Bioreactor nitrification with *Nitromosonas europaea* and *Nitrobacter winogradskyi* revealed sufficient halotolerance for urine treatment in space

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In long-term manned space missions, mass and economical limitations introduce the need for onsite food production. Astronauts' urine can be nitrified to obtain a stable liquid fertilizer for the production of food and oxygen through microalgae and plant crops. However, restrictions and challenges are inherently connected in using urine for food production in space. A mixed microbial community for urine nitrification as reported in literature cannot be applied because pathogens can endanger health of the space crew. The European Space Agency (ESA) suggested urine should be nitrified by a synthetic microbial community. Consequently, reactor operation must be sterile, including urine sterilization. Open nitrifying communities can be adapted to the high salt concentrations in urine (up to 75 mS cm^{-1}). However, the haloplasticity of a pre-defined nitrifying community with *Nitrosomonas europaea* ATCC 19718 and *Nitrobacter winogradskyi* Nb-255 ATCC 25391 has not been validated.
Gradual salt adaptation of this coculture was achieved in an axenically operated chemostat with synthetic hydrolysed urine. Nitrogen loading rates and salinity were increased by separate NH_4^+ and salt solutions.
Initially, the reactor was operated at 10 mS cm^{-1} with 42.5 mg N L^{-1} d^{-1} loading rate corresponding to an oxidation rate of 41 ± 18 mg NH_4^+-N L^{-1} d^{-1}. Higher loading rates caused free ammonia (FA) build-up. Predictive batch tests were performed to screen for the highest conductivity at which reactor activity could be maintained.
A first increase to 35 mS cm^{-1}, inhibited NH_4^+ oxidation for one day, indicated by a peak of 1.3 mg NH_3-N L^{-1}. No free nitrous acid could be detected, thus mainly *N. europaea* suffered from the salt. A subsequent increase to 45 mS cm^{-1} yielded a similar scenario with restabilization around 45 mg NH_4^+-N L^{-1} d^{-1}. In the predictive batch tests, *N. europaea* showed 35 and 28 mg N L^{-1} d^{-1} at 35 and 45 mS cm^{-1} salt shocks, respectively, which is lower compared to the reactor as there was no adaptation time. The activity of *N. winogradskyi* was 23 and 30 mg N L^{-1} d^{-1} during salt shocks of 35 and 45 mS cm^{-1}, respectively.
At 55 mS cm^{-1}, activity could be maintained for 7 days at 46 ± 9 mg NH_4^+-N L^{-1} d^{-1} after which gradual FA build-up indicated *N. europaea* could not maintain its activity. Rates dropped with 88% until 5 ± 9 mg N L^{-1} d^{-1} was reached in the reactor and 3.8 ± 0.2 mg N L^{-1} d^{-1} in the finalizing batch activity test. *N. winogradskyi* was also influenced by this salt increase as the batch test showed a decrease to 4.6 ± 0.1 mg N L^{-1} d^{-1}. Activities at lower salinities (10, 30, and 45 mS cm^{-1}) barely recoverd in the batch test for both *N. europaea* and *N. winogradskyi* with maximal activities of 9.6 ± 1.2 and 14.2 ± 1.2 mg N L^{-1} d^{-1} at 10 mS cm^{-1}, respectively.
In order to maintain nitrification activity a maximum salinity level of 45 mS cm^{-1} is advised, corresponding to a 50% urine dilution. Currently, additional reactor tests are running on real urine with an extended community.