UN(K)NOWN Project: Development of Anammox Microbial Inocula to Improve Nitrogen Removal Efficiency in Wastewater Treatment

Ribeiro H.[1,2], Soares-Santos V.[2], Machado A.[1,2], Costa-Dias S.[1,2], Wijaya I.M.W.[2,3], Salgado P.[1,2], Bordalo A.A.[1,2], Teixeira C.[1,2,*]

[1] CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, Matosinhos 4450-208, Portugal

[2] Institute of Biomedical Sciences, University of Porto (ICBAS-UP), Rua Jorge Viterbo Ferreira, 228, Porto 4050-313, Portugal

[3]Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember (ITS) Surabaya, Jawa Timur, 60111, Indonesia

*corresponding author: e-mail: catarina@icbas.up.pt

Abstract

The discovery of anaerobic ammonium oxidation (anammox) revealed the existence of a shortcut in the classic nitrogen cycle where ammonium is converted directly to dinitrogen gas. The anammox process can be used to develop more cost and energy-efficient sustainable nitrogen removal systems comparing to those existing today. However, large-scale applications are limited due to the slow growth rate of anammox bacteria described so far. The UNNOWN project proposes to explore the presence of these bacteria in different ecological niches, and select the appropriate seeding sludge starter as the inoculum for reactors. Anammox biomass is enriched in batch experiments as well as in laboratory scale bioreactor systems, such as Up-flow Biofilter, and Anaerobic Baffled Reactor. Afterwards, microbial community composition, optimum growth conditions, and nitrogen removal efficiency are studied. The ongoing project aims to investigate new anammox bacteria, and the relation between community structure and process activity. Ultimately, it is planned to develop microbial inocula to be used as seeding sources for anammox reactors, and to contribute to a wider application of anammox process in wastewater treatment.

Keywords: Anammox; Bioreactors; Ecology; Nitrogen removal; Wastewater

1. Introduction

Anthropogenic inorganic nitrogen inputs to aquatic systems are a major environmental concern in the EU and worldwide (Camargo and Alonso 2006). Conventional wastewater treatment technology for nitrogen removal makes the use of nitrification followed by denitrification, a cumbersome and expensive approach (Kartal et al. 2010). The anammox process converts ammonium to nitrogen gas by using nitrite as an electron acceptor under anoxic conditions (van de Graaf et al. 1995). This process is important for removing fixed N from both engineered and natural systems. The anammox process can be applied to wastewater treatment, in order to replace conventional treatment systems, being more cost effective (60 % reduction in costs), and environmentally friendly (90 % less CO_2 emissions) (Kartal et al. 2010). However, industrial applications are limited by a long start-up period owing to the low growth rate ($\mu max = 0.065\ d^{-1}$), and doubling time (11 days) of anammox bacteria (Strous et al. 1998).

We aim to investigate new anammox bacteria and the relationship between community structure and process activity, and to contribute to a wider application of the anammox process in wastewater treatment.

2. Main Activities

To achieve the proposed objectives, the ongoing UNNOWN project has the following activities planned, and their interactions are schematized in Figure 1.

2.1. Environmental screening for anammox bacteria

Several habitats will be screened for the presence of anammox bacteria. Different samples will be collected from estuarine sediments, freshwater sediments, groundwater, and agricultural soils. Locations will be selected based on environmental characteristics. Parameters such as temperature, conductivity, dissolved oxygen, oxidation-reduction potential, nitrate, nitrite and ammonium availability, and organic matter contents will be quantified. The presence, abundance, and diversity of anammox bacteria will be characterized by phylogenetic analysis and quantitative PCR targeting anammox bacteria, and specific functional genes (hydrazine oxidoreductase, *hzo*; and hydrazine synthase, *hzs*), using previously successfully described primer sets.

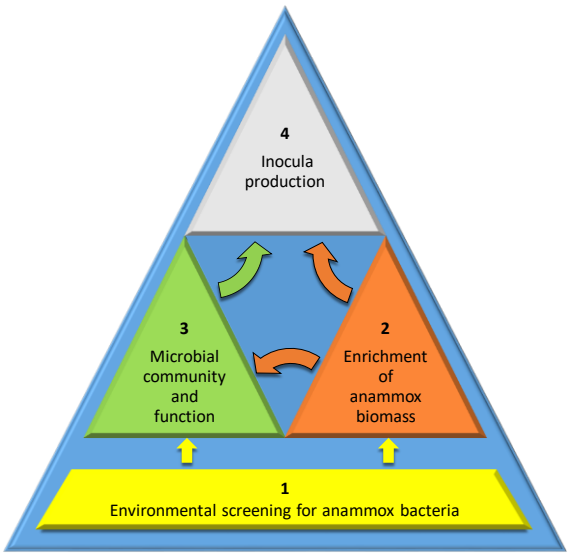

Figure 1. Structure and interactions among the planned activities within the scope of the UNNOWN project.

2.2. Enrichment of anammox biomass

Environmental samples with the most anammox potential will be selected as seedings for enrichment batch cultures, as well as laboratory scale bioreactor systems, such as Up-flow Biofilter, and Anaerobic Baffled Reactor. Anammox bacteria will be periodically enriched with a synthetic nutrient (ammonium and nitrite) medium under anaerobic conditions. Nitrogen compounds will be periodically monitored. Quantitative PCR targeting functional genes (*hzo* and *hzs*) will be used as an indicator for growth of the anammox population. Anammox activity will be measured using the N isotope pairing technique that involves performing incubations amended with different ^{15}N-^{14}N isotopic mixtures and the different N masses ($^{28}N_2$, $^{29}N_2$ and $^{30}N_2$), quantified by isotope-ratio mass spectrometry (IRMS), following the procedures described by Teixeira et al. (2016).

2.3. Microbial community and function

Total DNA recovered from environmental samples and enrichment culture samples, will be analyzed using automated rRNA intergenic spacer analysis to assess the microbial community structure. Based on the level of diversity obtained, bacterial community composition of selected samples will be further investigated using next generation sequencing and bioinformatics analysis. We are currently using Illumina MiSeq NGS platforms with respective bioinformatics pipelines. Bioinformatic analysis of raw reads will be performed using the metagenomic data analysis QIIME Illumina pipeline. This will provide insights into the phylogeny of the communities and estimate the catabolic genes present in each enrichment culture.

The data collected will be analyzed by means of several statistical methodologies. Multivariate data analysis using diverse statistical tools will be applied to explore relationships between molecular microbial data, operational parameters, and nitrogen removal activities.

2.4. Inocula production

Successfully enriched cultures will be maintained throughout the project. The links established between enriched cultures and function, will be matched with specific properties wastewater. Future work will be planned to use enriched cultures as seed sludge for anammox reactors to be applied to real scale wastewater treatment.

3. Final Remarks

In this study, we expect to contribute to a wider application of the anammox process in wastewater treatment by improving current available technology with fundamental knowledge of anammox ecology.

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