during the test, with sodium acetate. The biomass used in the experiment shown on Figure 8 carried out the second step of the denitrification process at a lower rate than that observed for the first step, as a consequence nitrite accumulated in the system. Although a correction can be made for the accumulated nitrite, this should be avoided by using biomass grown on a composite carbon source, rather than a specific compound.

Yield coefficient

Of the parameters liable of affecting the characterisation results using anoxic respiration, the yield coefficient (Yh) is the most difficult to assess and control. As defined previously, the Yh is the ratio of the amount of biomass produced over the total amount of organic matter consumed. Theoretically, there are two possible paths to determine Yh. The first approach would be the direct measurement of the amount of biomass produced and the amount of organic matter consumed. This approach has a strong inherent shortcoming which renders it inaccurate: the impossibility to accurately measure the active biomass growth during the test, specially at the very small concentration variations involved. The second approach is to measure the total amount of electron acceptor consumed (in this case, $N\text{-}NO_3$) and the total amount of organic matter consumed. This approach is consistent when specific carbon sources are used. The concentrations of these carbon sources can be measured directly by specific analytical methods. However, the yield coefficients thus established, cannot be generalised to the situations where wastewater is used as carbon source, as it has been shown that the yield coefficient varies according to the carbon source used. When domestic wastewater is being tested the approach of measuring specific compounds may no longer be valid because these measurements account only for 50-70% of the readily biodegradable COD available. The organic matter consumed has to be evaluated through the measurement of COD, which remains a global parameter. The shortcomings, here, stem from the imprecision of the hypothesis that the filtered COD measured represents the organic matter consumed and from the imprecision of the COD analytical method itself.

Despite the shortcomings stated, the second approach remains a valid procedure to evaluate the order of magnitude of the yield coefficient, specially when dealing with effluents for which little data are available (such is the case for some industrial effluents, for example). In practice, the respirometric tests proposed in the literature (aerobic and anoxic) agree that the choice of Yh = 0.67 mg COD/ mg COD, for domestic wastewater of classic characteristics, is representative.

Characterisation of domestic wastewater

The method was used to determine the Ss of a domestic wastewater presenting COD concentrations of around 500 - 600 $mg\ l^{-1}$. According to the procedure developed above, this would demand initial nitrate doses of the order of 125 - 150 $mg\ N\text{-}NO_3\ l^{-1}$ to obtain COD/N initial ratios of the order of four. As these high doses are not convenient in terms of the analytical methods used for nitrate measurements, the wastewater is diluted to obtain initial COD values of the order of 300 $mg\ l^{-1}$, which enables initial nitrate doses of 70 mg $N\text{-}NO_3\ l^{-1}$.

The values of Ss in the range of 20 - 30% of the total initial COD of the raw wastewater are in accordance with the values usually found for domestic wastewater. The values found for settled wastewater are, expectedly, higher. However, the nitrate versus time plots obtained with wastewater, are sometimes more difficult to interpret. As a result, the precision of the method becomes dependent on the experience of the operator.

Table 8. Ss determinations for domestic wastewater. RW = raw wastewater, SW = settled wastewater, CODt = total chemical oxygen demand, CODf = chemical oxygen demand of filtered sample.

Number of tests	Wastewater tested	CODt at t=0 ($mg\ l^{-1}$)	CODf at t=0 ($mg\ l^{-1}$)	$N\text{-}NO_3$ at t=0 ($mg\ l^{-1}$)	Yh (DCO/DCO)	Ss ($mg\ l^{-1}$)	% of Ss
10	RW	611 ± 63	335 ± 61	70/40	0.67	160 ± 27	26 ± 4
10	SW	318 ± 64	230 ± 52	40	0.67	177 ± 52	54 ± 9

Results for the continuous flow procedure

The sensitivity of the continuous system to the variations in the inlet Ss concentrations was tested by adding different concentrations of the external carbon source to the inlet stream and maintaining the same nitrate inlet concentration. Using the continuous system with inlet hydraulic surface load of 0.7 m h^{-1}, recirculation hydraulic velocity of 11.0 m h^{-1} and biomass concentration of 27.5 g SS m^{-2}, initial nitrate dosing was kept constant and different initial sodium acetate concentrations were added to the inlet stream. Nitrate limitation was avoided by keeping nitrate concentration in excess. For the range of inlet Ss concentrations tested, a linear relation was found between the eliminated nitrate loads and the incoming Ss (Figure 9).

The continuous procedure was used to evaluate the performance of an experimental volatile fatty acids producing process. The process consists of a sludge blanket fed with raw wastewater (without primary settling) and operated at retention times and anaerobic conditions which enable acidogenic fermentation to take place and the production of VFA to be optimised. Two continuous NUR systems, reactor A1 and A2, are fed with the inlet and outlet of the sludge blanket reactor, respectively. The feed stream of both NUR systems are dosed with the same nitrate concentration. The outlet nitrate concentrations obtained are shown in Figure 10.

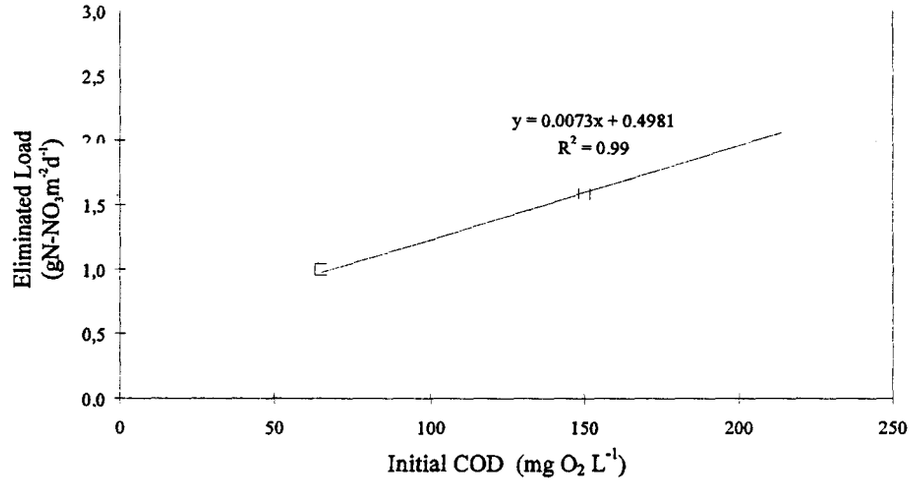

Figure 9. Eliminated load as a function of initial carbon source concentration.

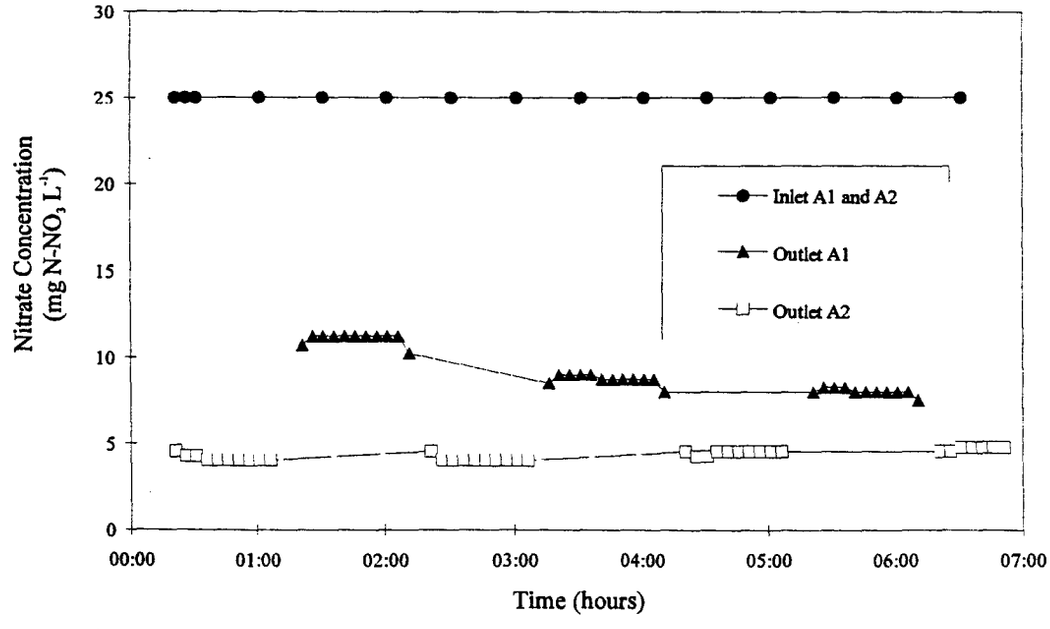

Figure 10. Denitrification capacities for the influent and effluent of an acidogenic fermenter fed with raw domestic wastewater. (Reactor A1 was fed with raw wastewater, Reactor A2 was fed with fermented wastewater rich in volatile fatty acids).

The qualitative analysis of the feed waters of each reactor is very explicit. Reactor A1, fed with raw wastewater, presents a clearly smaller denitrification capacity. In addition to this, the response of the system varies according to the variation of the organic matter concentration of the incoming wastewater. Reactor A2, fed with fermented wastewater rich in VFA, presents a higher and more stable denitrification capacity. The quantitative analysis presents greater difficulties due to the small precision in the determination of the yield coefficient. Adopting the literature value for Yh = 0.67 mg COD/ mg COD, the values calculated for Ss are too high in relation to the inlet and outlet concentrations of filtered COD. Accepting the hypothesis that only the filtered carbon source used is oxidised (that is, none is supplied by hydrolysis of Xs), the calculated Yh values are quite similar for both tests but lower than the usually adopted value.

The continuous system, at this stage of its development, is a practical tool for the qualitative analysis of the performance of processes aimed at producing VFA for biological nutrient removal treatment plants. Further development is necessary in order to enable accurate quantitative analysis, especially in regards to Yh determination.

CONCLUSION

The use of the standard suspended solids analysis as a measurement of the concentration of biomass was validated for biomass sampled from the same source. This approach is simple and adapted to the support media and to the type of biofilm used in this study. The specific denitrification rate obtained (0.37 g $N\text{-}NO_3$ g^{-1} SS d^{-1}) is in accordance with literature values for laboratory scale reactors under controlled conditions. Denitrification rates of 4.8, 3.2 and 0.8 g $N\text{-}NO_3$ m^{-2} d^{-1} found for acetate, glucose and starch, respectively, show that the NUR method on fixed biomass is adapted for the qualitative evaluation of the biodegradability of the effluent. An initial COD/N ratio of the order of four was determined to avoid a nitrate limited test, in which the change in kinetics necessary for data analysis would not be observed. If the biomass used for the test is sampled from a culture grown on a specific compound, such as methanol, nitrite accumulation may occur when other carbon sources are used. This problem was not encountered for cultures grown on domestic wastewater. Finally, the characterisation of domestic wastewater using this procedure gave Ss results compatible with literature results. However, the application of the method to complex effluents, such as domestic wastewater, lead to nitrate versus time plots which present less well defined change in kinetics than for specific substrates.

The continuous flow procedure proved to be simpler to perform and enables the use of an on-line nitrate sensor. This means that the test may be run automatically, which is not the case for the batch procedure. The procedure showed a good sensitivity to the biodegradability of the influent organic matter and to the concentrations of influent readily biodegradable organic matter. The continuous procedure avoids the large variations in concentrations of substrates and electron acceptor.

The methods presented are considered, in general, consistent tools for the evaluation of the denitrification capacity and Ss concentration of an effluent. However, the precision of the values determined is highly dependent on a somewhat neglected parameter, the yield coefficient. Adopted values are usually used for this parameter and little data are available in the literature for the yield coefficient of mixed cultures using wastewater. Most data available state yield coefficients determined for mixed or specific cultures growing on specific carbon sources. However, the yield coefficient has been shown to vary with the carbon source and the values determined for specific compounds cannot be transposed to composite carbon sources, such as wastewater. Given the influence of this coefficient on the results of respirometric methods, further work is necessary as to its determination and variation in different systems.

In spite of the doubts which might be formulated about the relative imprecision of the methods presented, the procedures enable a clear statement of whether a given effluent contains strong, average or low concentrations of readily biodegradable carbon source available to denitrifying organisms in fixed film systems.

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