

## Bioreactor operation shows haloplasticity of a synthetic nitrifying community, enabling urine treatment in space

Marlies E.R. Christiaens<sup>1</sup>, Chiara Ilgrande<sup>1</sup>, Peter Clauwaert<sup>1</sup>, Siegfried E. Vlaeminck<sup>1,2</sup>

<sup>1</sup>Ghent University, Center for Microbial Ecology and Technology, Coupure Links 653, 9000 Gent, Belgium

<sup>2</sup>University of Antwerp, Research Group of Sustainable Energy, Air and Water Technology, Groenenborgerlaan 171, 2020 Antwerpen, Belgium

In long-term manned space missions mass limitations introduce the need for on-site food production. A stable liquid fertilizer can be obtained from nitrifying astronauts' urine [1,2]. Urine nitrification by an open microbial community as reported in literature [3,4,5] can contain pathogens endangering the health of the space crew. A carefully assembled synthetic microbial community eliminates this risk. Consequently, reactor operation must be sterile. Open nitrifying communities can be adapted to the high salt concentrations in nitrified urine (up to 75 mS cm<sup>-1</sup> [2]). However, the haloplasticity of a pre-defined nitrifying community has not been validated.

Gradual salt adaptation of the selected coculture *Nitrosomonas europaea* ATCC19718 and *Nitrobacter winogradskyi* Nb-255 ATCC25391 was studied in an axenically operated chemostat with synthetic hydrolysed urine (no organics). Initial reactor operation at 10 mS cm<sup>-1</sup> yielded an oxidation rate of  $41 \pm 18$  mg NH<sub>4</sub><sup>+</sup>-N L<sup>-1</sup> d<sup>-1</sup> for 84 days. Increasing conductivity to 35 (8 days) and 45 (6 days) mS cm<sup>-1</sup> inhibited ammonium oxidation for one day. Absence of nitrite accumulation indicated that mainly *N. europaea* suffered the salinity increases. At 55 mS cm<sup>-1</sup>, the reactor activity dropped by 88% after 7 days and could not be recovered. A maximum salinity level of 45 mS cm<sup>-1</sup> is advised to maintain nitrification activity, corresponding to the low urine dilution of 40%. Hence, in the long term, a large haloplasticity (10-45 mS cm<sup>-1</sup>) was confirmed for the selected nitrifiers.

Despite of careful axenic operation, heterotrophs grew into the community. Analyses with 16S rRNA illumina sequencing indicated *Pseudomonaceae* as abundant contaminants (up to 50%). In a follow-up reactor run, the synthetic nitrifying community was supplemented with three ureolytic heterotrophs, including *Pseudomonas fluorescens* LMG 17943. This experiment on real human urine will reveal the ability of the selected heterotrophs to prevent contamination via pre-emptive colonisation and substrate competition.

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