Continuous nitrification of artificial urine with a bacterial co-culture in a packed-bed bioreactor

Justyna Barys[1,2,3], Christel Paille[1], Carolina Arnau[2], Francesc Gòdia[2], Nico Boon[3], Siegfried Vlaeminck[3,4], Peter Clauwaert[3]

1) Life & Physical Sciences and Life Support Laboratory, European Space Research and Technology Centre, European Space Agency, The Netherlands
2) MELiSSA Pilot Plant – Laboratory Claude Chipaux, Universitat Autònoma de Barcelona, Spain
3) Laboratory of Microbial Ecology and Technology, Faculty of Bioscience Engineering, Universiteit Gent, Belgium
4) Research Group of Sustainable Energy, Air and Water Technology, Department of Bioscience Engineering, Faculty of Science, University of Antwerp, Belgium

A life support system (LSS) in space aims at creating a safe and healthy environment for the astronauts. Nowadays LSS provides water and oxygen by physico-chemical methods. Nevertheless, current LSS cannot provide food, which has to be delivered from the Earth. Production of food can be achieved by using regenerative life support systems (RLSS). MELiSSA (Micro-Ecological Life Support System Alternative) aims at producing food via vegetable crops and edible phototrophic bacteria. To produce this phototrophic biomass and to provide sustainability to the system, conversion of urine, a waste stream containing ~85% of the crews' nitrogen intake, into a nitrate substrate should be achieved. Conversion of urine to nitrate is extremely important for the photoautotrophic elements, because nitrate is the main source of nitrogen for plants. Urine is a complex matrix composed of many organic and inorganic components, such as urea. For converting urea into nitrate, it will be necessary to use a bacterial consortium composed of strains allowing urea hydrolysis, ammonium and nitrite oxidation. Cell immobilization on a solid support is particularly interesting for nitrification purposes, as nitrifiers are slow growing microorganisms, as a packed-bed configuration allows biomass retention during continuous bioreactor operation. The main goal of the conducted research is to develop a synthetic microbial consortium to nitrify synthetic urine in the continuous system based on immobilised cells in a packed-bed bioreactor.

A consortium of three bacterial strains (*C. pinatubonensis, N. europaea* and *N. winogradskyi*) was tested to convert urea to nitrate in an immobilised packed bed bioreactor. Culture medium was an optimal medium for cultivation of *N. europaea* and *N. winogradskyi*, in which ammonium was exchanged for urea. It was demonstrated that in the conditions tested the synthetic microbial consortium hydrolysed urea and oxidized ammonium to nitrate. PVA gel bioreactor beads have been used as a biofilm support media and it proved to be a good carrier for biofilm formation for strains used.

Taking as a basis these preliminary results, a more systematic work was planned in order to identify the optimal conditions for the desired biotransformation, with the final target to test it in the MELiSSA Pilot Plant:

a) Selection of the optimal synthetic microbial consortium able to convert urea into nitrate in a synthetic urine medium,
b) Selection of an autoclavable, non-compressible biofilm carrier supporting good biofilm development,
c) Use of molecular biology techniques to characterize microbial distribution in biofilms,
d) Test of selected combinations of media, bacterial cells and biofilm support material in three bench scale packed-bed bioreactors,
e) Test the best conditions at pilot scale level.