Testing for the removal of tris(2-chloroethyl) phosphate under anammox conditions

Introduction:

As Earth's resources dwindle and the human population grows, there is an increasing need to maximize the efficiency of the planet's resource management. The current world population of 7.3 billion is expected to grow to 9.8 billion by 2050, according to the United Nations Population Prospects report. To illustrate the issue of resource scarcity, the United Nations "Zero Hunger" project claims that 795 million people around the world are currently undernourished, and the figure is projected to increase as competition for resources increases. Similarly, it is estimated that 783 million people currently lack access to clean drinking water. Due to clean water's crucial nature for human survival, it is important to preserve and maintain the resource for future generations.

Anaerobic ammonium oxidation (anammox) is a naturally occurring process whereby nitrite and ammonium ions are converted to nitrogen gas and water **[1]**.

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O.$$

There are ten species of anaerobic ammonium oxidizers which are often found in wastewater sludge (semi-solid material produced during the treatment of sewage water). Anammox bacteria are of great interest to researchers, partially because they provide a naturally occurring, environmentally friendly manner of denitrifying (effectively "cleaning") water bodies.

Tris(2-chloroethyl) phosphate (TCEP) is a chlorinated flame retardant which is commonly used in industrial fireproofing, and which is increasingly being used to fireproof everyday objects, such as furniture and toys **[2]**. TCEP is a carcinogen which is toxic in high concentrations, and which is increasingly found in wastewater and freshwater bodies, with concentrations of up to 10mg/L in freshwater **[3]** and 30mg/L in wastewater **[4]** being quoted by different studies.

Objectives:

Previous studies have demonstrated that estrone is degraded under anammox conditions**[5]**, therefore the objective of the study was to determine whether anammox bacteria degrade TCEP added to the reactor's influent solution.

Progress:

- Batch reactors were initially set up, but were not capable of sustaining anammox bacteria populations, and the bacteria ended up dying (likely due to the relatively long intervals between "feeding" cycles necessitated by the reactor design.)
- Liquid-liquid extractions were initially used to isolate TCEP in samples, but were slow and inefficient due to the volume limitations. SPE was found to be more efficient and effective.
- GC analysis was performed on TCEP samples in hexane and ethyl acetate, but the molecule was not detectable in the apparatus. GCMS analysis was successful in detecting TCEP in ethyl acetate.
- An SBR reactor was set up with a population of anammox bacteria, and was subsequently "fed" TCEP for analysis. This reactor was successful at sustaining the bacteria.

Figure 1- The final model of the SBR (sequencing batch reactor) used to conduct the experiment.

Results:

No TCEP degradation was detected at 100ppb TCEP concentration in the annamox reactor. Additionally, the relatively miniscule amount of TCEP added appeared to negatively impact the anammox process, causing denitrification activity to cease almost entirely (Figure 2). The experiment was stopped following the demonstrated toxic effect of TCEP so as to allow the bacteria in the reactor to survive and recover from the toxic shock. Future experiments will focus on introducing TCEP to the bacterial community in a more gradual manner in order to assess its threshold of toxicity.

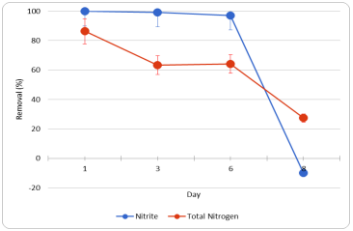

Figure 2- Nitrite removal (%) and total nitrogen removal (%) from influent to effluent in the reactor over the days of the experiment. TCEP was added on day 6, after which a noticeable decline in both removals is observed. Nitrite removal is shown to be less than 0 as there was a net production of nitrite in the effluent.

References:

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[2]Tris (2-chloroethyl) phosphate IARC evaluation, 1990.

[3]Ren, X., Lee, Y.J., Han, H.J., and Kim, I.S. 2008. "Effect of tris-(2-chloroethyl)-phosphate (TCEP) at environmental concentration on the levels of cell cycle regulatory protein expression in primary cultured rabbit renal proximal tubule cells." *Chemosphere* ***74****, 84-88.*

[4]Meyer, J., Bester, K. 2004. "Organophosphate flame retardants and plasticisers in wastewater treatment plants." Journal of Environmental Monitoring **6**, 599-605.

[5]Peterson, K.N., Tan, D.T., Bezares-Cruz, J.C., and Novak, P.J. 2017. "Estrone biodegradation in laboratory-scale systems designed for total nitrogen removal from wastewater." *Environ. Sci.: Water Res. Technol.*

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