Enyi, Francis Owuna (2017): Characterization of Planktonic Anammox-Containing Mixed Culture. Supervisors: Fischer, Adrian (Masterthesis).

ABSTRACT

Anaerobic ammonium oxidation (Anammox) is a promising alternative process to the conventional nitrification/denitrification process commonly used to remove nitrogen species in waste water. Despite its pivotal role in waste water treatment, the anammox bacteria responsible for this process have been known to grow extremely slowly, doubling once in every 3 to 10 days depending on species and conditions of growth.

In this thesis, anammox bacterium species *Kuenenia stuttgartiensis* co-culture was successfully grown in a continuously stirred tank reactor (CSTR) as suspended cells with doubling time of about 2.1 days, one of the shortest ever reported in literature. The use of CSTR is newly developed and has never been used to grow and enrich anammox bacteria.

Also in this thesis, inhibition experiment was performed with selected compounds, some of which are frequently seen in waste water, and which are potential inhibitors of anammox process. $NO_2^-$, salt (NaCl), $PO_4^{3-}$, sulphide ($H_2S$), chloramphenicol and methylene blue were tested to determine their inhibitory concentration using a pressure sensor. The inhibition experiment reveals that sulphide and methylene blue had the highest inhibition effect on anammox activity whereas phosphate - up to concentrations of 50mM - does not seem to affect the anammox activity significantly. Sulphide concentration as low as 30µM resulted in complete and methylene blue concentration as low as 100 µg/L caused more than 50% inhibition of anammox activity. Nitrite and salt were found to be moderate inhibitors. Chloramphenicol could be tolerated up to 10 mg/L.

Shortgun proteomics using nLC-MS/MS was performed to identify and quantify the proteins present in the anammox bacterial species *Kuenenia stuttgartiensis*. All proteins associated with anammox activity were found and discovered to be highly expressed except nitrite reductase (NirS) whose abundance was lower than the others.

The centrally located membrane of anammox bacteria, the anammoxosomes, where metabolic activities takes place was also isolated and proteomics performed on it. The design of an enzymatic activity test was attempted to determine the expression of one the key enzymes (nitrite reductase) in anammox process.

This study provides first insights of the use of CSTR in growing anammox bacteria. It also reveals the tolerance of planktonic anammox cells to compounds and substrates amongst some of which are routinely found in waste water.