Process Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Modeling the decay of nitrite oxidizing bacteria under different reduction potential conditions

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ARTICLE INFO

Keywords:
Ammonia oxidizing bacteria (AOB)
Decay rate
Nitrite oxidizing bacteria (NOB)
OUR
Respirometry

ABSTRACT

Autotrophic growth and decay rates of ammonium and nitrite oxidizing bacteria (AOB and NOB, respectively) have a significant impact on the design and on the process performance of wastewater treatment systems where nitrification occurs. Literature data on the separate decay rates of AOB and NOB is scarce and inconsistent. In this study, batch experiments based on respirometric techniques were conducted to determine the NOB decay rates under different oxidation-reduction potential conditions, in order to widen the understanding of nitrite dynamics.

The decay rate measured under anoxic conditions was 85% lower than under aerobic conditions, whereas under anaerobic conditions the decay rate reduction was 92%. A design and simulation tool was used to assess the impact of applying these results in differentiated areas of an activated sludge system. Simulations show a greater impact for systems with a sludge retention time under 10 days, for which up to a 16-fold increase in NOB biomass concentration and up to 86% and 80% reductions in ammonium and nitrite concentrations in the effluent were calculated.

Therefore, this work demonstrates that considering different decay rates for autotrophic biomass under different ORP conditions avoids underestimating system performance and over dimensioning new activated sludge schemes.

1. Introduction

The most used nitrogen removal strategy in wastewaters is the nitrification-denitrification process, through which ammonium is converted into nitrate (nitrification) and nitrate is converted to nitrogen gas (denitrification). Nitrification is a two-step process where two different groups of autotrophic bacteria take part: in a first step, ammonium oxidizing bacteria (AOB) convert ammonium into nitrite (Eq. (1)); in a second step, nitrite oxidizing bacteria (NOB) convert nitrite into nitrate (Eq. (2)). Denitrification is carried out by heterotrophic bacteria, which reduce nitrate and nitrite to nitrogen gas.

$$\text{Nitritation: } NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O \quad (1)$$

$$\text{Nitratation: } NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \quad (2)$$

For most municipal wastewater treatment systems under normal operating conditions, nitritation is the limiting step and there is practically no nitrite accumulation. Therefore, this component can be disregarded: traditionally, most activated sludge models include one-step nitrification in their structure (e.g. [1]). This simplification renders acceptable results for conventional operation of nitrification processes [2].

However, under certain conditions, nitrite peaks can be detected in activated sludge systems. They usually indicate a disturbance in the microbiological processes, which normally happens under unstable operation caused by a number of reasons: insufficient oxygen, low temperature, high temperature, low sludge retention time or presence of inhibitory compounds. Nitrite concentration in these cases needs to be controlled, since it can rise to toxic levels. One-step nitrification models cannot predict nor analyze these problems.

Besides the abovementioned cases, in the treatment of side streams and of industrial wastewaters, processes where nitrite formation is specifically promoted are increasingly used. In these processes (e.g. partial nitrification and anaerobic ammonium oxidation processes)

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https://doi.org/10.1016/j.procbio.2018.05.021
Received 19 February 2018; Received in revised form 24 May 2018; Accepted 28 May 2018

nitrite plays a fundamental role, and it becomes a key component to be measured and controlled. For this task, models which account for the two-step nitrification process have been developed or adapted in the last two decades [2–7]. These mathematical structures proposed by different authors to model nitrite show discrepancies about some of the aspects involved, such as how denitrification occurs or which nitrogen species are the active substrates. At the same time, significant variability of model parameter values among different studies can be found. In those studies, some of the adopted parameters were measured by the authors whereas some others were assumed from different literature sources. A review on nitrite modeling in wastewater treatment systems can be found in [2].

Growth and decay coefficients of autotrophic bacteria directly affect the performance of nitrification, since they determine the amount of bacteria in the system and, as a result overload and bacteria wash-out are phenomena that depend on them. Therefore, they are the most important parameters affecting the design and operation of activated sludge systems. Unlike growth, autotrophic decay is an uncertain process that has been seldom studied. Still, Koch et al. [8] identified it in the set of sensitive parameters for ASM3. The term decay represents the loss of bacterial activity, which includes maintenance, lysis and predation, and is proportional to biomass loss. Specifically differentiated AOB and NOB decay rates were not frequently measured in the studies referred above, although some authors have developed and calibrated specific models for nitratation, thus obtaining a decay coefficient for NOB [9].

It has been noted that NOB (and AOB) decay rates under anoxic conditions are smaller than under aerobic conditions [10–13], although the range of observed decay rate reduction ranges from 30 to 100% and therefore further research is needed to clarify this phenomenon. Expanding the knowledge of the activity kinetics of the NOB and their dependence on ORP conditions will allow for a better control of the nitrification process and will help adapting the design of wastewater treatment plants for nitrification.

The aim of this work is to determine the NOB decay rate (b_{NOB}, d^{-1}) in different reduction potential (ORP) conditions (aerobic, anoxic and anaerobic) by means of laboratory batch experiments using respirometric techniques, which are simple and reproducible. The studied biomass from which three different decay rates were obtained was obtained from a pilot scale wastewater treatment plant. On the other hand, simulations on an activated sludge system run with the software DESASS [14] were used to assess the extent to which the use of differentiated NOB decay rates influence the results (water quality) based on system parameters (mainly SRT and anoxic-anaerobic-aerobic volumes) as compared to using an unified decay rate.

2. Material and methods

2.1. Setup descriptions

2.1.1. Pilot plant

NOB used in this study were obtained from a pilot plant which was located within the full-scale WWTP "Conca del Carraixet" and treated its primary settler effluent. The pilot plant had a modified University of Cape Town (UCT) scheme for both organic matter and nutrient removal. Temperature was controlled, with a set point at 30 °C. The average hydraulic retention time (HRT) and the SRT for the pilot plant were maintained at 9.6 h and 7 days, respectively. The blower frequency was controlled to keep the dissolved oxygen (DO) concentration in the aerated compartment around a desired set point (2–2.5 $mgO_2 l^{-1}$). Fig. 1 shows the layout of this process.

2.1.2. Reactor 1

Reactor 1 consisted of an aerobic completely stirred tank reactor (CSTR) installed in the laboratory, with a total volume of 10 L. Temperature was kept at 30 °C with the aid of a thermostatic bath. Dissolved oxygen (DO) concentration in the tank was monitored with a Cellox 325 electrode (WTW, Germany) connected to an oximeter (Oxi 320, SET WTW, Germany). An air blower was switched on when DO went below 2 mg O_2/L, aerating the reactor through fine bubble diffusors installed at the bottom. The blower switched off when DO reached 5 mg O_2/L.

2.1.3. Reactors 2

Reactors 2Ae, 2Ax and *2An* were used for achieving different ORP conditions in the laboratory. Each had a working volume of 3 L. Their temperatures were controlled at 30 °C and the DO concentrations were monitored with a Cellox 325 electrode (WTW, Germany) connected to an oximeter (Oxi 320, SET WTW, Germany). *Reactor 2Ae* had an aeration system analogous to that one described for *Reactor 1*. Nitrate was added to *Reactor 2Ax* to sustain a concentration between 7 and 10 mg NO_3-N/L.

2.1.4. Batch reactor

The *Batch Reactor*, with a volume of 300 mL, was used to examine the activity of the NOB biomass present in the different reactors, by means of short specific respirometric experiments. This reactor was water jacketed for keeping temperature at 30 °C. An air blower aerated the samples at the beginning of each specific respirometry. The DO concentration was monitored like previously explained.

2.2. Experimental procedure

2.2.1. Bringing biomass to endogenous conditions

In the first place, the biomass from the pilot plant was brought to endogenous conditions in *Reactor 1*. For this, the aeration control system described above was switched on and biomass was therefore given enough oxygen (and time) to consume all possible substrate. The periods when the blower was in off mode were used to determine the Oxygen Uptake Rates (OURs), calculated (in Excel 2011) as the slope of the recorded DO concentrations regression line. Endogenous conditions were achieved when the OUR values remained practically constant, which happened after approximately 20 h.

After achieving endogenous conditions, the sludge in *Reactor 1* was split into reactors *2Ae*, *2Ax* and *2An*, being the ORP the only difference among them, as previously explained: in *Reactor 2Ae* the oxygen concentration remained over 2 mg O_2/L; in *Reactor 2Ax* nitrate concentration was kept over 7 mg NO_3-N/L; *Reactor 2An* remained under anaerobic conditions. There was no detectable nitrite in the reactors, and therefore, in the absence of substrate, there was no NOB growth. NOB activity from all three reactors was examined along the whole study, which lasted 160 h, by performing short respirometric studies as explained in Section 2.2.3 below.

2.2.2. Determination of NO_2 concentration required to achieve maximum growth rate

Prior to the respirometric studies, eight samples from the sludge under endogenous conditions in *Reactor 1* were used for determination of the required nitrite concentration to increase the growth rate up to its maximum. Each of these eight samples was transferred to the *Batch Reactor* and kept under aeration before adding a certain nitrite concentration ranging from 0.5 to 10 mg NO_2-L^{-1}. Each test was short enough (5 min) to assume that biomass concentration in the reactor remained constant. Since (different) substrate additions caused a (different) increase in the NOB activity, an increase in the OUR could be measured each time (calculated as the difference between the measured OUR before and after nitrite addition). The obtained values from the eight tests were represented along a substrate concentration axis and thus the nitrite concentration required to achieve the maximum growth rate could be determined. The affinity constant for nitrite could also be obtained by minimizing the sum of the squared errors between experimental and predicted data, which was done using the Solver