Potential Inhibition By Organic Matter In Cambi-THP Anammox Activity

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ABSTRACT

Anaerobic ammonium oxidation, also known as Anammox, is an important microbial process of the nitrogen cycle. Excess nitrogen in water can result in diverse harmful outcomes such as eutrophication of aquatic environments and toxicity to living organisms. Anammox-mediating bacteria, discovered in 1999, display an efficient removal of ammonium in water and this relatively new mechanism has actively been applied to wastewater treatment engineering for removing excess nitrogen from wastewater. In addition, an environmental consultant company, Cambi, developed the Cambi thermal hydrolysis pressure technology that further enhances the efficiency of the Anammox process when installed to wastewater treatment plants. Yet, inhibitory activity on the Anammox process was detected in Cambi-installed wastewater plants, and the source of inhibition is unknown. This study seeks to identify the inhibitor that impedes the Anammox process through multiple comparative experiments between wastewater collected from Cambi-installed plants and regular Anammox treatment plants. The experiments will involve the culturing of the Anammox bacteria in batch reactors, sterile syringe filtration of Cambi-centrate, and residue ion chromatography. The collected data was analyzed by graphing and comparing the general changes in Anammox activity rates at different conditions. In this study, the potential inhibitory activity by organic matter group on Cambi-THP centrate fed Anammox bacteria is exhibited.

KEYWORDS

Environmental engineering, wastewater treatment, microbiology, nitrogen removal, water quality

INTRODUCTION

The efficient removal of excess nitrogen from wastewater is a key solution to worldwide water-related issues. However, managing nitrogen can be challenging due to many forms, such as ammonia, nitrite, nitrate, and organically bound nitrogen (Kartal et al. 2012). Inorganic nitrogen provides a nutrient source for algae in receiving waters , where wastewater is discharged, and the combination of nitrogen and phosphorous can cause uncontrolled blooms, which block waterways (Kuenen et al. 2008). As the lower layers of algae die off, the decomposition can cause low dissolved oxygen (DO) conditions (Figdore et al. 2011). This condition is called eutrophication. Ammonia also creates a biological oxygen demand, which contributes to eutrophication in natural waters (Dapena-Mora et al. 2007). Furthermore, ammonia is toxic to many aquatic species. When discharged from a treatment plant, it can cause the death of aquatic organisms in the receiving stream (Kartal et al. 2012). Lastly, high concentration of nitrate poses a health risk in drinking water supplies (Jin et al. 2012). For all of these reasons, the removal of nitrogen from wastewater is increasingly important.

To address these issues, wastewater treatment has become a common engineering technique that aims to purify contaminated water to a cleaner state so that it is safe for people and other living organisms to intake. The application of anaerobic ammonium oxidation (Anammox) involves the conversion of harmful nitrite and ammonium into harmless dinitrogen gas by Anammox bacteria and is utilized in the removal of excess ammonium in wastewater treatment (Kartal et al. 2012). Anammox has successfully substituted the conventional method of nitrogen removal, nitrification-denitrification, which required high oxygen and additional carbon inputs (Strous et al. 2006). This alternative approach is a much more cost effective, robust and sustainable method for nitrogen removal from wastewater, with no oxygen input and no excessive carbon required. Anammox process, however, can be impeded by two main factors: 1) a variety of inhibitory substances, including substrate, organic matter, and heavy metals (Galloway et al. 2004), and 2) the slow growth rate of Anammox bacteria that lowers the efficiency of the whole process (Mulder et al. 1995). The improvement on these impeding factors will bring tremendous effects on the wastewater-engineering field.

In order to speed up the wastewater treatment using Anammox process, it is necessary to determine an optimal, inhibition-free environment, where Anammox bacteria will exhibit fast

growth rate as well as efficient removal of nitrogen. The Norwegian environmental consultant company, Cambi, has developed a unique steam explosion thermal hydrolysis process (THP), which allowed efficient use of energy and overcomes the slow growth rate of Anammox bacteria (Martinez-Espinosa et al. 2011). Since its first installment in 1996, Cambi THP has been used worldwide. Recently, however, it has been detected that unknown inhibitory activity in a Cambi-installed wastewater treatment plant is slowing down its process, indicating its potential nitrogen removal rate is actually higher (van Dongen et al. 2011). Elimination of the unknown inhibitor could further improve the present efficiency of Cambi THP. The government of the City and County of San Francisco has recently proposed its plan to further apply Cambi THP to wastewater treatment plants in the bay area other than San Francisco Southeast Wastewater, where Cambi THP is already installed (van Dongen et al. 2011).

The overarching goal of this study is to determine the unknown inhibitor that impedes the Anammox process in the sludge collected from a Cambi THP-installed wastewater treatment plant located in southeast San Francisco. I have designed hypotheses for three steps of the experiments: (1) the inhibition only occurs in Cambi-centrate fed batch reactor, provided that the identical adjustments (pH, temperature, and influent concentration) are made to all three reactors so that inhibition is not caused by substrates, (2) the source of inhibition in Cambi-centrate will no longer be present when it is filtered, indicating that the source of inhibition is in the residue, (3) the source of inhibition belongs to organic matter (OM) group. Three major sample types will be used in this study: 1) Sludge Wastewater (SWW), 2) Centrate, and 3) Cambi-centrate. The difference of these samples is that they are collected at different stages of treatment process at the SEP. SWW will be collected from the primary sedimentation stage. Centrate is the liquid that has been separated from the solid substances during the centrifuge stage, which comes right after the primary sedimentation stage. Finally, the Cambi-centrate will be collected from the Cambi THP processing stage. I will only proceed to the next hypothesis when the prior one is accepted. The following are the conclusions and suggestions I can make if the first and second hypotheses are rejected: (1) If the inhibition occurs in Cambi-centrate fed batch reactor and in any other batch reactors, it will indicate that the occurrence of inhibition is not necessarily due to Cambi THP technique. On the contrary, if the inhibition does not occur in Cambi-centrate fed batch reactor, this will be an important finding that counter-argues the previously proposed paper on Cambi THP inhibition, (2) If the source of inhibition in Cambi-centrate is in filtrate, not in the

residue, it implies that the source of inhibition is non-polar since the sterile syringe only filters out the non-polar compounds. In this case, a liquid chromatography technique can be used to separate the sample into its individual parts based on the interactions of the sample with the mobile and stationary phases. I will use t-test to evaluate the significance of data. The successful determination of the inhibitor will lead to an enhancement of current Cambi technique by speeding up the nitrogen removal process, and thus provide cleaner water at a much faster pace.

METHODS

Site description

Samples will be collected from San Francisco Southeast Treatment Plant (SEP). SEP is located in Bayview Hunters Point and is nestled in the midst of a mixed industrial, commercial and residential area (How 2015). The SEP is San Francisco's largest and oldest wastewater facility, responsible for treating nearly 80% of the City's flow. The SEP treats 57 million gallons per day (MGD) of wastewater (How 2015).

Built in 1952, many parts of the SEP facilities represent 1940's technology and are operating well beyond their useful lives (Resnik 1990). In the fall of 2013, The San Francisco Public Utilities Commission (SFPUC) initiated the planning and design efforts for the New Biosolids Digester Facilities Project at the SEP (How 2015). The Biosolids Project team has evaluated and recommended the Thermal Hydrolysis Process (THP) to pretreat solids upstream of traditional solids digestion. The installment of Cambi THP will reduce the number and volume of new digesters required, reduces pathogens and odors, reduces residual volumes, generates energy and will enable SFPUC to beneficially reuse 100% of the biosolids generated (How 2015).

Research design and Data collection

Three major sample types will be used in this study: 1) Sludge Wastewater (SWW), 2) Centrate, and 3) Cambi-centrate. The difference of these samples is that they are collected at different stages of treatment process at the SEP (Lex et al. 2012). SWW will be collected from the primary sedimentation stage. Centrate is the liquid that has been separated from the solid substances during the centrifuge stage, which comes right after the primary sedimentation stage. Finally, the Cambi-centrate will be collected from the Cambi THP processing stage. A minimum of 20L of each type of samples will be collected to provide a sufficient amount for analysis. For preservation purposes, the volume of 20L will be collected into 40 bottles of 500mL so that they can be kept in the freezer and each 500mL of sample will be defrosted right before its use and any leftovers of the defrosted sample is be disposed. The collected samples will serve as the influents that feed Anammox activity in three batch reactors.

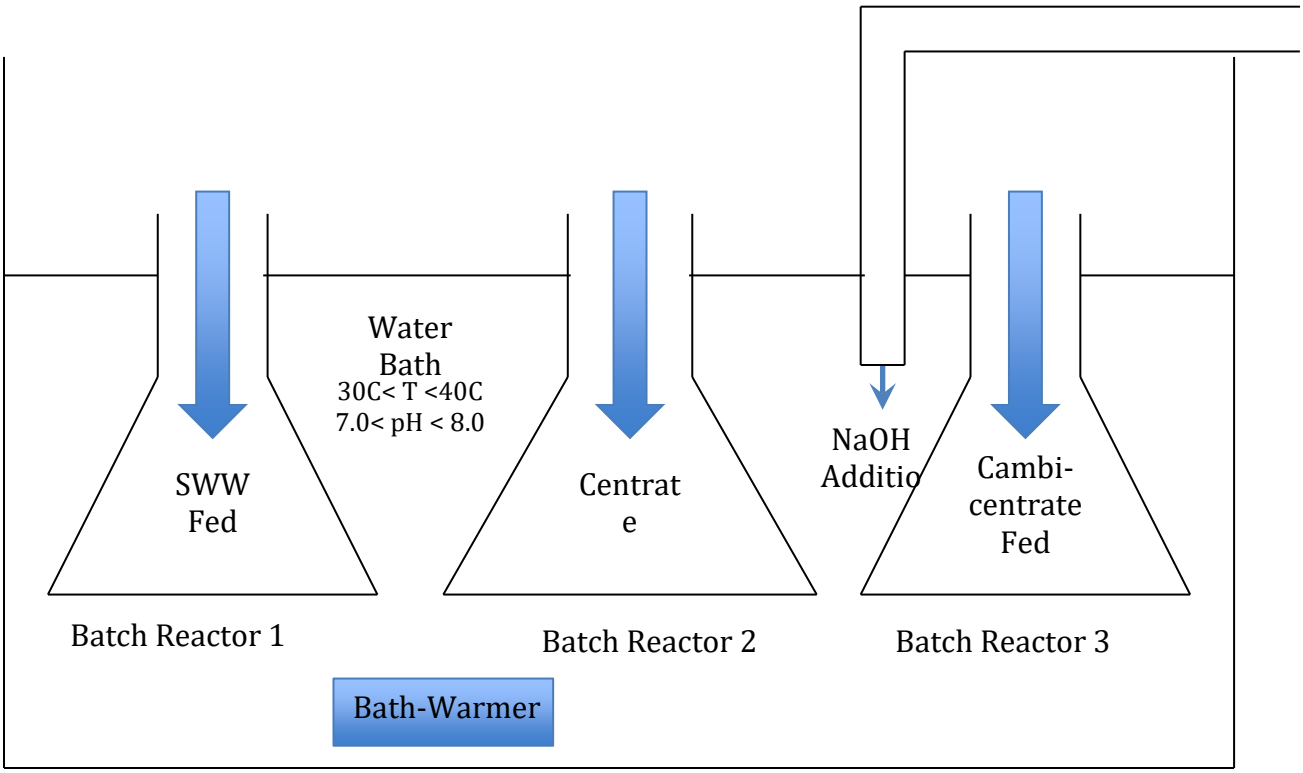

Figure 1: A sketch of set-up for Batch reactors. These three Batch reactors will be fed with three different influents. The influents will be SWW, regular centrate and Cambi-centrate.

Cambi-centrate inhibition

Batch reactors are chosen as the chemical reactor for this experiment because they are most adequate for small-scale production and laboratory applications (Ni et al. 2012). This part of the experiment tests the first hypothesis. In each batch reactor, three different influents will be the only inflow into the system. In order to ensure that the Anammox process is not inhibited by any other sources such as conditional factors and its own substrates (Strous 2006), careful adjustments will be made. The optimal pH and temperature for Anammox activity are 7.0 - 8.5 and 30 – 40 °C respectively (Menco et al. 2012). The following conditions are needed to prevent inhibition by the reactions' own substrates, NH_3 and HNO_2. These substrates are the true essence of` inhibition of anammox, hence providing optimal conditions to batch reactor will control the substrate level below the threshold values (Ni et al. 2012). The temperature will be adjusted by using the water bath and the pH can be adjusted by frequent addition of NaOH (alkaline chemical), since the reaction tends to turn acidic.

After giving 12 days of stabilization period (Bettazzi et al. 2010), the rate of Anammox activity will be measured by using Hach principles once a day for 3 months. This method allows me to obtain accurate concentrations of NO_2^-, NO_3^-, and NH_3 using analytical instruments and reagents in the batch reactors. During the anammox activity, NO_2^- and NH_3 are reactants and NO_3^- is the product (Kartal et al. 2010). Hence, low concentrations of NO_2^- and NH_3 (the reactants) and high concentration of NO_3^- (the product) will indicate high rate of Anammox activity, and vise versa. The concentrations of each compound will be plotted on a graph and compared for gradient analysis.

Cambi-filtrate

To test the second hypothesis, I applied a syringe filtration technique. Using 30mL syringe, each sample will be collected through sterile syringe filter with 0.2micrometer pore membrane (Jensen et al. 2007). Then using the same method used to test the first hypothesis, the rate of Anammox activity in the filtered sample (the filtrate) can be determined. In the same manner as above, I will do analysis of the data by comparing the rate of the Anammox activity in filtrate and original sample. If the rate of Anammox activity in the filtrate is faster than that of

original sample, it confirms that the source of inhibition was filtered out as a residue during the syringe filtration process.

Ion chromatography

The last hypothesis, *the source of inhibition belongs to one of the organic compound (OM) groups,* can simply be tested using ion chromatography. This process separates ions and molecules based on their affinity to the ion exchanger (Figdore et al. 2011). The residue collected after syringe filtration will undergo ion chromatography. Then, the list of molecular contents in the residue can be obtained. For analysis, I will eliminate the substances that are known to be harmless to Anammox activity from this list. Any other substances will each be added to filtrate solution of Batch (3) separately. I will then measure the rate of Anammox activity in these filtrate solutions in the same way as above. The rates will be recorded for 2 months. The filtrate solution with significant decrease in Anammox activity rate can be concluded as the one with inhibitor. I will use p-value statistical test to evaluate the significance. If the p-value is smaller than the alpha, the data is not significant; hence the hypothesis will be rejected.

RESULTS

Batch test

The cambi-centrate fed reactor exhibited the slowest rate of Anammox. The rate of Anammox activity gradually increased in all Batch reactors. The batch reactor (1) that is fed with SWW exhibited a slightly lower rate of Anammox activity than that of the batch reactor (2), fed with centrate.

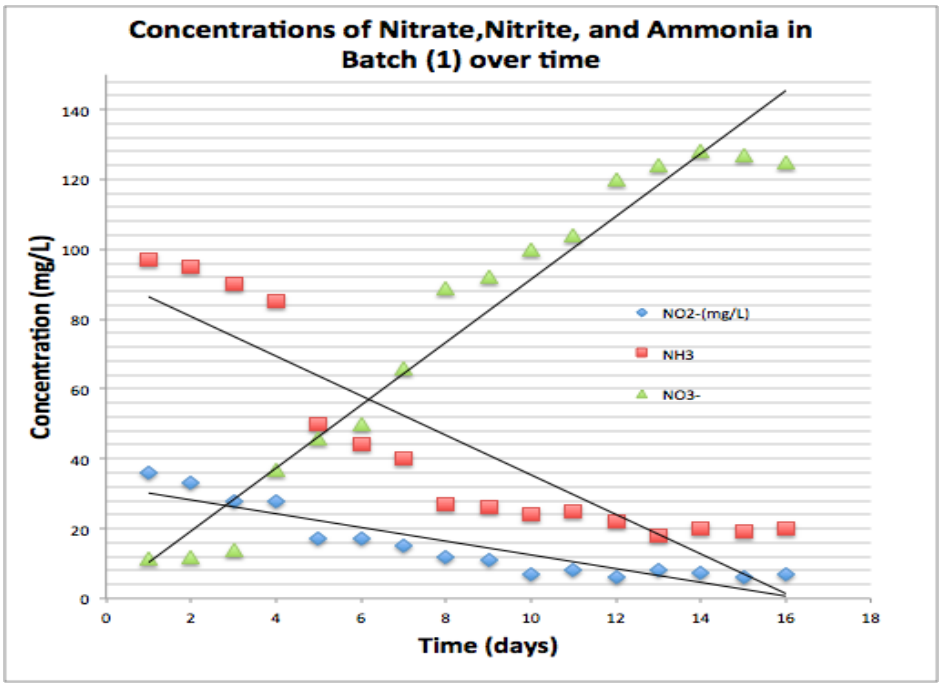

Figure 1: Change in concentrations of Anammox reactants and products over time. Nitrate concentration gradually increases whereas both reactants, nitrite and ammonia concentrations generally decreases.

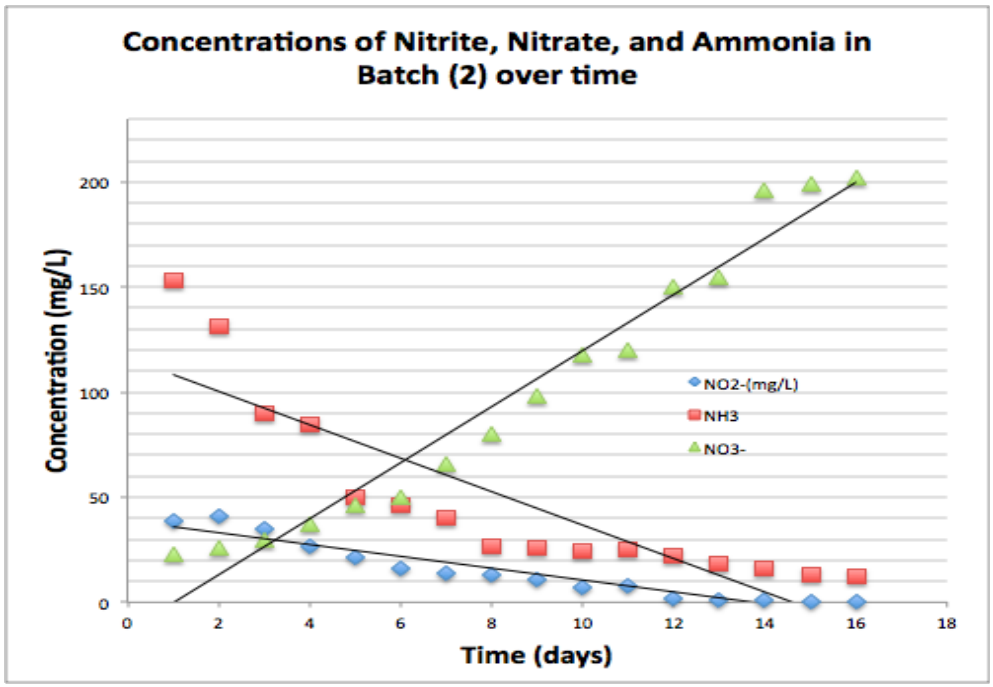

Figure 2: Change in concentrations of Anammox reactants and products over time in Batch reactor (2). Nitrate concentration increases at a relatively rapid rate whereas both products, nitrite and ammonia, decrease over time.

The centrate is further purified than the SWW since it is collected after the primary sedimentation. Batch (3) initially showed a slight increase in rate of Anammox activity, but soon started to slow down as the inhibitory activity started to impede Anammox process.

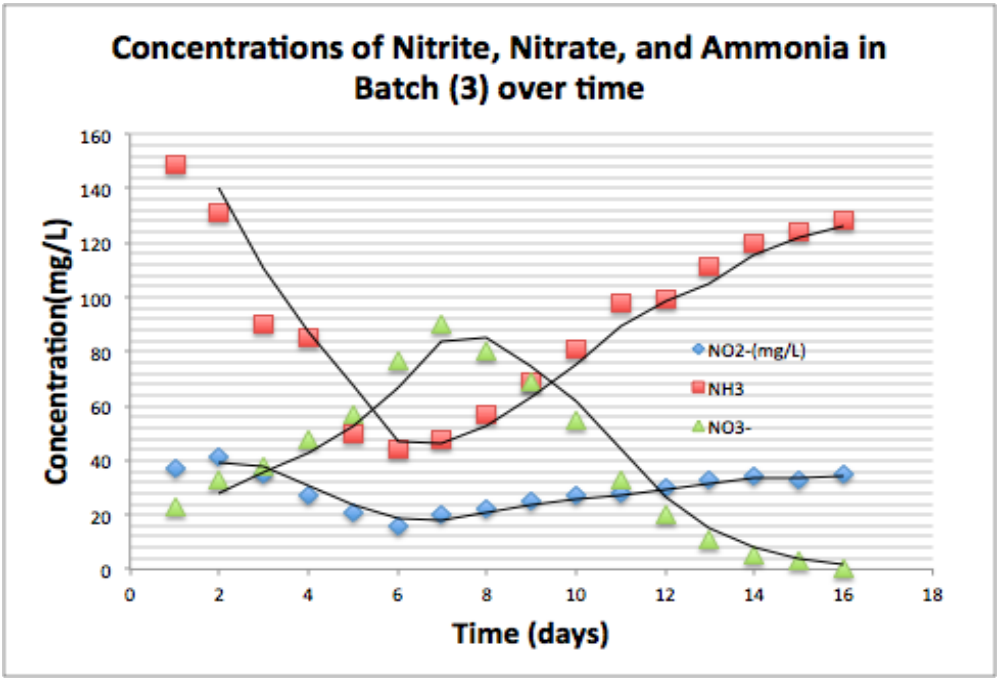

Figure 3: Change in concentrations of reactants and products of Anammox in Batch reactor (3). The concentration of the product, nitrate, initially increases but soon start to decrease. The reactants, on the other hand, decrease initially but increase eventually.

Cambi-filtration test

The source of inhibition was filtered out as a residue during the 0.2 micrometer syringe filtration. I recorded the nitrite, ammonia (reactants), and nitrate (product) concentration of 2 reactors (one fed with filtrate influent, another fed with residue influent) for 10 consecutive days and the data table below shows the average concentration values.

Average Concentration Data

	Reactor A (Filtrate)	Reactor B (Residue)
NO_2^-N (mg/L)	0.04	49.1
NH_3-N (mg/L)	94	380
NO_3-N (mg/L)	187	35
pH	7.043	7.126
Temperature (°C)	37.0	37.0
Flow (mL)	12.8	13.5
Pressure (atm)	0	0

I set identical environment in Reactor A and B by constant adjustment of pH, temperature, and pressure. The only difference between these two reactors was the type of influent that I was feeding them with. I fed Reactor A with the influent that had filtrate dissolved into it, while feeding Reactor B with the influent with residue dissolved into it. It is clear that the concentration of reactants in the Reactor B is significantly high in comparison to that of Reactor A. It indicates that the Anammox activity was not occurring as actively as it was in Reactor A. Therefore, the rate of anammox activity in Reactor B (fed with filtrate) was faster with the inhibitor removed.

Ion Chromatography

Lastly, through ion chromatography, a list of suspected inhibitors can be generated. The substances that are harmless to Anammox activity, such as NaOH, KCl, $NaHCO_3$, and $(NH_4)_3PO_4$ will be eliminated (Jetten 2009) and the remaining substances will likely belong to organic compound group. This is due to two major reasons. One is that wastewater often have high chemical oxygen demand (COD) value, indicating that there is high composition of organic carbon in water (Ni et al. 2012). The second reason is that organic compounds often interact with hydrazine and hydroxylamine, which are the intermediates that catalyze metabolic pathways of Anammox-mediating bacteria (Bettazzi et al. 2010). For these reasons, there is a high possibility that the inhibitor will be an organic compound.

DISCUSSION

Through multiple steps, I found the suspected inhibitor that impedes anammox activity by determining the rate of anammox reaction. I developed the setup procedure that provides the optimal environment for all the reactors to improve anammox procedure. This ensured that the source of inhibition solely came from Cambi-centrate. In this study, the efficiency of nitrogen removal by anammox activity largely depended on the influent content. I used the obtained effluent NO_3^- concentration and NO_2^- and NH_4^+ removal concentrations to calculate the Anammox reaction rate.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ = 1.02N_2 + 2.03H_2O + 0.26NO_3^- + 0.066CH_2O_{0.5} \quad \text{(a)}$$

The reaction equation (a) shown above suggests that every 1 mol of NH_4^+ requires 1.32 mol of NO_2^- to produce 0.26 mol of NO_3^-. In this reaction, we know that anammox bacteria derive their energy for growth from the 1:1 chemolithotrophic conversion of ammonium and nitrite into nitrogen gas. Bicarbonate serves as the sole carbon source for the synthesis of cell biomass ($CH_2O_{0.5}N_{0.15}$), making the organisms autotrophs (Jetten et al., 2009). Since N (nitrogen) is our core interest, I expressed NH_4^+, NO_2^-, and NO_3^- as nitrogen concentration (i.e. NO_3^-N = Nitrate as N). The relatively low concentration of NO_3^-N indicated that the anammox activity was not actively occurring in Cambi-centrate fed reactor. I compared the calculated rate of Anammox reaction in each of the experiments to determine which influent contained inhibitor.

Cambi-centrate

Low NO_3^-N concentration in effluents of Cambi-centrate fed reactor suggested the presence of inhibitor in the influent that slows the Anammox reaction rate. Concentrations of reactor effluents can be used to determine the rate of anammox reaction, using the relationship:

$$\text{rate} = -\frac{\Delta[\text{reactant}]}{\Delta t} \quad \text{or} \quad \text{rate} = \frac{\Delta[\text{product}]}{\Delta t}$$

Rate of the Anammox reaction suggests whether there is a factor that is impeding the metabolic reaction of Anammox bacteria. Low concentration of the product in Cambi-centrate fed reactor suggest the slow rate of Anammox reaction, hence suggests presence of inhibitor

Cambi filtration

Same theory applied to determine the rate of Anammox in filtrate and residue Residue presented slower rate of Anammox, narrowing down to the fact that inhibitor is in the residue, not in the filtrate of Cambi-centrate. Residue contains any substances larger than 0.2micrmeter. There was an unanticipated finding during this part of the experiment. Anammox bacteria's sensitivity to Potassium (K) concentration in the reactor

Ion Chromatography

Organic matter refers to the large pool of carbon-based compounds. Generally, wastewaters containing ammonia are not free from OM and actually some wastewaters contain high OM and nitrogen content (Waki et al., 2007). The presence of OM (organic matter) negatively affect anammox bacteria (Chamchoi et al., 2008), leading to less ammonia removal. This is because the anammox microorganisms can not compete with denitrifiers for nitrite under high OM concentration (Molinuevo et al., 2009). Due to efficient growth rate, nitrifying bacteria grew much faster than anammox sludge. Therefore, anammox bacteria were not able to outcompete with denitrifiers. What it indicates is that nitrite react with OM by denitrifiers under anoxic condition and inhibit anammox activity (Chamchoi et al., 2008)

Limitations

Culturing Anammox bacteria: Before starting Anammox research, I had to prepare sufficient amount of anammox bacteria with which I can test with. I planned out 6 months long anammox bacteria culturing period, which I thought would be more than enough, but due to constant failure in culturing CMFR reactors, my research was delayed for another 3 months. Working

with sensitive microbial sized bacteria left me with countless unexplainable failures. For many times, our bacteria were 'cooked', or got washed out as effluents. When I finally overcame these trial and errors, I had very little time to run batch reactors to determine the inhibitor, delaying the whole research by many days.

Preservation issue: It is nearly impossible to preserve the wastewater samples perfectly. During the delivery (from wastewater plant to laboratory), the composition of the wastewater can undergo certain amount of changes due to changing temperature and fluctuating amount of dissolved oxygen.

Future Directions

The best way to minimize the potential errors will be to shorten the delivery time as much as possible (avoiding rush hour), and prepare a heat insulator that will temporarily keep the inside environment as constant as possible.

Broader Implications

If the unknown inhibitor that impedes Anammox process in Cambi-installed plant is determined, it could ensure the high rate of wastewater treatment process. This will enable the cities with Cambi-installed wastewater plants to supply a greater amount of clean water at a given time. In particular, cities within the State of California, where severe drought prevailed since 1999, faster and more efficient wastewater treatment technique will provide a solution to a long drawn-out water shortage problem.

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