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High ammonium loading and nitrification modelling in a fixed-bed bioreactor

Nelly Cruvellier[a,*], Laurent Poughon[a], Catherine Creuly[a], C-Gilles Dussap[a], Christophe Lasseur[b]

[a] Université Clermont Auvergne, Institut Pascal, UMR CNRS 6602, TSA 60026, CS 60026, F-63178, Aubière cedex, France
[b] ESTEC, European Space Agency, Noordwijk, The Netherlands

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ABSTRACT

The aim of this study was to investigate ammonia load removable by a fixed-bed bioreactor. In this study, high ammonia loads, up to 4.5 kgN/m^3 d were applied on a fixed bed reactor colonised by two pures nitrifying bacteria: *Nitrosomonas europaea* ATCC® 19718 and *Nitrobacter winogradskyi* ATCC® 25391. The loads were similar to the highest values reported in literature especially with a two bacteria population. The complete ammonia removal efficiency in our installation has reached 2.5 kgN/m^3.d for a full nitrification. An N-tank in series model was used to represent the fixed bed reactor and to predict the nitrification in the installation. The developed model was accurate and representative of the nitrification assumed by *Nitrosomonas europaea* ATCC® 19718 and *Nitrobacter winogradskyi* ATCC® 25391. This model was also used to predict the biomass and oxygen concentrations during the experiment under various operational conditions. The biofilm fixed on the solid support (polysterene beads 2 mm diameter) was mainly present in the bottom of the reactor where the ammonia load was applied. It is demonstrated that in this region of the column the oxygen concentrations reached the lowest values.

1. Introduction

Nitrogen removal by bioprocesses has been studied for a long time. Traditionally focused on the nitrification-denitrification scheme, recent studies highlight new treatment processes. They are supposed to be more efficient and less energy-consuming [1]. The Annamox process developed in the 1990s has often been studied as a one-step nitrogen removal process: it allows the ammonium oxidation into nitrogen gas using nitrite [2]. The nitrification can then be coupled with Annamox degradation [3,4], or denitrification [5,6]. Many works on the short-cut nitrification have been published in recent years, with the objective to stop the nitrification at the nitritation step and enhance the accumulation of nitrite [1]. It is an interesting way to decrease the consumption of energy due to lower oxygen consumption by reducing required gas liquid transfer and therefore dissolved O_2 concentration in the culture broth. [5,7–12]. All these processes must support different nitrogen loads according to environmental requirements. High ammonia removal is a difficult to control process due to the free ammonia inhibition on the Ammonia Oxidizing Bacteria (AOB) [13–15]. Values of nitrogen load supported in different kinds of reactors and installations are reported. Maximal values of actual nitrogen loads are given in Table 1. Current processes reach up to 5 kgN/m^3.d but do not achieve total nitrification.

Higher nitrogen load are abated in short-cut nitrification (oxidation of ammonium into nitrite), up to 6.1 kgN/m^3 d [20] and in traditional nitrification – denitrification scheme, up to 3.5 kgN/m^3 d [26].

Biological nitrogen removal process varies in wastewater treatment plants. In confined environment, as space exploration, isolated life missions (Concordia Research Station or submersibles), the nitrogen removal processes must be adapted. The space domain requires perfect control of a bioprocess, including nitrogen treatment. For long-term space missions, the resupply of water, food and oxygen by closed Life-Support Systems (LSS) is required to maintain astronauts alive. The MELiSSA system (Micro-Ecological Life Support System Alternative) is a closed bioregenerative LSS (BLSS) developed by European Space Agency (ESA) [27], which consists of five biological compartments. It is designed on the basis of a lake ecosystem including the recycling of nitrogen by an N-cycle [28]. The nitrogen removal in MELiSSA is a fundamental issue considering that life support systems generally consider independently water, oxygen recovery and CO_2 elimination. MELiSSA is conceived as an integrated system for carbon, oxygen, nitrogen and water recycling. It is based on the autotrophic nitrification process. The objective of the nitrification unit operation is to produce nitrate by oxidation of the ammonium produced by the liquefaction of solid and

* Corresponding author.
E-mail address: nelly.cruvellier@uca.fr (N. Cruvellier).

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Table 1
Maximal nitrogen applied in nitrification process. ND: not determined.

Maximal load applied (kg/m^3 d)	Nitrification Efficiency (%)	Installation	Heterotrophic consortium	References
0.43	100	Fluidized reactor	yes	[16]
0.65	ND	Submerged up-flow biofilter process	yes	[17]
1	ND	Biofilm sequencing batch reactor	yes	[18]
1	100	Continuous biofilm reactor	no	[19]
1	100	Continuous granular reactor	yes	[20]
1.46	> 90	Continuous mixed reactor	yes	[21]
1.98	96	Submerged up-flow biofilter	yes	[22]
2.2	100	Continuous mixed reactor	no	[23]
2.5	100	Fluidized reactor	yes	[24]
4	100	Continuous mixed reactor	yes	[8]
5	50–80	Inversed turbulent bed reactor	yes	[25]

The studies working with heterotrophic consortium were not respecting axenic conditions.

liquid wastes. The main constraints of the nitrogen removal process applied in BLSS are: (i) to operate at low residence time (and high nitrogen load), (ii) to control a robust and low mass process and (iii) to avoid the accumulation of nitrite considering it is a toxic compound for humans [27]. To achieve the nitrification in MELiSSA, a fixed-bed nitrifying column is used as the central element of the N-cycle and biomass grows on polystyrene beads as a biofilm [19]. The daily nitrogen production by one human is around 0.008 kg d including urine (0.006–0.007 kgN d) and feces (0.001–0.0014 kgN d) [29]. In the terrestrial 1-man sized demonstrator of MELiSSA, the 10 L nitrifying compartment oxidises ammonium with a daily nominal load of 0.9 kgN/m^3 d and an 8 h liquid residence time [28]. According to this parameter, the working volume of the nitrification bioreactor must be 10 L which approximately corresponds to the volume necessary for recycling nitrogen for one human. In any case, this specific rate is lower than the maximum possible loads reported in Table 1, letting a sufficient safety margin. To meet the requirements of Biological Life-Support Systems (BLSS), each subsystem of the MELiSSA loop must be controlled by a rationale approach. Such an objective is achievable with the development of mechanistic and predictive models. Such an objective is achievable with the development of mechanistic and predictive models offering a certain degree of knowledge and understanding for anticipating the optimal set-points values for the action variables, such as concentrations and flow rates. For the nitrification process in MELiSSA, such a model requires to establish: (i) a bioreactor model including hydrodynamics and physical phenomena, (ii) a reliable kinetic model for the co-culture" The kinetic model for the *Nitrosomonas europaea* ATCC® 19718 and *Nitrobacter winogradskyi* ATCC® 25391 co-culture has already been defined and validated [30]. The hydrodynamic model for the MELiSSA nitrifying compartment has already been published [19,31].

In this study, the MELiSSA fixed bed N-tank in series model is combined to the biological nitrification model [30]. The model is used to describe a long-term experiment (more than 300 days) for a fixed-bed bioreactor colonised by *Nitrosomonas europaea* ATCC and *Nitrobacter winogradskyi* ATCC. Various nitrogen loads are applied (up to 4.5 kgN/m^3 d) and high nitrogen removal is observed. This high nitrogen removal is similar to the highest values found in the literature for a nitrification process.

2. Material and methods

2.1. Strains and culture media

Nitrosomonas europaea ATCC® 19718 (Institut Pasteur, Paris, France) and *Nitrobacter winogradskyi* ATCC® 25391 (Institut Pasteur, Paris, France) mixed cultures were axenically grown in autotrophy in the medium composed of: $(NH_4)_2SO_4$, 1.32 g/L; $MgSO_4{\cdot}7H_2O$, 0.0043 mg/L; $CaCl_2$, 0.74 mg/L; $CuSO_4$, 0.004 mg/L; $FeSO_4$, 2.5 mg/L; KH_2PO_4, 0.68 g/L; Na_2HPO_4, 0.71 g/L; $(NH_4)_6Mo_7O_{24}$, $4H_2O$, 0.18 g/L; $ZnSO_4.7H_2O$, 0.0043 mg/L; $NaHCO_3$, 0.8 g/L [32].

2.2. Maintenance of cultures

Mixed cultures were performed in 500 mL Erlenmeyer flasks incubated in dark and at 28 °C with 100 rpm shaking. Then, a subculture at 10% was performed every three weeks to avoid the nitrate concentration to reach the toxicity threshold of 3.84 gN-NO_3^- L^{-1} for *Nitrobacter winogradskyi* [33].

2.3. Experimental reactor set-up and operation

The lab-scale reactor with a working volume of 1 L was made of glass and operated under oxic and dark conditions. The internal diameter and working height of the reactor were 6 cm and 43 cm, respectively. Compressed air and influent were injected through the bottom of the reactor. Mineral media, used for the mixed cultures of the two nitrifying bacteria selected and described in 2.1, was used as the influent under continuous mode. Part of the effluent was returned to the inlet of the reactor with the liquid recirculation loop and allowed better liquid mixing in the reactor. Polystyrene beads, Biostyr®, were used as carriers for the biofilm attachment. The pH was set at 7.9 and controlled with a KOH 1M solution. The temperature was kept at 28 °C with thermostatic heater. During the operation, on-line follow-up of the pH and dissolved oxygen in reactor (pO2) were performed with probes. The start-up of the reactor was made at a residence time (T_R) of 50 h and was gradually decreased during the experiment. Dissolved oxygen and the CO2 (carbon source) were only provided by air flow. To avoid an oxygen and so oxidation limitation, air was continuously provided at 0.45 L_{air}/min flow rate. The volumetric gas/liquid transfer coefficient was 155 h^{-1} and the superficial gas velocity about 0.01 m/s.

2.4. N-compound analysis

Ammonium, nitrite and nitrate concentrations were assessed by Metrohm high-performance liquid chromatography. For the follow-up of the ammonium ions, an 882 Compact IC plus-Cation, equipped with a negative resin column Metrosep C 4250 was used. The mobile phase consisting of nitric acid (65% at 117.5 µL/L) and 0.7 mM of dipicolinic acid in ultrapure water was degassed before use. Then, it was pumped at 0.9 mL/min. The time of the analysis was fixed to 25 min. Nitrite and nitrate concentrations were monitored using an 882 Compact IC plus-Anion, equipped with a column with a positive resin Metrosep A Supp 5. The mobile phase consisted of 1 mM of sodium bicarbonate and 3.2 mM of sodium carbonate in ultrapure water and was degassed before use. Then, it was pumped at 0.7 mL/min. The time of the analysis was fixed to 35 min.