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Enrichment of nitrite-dependent anaerobic methane oxidizing bacteria in a membrane bioreactor

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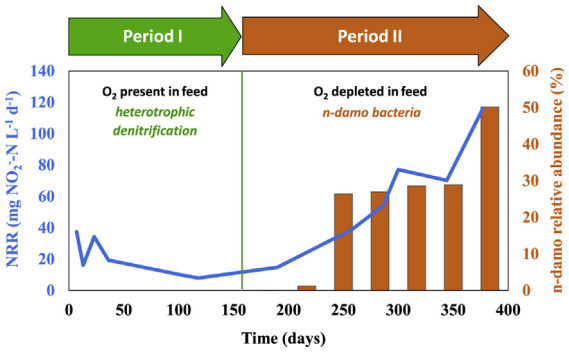
HIGHLIGHTS

- The biomass enrichment on n-damo bacteria was successfully achieved.
- High specific n-damo activities were attained.
- Biomass accumulation was not detected during the operation.
- Ammonium was added as an extra nitrogen source for n-damo bacteria growth.
- Correlations between nitrite permeate concentration and N_2O production were found.

GRAPHICAL ABSTRACT



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ABSTRACT

The use of nitrite-dependent anaerobic methane oxidation (n-damo) processes could represent an innovative technology in order to minimize the environmental impact of anaerobic sewage effluents at low temperatures, since these biological processes are able to simultaneously remove nitrite and dissolved methane in anaerobic conditions. Nevertheless, n-damo bacteria are well-known by their reported low activity and slow doubling times which hinders a practical application. On this study, the enrichment on these bacteria was successfully achieved in a membrane bioreactor system at 28 °C. Despite biomass accumulation was not detected, a high apparent specific n-damo activity of 95.5 mg NO_2^--N g^{-1} MLVSS d^{-1} was achieved after 388 days of operation, being one of the highest nitrite removal rates reported in the literature for n-damo cultures to date. Additionally a slow doubling time of 11.5 d was estimated. 16S rRNA gene amplicon sequencing analysis indicated that *Candidatus* Methylomirabilis became the most abundant bacterial organism by day 344 with a relative abundance of 50.2%. During the entire experiment ammonium was continuously added to the system as an alternative nitrogen source, to avoid biomass growth limitations. Finally, a relation between permeate nitrite concentrations and nitrous oxide production was found, which allows to optimize the process in terms of the minimization of both nitrogen species. The nitrous oxide emissions represented between 0 and 3.7% of the denitrified nitrogen.

Abbreviations: Anammox, anaerobic ammonium oxidation; CL, cluster; COD, chemical oxygen demand; CSTR, continuously stirred tank reactor; DAMO, denitrifying anaerobic methane oxidation; DO, dissolved oxygen; DOC, dissolved organic carbon; GHG, greenhouse gas; GWP, global warming potential; FISH, fluorescence *in situ* hybridization; HRT, hydraulic retention time; MBfR, membrane biofilm reactor; MBR, membrane bioreactor; MLTSS, mixed liquor total suspended solids; MLVSS, mixed liquor volatile suspended solids; MSGLR, magnetically stirred gas lift reactor; N-damo, nitrite-dependent anaerobic methane oxidation; NLR, nitrite loading rate; NRR, nitrogen removal rate; OTU, operational taxonomic units; SBR, sequencing batch reactor

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1. Introduction

Anaerobic sewage treatment is widely used in warm and temperate climate regions because of different advantages such as the low sludge production and the energy recovery among others, in comparison to aerobic processes. Nevertheless, a large fraction of methanized chemical oxygen demand (COD) is present dissolved in anaerobic bioreactors effluents, especially at low temperatures. Since methane is a strong greenhouse gas (GHG), with a global warming potential (GWP) 28 times higher than the CO_2 for a hundred year time horizon [1], the removal of dissolved methane from anaerobic effluents should be achieved preventing its direct release to the atmosphere [2]. Another important challenge for these effluents is their high total nitrogen concentrations. An interesting strategy to deal with both problems is the utilization of bioprocesses, in which methane is used as inexpensive electron donor for denitrification. In this scenario two main microbiological pathways can be distinguished: aerobic and anaerobic. In the first pathway, aerobic methane oxidation is coupled to denitrification, through a consortium between aerobic methanotrophs and conventional denitrifiers [3]. In the second, bacteria affiliated with the candidate NC10 phylum, such as "*Candidatus* Methylomirabilis oxyfera" [4] are able to anaerobically oxidize methane by using nitrite as electron acceptor, n-damo bacteria (Eq. (1)). Besides, archaea like "*Candidatus* Methanoperedens nitroreducens" [5] are also able to oxidize methane in the same conditions but reducing nitrate to nitrite, damo archaea (Eq. (2)). Both anaerobic pathways are collectively called denitrifying anaerobic methane oxidation (DAMO) processes.

Nowadays, n-damo processes are too far to be implemented at full-scale plants and further investigation is needed, mainly due to their reported low activities (Table 1) and their slow doubling times of 1–2 weeks [4]. A better understanding, in terms of physiology and kinetics, would be necessary to facilitate a technological application [6].

$$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 4N_2 + 10H_2O \quad (1)$$

$$2CH_4 + 8NO_3^- \rightarrow 8NO_2^- + CO_2 + 4H_2O \quad (2)$$

For the last several years, different studies involving n-damo microorganisms have been carried out. Luesken et al. [7] proposed the use of an anaerobic ammonium-oxidizing bacteria (anammox) and n-damo bacteria co-culture in a sequencing batch reactor (SBR), to simultaneously remove from wastewater nitrite, ammonium, and dissolved methane. An apparent nitrite removal of 33 mg NO_2^--N L^{-1} d^{-1} (Table 1) was achieved for n-damo bacteria. Kampman et al. [8] studied a new concept to reduce the impacts of effluents from methanogenic reactors at low temperatures with presence of a considerable amount of dissolved methane [9] and nitrogen, by using n-damo processes in a SBR at 30 °C. A nitrogen removal rate (NRR) of 37.8 mg NO_2^--N L^{-1} d^{-1} was attained, although biomass was washed out from the system throughout all the experimentation. In addition, Hu et al. [10] studied the impact of different reactor configurations in n-damo bacteria enrichments: continuously stirred tank reactor (CSTR), sequencing batch reactor (SBR), and a magnetically stirred gas lift reactor (MSGLR), achieving NRRs of 26.4, 11.4 and 76.9 mg NO_2^--N L^{-1} d^{-1}, respectively. These authors suggested that the higher nitrite removals observed in the MSGLR were the result of an improvement in the gas (CH_4)-liquid mass transfer, however, and important biomass washout was also observed with this configuration. In order to avoid the biomass washout in a n-damo enrichment culture (20 °C), Kampman et al. [11] utilized a membrane bioreactor (MBR), and, in spite of the complete biomass retention, a decrease in the nitrite consumption was observed after reaching a maximum NRR of 36 mg NO_2^--N L^{-1} d^{-1}. Shi et al. [6] indicated that the low aqueous solubility of CH_4 limits n-damo activity and, in order to enhance its transfer, the use of a potential novel technology with presence of gas diffusive membranes was proposed, the membrane biofilm reactor (MBfR). The membrane surface would also promote biofilm development. These authors demonstrated for the first time the feasibility of nitrogen removal by combining damo and anammox processes, obtaining an NRR of 190 mg NO_3^--N L^{-1} d^{-1} and 60 mg NH_4^+-N L^{-1} d^{-1}. Cai et al. [12], also in a co-culture of damo and anammox microorganisms in a MBfR, obtained a surprising nitrate and ammonium removal rate of 684 mg NO_3^--N L^{-1} d^{-1} and 268 mg NH_4^+-N L^{-1} d^{-1}, respectively.

Nitrogen is an essential nutrient for microorganisms due to its presence in macromolecules, such as, proteins and nucleic acids. In previous n-damo bacteria enrichments, nitrite was used as the only nitrogen source [8,10,11]. However, microorganisms preferentially assimilate the reduced form, ammonium, since the transformation into organic forms requires less energy than the oxidized nitrogen species [13]. In addition, authors such as Ma et al. [14] observed that low concentrations of ammonia (1–10 mg L^{-1}) stimulates the activity and the growth of *Nitrobacter winogradskyi*, a nitrite-oxidizing bacteria. On this study, despite nitrogen assimilatory pathways of n-damo bacteria has not been characterized yet, in order to avoid a possible nitrogen limitation and to guarantee a proper biomass performance, it was decided to add ammonium into the system to provide an extra nitrogen assimilation source, besides nitrite.

The main goal of this study was to promote the development of an enrichment culture of n-damo bacteria in a fully-monitored MBR, operating at 28 °C and using nitrite and methane as primary substrates. Additionally, a small amount of ammonium was fed as an alternative nitrogen source to avoid microbial growth limitations. The reactor operation was evaluated in the long-term especially focusing on the N-species removal rates (specific and volumetric) and the biomass evolution (concentration and microbial composition). Additionally, the evolution of nitrous oxide, a strong GHG, was also studied, as well as its relation to the occurrence of inhibition events.

Table 1
Overview of the nitrogen removal rates reported in literature for n-damo bacteria.

References	Reactor configuration	Temperature (°C)	HRT (d)	Nitrogen Removal Rate (mg N L^{-1} d^{-1})
[41]	SBR	25	5	Nitrite: 15.3
[15]	SBR	30	13–30	Nitrite: 29.3
[7]	SBR	30	50–15	TN: 110
[8]	SFBRs	30	1.3–1.5	Nitrite: 33.5–37.8
[6]	MBfR	22	3	Nitrate: 190 Ammonium: 60 TN: 250
[42]	SBR	35	–	Nitrate: 67.7 Ammonium: 57 TN: 124.7
[11]	MBR	30	1.3	Nitrite: 36
[10]	CSTR SBR MSGLR	30	2	Nitrite: 26.4 Nitrite: 11.4 Nitrite: 76.9
[12]	MBfR	22	3–1.5	Ammonium: 354 Nitrate: 684 TN: 1038
[43]	SBR	35	29	Nitrite: 46
[32]	MBfR	35 ± 2	4	Nitrate: 78.3 Ammonium: 26
This study	MBR	28	1	Nitrite: 116 Ammonium: 9.7 TN: 125.7