Denitrification of drinking water using *Saccharum spontaneum* L. as a natural organic solid substrate

Euclides M. Deago and Gonzalo E. Pizarro

ABSTRACT

There is a global concern regarding the increasing pollution of natural water bodies by nitrate as a result of anthropogenic activities. To address this situation, natural organic solid substrates (NOSS) have been investigated as carbon sources in denitrification. Despite advances in this field, a lack of knowledge about the kinetics involved in the denitrification processes using NOSS still exists. For this reason, we have studied denitrification using *Saccharum spontaneum* L. (*S. spontaneum*) as NOSS to better understand these kinetic processes. This paper presents experimental results obtained for the release of organic carbon from *S. spontaneum*, and for its use by denitrifying bacteria. Laboratory tests were developed under anoxic conditions in batch reactors. Our results showed that the kinetics of release of organic carbon from the *S. spontaneum* was first order (0.08 d^{-1}). Furthermore, the maximum rate of utilization of substrate (5.61 mg $N\text{-}NO_3^-$ $mgVSS^{-1}$ day^{-1}) and the denitrification rate (327 mg $N\text{-}NO_3^-L^{-1}$ d^{-1}) were high (VSS = volatile suspended solids). We demonstrated that it is possible to obtain high yields of denitrification using organic carbon released from *S. spontaneum*. This study improves the knowledge on the use of NOSS, as alternative sources of carbon for denitrification.

Key words | denitrification, drinking water, kinetic, natural organic solid substrates, *Saccharum spontaneum* L.

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INTRODUCTION

The presence of nitrate in drinking water is associated with the disease methemoglobinemia or blue-baby syndrome, which generally affects infants under 6 months of age (Chambon *et al.* 1998). For this reason, the World Health Organization (WHO) established 50 mg L^{-1} as the maximum allowed amount of nitrate in drinking water (Chambon *et al.* 1998). Nitrate can be removed from water using denitrification.

Denitrification is a technology that takes advantage of the ability of bacteria to use nitrate as an electron acceptor. It is simpler and more economical on a large scale than other processes such as reverse osmosis or ion exchange (Mateju *et al.* 1992). A requirement for denitrification to occur is the provision of a substrate, whether organic or inorganic, to supply the electrons needed to complete the reduction process (Madigan *et al.* 2003). It has been determined that denitrification is more efficient using organic substrates, catalyzed by heterotrophic bacteria (Mateju *et al.* 1992).

Traditionally, denitrification of drinking water is performed using liquid organic substrates. Substrates such as ethanol, methanol and acetic acid are commonly used to remove nitrate in conventional systems, yielding efficiencies between 70 and 95% (Shrimali & Singh 2001). However, the denitrification may be restricted by factors such as the proper dosage of soluble substrate, the use of substrates that pose health risks (Shrimali & Singh 2001) and high cost (Rittmann & McCarty 2001). Due to the limitations of the denitrification, several studies have suggested that it would be worthwhile (or economical or of value) to explore new carbon sources and design innovative and robust systems for obtaining lower operating costs in the denitrification (Soares 2000).

Recently, researchers have begun studying natural organic solid substrates (NOSS) as carbon sources for the denitrification. The NOSS are plant materials that can be

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obtained from nature or agricultural processes. Several NOSS that have been previously studied are wheat straw, grasses, barley stalk, rice husks and palm leaves (Aslan & Turkman 2003; Ovez *et al.* 2006; Hashemi *et al.* 2011). Results of these studies have shown that it is possible to implement systems to remove nitrate from wastewater and drinking water. Other materials such as sawdust have been studied *in situ* to remove nitrate (Robertson *et al.* 2000; Schipper & Vojvodic-Vukovic 2000). However, to improve the design of such systems it is necessary to know the kinetics for the removal of nitrate using the NOSS.

In this research, we selected *Saccharum spontaneum* which is an abundant grass in tropical climates considered an invasive species difficult to control (Park *et al.* 2010). Recently *S. spontaneum* has been studied as biomass for bioethanol production (Scordia *et al.* 2010). The objectives of our research were: (i) to study the release of organic carbon from *S. spontaneum* due to leaching and hydrolysis; (ii) estimate kinetic and stoichiometric parameters of denitrification using organic carbon released from *S. spontaneum*; and (iii) estimate the rate of removal of nitrate using organic carbon released from *S. spontaneum*.

This study considered *S. spontaneum* as a potential carbon source for the biological removal of nitrate, due to its low cost, accessibility, and abundance.

MATERIALS AND METHODS

Physico-chemical analysis of NOSS used in this study

The *S. spontaneum* was collected on the campus of the Technological University of Panama. The detrital material of *S. spontaneum* consists of two main parts: the cortex and pith (Audesirk & Audesirk 1995). Detrital material was evaluated for a number of properties, such as percentage of dry mass, the lignin, cellulose, and hemicellulose content, the nitrogen content, biodegradable fraction and the amount of organic carbon available.

The moisture content was determined by drying the sample in an oven at 105 °C for 24 h. The fiber content (lignin, cellulose, and hemicellulose) was obtained using the Van Soest method (Van Soest 1963). The total nitrogen was determined by the Kjeldahl method, and the total carbon was obtained by the combustion method. The content of cations in *S. spontaneum* was determined using nitric acid digestion and inductively coupled plasma (ICP) detection. The biodegradable fraction and available organic carbon were estimated by applying the methodology proposed by Van Soest (1996). The equations proposed by Van Soest for estimating these parameters are described in Appendix A (available online at http://www.iwaponline.com/jws/062/115.pdf).

Leaching test

The leaching test was conducted to evaluate the release of soluble compounds from the *S. spontaneum*, by the physical action of water (autolysis). Autolysis is the outbreak of the vacuoles of plant cells due to water saturation. This occurs in the decomposition of submerged plant material (Reddy & DeLaune 2008).

The test was performed on three types of reactor: (i) one containing the cortex of *S. spontaneum*; (ii) one containing pith of *S. spontaneum*; and (iii) one containing complete sections of *S. spontaneum* (pith and cortex). Equal masses of the three materials (4 g measured as dry weight) were added in the respective reactors. These materials were cut into pieces of 15 mm. Water and bottles used in the leaching test were sterilized and the detrital material was washed thoroughly to remove impurities. The volume of water used in the test was 200 mL. The reactors were sealed and operated during 4 days at a temperature of 30 ± 1 °C. The water analyses conducted in these experiments included nitrate ($N\text{-}NO_3^-$) and nitrite ($N\text{-}NO_2^-$) content, ammonium ($N\text{-}NH_4^+$) content, chemical oxygen demand (COD), and cations.

Batch experiments

Upon completion of the leaching test, complete *S. spontaneum* (cortex and pith) showed the best performance; therefore, this material was selected for the following batch experiments. The pH in the reactors was maintained between 6.5 and 7, using 3 g L^{-1} of KH_2PO_4 and 3 g L^{-1} K_2HPO_4. Reactors were continuously agitated at 150 rpm and were injected with nitrogen gas for 5 min to maintain anoxic conditions (dissolved oxygen <1.0 mg L^{-1}). The tests were conducted at a temperature of 30 ± 1 °C.

Evaluation of carbon release from *Saccharum spontaneum* L. in anoxic conditions

The aim of the experiment was to study the behavior of organic carbon release from the *S. spontaneum* in anoxic conditions (denitrification).

Experimental test

An experimental test was conducted in three 500 mL amber glass bottles using 400 mL of deionized water unsterilized and 12.7 g (as dry mass) of *S. spontaneum* unwashed. $NaNO_3$ was used to obtain a concentration of 100 mg $N\text{-}NO_3^-$ L^{-1}. Denitrification was promoted by the indigenous bacteria attached to *S. spontaneum*, which means that no bacteria were inoculated in this assay. The use of indigenous bacteria attached to solid substrates has been applied in similar research (Gibert *et al*. 2008). In this experiment, no micronutrients were added because it was considered that these would be obtained by bacteria from the *S. spontaneum*. Periodically, 25 mL of water were taken from each reactor. This water was analyzed for nitrate, COD, pH, and dissolved oxygen (DO). The volume of water removed was replaced with fresh water and $NaNO_3$ was dosed to maintain a nitrate concentration of 100 mg L^{-1}.

Determination of first-order hydrolysis rate constant

During this study, first order kinetics was considered for the release of organic carbon from the *S. spontaneum* (Equation (1)). The first order kinetic equation has been commonly used in studies of degradation of complex substrates (Vavilin *et al*. 1996; Veeken *et al*. 2000). The release constant of organic carbon (k_h) was obtained by linear regression.

$$C(t) = C_o * \exp^{-kh*t} \quad (1)$$

$C(t)$ is the residual COD in the detrital material at the end of each measurement period and is obtained using Equation (2).

$$C(t) = C_o - \mathrm{COD}(t) \quad (2)$$

$\mathrm{COD}(t)$ is accumulated COD during the test. $\mathrm{COD}(t)$ is the mass of organic carbon released from the *S. spontaneum* and is calculated as follows:

$$\mathrm{COD}(t) = \sum(\mathrm{COD}r + \mathrm{COD}c) \quad (3)$$

where COD*r* corresponds to the mass of soluble COD measured in the supernatant during the tests; and DQO*c* corresponds to the mass of equivalent COD used by bacteria to reduce nitrate (electron acceptor). To obtain this equivalent COD, nitrate consumed by the bacteria during the test was multiplied by the factor 2.225 g COD/g $N\text{-}NO_3^-$ (obtained from Equation (4)).

$$CH_2O + 0.232NO_3^- + 0.142NH_4^+ + 0.142HCO_3^- + 0.232H^+ \rightarrow 0.142C_5H_7O_2N + 0.432CO_2 + 0.116N_2 + 0.974H_2O \quad (4)$$

The criteria used to obtain Equation (4) are described in Appendix B (available online at http://www.iwaponline.com/jws/062/115.pdf).

Denitrification rate using OxiTop system

This experiment was established to determinate the denitrification rate in batch reactors, using *S. spontaneum* as an organic carbon source. It was carried out in the OxiTop system (WTW Measurement Systems, Germany). This method is based on measurements of pressures generated by gases formed in decomposition processes. In this study, the gases formed were N_2 and CO_2 (according to Equation (4)). The test was conducted for a period of 7 days in three 500 mL bottles with equal volume of water (200 mL), and equal mass of detrital material of *S. spontaneum* (4 g as dry mass) (Figure 1). In this test, unwashed detrital material (i.e. with endogenous bacteria) was also used. The nitrate concentration used was 100 mg $N\text{-}NO_3^-$ L^{-1}. The water of the reactors was analyzed for nitrate, nitrite, ammonium and COD.

Determination of kinetic parameters

First, a leaching test was conducted according to the setup described previously. After the leaching test, the supernatant

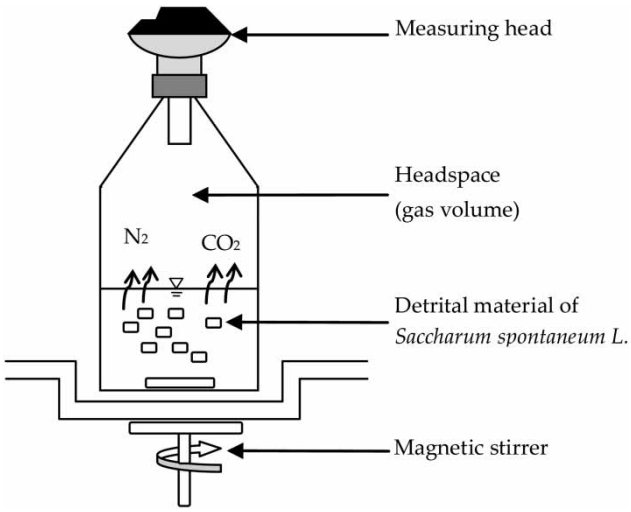

Figure 1 | Schematic representation of denitrification test using the OxiTop system.

was filtered and inoculated with bacteria obtained from previous tests. The bacteria were acclimated for 24 hours with a nitrate concentration of 200 mg $N\text{-}NO_3^-$ L^{-1}. The volume of water used in each reactor was 200 mL. The initial concentration of nitrate and COD in the test was 140 mg $N\text{-}NO_3^-$ L^{-1} and 479 mg L^{-1}, respectively.

The reduction rate of nitrate was assumed to follow a Monod-type kinetic. Nonlinear regression was applied to estimate the kinetic parameters Monod half saturation (K_s) and maximum nitrate utilization rate (q_{max}). Moreover, the net yield of bacteria (Y) was another important parameter determined by the kinetic assay, which was calculated using Equation (5), based on the reduced nitrate and COD consumed in the assay (Cokgor *et al.* 1998):

$$Y = 1 - 2.86 * (\mathrm{N/COD}) \tag{5}$$

Analytical techniques

Before each analysis, all samples were filtered through 0.45 μm cellulose nitrate membranes – to determine the dissolved components. $N\text{-}NO_2^-$, $N\text{-}NH_4^+$, and COD were analyzed using a HACH DR/2400 spectrophotometer (HACH Company, USA), applying the Ferrous Sulfate method (HACH 8153), Nesler method (HACH 8038), and colorimetric method (HACH 8000), respectively. DO was measured with a luminescence electrode coupled to HACH HQ40d portable (HACH Company, USA). The pH was measured with glass electrode (Thermo Scientific, USA). Nitrate was analyzed with ion selective electrode (Cole Palmer, USA). Both electrodes were coupled to a multiparameter Orion Star 5 (Thermo Scientific, USA). The cations were determined by ICP spectrometry (APHA *et al.* 2005). The biomass (as mg COD L^{-1}) was calculated using Equation (6) (Grady *et al.* 1999):

$$\mathrm{COD}_B = \mathrm{COD}_T - \mathrm{COD}_S \tag{6}$$

where COD_T is total COD measured in the supernatant (unfiltered samples) and COD_S correspond to soluble COD measured in the supernatant (filtered samples).

RESULTS AND DISCUSSION

Properties of *Saccharum spontaneum* L. used in the study

Detrital material was chosen because it contains the highest lignocellulose concentration in the plant (Reddy & DeLaune 2008). To evaluate the properties of *S. spontaneum*, the cortex and pith were separated. The carbon and nitrogen content were higher in the pith; however, the C/N ratio was higher in the cortex (Table 1). The cortex had higher concentrations of lignin and cellulose, while the pith had the highest hemicellulose content (Table 1). The pith had the highest content of nutrients, particularly for calcium and potassium which were at levels 10 times higher than those found in the cortex.

The C/N ratio and lignocellulose composition suggest that the pith is more biodegradable than the cortex. Reddy & DeLaune (2008) reported that materials with lower C/N ratio and lower lignin content are more biodegradable. Furthermore, the biodegradable fraction estimated by pith indicates that almost all of the pith was bioavailable (95%). Therefore, we can infer that most probably bacteria obtained the organic carbon from the pith of *S. spontaneum*.

Leaching test

The releases of organic carbon (in terms of COD) were high, especially in the reactor containing cortex (Table 2). The

Table 1 | Physical-chemical properties of *Saccharum spontaneum* L.

Parameters	Cortex	Pith
Ca (mg g^{-1})	0.21	1.33
Fe (mg g^{-1})	0.12	0.07
K (mg g^{-1})	10.17	48.86
Mg (mg g^{-1})	0.28	0.35
Na (mg g^{-1})	0.05	0.07
P (mg g^{-1})	2.33	3.89
N (%)	0.55	2.10
C (%)	36.62	45.54
C/N	66.58	21.69
Celullose (%)	46.98	30.02
Hemicellulose (%)	22.19	25.51
Lignin (%)	8.72	0.62
Organic carbon (%)	26.96	44.05
Biodegradability fraction, f_b(%)	66.08	94.17
Density (g/mL)	0.76	0.81
Dry material (%)	78.62	58.33
Ash (%)	4.68	12.76

high values of COD should be evaluated with caution, because this is a limitation if we consider the use of *S. spontaneum* as a substrate for biological removal of nitrate from drinking water. Nitrogen released by leaching from *S. spontaneum* was negligible (Table 2).

The values obtained of elements (Ca, K, Mg, Na, Si) were low (Table 2). Angelidaki & Sanders (2004) indicate that elements such as Ca, Na, K, and Mg are used by bacteria in enzymes, cell walls, ribosome and nucleic acids. Potassium (K) was the compound that had the highest concentration in the water. This result was consistent with the K content detected in detrital material (Table 1). The concentrations of the analyzed elements are not a risk of contamination. However, caution should be exercised in the use of *S. spontaneum* to remove nitrate for drinking water, because the WHO states that the maximum allowable concentration of K is 10 mg L^{-1} (Chambon *et al.* 1998).

According to the results of leaching test, it was concluded that the complete detrital material of *S. spontaneum* was better suited as a carbon source for denitrification assays than the pith or cortex. Detrital material used without removing the cortex reduces the effect of autolysis, because the cortex acts as an impermeable wall that restricts the contact of water with labile material. Therefore, the water will act only on the cross sections of the pieces of detrital material of *S. spontaneum*. The slower release of soluble substrates from *S. spontaneum*, will allow removal of nitrate over a longer period.

Behavior of carbon release from *S. spontaneum*

A greater content of detrital material was used than that in the leaching test, due to several conditions: (i) first, the time used in this test was greater; (ii) it was assumed that organic carbon would be obtained from part of material with less lignin content (pith); and (iii) it was also assumed that the bacteria would use only the cellulose present in the pith of the *S. spontaneum*. The biodegradation of detrital material is characterized by a relatively rapid loss of cellulose (Melillo *et al.* 1989), but this biodegradability depends on the content of lignin (Chandler *et al.* 1980).

The maximum soluble COD measured during the test was obtained on the third day of the batch reactors operation (Figure 2(a)). After the third day, there was a steady decline until the end of the trial. Based on these results we

Table 2 | Mass of nitrogen, COD and others elements released per gram of mass of *Saccharum spontaneum* L. used in the leaching test

	Parameters (mg g^{-1} dry mass)								
Reactors	$N\text{-}NO_3^-$	$N\text{-}NO_2^-$	$N\text{-}NH_4^+$	COD	Ca	*K*	Mg	Na	Si
Complete *S. spontaneum*	0	0	0.029	30.00	0.051	1.120	0.014	0.012	0.074
Pith *S. spontaneum*	0	0	0.029	33.75	0.107	2.264	0.020	0.012	0.094
Cortex *S. spontaneum*	0	0	0.030	37.50	0.207	2.714	0.026	0.012	0.137

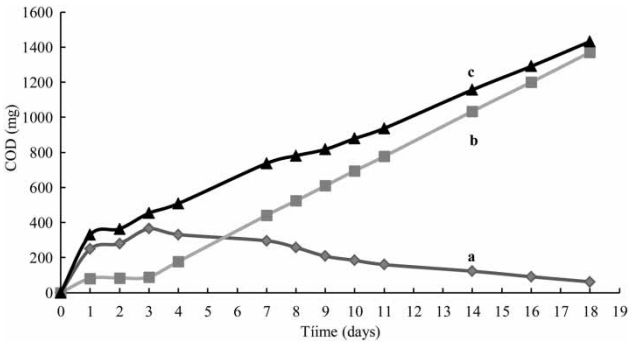

Figure 2 | (a) COD measured directly in the water, (b) COD obtained from nitrate equivalent electrons consumed in the assays, (c) total COD released from *Saccharum spontaneum* L.

can indicate that the release of the organic carbon from *S. spontaneum* occurred in two phases: leaching phase, which occurred within the first 3 days of testing, and hydrolysis phase, started after the third day. Reddy & DeLaune (2008) indicate that leaching is the first phase in the degradation of submerged detrital material, and the hydrolysis is the breakdown of macromolecules (such as cellulose) to simpler compounds (monomers) (Vavilin *et al.* 1996; Reddy & DeLaune 2008).

Gradual reduction of COD from day 3 in batch reactors (Figure 2(a)), suggests that the bacteria had favorable conditions in terms of electron donor and nutrient content, provided by the leaching phase. Reddy & DeLaune (2008) indicated that soluble substrates released by leaching from detrital material, can be directly used by heterotrophic bacteria. The behavior described in our experiment (Figure 2(a)) has been reported in similar studies (Volokita *et al.* 1996; Xu *et al.* 2009).

The values of the carbon accumulated during the assay (Figure 2(c)) represent the carbon released from the *S. spontaneum*. The maximum carbon concentration obtained was 1,432 mg COD (Figure 2(c)). During the leaching stage, 32% of this carbon was released, while the remaining 68% was due to hydrolysis. Released carbon represents 78% of the cellulose mass available in the pith of *S. spontaneum*. Cellulose mass used during the test was 1,262 mg (measured as COD).

The behavior observed in the data of Figure 2(c), suggests that there was first order release of carbon. The hydrolysis constant (k_h), which describes the behavior of carbon release from the *S. spontaneum*, was obtained by applying linear regression to the plotted data obtained from the linearization of Equation (1). In these calculations, the mass of carbon released by leaching was not considered. The k_h obtained with linear regression was 0.08 d^{-1}. Our results confirm that the release of organic carbon from *S. spontaneum* was first order ($R^2 = 0.96$). Angelidaki & Sanders (2004) indicated that the release of carbon in anoxic conditions depends on the availability of electron acceptor such as nitrate. This k_h was in the range of values reported in the degradation of similar materials (Reddy & DeLaune 2008; Vavilin *et al.* 2008).

In this experience, 1 gram of *S. spontaneum* (measured as dry weight of pith) was required to remove 0.15 g of nitrate. This ratio and the k_h are variables that can be used for determining the dimensions of water treatment systems to remove nitrate. Furthermore, these variables are useful for mathematical modeling of the denitrification using NOSS.

Denitrification test using OxiTop system

Behavior of $N\text{-}NO_2^-$, $N\text{-}NH_4^-$ and COD

In the denitrification assay, we monitored the behavior of the concentration of $N\text{-}NO_2^-$, $N\text{-}NH_4^+$, and COD. The measured concentration of nitrite was below 1 mg L^{-1} for all three reactors (Figure 3). These results indicate that conditions within the reactors favored limited nitrite production; these conditions included the absence of dissolved oxygen and adequate concentration of electron donor (Rittmann & McCarty 2001).

Ammonium content in the reactors was graphed over the course of the experiment (Figure 3), and results indicated low concentration of ammonium in the reactors containing (<0.7 $N\text{-}NH_4^+$ mg L^{-1}). The ammonium is

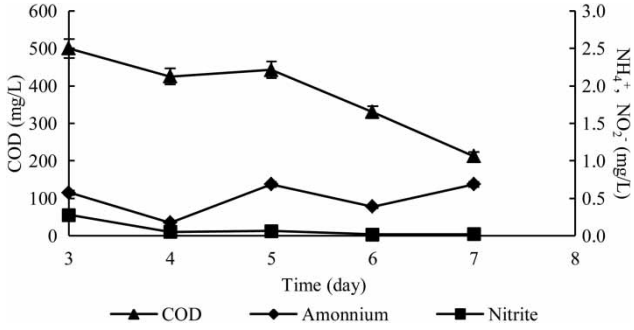

Figure 3 | Behavior of COD, ammonium, and nitrite in the denitrification test.

attributed to the ammonification of nitrogen present in the detrital material of *S. spontaneum*, and according to the literature, a nitrogen-rich material promotes ammonification (Reddy & DeLaune 2008). COD had its maximum concentration at day 3 (500 mg L^{-1}), and the lowest concentration (212.5 mg L^{-1}) was measured at the end of the trial. The maximum concentration of COD was in agreement with results obtained in previous trials (Figure 2(a)), which indicates that the COD was provided by the soluble substrates delivered from *S. spontaneum*. From day 3, the released COD is associated with the hydrolytic activity of attached bacteria in the detrital material of *S. spontaneum*.

Based on the results obtained for COD, ammonium and nitrite content, we confirm that the detrital material of *S. spontaneum* provided the concentration of nutrients needed for denitrification.

Evolution of denitrification

The evolution of denitrification was evaluated by measuring the pressures of gases formed (N_2 and CO_2) using the OxiTop system. The pressure in the batch reactors reached a constant value of 47.33 ± 5.69 hPa between 420 and 440 minutes from the beginning of the test (Figure 4), which suggested that the production of gaseous nitrogen had ended. It was verified with measurements of nitrate at the end of the trial, which were zero. Furthermore, using the general gas law, it was possible to check that the sum of the partial pressures of the gases formed in denitrification (Equation (4)), was equal to the measured pressures at 440 min (end of denitrification). These partial pressures were calculated using the moles of N_2 and CO_2 (Equation (4)), a temperature of 30 °C and the volume of gas or headspace (Figure 1), which was 292 mL.

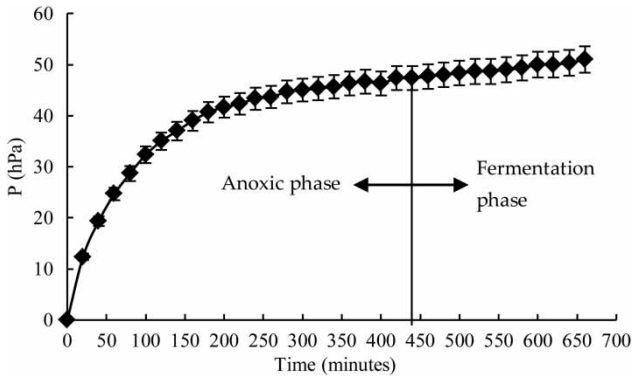

Figure 4 | Behavior of the pressures measured by the OxiTop system.

The increase in the pressures measured after 440 minutes (Figure 4) suggest the presence of gases due to fermentation. This behavior confirms the supposition that the hydrolysis process is responsible for release of organic carbon from the *S. spontaneum* during denitrification.

In this assay, the nitrate was removed at a rate of 327 mg $N\text{-}NO_3^-$ L^{-1} d^{-1}. This rate was higher than those reported by other studies that used similar NOSS (Table 3). These results indicate that *S. spontaneum* may be an efficient carbon source for biological removal of nitrate from water.

Kinetic and stoichiometric parameters of denitrification

Complete denitrification occurred by 325 minutes following reaction initiation (Figure 5). In this test, 200 mg COD L^{-1} were needed to reduce 140 mg NO_3^- L^{-1} of dosed nitrate. The observed $COD_{consumed}/N_{reduced}$ ratio was 6.31 g COD/g N. This value is slightly higher than the stoichiometric value obtained from Equation (4) (6.11 g COD/g N). This condition was consistent with that reported in the literature, which indicates that complete denitrification occurs when the ratio COD/N approaches the stoichiometric value (Cuervo-López *et al.* 2009).

Table 3 | Maximum denitrification rates obtained using NOSS as electron donors

Substrates	Description system	Concentration of nitrate (mg $N\text{-}NO_3$ L^{-1})	Denitrification rate (mg $N\text{-}NO_3$ L^{-1} d^{-1})	Test temp. (°C)	Reference
Wheat straw	Column	100	235	25	Soares & Abeliovich (1998)
Glycyrrhiza glabra	Column	100	167	20	Ovez *et al.* (2006)
Arunda donax	Column	100	102	20	Ovez *et al.* (2006)
Saccharum spontaneum L.	Batch	100	327	30	This study

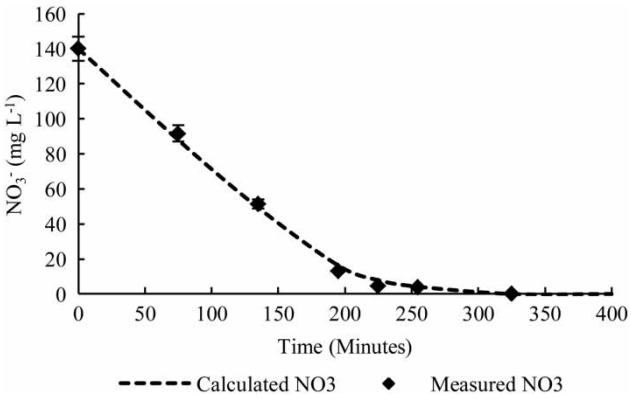

Figure 5 | Denitrification kinetics obtained based on organic carbon released from *Saccharum spontaneum* L.

Nonlinear regression of the Monod equation was adjusted to fit the kinetic of the experimental data by using organic carbon released from *S. spontaneum* (Figure 5). The values of q_{max} and K_S obtained were 5.61 mg N-NO_3^- mg VSS^{-1} d^{-1} and 20.15 mg N-NO_3^- L^{-1}, respectively (VSS = volatile suspended solids). The q_{max} was higher than the values reported for other studies of denitrification (Table 4). This could be due to two factors: ammonium consumption and high temperature during the test. The bacteria use ammonium as their first option as source of nitrogen for cell synthesis (Rittmann & McCarty 2001); and according to the literature, q_{max} is increased to twice its value when the temperature is increased by 10°C (Rittmann & McCarty 2001; Reddy & DeLaune 2008).

The value of K_s obtained in this study varied considerably compared with values reported in the literature (Table 4). Rittmann & McCarty (2001) reported that the K_S parameter is highly variable.

The net yield (Y) of bacteria was calculated using values of reduced nitrate and COD consumed in the batch test. The Y obtained was 0.55 mg VSS/mg COD, which was higher than those reported in other studies that used soluble substrates (Table 4). These results suggest that organic carbon obtained from *S. spontaneum* allowed greater efficiency of bacteria.

CONCLUSIONS

The denitrification was studied in batch reactors using *S. spontaneum* as a NOSS. The results of this study demonstrated that the release of organic carbon from the *S. spontaneum* in anoxic conditions began with a leaching of soluble components; subsequently detrital material was hydrolyzed by bacteria with first order kinetics. High values were obtained for nitrate removal rate, kinetic and stoichiometric parameters. These results were greater than the values reported for similar NOSS and conventional substrate. This high performance reflected a positive adaptation of bacteria to the microenvironment, which allowed obtaining the electron donor and nutrients necessary for their development. Our results demonstrated the potential of the *S. spontaneum* as sole substrate for nitrate removal from drinking water.

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Table 4 | Kinetic and stoichiometric parameters of denitrification obtained with organic carbon released from *Saccharum spontaneum* L. vs. those reported in the literature for conventional organic carbon

q_{max} (mg N-NO_3^- mgVSS^{-1} d^{-1})	K_s (N-NO_3^- L^{-1})	Y (mg COD/mg COD)	Test temp. (°C)	Organic carbon sources	Reference
3.14	18.20	0.21	23	Methanol	Rabah *et al.* (2007)
3.65	0.71	0.34	25–26	Glucose	Lin (2008)
0.65	24.15	0.41	20	Methanol	Cherchi *et al.* (2009)
1.45	58.97	0.50	20	Acetate	Cherchi *et al.* (2009)
5.61	20.15	0.55	30	*S. spontaneum*	This study

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