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Articles

Denitrification modeling using natural organic solid substrates as carbon sources

Modelación de desnitrificación usando sustratos sólidos orgánicos naturales como fuentes de carbono

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Abstract

In recent years, Natural Organic Solid Substrates (NOSS) have been studied as economical and safe carbon sources for use in water denitrification. Although the denitrification results with NOSS have been satisfactory, the information of the processes involved in the NOSS-biofilm interface is still limited. To understand these processes, we developed a one-dimensional mathematical model of counter-graduate denitrification, with which we evaluated: the behavior of the biofilm adhered to NOSS and its effect on carbon release; transport and consumption of carbon; and transport and consumption of nitrate. For this purpose, Saccharum Spontaneum L. was used as a reference NOSS, of which biokinetic and hydrolysis parameters were generated in previous denitrification studies. This paper presents the results of the mathematical modeling of denitrification, with NOSS as carbon sources, which was simulated with the AQUASIM program. The assumptions and simplifications defined allowed the theoretical prediction of denitrification in batch reactors and completely mixed with flow. The best simulations of the study were obtained under biofilm detachment conditions, where denitrification was greater than 80%. The most significant parameters of this mathematical modeling were the net yield of bacteria (Yh) and hydrolysis (k_h). These findings demonstrate the importance of posing conceptual and mathematical models of denitrification using NOSS. This study would be a first approximation of a mathematical model of counter-graduate denitrification, using NOSS as a carbon source and biofilm carrier.

Keywords: Mathematical modeling, denitrification, biofilm, counter-gradient, natural organic solid substrates.

Resumen

En los últimos años se han estudiado sustratos sólidos orgánicos naturales (SSON) como fuentes de carbono económicas y seguras para su uso en la desnitrificación del agua. Aunque los resultados de desnitrificación con SSON han sido satisfactorios, aún es limitada la información de los procesos que intervienen en la interfaz SSON-biopelícula. Con el objetivo de comprender dichos procesos, desarrollamos un modelo matemático unidimensional de desnitrificación en contragradiente, con el cual evaluamos el comportamiento de la biopelícula adherida al SSON y su efecto en la liberación del carbono; transporte y consumo de carbono; transporte y consumo de nitrato. Para tal fin, se usó *Saccharum spontaneum L.* como SSON de referencia, de la cual se tienen parámetros biocinéticos y de hidrólisis generados en estudios previos de desnitrificación. En este artículo se presentan los resultados de la modelación matemática de desnitrificación con SSON como fuentes de carbono, lo cual se simuló con el programa *AQUASIM*. Los supuestos y simplificaciones definidas permitieron predecir teóricamente la desnitrificación en reactores batch y completamente mezclado con flujo. Las mejores simulaciones del estudio se obtuvieron en condiciones con desprendimiento de biopelícula, donde la desnitrificación fue superior al 80 %. Los parámetros más significativos de esta modelación matemática fueron el rendimiento neto de bacterias (Y_h) y la hidrólisis (k_h). Estos hallazgos demuestran la importancia de plantear modelos conceptuales y matemáticos de desnitrificación usando SSON. Este estudio sería una primera aproximación de un modelo matemático de desnitrificación en contragradiente usando a los SSON como fuente de carbono y portador de biopelícula.

Palabras clave: modelación matemática, desnitrificación, biopelícula, contragradiente, sustrato sólido orgánico natural.

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Introduction

In recent years, mathematical modeling has gained relevance in the study of biological processes for water treatment, where bacteria are used in suspension or attached growth (biofilm). For systems in suspension, mathematical modeling plays an important role, since it allows diagnosing problems and optimizing processes (Shi, Long, Meng, and Raajeevan, 2008). Regarding systems that use bacteria with adhered growth, mathematical modeling is also essential, because it allows integrating components and processes that occur in this microsystem, allowing the understanding of their behavior (Wanner *et al*., 2006).

One of the processes that have been mathematically modeled is biological denitrification, where both systems in suspension (wastewater and groundwater) have been studied (Calderer, Jubany, Perez, Marti, and De Pablo, 2010; Henze, Gujer, Mino, and van Loosdrecht, 2000; Shi *et al*., 2008); likewise, adherent growth denitrifying systems have been studied using membrane reactors (Matsumoto, Terada, and Tsuneda, 2007) and in fluidized reactors and biofilters (Horn and Telgmann, 2000;

Lin, 2008. The common factor in each treatment condition (in suspension or adhered) is the dosage of organic carbon, which will also be used as an energy source (Mateju, Cizinska, Krejci, and Janoch, 1992). Common organic carbons used in the denitrification study are glucose, ethanol, and methanol (Horn and Telgmann, 2000; Lin, 2008; USEPA, 1993; Mateju *et al*., 1992). This constant carbon dosage simplifies the initial and edge conditions when making a mathematical statement.

Although the condition of constant carbon dosage simplifies the mathematical modeling of denitrification processes, the costs associated with the operation of denitrifying systems have generated a foray into studies of alternative, cheaper sources. This confirms what was stated by Garzón-Zúñiga (2005), which points to the need for more research on unconventional mechanisms for nitrate treatment. In this sense, Natural Organic Solid Substrates (NOSS) have become alternative sources of carbon for denitrification due to certain characteristics that could be used. Boley, Muller and Haider (2000), point out that one of the advantages of having a solid biodegradable substrate, from which the bacteria obtain their source of carbon and energy, is that the need to dose soluble carbon is eliminated and they also function as a carrier or support for the bacteria that form a biofilm. This has been demonstrated in performance studies (Table 1).

Table 1. Comparison of costs and yields of conventional substrates and natural organic solid substrates.

Substrates	**Substrate cost ($/kg)**	**Cost per Kg of nitrate removed ($/kg N-$NO_3^-$)**	**Substrate consumption (Kg/kg N-NO_3^-)**	**Denitrification Rate (Kg N-NO_3^-/m^3-day)**	**References**
Methanol	1.13	2.26 - 4.52	2.08 - 3.98	3.48	Boley, Muller and Haider (2000)
Ethanol	1.36	2.71	2.0	1.29-1.59	
Cotton	0.53	1.48	2.8	2.8	Soares *et al*. (2000); Volokita *et al*., (1996a)
Gracilaria verrucosa	1.13	1.05 - 1.62	0.93-1.43	0.27	Ovez, Mergaert and Saglam (2006a)

In addition to NOSS studies such as those presented in Table 1, experimentally much research has continued the use of NOSS. Among the works with NOSS carried out are cotton (Della-Rocca, Belgiorno, and Meric, 2007; Della-Rocca, Belgiorno, and Meric, 2005; Soares and& Abeliovich, 1998); sawdust (Healy, Rodgers, and Mulqueen, 2006; Schipper & Vojvodic-Vukovic, 2000); tree chips and bark (Aslan and Turkman, 2003; Gibert, Pomierny, Rowe, and Kalin, 2008; Saliling, Westerman, and Losordo, 2007); wheat straw (Aslan and Turkman, 2003; Aslan and Turkman, 2004; Aslan and Turkman, 2005); marine algae (Ovez, Ozgen, & Yuksel, 2006b); and grasses (Deago and Pizarro, 2013; Deago and Pizarro, 2015). This research shows that it is possible to remove nitrate, using NOSS as a carbon source for both wastewater and drinking water.

It has been experimentally verified that the release of organic carbon in denitrification tests using NOSS, occurs in a series of sequential processes starting with leaching; then colonization of NOSS by the bacteria that form the biofilm; the enzymatic hydrolysis process occurs by a specialized consortium of bacteria that degrade bioavailable carbon; and finally, biological denitrification is presented (Deago and Pizarro, 2015). Under these conditions, hydrolysis is the limiting factor in the biodegradation of NOSS (Angelidaki and Sanders, 2004). However, the mathematical modeling of denitrification using NOSS is a pending task necessary for a better understanding of how the processes of carbon release are, nitrate reduction and the evolution of adhering bacteria around these materials occur.

Therefore, for this work, *Saccharum spontaneum L.* (Paja Canalera) was used as the reference NOSS, which served as a carrier or support for the adhered growth of bacteria (biofilm) and as the only carbon source. The data of *Saccharum spontaneum L.* were obtained from the work of Deago and Pizarro (2013). The objective of the research was to evaluate denitrification, through a mathematical approach of adhered growth systems with a counter-adherent behavior of the organic substrate (electron donor) and nitrate (electron acceptor). For this, the simulation of the mathematical approach was carried out with the free software AQUASIM.

Materials and methods

Development of the mathematical model

The conceptual representation of denitrification using Natural Solid Organic Substrates (NOSS) is based on results obtained from studies in batch reactors (Deago and Pizarro, 2013; Deago and Pizarro, 2015) (Figure 1). For this conceptual model of denitrification, the following components have been identified: a) NOSS that acts as a carrier or support for the biofilm and in turn the source of organic carbon; b) hypothetical permeable membrane located between the NOSS and the biofilm, to represent the carbon flux (J_{COD}); c) the biofilm that grows around the NOSS; d) a diffuse layer of water between biofilm and the water body, and e) the body of water (Figure 1).

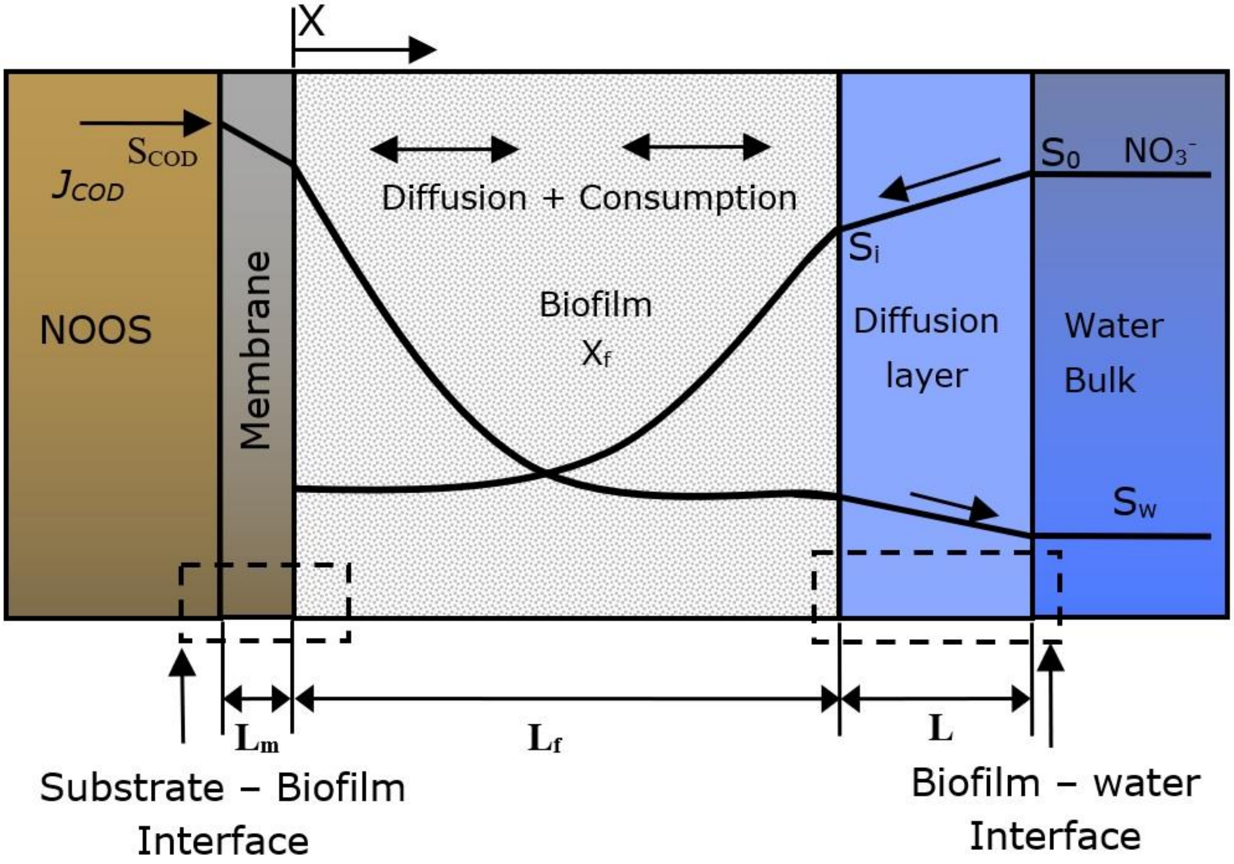

Figure 1. Micro-level scheme of electron donor and acceptor transfer and consumption at the NOSS-biofilm-water interface; where X_f is the density of the active biofilm; L_f, the biofilm thickness; L_m, hypothetical membrane thickness; L is diffuse layer thickness; S_{COD} is the concentration of carbon in NOSS measured as COD.

Denitrification of water using NOSS is a biodegradation process where nitrate is used as the electron acceptor. Angelidaki and Sanders (2004), mention that biodegradation is a function of the availability of the electron acceptor and that the release of energy from redox reactions is used for the growth of microbial populations. This type of denitrification processes occurs in natural systems such as wetlands, where the biodegradation of detrital materials occurs underwater (Reddy and DeLaune, 2008).

For the development of the mathematical model, three aspects were considered: compartments (previously described), components (particulate and dissolved), and processes (transformation and transport) (Wanner *et al*., 2006). The components were divided into soluble and particulate. The soluble compounds are represented by the electron acceptor (NO_3^-) and electron donor, which is the organic carbon released from NOSS (represented as COD). Meanwhile, the particulate component corresponds to biomass (Biofilm). Finally, the processes of the system are defined as follows: electron donor and acceptor consumption; growth and decay of bacteria; transportation (diffusion); and the hydrolysis of structural carbon from NOSSs.

For the mathematical development of denitrification, a series of assumptions and considerations for the compartments, processes, components, and operating conditions were raised, which are detailed below.

Compartments

It is established that the NOSS is uniform in its structure, that is, it is considered homogeneous. The biofilm will completely cover the NOSS and will have a constant thickness (1D model) at a steady state. Rittmann *et al*. (2018), point out that a good approximation for biofilm models is that it be considered one-dimensional (1D). This biofilm will be considered deep, since it is a system in the absence of oxygen and will have a behavior similar to the oxidation of carbon at the bottom of a drip filter, whose biofilm thickness is in the range of 100 μm (Rittmann and McCarty, 2001; Rittmann *et al*., 2018). To represent the contribution of organic carbon from the NOSS, which will be represented by the COD, it was assumed that the connection with the biofilm would be a membrane, where the movement of the substrate will be based on the first Fick's Law.

Processes

Within the biofilm, the transformation of both the electron donor (COD) and the electron acceptor (NO_3^-) and the counter-gradient diffusion movement of both compounds were considered. The transformation of electron donors and acceptors was defined as double Monod because both substrates can be limiting factors of the system (USEPA, 1993). This Monod kinetics captures the effect of substrate concentrations on the behavior of the synthesis rates of bacteria (Wanner *et al.*, 2006).

Transport within the biofilm was considered by counter-gradient diffusion and in a normal direction to the plane of the biofilm; where the

COD has the direction from the NOSS towards the biofilm and the nitrate moves from the water mass towards the biofilm (Figure 1). These transformations and transport conditions are assumed to occur under conditions like a membrane biofilm reactor. According to Nerenberg (2016), this type of counter-gradient system is very favorable because it allows processes to occur in the central part of the biofilm.

Components

The biofilm was assumed to have a constant density. This assumption is consistent with the reports of systems in counter-gradient of membrane guards, where a single microbial structure develops (Nerenberg, 2016). Carbon released (COD) from NOSS will be represented by the J_{COD} flux, like modeling in membrane biofilm reactors (Matsumoto *et al*., 2007; Zhu *et al*., 2018). It was established that organic carbon will be contributed by the hydrolysis of structural carbon present in NOSS. It was assumed that the release of COD from NOSS will be homogeneous; that is, at any point in the NOSS-biofilm interface it will have the same concentration. Furthermore, it was established that nitrate will be the only electron acceptor and nitrogen source since the system operates under anoxic conditions.

Operating conditions

The operating temperature will be a constant 30 °C and, therefore, the inhibitory effect of the variation of this parameter is neglected. The system was operated at a pH between 6.5 and 8.5. This pH range has been defined for denitrification with NOSS as a carbon source because several researchers have verified that bacteria have adapted well in this range of values (Gibert *et al*., 2008; Ovez, 2006; Xu, Shao, Yin, Chu, and Yao, 2009). The diffusion coefficients within the biofilm for the substrates were considered constant and equal to 0.8 times the diffusion coefficient in water (Wanner *et al*., 2006). The diffusion of particulate material through the biofilm is ruled out.

For the case of denitrification modeling in batch reactors, biofilm detachment due to shear forces is ruled out, given that the simulation period was short (< 24 hours). Meanwhile, for continuous reactors, shear force detachment is also neglected, because low flow rates were used. In this case, it is detached by the aging of the biofilm. Rittmann and McCarty (2001) describe the shedding of bacteria by aging and its influence on adherent growth processes.

With these considerations, the balance equations were structured at the level of the biofilm (microscale) and the global system (reactors).

Mass balance in biofilm

At the biofilm scale, the mathematical approach was initially established based on the matrix of stoichiometric and kinetic coefficients of the system, considering the consumption of substrates (COD and nitrate), growth, and decay of bacteria (Table 2). The behavior of COD release from NOSS is considered first order (hydrolysis) and is reflected in the boundary conditions.

Table 2. Matrix of stoichiometric kinetic coefficients of the denitrification system.

Component, → i **J processes, ↓**	**1** **X_b**	**2** **X_I**	**3** **S_{COD}**	**4** **S_{NO3}**	**Reaction rates, ($M\ L^{-3}\ T^{-1}$)**
Increase	1		-1/Y	$-\left(\frac{1-Y}{2.86*Y}\right)$	$\mu_{max}*\left(\frac{S_{NO_3^-}}{K_{NO_3^-}+S_{NO_3^-}}\right)\left(\frac{S_{COD}}{K_{COD}+S_{COD}}\right)X_f$
Decay	-1			-1	bX_f
Inactivation		-1		-1	k_HX_f

From the matrix, the differential equations were derived that describe the processes of nitrate consumption and bacterial growth (Table 2).

Use of COD in biofilm:

$$\frac{\partial S_{COD}}{\partial t} = D_{COD}\frac{\partial^2 S_{COD}}{\partial z^2} - Z \cdot q_{max} \cdot X_f \left(\frac{S_{NO_3^-}}{K_{NO_3^-}+S_{NO_3^-}}\right)\left(\frac{S_{COD}}{K_{COD}+S_{COD}}\right) \tag{1}$$

Nitrate utilization in biofilm:

$$\frac{\partial S_{NO_3^-}}{\partial t} = D_{NO_3^-}\frac{\partial^2 S_{NO_3^-}}{\partial z^2} - \left(\frac{1-Y}{2.86 \cdot Y}\right) \cdot Y \cdot q_{max} \cdot X_f \left(\frac{S_{NO_3^-}}{K_{NO_3^-}+S_{NO_3^-}}\right)\left(\frac{S_{COD}}{K_{COD}+S_{COD}}\right) \tag{2}$$

Biomass kinetic process:

$$\frac{dL_f}{dt} = \int_0^{L_f}\left(Z \cdot Y \cdot q_{max} \cdot X_f \left(\frac{S_{NO_3^-}}{K_{NO_3^-}+S_{NO_3^-}}\right)\left(\frac{S_{COD}}{K_{COD}+S_{COD}}\right) - b \cdot X_f - k_H \cdot X_f\right) d_z \tag{3}$$

For these systems of differential equations, the boundary conditions are as follows:

Boundary conditions for substrate (COD):

$$z = 0 \quad \rightarrow \quad D_{COD}^{S}\frac{\partial S_{COD}}{\partial z} = D_{COD}^{f}\frac{\partial S_{COD}}{\partial z} \tag{4}$$

$$J_{COD} = \frac{D_{COD}^{f}}{L_{NOSS}} \frac{\partial S_{COD}}{\partial t} = \frac{D_{COD}^{f}}{L_{NOSS}} \cdot S_{COD_{max}} \cdot \left(1 - exp^{(-k_h \cdot t)}\right) \text{ (batch system)} \qquad (5)$$

$$J_{COD} = \frac{D_{COD}^{f}}{L_{NOSS}} \frac{\partial S_{COD}}{\partial t} = \frac{D_{COD}^{f}}{L_{NOSS}} \cdot \frac{S_{COD_{max}}}{(1 + k_h \cdot t)} \qquad \text{(continuous system)} \qquad (6)$$

$$z = L_f \quad \rightarrow \quad -D_{COD} \frac{\partial S_{COD}}{\partial z} = -D_L \frac{\partial S_{COD}}{\partial z} \qquad (7)$$

Boundary conditions for nitrate:

$$z = 0 \quad \rightarrow \quad \frac{\partial S_{NO_3^-}}{\partial z} = 0 \qquad (8)$$

$$z = L_f \quad \rightarrow \quad -D_{NO_3^-} \frac{\partial S_{NO_3^-}}{\partial z} = -D_L \frac{\partial S_{NO_3^-}}{\partial z} = J_{NO_3} = -D_L \frac{(S_i - S_0)}{L} \qquad (9)$$

General reactor mass balance

Once the differential equations that describe the denitrification process was defined, the mass balance for the reactor was carried out, where Equation (1), Equation (2), and Equation (3) were integrated. Nitrate

(J_{NO3}) and COD (J_{COD}) fluxes were defined for the liquid phase at the level of the entire system. Nitrate flow is from the water body to the biofilm (Equation (10)) and the COD flow is in the opposite direction, from the biofilm to the water body (Equation (11)):

$$J_{NO_3^-} = -D_L \frac{dS_{NO_3^-}}{dZ}\bigg|_{Z=L_f} \tag{10}$$

$$J_{COD} = -D_L \frac{dS_{COD}}{dZ}\bigg|_{Z=L_f} \tag{11}$$

It is assumed that the COD that is released into the water body is that which passes through the biofilm and was not consumed by the bacteria. This condition is raised because in denitrification studies with NOSS, organic carbon concentrations were recorded in the water body (batch reactor) or the effluent (continuous reactor) (Della-Rocca, Belgiorno, and Meric, 2006; Gibert *et al*., 2008; Soares and Abeliovich, 1998; Volokita, Abeliovich, and Soares, 1996a). Therefore, once the nitrate and COD fluxes were defined, the mass balance for the reactor (macro-scale) was established, as follows (Equation (12)):

$$V_B \frac{dS_B}{dt} = Q_{in}S_{in} - Q_{out}S_{out} + J_{DQO}A_F + r_BV_B \tag{12}$$

Where VB is the volume of water in the reactor; Q_{in} and Q_{out} are the outflows, respectively; S_{in} and S_{out} are the COD concentrations at the inlet and outlet of the reactor. A_F is the biofilm area; and r_B is the reaction that occurs in suspension (in the body of water). If the inflow and outflow flows are considered equal ($Q = Q_{in} = Q_{out}$), we have:

$$V_B \frac{dS_B}{dt} = Q(S_{in} - S_{out}) + J_{COD}A_F + r_B V_B \tag{13}$$

For a batch reactor, Equation (13) is as follows:

$$\frac{dS_B}{dt} = J_{COD}\frac{A_F}{V_B} + r_B \tag{14}$$

For a continuous flow reactor, reactions in the water body were ruled out, because it was assumed that the bacteria in suspension are quickly eliminated from the reactor by the flow; therefore, Equation 13 is as follows:

$$\frac{dS_B}{dt} = \frac{Q}{V_B}(S_{in} - S_{out}) + J_{COD}\frac{A_F}{V_B} \tag{15}$$

Application of the AQUASIM program for the simulation of the proposed mathematical model

The modeling of the system of differential equations described for denitrification was carried out with the AQUASIM program (Reichert, 1998c). This program was designed to simulate aquatic systems and for this, it uses the finite difference scheme to solve the differential equations (Reichert, 1998a; Reichert, 1998b).

For the simulation in the AQUASIM program of the NOSS-biofilm-water system (Figure 1), a first reactor was defined that corresponds to the NOSS, from which the COD will be provided and which is of the completely mixed type with the flow (Reichert, 1998c); and a second reactor, where the processes of transformation and consumption of electron acceptor and donor are presented (Reichert, 1998c). To link these reactors, the permeable membrane option of the AQUASIM program was used (Reichert, 1998c), since it is the way to represent the flow of COD from the NOSS.

The NOSS reactor that represents the volume of NOSS, which is inside the general reactor, was considered as completely mixed to represent the COD flow obtained by the hydrolysis of the organic carbon present (Equation (5)). The COD release behavior was considered first order (hydrolysis), due to reports of studies using NOSS as a carbon source (Deago and Pizarro, 2013; Deago and Pizarro, 2015).

Even though the diffuse layer between the biofilm and the water body was established in the conceptual model (Figure 1), for this first mathematical approximation this layer was omitted. It was defined to simplify this diffuse layer since it is one of the simplest biofilm models to simulate (Wanner *et al.*, 2006). Besides, to simulate the electron acceptor and donor counter-gradient system using AQUASIM, kinetic and stoichiometric parameters selected from denitrification studies were used

(Table 3). Regarding the batch and completely mixed with flow rectors, their characteristics are described below (Table 4).

Table 3. Parameters used in mathematical modeling obtained from similar studies.

Parameters	**Values**	**Reference**
Nitrate diffusion coefficient, D_{fNO3} (m^2/day)	0.00016	Picioreanu, Kreft and van Loosdrecht (2004)
COD diffusion coefficient, D_{fCOD} (m^2/day)	0.00015	Matsumoto, Terad and Tsuneda (2007)
Inactivation coefficient, b (d^{-1})	0.03	Horn and Telgmann (2000)
Endogenous depletion coefficient, k_H (d^{-1})	0.04	Horn and Telgmann (2000)
Initial biofilm thickness, L_f (µm)	100	Rabah *et al*. (2007)
Active biomass density in biofilm, X_f (g/m^3)	65 000	Horn and Telgmann (2000)
Monod's affinity constant of substrate K_{COD} (g COD/m^3)	19.50	Deago and Pizarro (2013)
Organic carbon release constant, ($_{kh}$) (d^{-1})	0.0837	
Monod's affinity constant (K_S) (g N-NO_3^-/m^3)	20.15	
Maximum nitrate assimilation rate (q_{max}) (g N-NO_3^-/g VSS/d)	5.61	
Net yield of bacteria (Y_H) (g COD/g COD)	0.55	
Stoichiometric ratio COD/NO_3^- (Z) (g COD/g N-NO_3^-)	2.225	Deago and Pizarro (2015)

Table 4. Physical characteristics of the modeled reactors.

Parameters	**Values**
NOSS reactor volume (m^3)	1.6E-5
General reactor volume (m^3)	0.00015
Membrane area (m^2)	0.0008
Biofilm area (m^2)	0.0008
Hypothetical membrane thickness between NOSS and biofilm (m)	0.0001
Flow in NOSS reactor (m^3/d)	0.0004
Flow rate in the main reactor completely mixed (m^3/d)	0.007

Sensitivity analysis

Sensitivity analysis is a way to evaluate or quantify the relationship of dependence of parameters used in models, concerning input conditions of a system (De-Pauw and Vanrolleghem, 2003). For the sensitivity analysis of the denitrification biofilm system using NOSS, the relative-absolute sensitivity function of the AQUASIM program was applied (Reichert, 1998c). This function is one of the most used because its measurement units are independent of the units of the parameters considered (Reichert, 1998a).

When applying this sensitivity function, 100 % changes were applied to the parameters to be evaluated, concerning the concentrations of donor and electron acceptor of the system. The sensitivity analysis was applied to the kinetic and stoichiometric parameters (K_s, q_{max}, Y_H), as well as the hydrolysis constant (k_h). The sensitivity analysis of these parameters was made for the input values of the system, which were nitrate (electron acceptor) and COD (electron donor). This type of sensitivity analysis using AQUASIM has been applied in similar biofilm studies (Arcangeli and Arvin, 1999; Matsumoto *et al*., 2007).

Results and discussion

Simulation results of batch reactors

The simulation of the batch reactor that was compared with the results of experimental tests carried out by Deago and Pizarro (2013), which contained pieces of the detrital material of *Saccharum spontaneum L.*, used as a source of carbon and support for the growth of biofilm. As it is a batch reactor, one of the simplifications of the system was to eliminate the inlet and outlet flow, leaving the mass balance as presented in Equation (14). Meanwhile, to represent the contribution of carbon from *Saccharum spontaneum L.*, the equation that represents the boundary conditions of batch systems was included (Equation (5)) (Zaman, 2010).

The parameters of Tables 3 and 4 were entered in the AQUASIM program and the system was modeled assuming that all the processes were carried out inside the biofilm.

Nitrate behavior in the supernatant

The denitrification simulation in the batch reactor was for a nitrate pulse with a concentration of 100 g/m^3. Nitrate is shown to decrease in the body of water concerning time. Nitrate depletion in the batch reactor occurred 340 minutes after starting the simulation (Figure 2). The results of the modeling were very close to the experimental values.

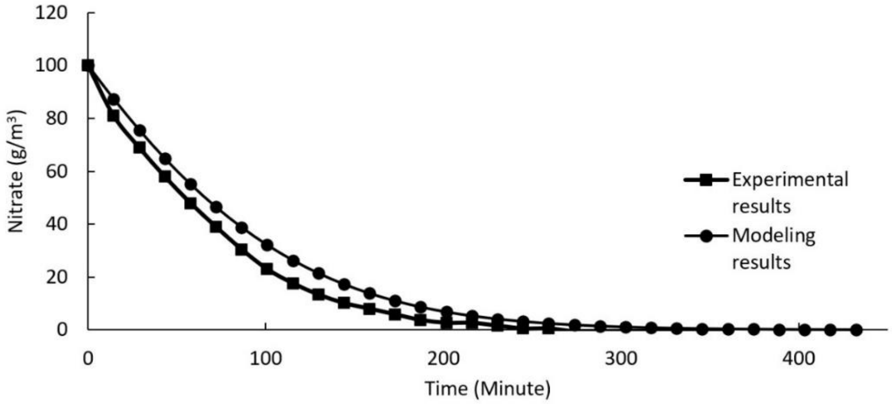

Figure 2. Mathematical and experimental results of nitrate depletion in batch reactor supernatant, having Saccharum spontaneum L. as a carbon source.

The results of the simulation of nitrate removal were closely adjusted to the experimental results, since the values obtained by Deago and Pizarro (2013) for the biokinetic parameters (q_{max}, K_s and Y_H), corresponded to tests with Organic carbon released from the detrital material of Saccharum spontaneum L. Similarly, the assumptions and assumptions allowed to adjust the mathematical model.

Simulation of COD release from NOSS

The COD simulation showed a rapid release in the first hours towards the batch reactor supernatant (Figure 3). This behavior is associated with the establishment of low biofilm thickness and therefore there is a lower denitrification capacity. Then there is a reduction in said COD level because it was used in denitrification. Once the nitrate in the supernatant is exhausted, close to the 5-hour simulation, an accumulation of COD begins.

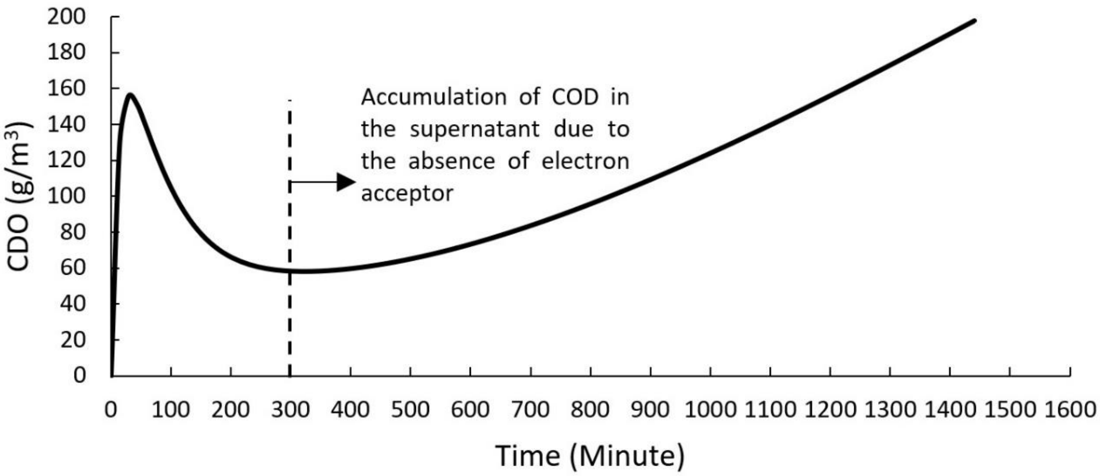

Figure 3. Results of the simulation of COD release from Saccharum spontaneum L. These values correspond to the theoretical COD accumulated in batch reactor supernatant.

The results of the simulation of organic carbon (represented by COD) released from *Saccharum spontaneum L.* correspond to a theoretical approach since at an experimental level it was not possible to determine the COD values in the range of hours. However, the simulation of the COD release behavior was like experimental results with *Saccharum spontaneum L.* reported by Deago and Pizarro (2013), where an accumulation of organic carbon in the batch reactor supernatant was observed. Deago & Pizarro (2013) indicate that the COD accumulated in the supernatant corresponds to the excess COD released from *Saccharum spontaneum L.* and that it was not consumed by the bacteria. This accumulation of organic carbon was also reported in other studies (Zhang, Feng, Hong, Hao, & Yang, 2012), which is an issue that needs to be controlled if NOSS is to be used in the denitrification of drinking water. If this release of carbon into the water is not controlled, it could become secondary pollution (Zhang *et al*., 2012).

Evolution of biofilm thickness

For the simulation of the biofilm in AQUASIM, an initial density and thickness of 65000 mg VSS/L and 100 μm, respectively, were defined (Table 3). The behavior of the thickness of the biofilm showed rapid growth in the first 200 minutes of simulation and then reaches a brief condition of steady-state until 300 minutes (Figure 4). After 300 minutes of simulation, there is a slight decrease in the thickness of the biofilm (Figure 4).

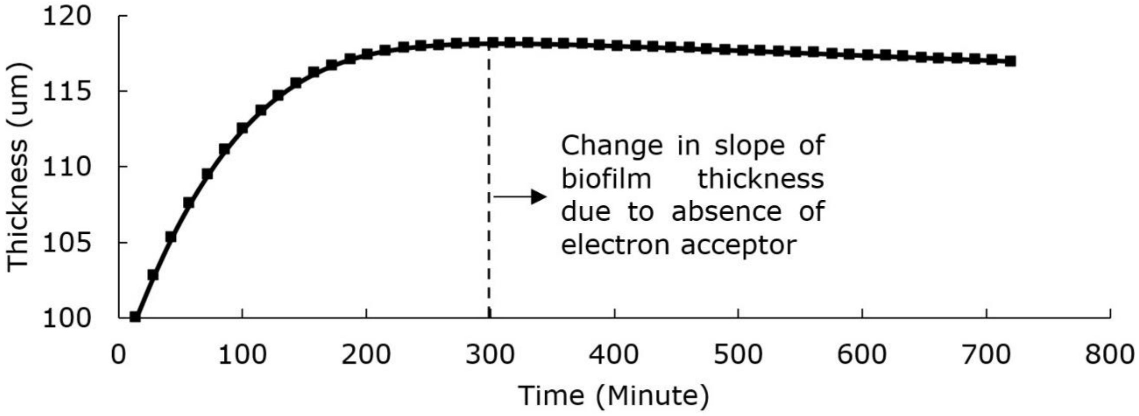

Figure 4. The behavior of the biofilm thickness reflects their dependence on nitrate, which represents the limiting factor in a batch reactor.

The decrease in biofilm thickness indicates that in batch reactors the nitrate concentration is a limiting factor. If the nitrate were kept constant, the behavior of the biofilm could reach a steady state, if the carbon present in the *Saccharum spontaneum L.* is not depleted. This was carried out and verified in the simulation of the fully mixed reactor. For the batch system, no inactivation or shedding of bacteria was considered, since the simulation time was short (<24 hours) and it was only evaluated for a nitrate pulse. This investigation is similar to that reported in the study of denitrification of groundwater (Calderer *et al*., 2010).

Simulation of fully mixed reactors with flow

For the simulation of a fully mixed reactor with the flow, it was established that the maximum bioavailable COD in *Saccharum spontaneum L.* was 3638.5 g COD/m^3 (Deago and Pizarro, 2013). The volume of this reactor was equal to that used in the batch test (Table 4). A flow rate of 0.0007 m^3/d was established to maintain a hydraulic retention time equivalent to the time in which the nitrate was completely consumed in the batch reactors (300 minutes). In this simulation, conditions with and without biofilm detachment were considered. Besides, the release of COD from *Saccharum spontaneum L.* was represented by Equation (6), for continuous systems in steady-state (Mata-Alavarez, 2003).

Nitrate simulation in the supernatant of a fully flow-mixed reactor

The nitrate concentration in the influent of the continuous system is the same as that used in batch tests (100 g N-NO$_3^-$/m^3). During the simulation in the AQUASIM program, the behavior of nitrate decreased rapidly in the first 8 hours to a concentration of 20 g/m^3 (Figure 5b); then the nitrate decreased slightly to a minimum value of 18 g/m^3 and remained in a steady-state condition for the case with a detachment of biofilm up to 10 days of simulation (Figure 5b). After the tenth day, a rise in the nitrate concentration in the fully mixed reactor effluent began to reach an approximate value of 80 g/m^3 towards the 100-day simulation period (Figure 5a).

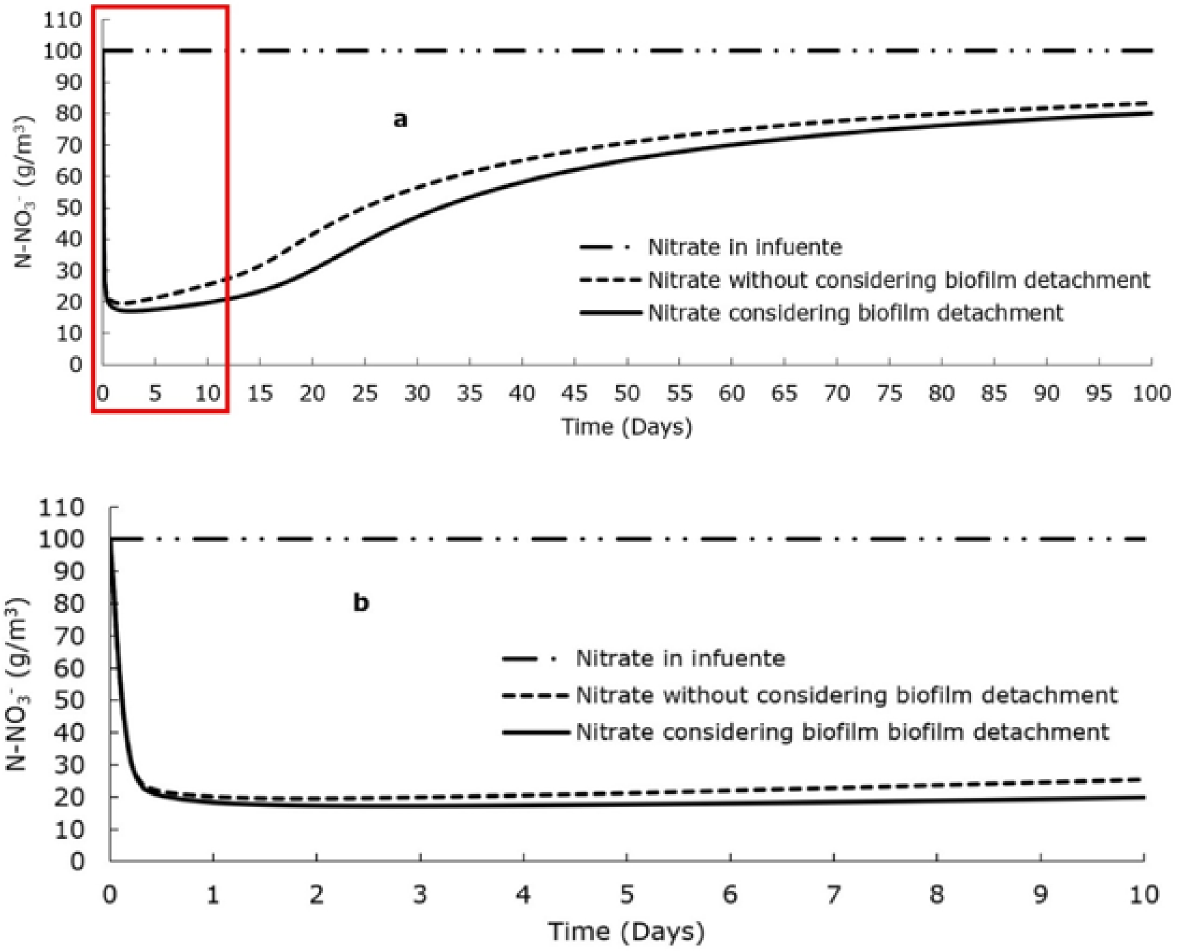

Figure 5. Nitrate simulation at completely mixed rectors with and without biofilm detachment: a) nitrate increased over time, due to the shortage of organic carbon; b) in the first eight hours of operation of the reactor there was a rapid reduction of nitrate.

The results of the modeling, for the behavior of nitrate in the effluent, reflect that the greatest removal occurs in the condition with biofilm detachment (Figure 5). This is associated with lower resistance to penetration into the biofilm, which depends on its density and thickness. Matsumoto *et al.* (2007) carried out mathematical modeling of a membrane reactor for simultaneous nitrification-denitrification processes, finding that biofilm with thick thicknesses was unfavorable for the system. Lakshmi and Setty (2008) found that the external nitrate transfer coefficient (K_L) increased with increasing biofilm thickness. According to Martin and Nerenberg (2012), the effect of biofilm thickness is a limitation in counter-gradient biofilm systems, since it slows the flow of substrates within the biofilm, limiting metabolic activity. This characteristic must be studied in greater detail for denitrifying systems, which use NOSS as a carbon source and support for biofilm growth.

The behavior of nitrate in the effluent (Figure 5) can be attributed to several aspects: the first factor to influence the reduction of the denitrification rate is the retention time defined in the reactor. Hydraulic retention time is a factor that affects the efficiency in nitrate removal (Hashemi, Heidarpour, & Mostafazadeh-Fard, 2011; Soares, 2000; Wang and Wang, 2013). The other aspect that would affect nitrate removal is the amount of available NOSS. If the mass of NOSS is increased, the longer the nitrate removal time will be, that is, the useful life of the NOSS will be extended. Ovez (2006) indicates that the greater the mass of NOSS, the greater it's surface area, and therefore there will be greater growth of biofilm, which will increase nitrate consumption. The greater available area of NOSS results in greater hydrolysis of carbon, which is released at a constant rate.

Simulation of COD behavior in the fully mixed reactor effluent

For the continuous reactor, the COD simulation showed high concentrations in the effluent on the first day; then there was a rapid decline until reaching zero around day 30 (Figure 6). The COD varied little with the condition of shedding of bacteria, concerning that in which shedding was not considered. This behavior indicates that, in the first days, there was a higher concentration of COD in the effluent than the bacteria could assimilate. Later, when generating an increase in the thickness of the biofilm (Figure 6), the COD decreased in the completely mixed reactor effluent. This suggests that all the COD is assimilated by bacteria within the biofilm, for nitrate reduction and cellular synthesis.

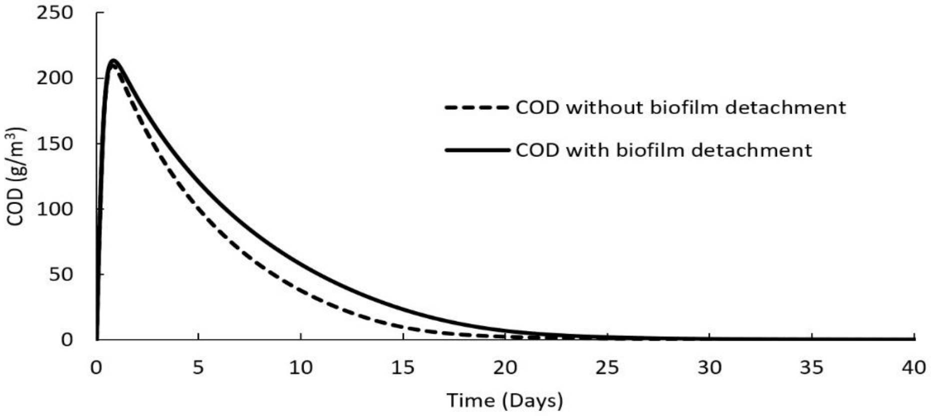

Figure 6. Results of mathematical modeling for COD in the fully mixed reactor effluent. The results are similar assuming conditions with and without biofilm detachment.

This counter-gradient system where the COD comes from NOSS and nitrate from the water body is like what occurs in nitrate removal in biofilm reactor systems with membranes, where a double Monod equation system is proposed. Martin and Nerenberg (2012) determined that in these counter-gradient membrane systems the condition of relative activity is met, which occurs when one of the substrates (acceptor or electron donor) is exhausted, said relative activity becomes zero.

Unlike membrane-based systems, the limiting factor in denitrification using NOSS is the constant contribution of organic carbon over time. This release of organic carbon depends on the lignin content (Chandler, Jewell, Gossett, Vansoest, & Robertson, 1980) and the ability of heterotrophic bacteria to colonize and hydrolyze the bioavailable carbon in the surface area of materials (Vavilin, Rytov, and Lokshina, 1996).

Simulation of biofilm evolution in fully mixed flow reactors

The simulation in AQUASIM of the growth of the biofilm has not achieved a condition of steady-state in the condition without detachment; while in the condition with detachment there was a decrease in the slope of the graph towards the end of the simulation, which shows a tendency to stabilize (Figure 7). At the end of the simulation, the biofilm thickness with the condition without detachment was 2150 µm, while considering bacterial detachment the final thickness was 1500 µm.

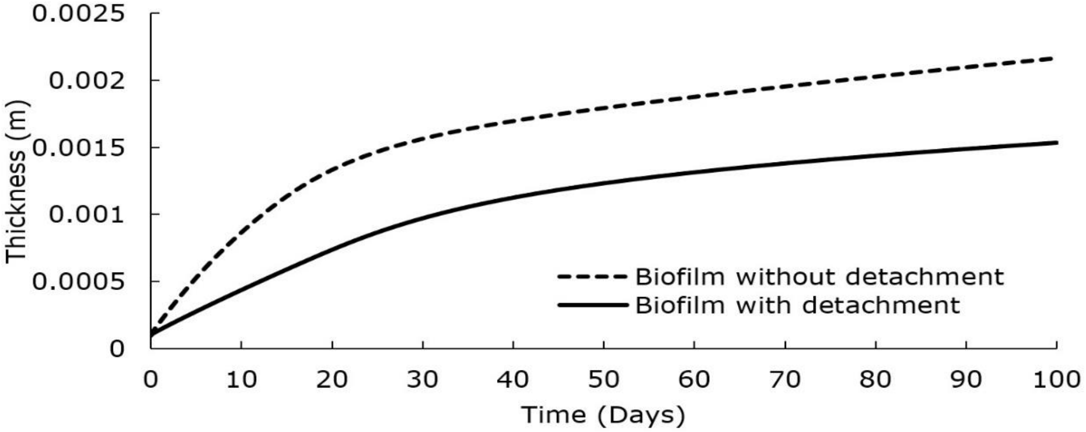

Figure 7. Results of the simulation of the biofilm adhered to *Saccharum spontaneum L.* in a reactor completely mixed with flow. The COD in the

reactor without biofilm detachment had a higher growth for the given condition.

The process of biofilm detachment has been modeled in denitrifying systems using AQUASIM (Horn and Telgmann, 2000). This tool is very useful for estimating biofilm thickness, as this is one of the most difficult parameters to determine under experimental conditions. For example, with this modeling, it is possible to know how biofilm growth behavior is. In the case of the fully mixed reactor, it is observed that there is a rapid growth of the biofilm, especially in the case of the reactor without shedding (Figure 7). This behavior is associated with a decrease in the contribution of soluble organic carbon (Figure 6).

The detachment phenomenon must be considered in biofilm-based treatment systems, despite low water flows. Rittmann and McCarty (2001) state that the shear stresses generated by the speed of the water on the surface of the biofilm are due to erosive effects; while anaerobic conditions, deep in the biofilm, can be the cause of the detachment of large portions of biomass. This detachment process contributes biomass from the biofilm matrix to the water body (Wanner *et al.*, 2006). The release of bacteria from denitrifying systems, towards the effluent of experimental tests has been reported (Soares, 2000; Soares and Abeliovich, 1998; Volokita, Belkin, Abeliovich, and Soares, 1996b).

The effect of biofilm thickness was not relevant for the case of COD behavior in the effluent (Figure 6), since by simulation day 30 the COD behavior was very similar in both conditions. However, in the case of nitrate, biofilm thickness had a greater influence (Figure 5), which is associated with resistance to the mass transfer of the biofilm. For example, after 30 days of operation in the system with detachment, the nitrate concentration in the effluent was 47 g/m^3, while in the scenario without detachment it was 56 g/m^3 (Figure 5a). This agrees with what is indicated by Di-Capua, Papirio, Lens and Esposito (2015), who point out that nitrate mass transfer is severely affected, due to a greater increase in biofilm thickness.

Counter-gradient simulation of nitrate and COD within the biofilm in a fully flow-mixed reactor

In the previous sections, the behavior of the electron donor and acceptor was shown at a general level or macro scale; that is, the compartment of the volume of water or effluent. However, to understand the system totally, it is necessary to know what happens at the biofilm level (micro-scale). In this sense, the simulation with AQUASIM allowed obtaining concentration profiles, both electron donor (COD) and the electron acceptor (nitrate). The COD results show a decrease as the biofilm thickness increased (Figure 8a). Meanwhile, the nitrate profiles reflected that, as the simulation time elapsed, the nitrate reduction capacity decreased; that is, nitrate was quickly consumed in the most superficial layers of the biofilm, but its concentration was higher at the surface of the biofilm (Figure 8b).

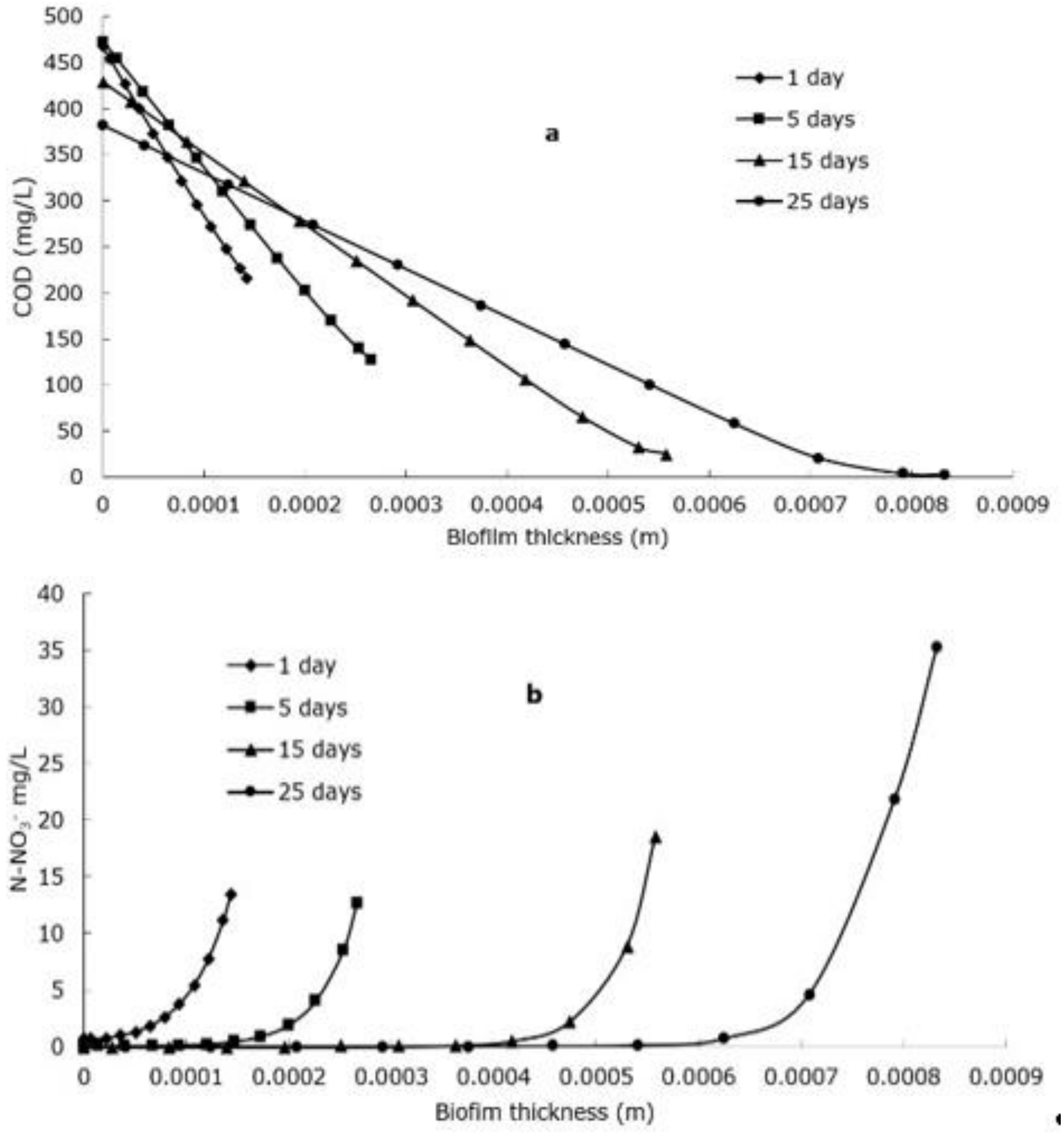

Figure 8. Profile of COD (a) and nitrate (b) concentrations in the biofilm for different simulation times.

The consumption of COD was higher within the biofilm as its thickness increased, which is associated with the favorable adaptation of the bacteria that comprise it. Meanwhile, the behavior of nitrate showed that as the thickness of the biofilm increased, its concentration became zero each time more distant from the origin (Figures 8b). When comparing the profiles of COD and nitrate within the biofilm (for example, on day 5), it is observed that COD decreases linearly, while nitrate rapidly decreased exponentially (Figure 9).

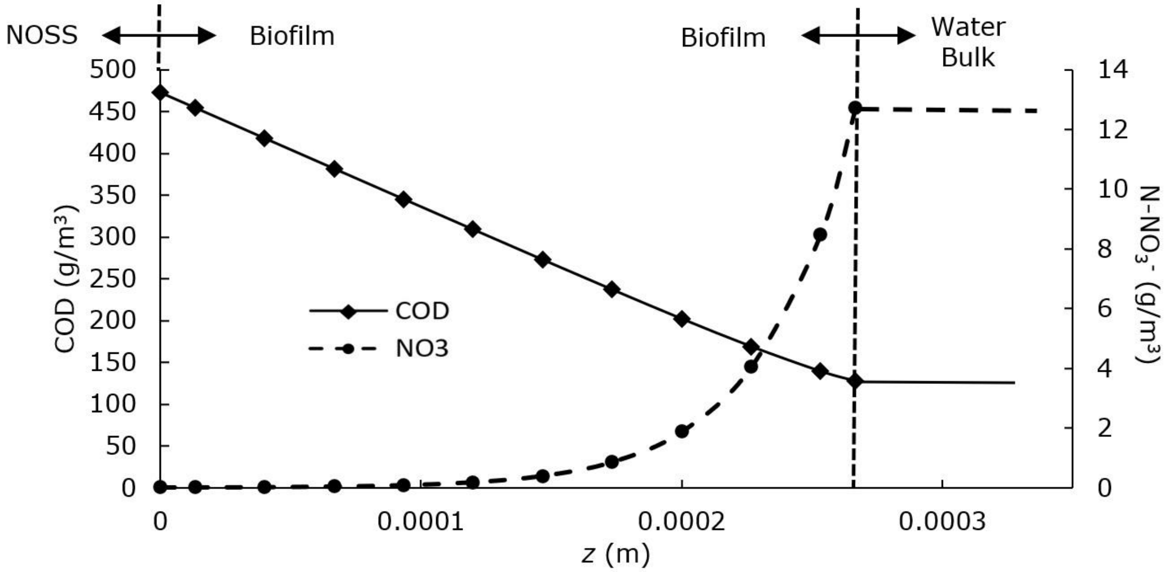

Figure 9. Comparison of the COD and nitrate concentration profiles within the biofilm on the fifth day of simulation.

It is presumed that these COD and nitrate behaviors within the biofilm are caused by the limitation of mass transfer, due to the increase in the thickness of the biofilm. On a micro-scale, what is found in the general system is reflected, where greater resistance to mass transfer of the nitrate provided from the water mass is evidenced; thus, corroborating what was indicated by Di-Capua *et al.* (2015). Similarly, Nicolella, Pavasant and Livingston (2000) found that the removal efficiency of a substrate in a counter-gradient membrane system decreases with increasing thickness of the biofilm.

Sensitivity analysis

The degree of sensitivity for the control variables (nitrate and COD) was weighted according to Arcangeli and Arvin (1999): great effect, significant, moderate, and insignificant. These weights are defined in Table 5. Similarly, the sensitivity analyzes for each parameter evaluated are presented graphically (Figure 10 and Figure 11).

Table 5. Assessment of the sensitivity analysis of evaluated parameters.

Parameters	COD	Nitrate
Net yield of bacteria (Y_H) g VSS/g COD	+++	+++
Organic carbon hydrolysis constant, k_h (d-1)	+++	+++
Maximum specific growth rate (μ) (d^{-1})	+	++
Monod's affinity constant (Ks) (g N-NO$_3^-$/m^3)	-	++

Great effect (+++); Significant (++); Moderate (+); insignificant (-)

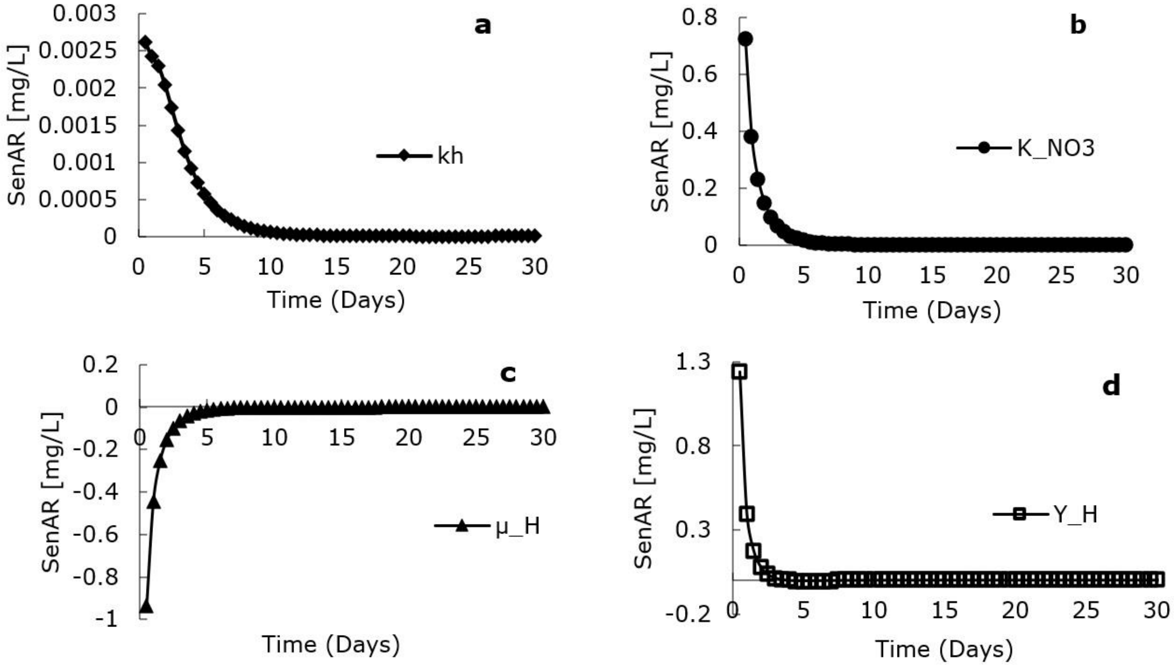

Figure 10. Results of sensitivity analysis for nitrate concentrations in the biofilm matrix concerning the parameters: a) kh; b) K_NO3; c) μ_H, and d) Y_H.

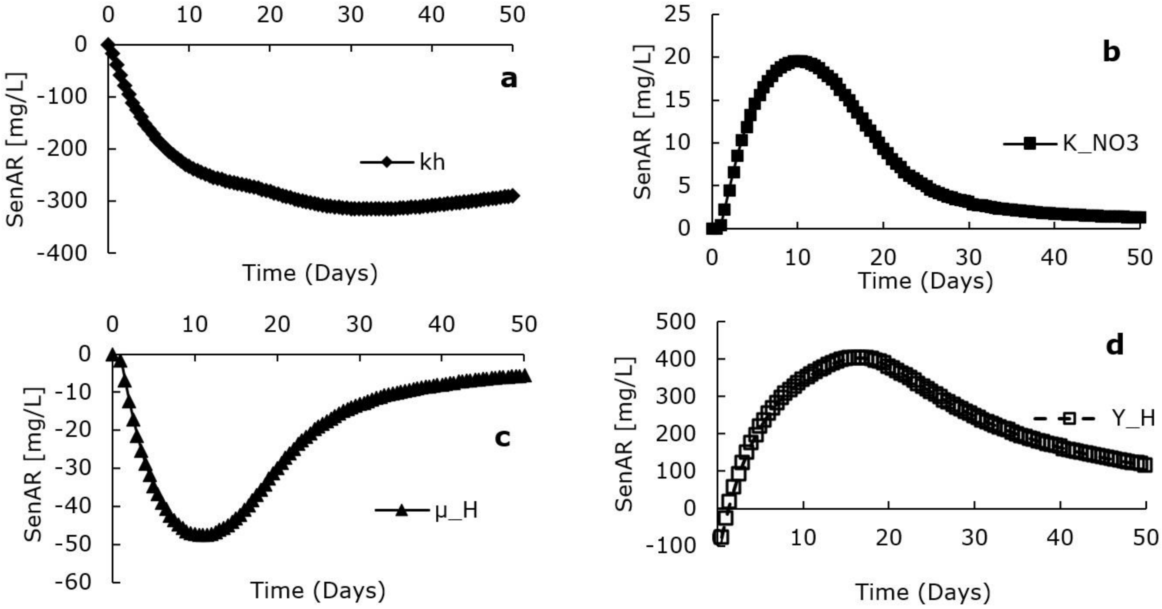

Figure 11. Results of sensitivity analysis for COD concentrations in the biofilm matrix concerning the parameters: a) kh; b) K_NO3; c) μ_H, and d) Y_H.

The sensitivity analysis obtained with the AQUASIM model showed that the significance for nitrate in descending order of the evaluated parameters was: Y_H, k_h, μ_H, and K_{NO3} (Table 5). The parameters Y_H and k_h are highly significant, while the parameter μH has a moderate significance. For the K_{NO3} parameter, the degree of sensitivity is negligible. Graphically, it is observed that the sensitivity of the nitrate concentration concerning the parameters Y_H, k_h, and K_{NO3} is a maximum close to zero, and all present a decreasing exponential behavior until reaching values of zero at different times (Figure 10d, Figure 10a, and Figure 10b). The sensitivity of the nitrate concentration for μH also has an exponential behavior, but its maximum value is negative (Figure 10c).

The importance or significance of the analyzed parameters, to the COD concentrations, has the same order as that obtained for nitrate, which was Y_H, k_h, μ_H, and K_{NO3}. However, for COD all of them have a degree of sensitivity from very significant to significant (Table 5). For the case of the Y_H parameter, the sensitivity function starts from zero to a negative value and then increases to a maximum and subsequently decreases (Figure 11d). For the parameter k_h, the values of the sensitivity function start from zero and increase negatively (Figure 11a). In the case of the μ_H parameter, it is observed that it also starts from zero and increases negatively up to a time close to 10 days and then decreases (Figure 11c). While the graphical result of the analysis for the K_{NO3} parameter showed that it starts from zero and increases positively towards a maximum value, decreasing later (Figure 11b). This behavior was like that obtained with the Y_H parameter.

As described, the parameters Y_H, k_h, and μ_H are fundamental to adequately represent the mathematical model of denitrification in a counter-gradient biofilm system, using NOSS as the carbon carrier and source. This sensitivity analysis corroborates the importance of hydrolysis, as a process that controls the release of organic carbon, for nitrate reduction. The behavior of the sensitivity analysis was similar to the AQUASIM modeling of membrane reactors with biofilms, where the μ_H and Y_H parameters were the most significant and the K_{NO3} parameters were the least significant (Matsumoto, *et al.*, 2007).

The simulation results allow us to visualize advantages and limitations such as those that have been described. In the case of the presence of COD in the treated effluent, it was observed that in the first 5 days (Figure 6) the concentrations were high. This information would allow evaluating the possibility of applying subsequent treatments, such as the use of sand filters; which have been tested to control excess COD and biofilm released in the effluent from denitrifying systems (Aslan, 2005; Aslan & Cakici, 2007; Della Rocca, *et al.*, 2005).

Conclusions

With this research it was possible to generate a mathematical approximation of denitrification, using NOSS as a biofilm carrier and the only source of organic carbon. Although assumptions were established and simplifications were made, it was possible to predict the behavior of the release and depletion of organic carbon from the NOSS. Similarly, nitrate removal was simulated based on the behavior of carbon, and the biofilm formed around NOSS. These results allowed us to demonstrate that mathematical modeling is a useful tool in the study of denitrification, using NOSS as a support for the growth of bacteria and a source of organic carbon; as well as understanding the behavior in the interaction between NOSS, biofilm, and nitrate. Similarly, is a predictive tool to estimate the useful life of NOSS as sources of organic carbon. All these simulations were possible with the support of the free software AQUASIM.

In these simulations, it was identified that, in batch reactors, the limiting factor was the nitrate concentration and in continuous reactors, it was the contribution of COD from NOSS. In batch reactors the nitrate dosage was punctual and once the COD consumption was exhausted, it was stopped. Subsequently, this COD accumulated in the supernatant. Meanwhile, in continuous reactors there was continuous dosing of nitrate, which stimulated the hydrolyzation of the bioavailable carbon in the NOSS; but late in the simulation time, this carbon was exhausted and consequently, the reduction of nitrate decreased, increasing its concentration in the effluent.

With the sensitivity analysis carried out, it was shown that the most significant parameters were the net yield of bacteria (Y_H) and the hydrolysis constant (k_h). The significance of these kinetic parameters shows the relevance of deepening the study of the interaction between NOSS and the biofilm that acts on these materials. Also, it is required to further strengthen the understanding of NOSS as a carbon source for denitrification, studying scenarios with variations in flow, temperature, nitrate loading, and mass of NOSS used.

With the development of this mathematical model, denitrification simulations can be performed in wastewater treatment systems using NOSS as a carbon source. Similarly, monitoring of drinking water systems could be carried out, evaluating, for example, carbon release rates from NOSS, since this is a risk if not controlled.

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