

Background

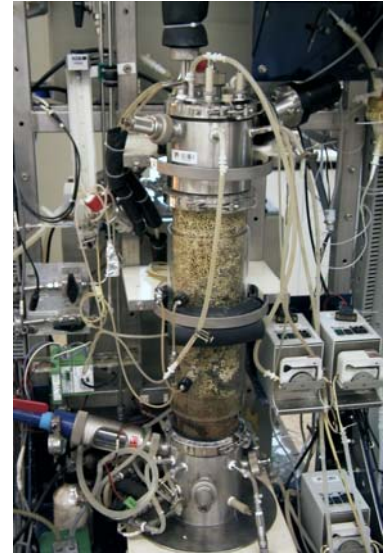
Since 1988, the Expertise group of Molecular and Cellular Biology (MCB) is an important partner in the development of the Micro-Ecological Life Support System Alternative (MELiSSA). The MELiSSA was designed to allow a small crew to survive on an Antarctic, lunar or Mars outpost, and is a joint research project currently fostered by the European Space Agency, ESA. The MELiSSA functions through a series of five interconnected compartments, of which four are microbial bioreactors and was engineered to degrade organic waste, regenerate the outpost's atmosphere and water, and provide the crew with an additional vegetarian diet.

The bioreactor of the third compartment provides the edible cyanobacteria and plants of the fourth compartment with nitrate instead of ammonium as a source of nitrogen. The two bacteria responsible for the biological transformation of ammonium to nitrate (nitrification) are *Nitrosomonas europaea* and *Nitrobacter winogradskyi*. Since all MELiSSA-reactors are to be relied on for a period of several years, reactor operation is to be studied exhaustively to allow optimal process- and reactor performance. Therefore, a pilot reactor for the third compartment was engineered and constructed at the Universitat Autònoma de Barcelona (UAB), where the pilot plant of the MELiSSA is installed. The reactor was able to perform nitrification with high efficiency for the entire trial period of nearly five years and was the subject of this study.

Objectives

Collaboration between the unit of MCB and researchers at Universitat Autònoma de Barcelona (UAB) allowed the development and validation of a mathematical model for the third compartment of the MELiSSA. A mathematical model will allow optimizing reactor operation and reactor performance even further. A Real-Time Quantitative Polymerase Chain Reaction (Q-PCR) was developed at MCB that allowed the quantitative assessment of the relative distribution of the two autotrophic nitrifying bacterial species along the reactor's packed-bed. Thus, the experimentally obtained distribution profile could be compared with the mathematically predicted distribution profile.

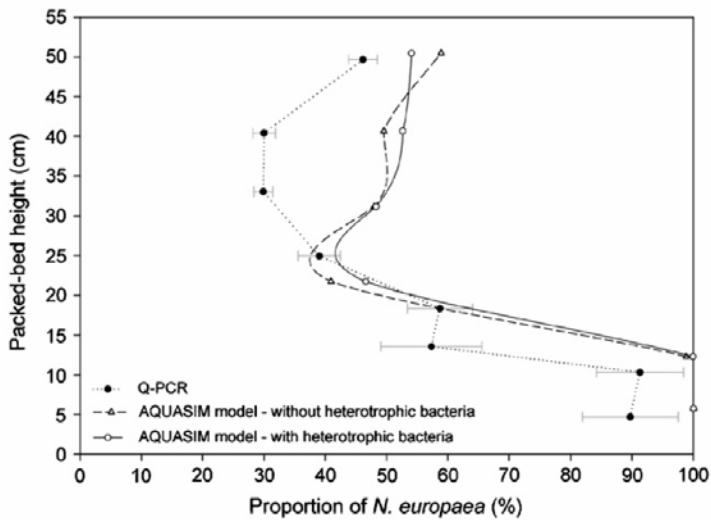
Nitrifying packed-bed biofilm reactor after 1750 days of reactor operation at the Universitat Autònoma de Barcelona



Principal results

At UAB, a mathematical model was constructed using AQUASIM software, which takes into account aeration, biomass detachment from the biofilm, diffusion of substrates within the biofilm, and bacterial cell kinetics as dynamic parameters. The bacterial kinetic processes taken into account were cellular maintenance, cellular division and decay for both bacterial species.

Using the data generated during reactor operation and after reactor dismantling, the model predicted biomass generation over the packed-bed length, total substrate conversion, and the distribution of both bacterial species on the packed-bed over a period of nearly five years. The calculated results for the distribution of both bacteria along the packed-bed were subsequently compared to the experimental data, provided by the MCB at SCK•CEN.



Graph illustrating the relative distribution of both nitrifying bacteria (*Nitrosomonas europaea* and *Nitrobacter winogradskyi*) along the packed-bed height. Both approaches show the numerical dominance of *Nitrosomonas europaea* over *Nitrobacter winogradskyi* at the bottom of the reactor.

Because of the good correlation with the experimental Q-PCR data, it was concluded that the mathematical model could accurately predict the distribution of both nitrifying bacteria. However, the distribution profile generated with the mathematical model slightly overestimated the relative presence of *Nitrosomonas europaea* over *Nitrobacter winogradskyi*. Therefore, the influence of a number of parameters was studied more thoroughly. Thus, it was determined that the maximum density of the biofilm, biofilm porosity, and biofilm thickness only had minor influences on the relative distribution of both nitrifying bacteria, while liquid phase flow pattern, growth yield ratio between *N. europaea* and *N. winogradskyi*, the oxygen concentration and, to some extent, the presence of a heterotrophic population of bacteria, had a more important impact on the distribution of both autotrophic nitrifying bacteria. These parameters did not allow to completely model the profile. It could also be possible that other micro-organisms, playing

a certain role in ammonium conversion, may have established themselves in the biofilm. Future research will hence be necessary to elucidate the identity and quantity of the other organisms residing in the biofilm to answer this question.

Future developments

Because the Q-PCR approach has proven to be a powerful tool to profile bacterial communities, its worth in this application, the technique will be further improved and expanded to fulfil future requirements, e.g. quantification of other nitrifying bacteria or quantifying contaminants in any of the MELiSSA reactors.

A fruitful collaboration, between MCB of SCK•CEN and the academic research partners, UAB and UGent, was established and will aid the further development of the MELiSSA.

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Main reference

Montras, A., B. Pycke, N. Boon, F. Godia, M. Mergeay, L. Hendrickx and J. Perez (2007). Distribution of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* in an autotrophic nitrifying biofilm reactor as depicted by molecular analyses and mathematical modelling. *Water Research*. In press.