



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 $2.97 \text{ kg m}^{-3} \text{ d}^{-1}$ 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.

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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^- -N L^{-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 ($\text{mg}(\text{mg O}_2)^{-1}$)
Y_n	anoxic bacterial yield coefficient ($\text{mg}(\text{mg NO}_3\text{-N})^{-1}$)
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 ($\text{mg mL}^{-1} \text{d}^{-1}$)
VOR_{max}	maximum volumetric oxygen removal rate ($\text{mg mL}^{-1} \text{d}^{-1}$)
K_x	half saturation bacteria constant (mg L^{-1})
T_N	nitrate consumption rate ($\text{mg NO}_3\text{-N L}^{-1} \text{d}^{-1}$)
T_O	oxygen consumption rate in the denitrification unit ($\text{mg NO}_3\text{-N L}^{-1} \text{d}^{-1}$)
O	oxygen concentration (mg L^{-1})
N	nitrate nitrogen ($\text{NO}_3\text{-N}$) 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	$\text{mg PHA} (\text{mg NO}_3\text{-N})^{-1}$
PHA_n	$\text{mg PHA} (\text{mg NO}_3\text{-N})^{-1}$
PHA_o	$\text{mg PHA} (\text{mg O}_2)^{-1}$
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).

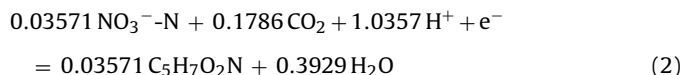
1.1. Stoichiometry

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 $\text{NO}_3^- \text{-N}$ 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).

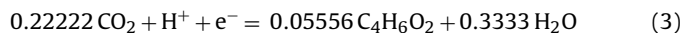
Electron acceptor:



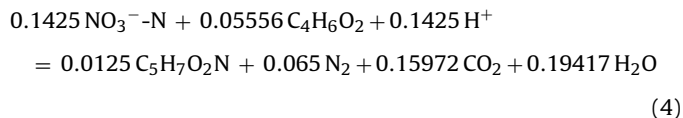
Cell synthesis:



Electron donor:



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



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



From Eq. (4) a ratio of 2.54 g PHB ($\text{g-NO}_3\text{-N})^{-1}$ reduced in the denitrification process is obtained. From Eqs. (1) and (5) an equivalent of oxygen of 2.86 g O_2 ($\text{g NO}_3\text{-N})^{-1}$ 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

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