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Development of a model for PHA-based denitrification in a packed bed reactor

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ABSTRACT

A model of the denitrification on a polyhydroxyalkanoate (PHA) based reactor for recirculating aquaculture was developed. PHA is a family of non-water soluble bioplastics produced by bacteria. The PHA formulation used in this work was polyhydroxybutyrate (PHB). The model considered nitrate concentration, dissolved oxygen, organic carbon and biomass concentration as the most significant variables. The developed model represents adequately the nitrate reduction with the medium used, for nitrate under 100 ppm NO_3^--N.

In the conditions tested, an average ratio of 2.92 g PHA to 1 g NO_3^--N reduced was found. The model results showed a denitrification rate of 2.97 kg m^{-3} d^{-1} for ranges from 10 to 50 mg NO_3^--N L^{-1}. Using this model as a management tool, the required size of denitrification units and PHA recharging time can be predicted based on the expected nitrate loading and the time between PHA recharges desired. The unit sizing should be done for the maximum load expected. The slow rate and the energy required for PHA hydrolysis, make it unavailable as electron donor after the nitrate is consumed, so it will not promote the formation of sulfides. The model can be modified for other biodegradable non-water soluble medium by changing the hydrolysis constant, which must be determined experimentally.

1. Introduction

Increasing regulations and the need for a reliable supply of good quality water has promoted the expansion of water reuse in aquaculture. Recirculating aquaculture systems (RAS) allow the water to be reused for prolonged periods of time and reduce the need of water exchange. This reduction decreases the possibility of introducing pathogens to the culture systems and the release of biological contaminants to the environment. In order to achieve a high level of reuse, the water must be treated before returning it to the culture system to remove waste products. These products include organic matter and nitrogenated waste including ammonia, nitrite and nitrate.

Removal or conversion of organic compounds and ammonia is generally achieved through biofiltration. In the most common configuration, aerobic biofiltration is used to oxidize organic compounds to CO_2 and nitrogenated waste to nitrate (NO_3^-). Nitrogenated waste production is directly related to the amount and protein introduced in the system (Losordo et al., 1998). The CO_2 is released as gas, but nitrate stays in the system as salt. The increase in the hydraulic retention times in RAS causes an accumulation of nitrates in the system, reducing the suitability of the water for reuse. Although nitrate has been deemed as less harmful than other nitrogenated compounds like nitrite and ammonia in natural waters, the high levels they can achieve in recirculating systems needs to be considered (Tórz et al., 2010).

Aquatic species at different life stages are impacted in diverse ways by nitrates. High nitrate content can cause slower growth, low reproduction rates, low hatching, increasing incidence of diseases, delayed hatching times and higher mortality (Morris et al., 2011; Shimura et al., 2002; Tsai and Chen, 2002). In marine species, the concentration at which toxic, lethal or sub-lethal effects have been detected can vary from 2.2 to more than 5000 mg-NO_3^--NL^1, with larvae and brood stock as the most sensitive stages (Canadian Council of Ministers of the Environment, 2012). Additionally, nitrate can be reduced to a more toxic form (nitrite) in low oxygen conditions.

Besides the direct effect on aquaculture species, nitrate rich waters discharge can induce eutrophication of the receiving waters,

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Nomenclature

Y_o	aerobic bacterial yield coefficient ($mg(mg\,O_2)^{-1}$)
Y_n	anoxic bacterial yield coefficient ($mg(mg\,NO_3\text{-}N)^{-1}$)
K_n	nitrate half saturation constant ($mg\,L^{-1}$)
K_o	oxygen half saturation constant ($mg\,L^{-1}$)
K'_o	oxygen inhibition constant ($mg\,L^{-1}$)
K_d	bacteria decay rate (d^{-1})
VDR_{max}	maximum volumetric conversion rate ($mg\,mL^{-1}\,d^{-1}$)
VORmax	maximum volumetric oxygen removal rate ($mg\,mL^{-1}\,d^{-1}$)
K_x	half saturation bacteria constant ($mg\,L^{-1}$)
T_N	nitrate consumption rate ($mg\,NO_3\text{-}N\,L^{-1}\,d^{-1}$)
T_O	oxygen consumption rate in the denitrification unit ($mg\,NO_3\text{-}N\,L^{-1}\,d^{-1}$)
O	oxygen concentration ($mg\,L^{-1}$)
N	nitrate nitrogen (NO_3-N) concentration ($mg\,L^{-1}$)
X	denitrification bacteria (mg)
X_t	total bacteria (mg)
V_{PHA}	volume of PHA (mL)
V	volume of the water in the denitrification reactor (mL)
PHA	mg PHA ($mg\,NO_3\text{-}N$)$^{-1}$
PHA_n	mg PHA ($mg\,NO_3\text{-}N$)$^{-1}$
PHA_o	mg PHA ($mg\,O_2$)$^{-1}$
δV	incremental volume of water (mL)
δz	length of the model reactor cell (cm)
A	cross sectional area of the reactor (cm^2)
Q	flow of water in the reactor

promoting algal blooms and high oxygen consumption as the algae dies off (Rabalais, 2002). In freshwater, nitrate discharges can also impact the quality of potable water sources. This has lead agencies such as U.S. Environmental Protection Agency (USEPA) to maintain a limit to the nitrate and nitrite nitrogen concentration in potable water to 10 and 1 mg-N L^{-1} respectively and has considered its control a priority (USEPA, 2010). In a 2004 Final Rule, the USEPA established effluent limitation guidelines for aquaculture facilities. This rule indicates that nitrate in aquaculture effluents is often above the maximum contaminant level (MCL) established by USEPA.

To reduce the amount of nitrate discharged from aquaculture systems and the accumulation of this compound in recirculating water, several approaches have been used. The most commonly used process for nitrogen removal is biological denitrification (Van Rijn et al., 2006). Nitrate is reduced to nitrogen gas through a series of steps carried out by bacteria. Although there is a large microbial diversity present in the denitrification units, for most of the microbes the process requires an energy rich carbon source. Traditionally, water soluble carbon sources such as methanol or acetate have been used as electron donors for denitrification. This approach speeds the acclimation of the bacteria population providing them with a readily available carbon source. The use of soluble carbon sources creates problems such as the need for sophisticated dosing control to prevent overdosing of carbon (Lee et al., 2000). An excess of carbon in the absence of nitrate in an anaerobic environment can reduce the redox potentials. Low redox potentials promote the reduction of sulfates and the production of toxic sulfides. The use of a non-water soluble source of organic carbon can support the denitrification process. PHA, a non-water soluble bacterially produced bioplastic is a viable carbon source for denitrification (Boley et al., 2000). The hydrophobic characteristic of the bioplastic prevents the excess release of carbon to the recirculating water that can occur with soluble carbon sources, simplifying the dosing control. The most common forms of PHA are PHB (polyhydroxybutyrate) and PHV (polyhydroxyvalerate) and their co polymers (PHBV).

In this paper, the nitrate reduction with PHA in the form of PHB as a carbon source was modeled, taking into account the main effects controlling this process: nitrate concentration, dissolved oxygen, organic carbon and biomass concentration. Other important parameters such as temperature and salinity have been taken into account through a modification of the constants of the model (Gutierrez-Wing et al., 2012).

1.1. Stoichiometry

Half reactions for the denitrification process with PHA (in the form of polyhydroxybutyrate or PHB) as electron donor or carbon source and nitrate as electron acceptor were calculated. The stoichiometric ratios of NO_3^--N and O_2 to PHB consumption were used for the initial model setup. The reactions assume a reduction to nitrogen gas and complete oxidation of the electron donor. The model also assumes that the denitrification unit is located after a biological filter, where the ammonia is oxidized to nitrate. The half reactions for nitrate reduction and cell synthesis are as described by Metcalf and Eddy (Tchobanoglous et al., 2003).

Electron acceptor:

$$0.2\,NO_3^-\text{-}N + 1.2\,H^+ + e^- = 0.1\,N_2 + 0.6\,H_2O \quad (1)$$

Cell synthesis:

$$0.03571\,NO_3^-\text{-}N + 0.1786\,CO_2 + 1.0357\,H^+ + e^- = 0.03571\,C_5H_7O_2N + 0.3929\,H_2O \quad (2)$$

Electron donor:

$$0.22222\,CO_2 + H^+ + e^- = 0.05556\,C_4H_6O_2 + 0.3333\,H_2O \quad (3)$$

The total reaction considering PHB as the electron donor and 35% conversion to cells and 65% for energy is:

$$0.1425\,NO_3^-\text{-}N + 0.05556\,C_4H_6O_2 + 0.1425\,H^+ = 0.0125\,C_5H_7O_2N + 0.065\,N_2 + 0.15972\,CO_2 + 0.19417\,H_2O \quad (4)$$

The half reaction per mole of e^- transferred for oxygen (Tchobanoglous et al., 2003) is:

$$0.25\,O_2 + H^+ + e^- = 0.5\,H_2O \quad (5)$$

From Eq. (4) a ratio of 2.54 g PHB ($g\text{-}NO_3\text{-}N$)$^{-1}$ reduced in the denitrification process is obtained. From Eqs. (1) and (5) an equivalent of oxygen of 2.86 g O_2 ($g\,NO_3\text{-}N$)$^{-1}$ is calculated for the aerobic reaction for PHB oxidation.

2. Methods

2.1. Development of the denitrification model

The biological denitrification process follows the kinetic equations used by many authors to describe heterotrophic growth and substrate utilization (Tchobanoglous et al., 2003) with one important difference: the inhibitory effect of dissolved oxygen (Hartsock and Shapleigh, 2010; McKenney et al., 1994; Patureau et al., 2000; Shapleigh, 2011; Strong and Fillery, 2002; Tchobanoglous et al., 2003).

PHA, a non-water soluble carbon source, can be used as the basis of a self-regulating denitrification system. The use of PHA as a carbon source and support medium differs from the equations used to

describe a soluble carbon source, like the commonly used methanol. For soluble carbon, the medium bed volume is constant. In contrast, the PHA bed is reduced as the denitrification proceeds, so the support medium for the bacteria is proportional to the amount of PHA present in the reactor and the amount of bacteria. The growth of the bacteria occurs in two distinct phases: aerobic and anoxic. The denitrification unit used is a packed bed column that may act as a plug flow reactor. First the oxygen then nitrate is reduced as the water flows through the column. The model is based on a maximum volumetric denitrification rate (VDR_{max}), maximum oxygen consumption rate (VOR_{max}) and a limitation on the maximum bacterial growth rate. The oxygen concentration has an inhibitory effect on the denitrification process and on the nitrate consumption rate. The representation of this effect takes the form of an inhibition term:

$$\frac{K_o'}{K_o' + DO} \tag{6}$$

The development of a model for recirculating aquaculture denitrification with PHA as a carbon source can be based on Eqs. (1)–(6), provided certain conditions are met. The conditions considered in this model, which represent observations from experimental work, are:

a. The input flow is small enough to reduce the oxygen input.
b. The volume of the water in the denitrification reactor is small compared to the total volume of water from the recirculating system.
c. The amount of biodegradable soluble chemical oxygen demand (bsCOD) that is not provided by the PHA is negligible (representing the scenario were the denitrification unit follows an aerobic biofilter).
d. The filter is managed to maintain a thin biofilm cover on the medium to prevent clogging.
e. The carbon release from the PHA is related to bacteria concentration and all of the hydrolyzed material stays in the biofilm.
f. The total bacterial biomass is limited by the specific surface area.

The denitrification biomass is composed of heterotrophic facultative anaerobic bacteria, that can switch between oxygen and nitrate as electron acceptor. Oxygen is used first as a more energetically favorable electron acceptor followed by nitrate. Based on the kinetic equations (Eqs. (1)–(5)), the rate of change of the nitrate, oxygen, carbon source and biomass in the denitrification unit for the system shown in Fig. 1 are described. The system has a culture tank, an aerobic biofilter for the conversion of ammonia to nitrate and a denitrification unit, to convert nitrate to nitrogen (Gutierrez-Wing et al., 2012). Only the culture tank and the denitrification unit were modeled. The denitrification unit in a commercial unit may work as a plug flow reactor. The data used to develop and test this model were obtained in a series of five reactors. Each one of the reactors can be modeled as a completely mixed reactor and a series of the complete mixed reactors can be used to model a system operated as a plug flow. The numerical solution for a plug flow simulation is closer to the analytical solution as the number of reactors is increased (Chapra, 1997). For this study, the model was designed in five contiguous cells with length increments of 5 cm to match the conditions of the data collection. A separate module was considered for the culture tank. This module acted as a mixing tank and had an oxygen transference component. The PHA consumed was calculated for the complete unit and for the individual cells. The process is controlled by the concentrations of PHA, nitrogen and oxygen in the denitrification unit. For system management, the target is the concentration in the culture tank (Fig. 1). The model was developed using the Stella software® from High Performance Systems Inc.

In a completely mixed reactor, the rate of nitrate utilization, with an inhibition term due to the oxygen, depends on the nitrate and biomass concentration (Eqs. (7) and (8)). VDR_{max} is defined as the maximum volumetric nitrate conversion rate (g-NO_3^--N converted (m^3 PHA $d)^{-1}$) and represents the maximum observed volumetric denitrification rate. T_N is the nitrate consumption rate in the denitrification unit that depends on oxygen, nitrate and bacteria concentration and the PHA volume.

$$\frac{\partial N}{\partial t}V = Q(N_i - N) - T_N V \tag{7}$$

$$\frac{\partial N}{\partial t}V = Q(N_i - N) - \mathrm{VDR}_{max} V_{PHA}\left(\frac{N}{K_n + N}\right)\left(\frac{K_o'}{K_o' + O}\right)\left(\frac{X_T}{K_x + X_T}\right)V \tag{8}$$

The limiting factors considered are nitrogen and biomass that appear as Monod-type terms in Eq. (8), with the effect of oxygen represented as an inhibition term. The biomass is expressed as a Monod relation as observations of denitrification units indicate that the denitrification increases with the increase of bacterial biomass until the biofilm thickness limits the diffusion of carbon and the water flow. The oxygen mass balance (Eqs. (9) and (10)) is dependent mainly on the oxidation of available carbon substrate and the exchange with the recirculating system. VOR_{max} is the maximum volumetric oxygen removal rate. T_O is the oxygen consumed in the PHA bed, which depends on oxygen and bacteria concentration and the PHA volume in the reactor.

$$\frac{\partial O}{\partial t}V = Q(O_i - O) - T_O V \tag{9}$$

$$\frac{\partial O}{\partial t}V = Q(O_i - O) - \mathrm{VOR}_{max} V_{PHA}\left(\frac{O}{K_o + O}\right)\left(\frac{X_T}{K_x + X_T}\right)V \tag{10}$$

The biomass balance has two components, one aerobic and one anaerobic, based on bacterial growth under anoxic and aerobic regimes. The dead biomass is consumed in the same reactor and is not harvested for long periods of time, so a harvest term is not included. The anoxic growth is dependent on the nitrate reduction in the reactor (T_N in Eq. (7)). Oxygen is the preferred electron acceptor for the facultative anaerobes, and in previous experimental work, no influence of nitrate concentration on the oxygen utilization was noted, so the aerobic growth depends only on the oxygen consumption rate (T_O in Eq. (9)).

$$\frac{\partial X_T}{\partial t}V = \mathrm{VOR}_{max} V_{PHA}\left(\frac{O}{K_o + O}\right)\left(\frac{X_T}{K_x + X_T}\right)\frac{1}{Y_O}V + V_{PHA}\mathrm{VDR}_{max}\frac{1}{Y_N}\left(\frac{N}{K_n + N}\right)\left(\frac{X_T}{K_x + X_T}\right)\left(\frac{K_o'}{K_o' + O}\right)V - K_d X_T V \tag{11}$$

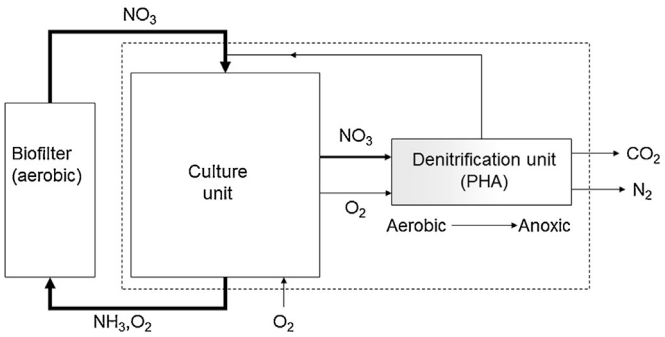

Fig. 1. Diagram of the denitrification system. The shadow in the denitrification unit represents the direction of the oxygen and nitrate gradients. Dashed outline represents the modeled components.

Substituting Eqs. (8) and (10) in Eq. (11), the two terms for aerobic and anoxic biomass growth are obtained:

$$\frac{\partial X_T}{\partial t}V = V(T_N(Y_N) + T_O(Y_O) - K_d X_T) \quad (12)$$

The PHA consumption rate is proportional to the bacterial growth rate, and depends on the yield of bacteria per unit of PHA:

$$\frac{\partial \mathrm{PHA}}{\partial t} = -\frac{1}{Y_{\mathrm{PHA}}}\frac{\partial X_T}{\partial t}V = -V\left(T_N\left(\frac{Y_n}{Y_{\mathrm{PHA}}}\right) + T_O\left(\frac{Y_O}{Y_{\mathrm{PHA}}}\right)\right) \quad (13)$$

Eq. (11) can be related to the consumption of PHA by the ratio of PHA consumed/NO_3^--N reduced and PHA consumed/O_2 consumed. These values can be determined by stoichiometry or experimentally. Substituting these values in Eq. (13), the consumption of PHA will be:

$$\frac{\partial \mathrm{PHA}}{\partial t} = -V(T_N \mathrm{PHA}_N + T_0 \mathrm{PHA}_o) \quad (14)$$

3. Model calibration

In the model development, constants based on completely mixed reactors were used. The values reported in literature have large variations from 1 to 100 mg L^{-1} nitrogen (Plósz et al., 2003; Strong and Fillery, 2002). The model was developed based on five completely mix cells, a single cell can simulate a complete mixed reactor, while the series of cells can simulate a plug flow reactor.

Averages of the constants obtained in literature were used as initial points for calibration (Table 1). The constants are based on daily rates and are mg L^{-1} concentrations. The rates obtained in the model are mg L^{-1}-d.

To calibrate the model, results of experiments conducted in five reactors of 5-cm diameter each with 50 cm^3 of medium connected in series, for a total of 250 cm^3 of PHB in the form of 4 mm pellets as described elsewhere (Gutierrez-Wing et al., 2012) were used. The methods are described here briefly. Synthetic sea water at 15 mg g^{-1} (ppt) salinity was prepared with Instant Ocean™ in 40 L tanks. Sodium nitrate was added to obtain a concentration of 50 mg NO_3^--N L^{-1}. Phosphorus as 0.5 ppm of PO_4-P was supplied based on preliminary trials that showed no difference in denitrification above this value, concurring with other authors (deBarbadillo et al., 2006; Sundareshwar et al., 2003). The average flow rate during the experimental work was 80 mL min^{-1}, resulting in a flow velocity of 60 m^3 m^{-2} d^{-1}, common for anaerobic reactors (Tchobanoglous et al., 2003). After the medium was acclimated and denitrification was observed, the artificial sea water was substituted with a new batch, prepared in the same way to remove the acclimation phase, which was not modeled. NO_3-N and PO_4-P were measured by ion chromatography and NO_2-N by spectrophotometry as per Standard Methods 4500 (APHA, 1999). During the lag phase the nitrate was measured only by colorimetric method, until denitrification started.

The sensitivity of the model to VDR_{max}, VOR_{max}, K_o, K'_o, K_n and K_x was analyzed. The model was calibrated initially with a set of data obtained at 15 ppt salinity and 20 °C and PHA medium W with molecular weight over 500,000 (Fig. 2). The calibration was done by changing the values of the parameters that influenced the most the denitrification rates and the PHA consumption, to reduce the root mean squared error of prediction.

The RMSEP or root mean squared error of prediction (Esbensen et al., 2002) was calculated as per Eq. (19).

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_1^n (N_{\mathrm{meas}} - N_{\mathrm{calc}})^2}{n}} \quad (19)$$

After calibration, the model was tested with three additional data sets from denitrification units operated at 0 ppt salinity with the same media W and at 30 ppt salinity using non-pelletized PHA media of molecular weights close to 50,000 and 7000 (Media A and B) to compare the model and determine the suitability of use under different conditions. The RMSEP was calculated for all data sets.

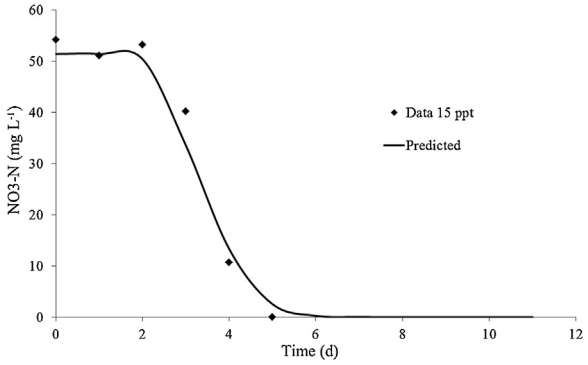

Fig. 2. Simulation results on a batch reduction of nitrate from an experimental run with a flow rate of 80 mL min^{-1}. The standards error of prediction was 9.8%.

4. Results

The RMSEP for the calibration dataset was 3.48, with an average percent error of 9.78% (Fig. 2). The resulting constants are presented in Table 1. The values obtained for all the constants are within the ranges reported in literature for diverse environments (Plósz et al., 2003; Tchobanoglous et al., 2003; Tiedje et al., 1983; Wu et al., 2001).

The sensitivity analysis of the model for different parameters showed that the maximum denitrification rate (VDR_{max}) has the highest impact on the slope of the nitrate reduction curve (Fig. 3). This parameter also impacts the rate of consumption of PHA (Fig. 4).

The oxygen half saturation constant K_o, has an effect on the length of the exponential phase (not shown) but the effect was negligible on the denitrification slope or the PHA consumption (Fig. 5). K_n affected the slope of the nitrate removal and the PHA consumption curves.

The K_x value had a large effect on the lag of both the start of denitrification and of PHA consumption. As this parameter affects the growth of the bacteria, the larger the value, the longer the lag was (Fig. 6).

The RMSEP were 1.06 for the W media in 0 ppt salinity, and 5.13 and 4.62 for media A and B at 30 ppt (equivalent to 4.12, 13.9 and 11.9% respectively). An example of the calibration data and

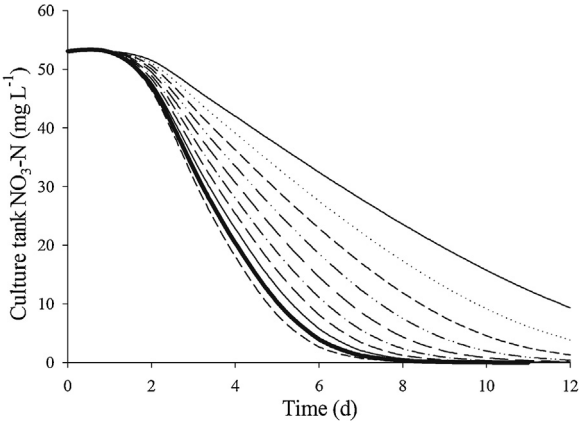

Fig. 3. Sensitivity analyses for VDR_{max} effect on NO_3-N concentration. The VDR_{max} was evaluated in a range of 2–14 mg mL^{-1} d^{-1}. The thick line represents the calibrated value of 12.0 mg mL^{-1} d^{-1}.

Table 1
Initial and calibrated values for the constants used in the model.

	Initial value[a]	Source	Calibrated value
Y_o	0.45 mg $(mg\ O_2)^{-1}$	Tchobanoglous et al. (2003)	0.45 mg $(mg\ O^2)^{-1}$
Y_N	0.45 mg $(mg\ NO_3\text{-}N)^{-1}$	Tchobanoglous et al. (2003)	0.4 mg $(mg\ NO_3\text{-}N)^{-1}$
K_n	10.3 mg L^{-1}	Plósz et al. (2003), Robinson and Tiedje (1983) and Wu et al. (2001)	10.3 mg L^{-1}
K_o	0.5 mg L^{-1}	Plósz et al. (2003)	0.5 mg L^{-1}
K'_o	0.2 mg L^{-1}	Tchobanoglous et al. (2003) and Plósz et al. (2003)	0.4 mg L^{-1}
K_d	0.02 d^{-1}	Plósz et al. (2003)	0.02
VDR_{max}	12.0 mg mL^{-1} d^{-1}	Experimental	12.0 mg mL^{-1} d^{-1}
VOR_{max}	7 mg mL^{-1} d^{-1}	Experimental	7 mg mL^{-1} d^{-1}
K_x	0.8 mg L^{-1}	Experimental	0.8
PHA_n	2.54 mg PHA $(mg\ NO_3\text{-}N)^{-1}$	Stoichiometry	2.2 mg PHA $(mg\ NO_3\text{-}N)^{-1}$
PHA_o	7.26 mg PHA $(mg\ O_2)^{-1}$	Stoichiometry	20.8 mg PHA $(mg\ O_2)^{-1}$

[a] Initial values are averages of those found in literature, calculated by stoichiometry or determined experimentally.

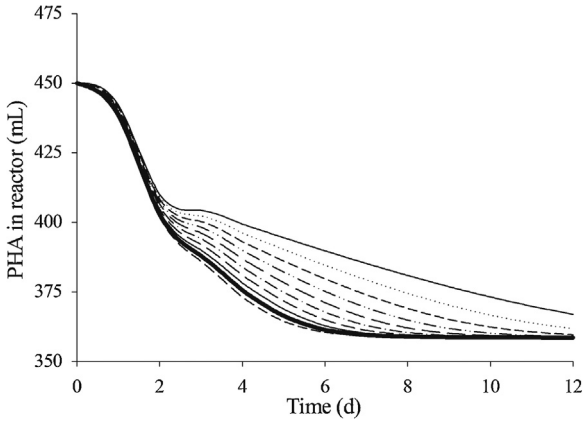

Fig. 4. Sensitivity analysis for VDR_{max} effect on PHA consumption. The VDR_{max} values represented in the graph range from 2 to 14 mg mL^{-1} d^{-1}. The thick line represents the calibrated value of 12.0 mg mL^{-1} d^{-1}.

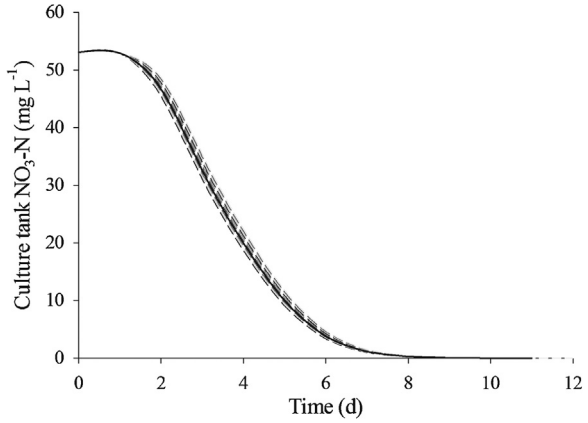

Fig. 5. Sensitivity analyses of the effect of K_o on nitrate reduction. The K_o values represented range from 0.1 to 1 mg L^{-1}. The thick line represents the calibrated value of 0.5 mg L^{-1}.

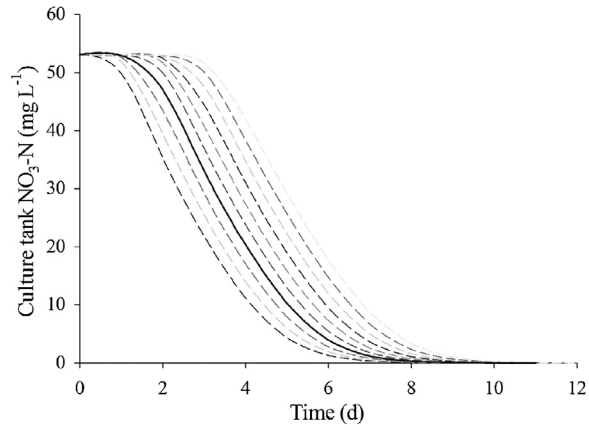

Fig. 6. Sensitivity analysis of K_x on nitrate. The lag increases proportionally with K_x. The K_x values represented range from 0.2 to 2 mg L^{-1}. The thick line represents the calibrated value of 0.8 mg L^{-1}.

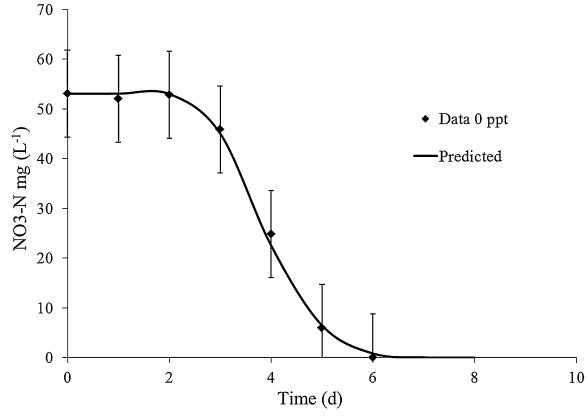

Fig. 7. Measured values compared with the model prediction for 0 ppt salinity. The error of prediction was 4.12%.

the values predicted by the model is presented in Fig. 7. The predicted rates on the exponential phase are within 20 percent of the observed values for all the data sets (Fig. 8). It was observed that the model underestimates the slope from 0 ppt, and this was also observed in the data sets from medium different from the one used for calibration. The W medium had the lowest denitrification rate observed experimentally.

5. Discussion

The characteristic of this system, based on a carbon source that is at the same time the support medium for the bacteria, modifies the availability of the carbon substrate. Soluble carbon denitrification systems were the nitrate and the carbon source are diffused from the water to the biofilm have been modeled (Boley et al., 2000; Casasús et al., 2005; Nielsen et al., 1990; Plósz et al., 2003; Van Rijn et al., 2006; Widdowson et al., 1988). In these systems, the supply of carbon from the water promotes the growth of the bacteria away from the support medium and creates areas with no nutrients close to the support. The bacteria closest to the substrate will slough off, removing from the support the active biofilm. In some cases, exopolymeric substances can store excess nutrients in the biofilm for differed use (Tolker-Nielsen et al., 2000) but the transference to the internal part of the biofilm will depend on the diffusion efficiency. In the PHA based system, the carbon

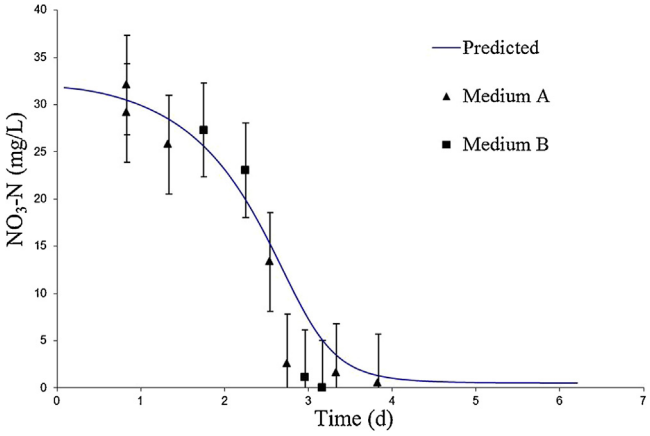

Fig. 8. Measured values with media with two different molecular weights (more than 50,000 an approximately 7000 Da) compared with the model prediction. The error of prediction was 13.9 and 11.9% respectively.

is hydrolyzed in the surface of the support substrate. If the bacteria hydrolyze more carbon than needed, it is trapped within the biofilm. The biofilm is limited by the distance that the carbon can reach, so no growth occurs too far from the support, resulting in a thinner biofilm (Gutierrez-Wing et al., 2012). The diffusion of nitrate in the conditions studied does not appear to have limited the process. Given this characteristics, the model used gave a satisfactory prediction of the denitrification rate in the exponential phase using as inputs the PHA volume, initial nitrate and oxygen concentrations and the maximum volumetric reduction factors for nitrate and oxygen (VDR_{max} and VOR_{max}). These last variables can be fine-tuned to the specific medium being used. The prediction using the calibrated constant gave results within 20% of the real data for the validation datasets.

The variability observed in the experimental sets is reflected in the model. Changes in the bacterial growth parameters, including maximum denitrification rate (VDR_{max}) and K_x have a great impact on the denitrification performance and the initial lag phase. These results concur with the data reported in literature were a change in the bacteria population can increase or collapse the denitrification rates. The denitrification rates can vary from 0 to 170 g $(m^3\text{-d})^{-1}$ or more, depending on the bacterial species and strain (Gomez et al., 2003; Patureau et al., 2000). The variability on the denitrification rates is also related to the abundance of bacteria capable of using PHA as a carbon source for denitrification (Hiraishi and Khan, 2003). These bacteria are widely distributed in the environment, but the species that act at each environmental condition are different. The PHA utilization rate in our study indicates that bacteria with PHA depolymerase are present at all salinities and with all medium tested. The low effect of the K_o in both the denitrification rates and the PHA consumption reflect the low oxygen observed after the first few centimeters in the reactor.

The effect of the PHA used did not significantly impact the denitrification rate, even when the specific surface area was different. This may be due to the reduction of the surface area after the formation of the biofilm, as it tends to cover the pores of the media and reduce the specific area. This was observed in SEM micrographs (not shown). It is expected that if other solid carbon source with very different hydrolysis behavior or configuration is used, the efficiency and the substrate consumption for denitrification will change. These changes can be accounted for determining experimentally the kinetic constants for the model.

The developed model can predict adequately the denitrification activity of the PHA based reactor. The results are more accurate if an acclimated filter is used. The initial lag phase of the denitrification curve is highly dependent on the initial bacterial concentration, and the estimation of the length of this phase cannot be predicted adequately.

With the calibrated model, a ratio of 2.92 g PHA: 1 g NO_3^--N removed predicts adequately the PHA consumption. The simulation gave an estimated removal rate of 600 mg NO_3^--N d^{-1} with 201 cm^3 of PHA as initial volume, for a volumetric rate of 2.97 kg NO_3^--N m^{-3} d^{-1}.

6. Conclusions

The developed model represents adequately the nitrate reduction with the medium used, in ranges of nitrate under100 ppm NO_3^--N. The denitrification rates at steady state can be adequately represented with this model. The model can be used as a tool to estimate the denitrification rates with different size reactors, as the tank cells allow the use of different volume systems and filters.

In the conditions tested, a ratio of 2.92 g PHA to 1 g NO_3^--N reduced was found. The model results showed a denitrification rate of 2.97 kg m^{-3} d^{-1} for ranges from 10 to 50 mg NO_3^--N L^{-1}. Using this model as a management tool, the required size of denitrification units and PHA recharging time can be predicted based on the expected nitrate loading and the time between PHA recharges desired. An oversizing of the unit will not promote the formation of sulfides as the carbon source is not available after the nitrate is consumed, so the sizing can be done for the maximum load expected.

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