The Application of Partial Nitrification in Nitrogen Removal Process: The Potential Inhibitory Effect of Free Ammonia and Cambi-THP Pretreated Centrate

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ABSTRACT

Cambi Thermal Hydrolysis Process(Cambi-THP) is a high-temperature and high-pressure pretreatment process that can greatly reduce the energy cost of the anaerobic digestion process. However, previous studies have indicated that the Cambi-THP centrate can cause an inhibitory effect on ammonia-oxidizing bacteria(AOB). Since the AOB is the major bacteria used in Partial nitrification(PN) process, Cambi-THP centrate can result in the reduction of the treatment efficiency. Still, the correlation between the concentration of Cambi-THP pretreated centrate and the inhibitory effect is not well understood. Under the sponsorship of SFPUC, we conducted this research to find out the correlation between the percent of the Cambi Centrate used and the level of inhibitory effect. Also, we studied the inhibitory effect of free Ammonia(FA) on AOB in search of the FA and ammonium concentration at which we can get the highest nitrogen removal rate in PN process. To achieve the two research objectives, we conducted several sets of batch experiments for synthetic, regular and Cambi centrate at the ammonium concentration ranging from 0 to 2170 mg/L $NH_4^+ - N$. The result indicated that we can achieve the highest nitrogen removal rate at 542 mg/L $NH_4^+ - N$ and 9.7 mg/L FA-N. Also, the result of our study suggested that Cambi centrate didn't have a statistically significant inhibitory effect on AOB, which contradicts the results from Figdore et al. (2011). Overall, our research focused on the potential inhibitory effects in PN process and provided experimental data that can be used as a reference for the operation of PN reactor.

KEYWORDS

biological wastewater treatment process, treatment efficiency, ammonia oxidizing bacteria, batch experiments, ammonium removal rate

INTRODUCTION

Human populations are growing and releasing an increasing amount of nitrogen into the natural environment. Anthropogenic activities like food and energy production significantly increase nitrogen availability (Galloway et al.,2004). Since excessive nitrogen in the ecosystem can cause a number of environmental problems including water eutrophication, greenhouse gas emissions, and soil acidification, nitrogen accumulation is an important issue to address (Galloway et al., 2004). In order to match up with the increasing amount of nitrogen discharge, the engineers in wastewater industry developed a variety of physicochemical and biological treatment process to remove nitrogen from wastewater. Since biological treatment processes are more cost-effective and relatively inexpensive compared to physicochemical treatment process, they become the most commonly used methods of nitrogen removal (Ahn 2006).

Since the discovery of anaerobic ammonium oxidation (Anammox) in 1995, it has largely replaced the traditional Nitrification/Denitrification(N/DN) biological nitrogen removal process (Ahn 2006). The Anammox process is the anoxic oxidation of ammonium with nitrite as the electron acceptor (Van de Graaf et al., 1996). The stoichiometric equation for anammox process is shown below (Sultana 2014):

$$NH_4^+ + 1.32\,NO_2^- + 0.066\mathrm{HC}O_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_2^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$$

Compared to conventional nitrification/denitrification, Anammox can reduce the aeration and carbon-source demand by 50 to 100% (Fux et al., 2002). Even though anammox process is already a great advancement from traditional N/DN process, it is still not optimized regarding cost-efficiency. In order to further improve the nitrogen removal efficiency, researchers invented the Partial Nitrification/Anammox process (PN/A) by coupling the partial nitrification process and the Anammox process in a single treatment plant. (Sultana 2014). As we discussed above, anammox process requires both ammonia and nitrite as reactants, and ideally, the rate of ammonia to nitrite should be 1:1.32 according to the stoichiometric equation of Anammox process. Since municipal wastewater usually contains a high concentration of ammonia and very low concentration of nitrite

(Ahn 2006), We can further improve the treatment efficiency of anammox by balancing the nitrite and ammonia concentration in the influent of wastewater.

The treatment process that engineers adopted to achieve the goal of partially convert ammonia to nitrite is partial nitrification(PN) process. The stoichiometric equation for partial nitrification process is shown below:

Partial Nitrification: $$NH_4^+ + 1.5O_2 \xrightarrow{AOB} NO_2^- + 2H^+ + H_2O$$

By mixing the effluent from partial nitrification reactor and original municipal wastewater, we can achieve the objective to balancing ammonia and nitrite in the wastewater, and, therefore, treatment efficiency of Anammox process can be further improved (Ahn 2006).

Currently, the PN/A process has already been successfully applied to treat ammonium rich water, and its economic benefit is widely accepted (Sultana 2014). Since anammox process requires low carbon to nitrogen ratio, PN/A saves about 60% of the aeration cost, 90% of the sludge handling and transport cost. Furthermore, because no additional carbon is needed, PN/A process can save 100% cost regarding the external use of carbon compared to conventional nitrification/denitrification (Mulder 2003). Nevertheless, PN/A treatment process is still far from perfect. Scholars are still conducting researches to further improve the efficiency of the nitrogen removal process in two promising directions, inhibition of the bacteria and the pretreatment process. (Sultana 2014)

Free ammonia(FA) is one of the major inhibitors in PN/Anammox process. Previous research indicated that the PN/A process is mainly hindered by the realization of partial nitrification process. (Wang 2016). Since the partial nitrification process is realized by the metabolism of ammonia-oxidizing bacteria (AOB) (Sultana 2014), AOB's inhibitors come to a heated research topic for improving the nitrogen removal efficiency. FA is the deionized form of ammonium. Therefore, it is an inevitable inhibitor in ammonia rich wastewater. According to literature, FA is observed to have an inhibitory effect on AOB that can cause 50% activity loss with an ammonium concentration higher than 770mg/L (Jin et al., 2012). Moreover, the inhibitory effect is positively related to the ammonium and FA concentration (Jin et al., 2012). On the other hand, when we set free ammonia aside, increasing ammonium concentration is verified to have a positive effect on nitrogen removal rate. Therefore, in order to balance the inhibition effect of FA and the positive effect of increasing ammonium concentration, we conduct this study to find out the concentration of ammonium and FA at which we can achieve the highest ammonium removal rate in partial nitrification reactor,

In addition to eliminating the inhibitor of PN/A, we also studied the inhibitory effect of a newly developed pretreatment process: Cambi Thermal Hydrolysis Process (Cambi THP). Cambi THP is a high-temperature and high-pressure pretreatment process that can greatly reduce the energy cost in the anaerobic digestion process. However, a study on deammonification of Cambi-digestion indicated that this pretreatment could cause a significant reduction in nitrogen removal rate (Figdore et al., 2011). Therefore, further studies focused on the cause of the inhibition and potential improvement in treatment efficiency. For our research, we focused on the mechanism of inhibition by cambi pretreated centrate under the sponsorship of San Francisco Public Utility Commission (SFPUC).

The objective of my research is to set up a partial nitrification reactor and study the FA and Cambi pretreated centrate inhibition on AOB. The first goal of this study is to find out the correlation between the concentration of FA and the level of inhibitory effect in partial nitrification reactors. Since the high concentration of FA can promote ammonium removal rate but inhibit AOB metabolism, I hypothesized that the ammonium removal rate would increase with FA concentration to a certain point and then drastically decrease to zero. The second goal of this research is to find out the correlation between the concentration of Cambi-THP pretreated centrate and level of inhibition in partial nitrification reactors and also verify the inhibitory effect caused by Cambi-THP pretreated centrate. I hypothesized that high Cambi centrate concentration could significantly impair the AOB activity and it has a much more significant inhibitory effect on AOB compared to that of regular centrate. In order to test both of the hypotheses, we first incubated the bacteria in a partial nitrification reactor. Next, we conducted batch experiments for synthetic, regular, and Cambi pretreated centrate at the initial ammonium concentration ranging from 0 to 2170 mg/L $NH_4^+ - N$. We used the ammonium removal rate as a measurement for the level of inhibitory effect for all batch experiments and conducted t-test to verify the inhibitory effect of Cambi pretreated centrate.

METHODS

Bacteria incubation

To run the experiments on measuring the inhibition of FA and Cambi Pretreated Centrate on AOB, we set up an AOB reactor and incubated the bacteria until the AOB became the majority of the bacteria in the reactor. We collected the seed of the AOB from the anammox reactor of the Southeast Wastewater Treatment Plant, located at San Francisco's Bayview District, 750 Phelps Street, 94124. The Southeast Wastewater Treatment Plant collects and treats 80% of municipal wastewater from the area along the eastern shore of San Francisco Bay. After the treatment, the wastewater is discharged into San Francisco Bay. On average, The Southeast Wastewater Treatment Plant treats about 63 million gallons of wastewater every day. Since the WWTP treats huge amounts of wastewater every day, the seed we got was in bright brown color, which means that the bacteria were very vigorous.

The reactor we chose to incubate the AOB was the SBR (sequential batch reactor). The SBR reaction process consists of four stages: fill, react, settle and decant. In the filling stage, the inlet pump opens, and the reactor is filled by the wastewater. Then, in the reacting stage, the stirrer in the reactor begins, and the bacteria react with the wastewater. In the settling stage, the stirrer stops and the suspended bacteria settled to the bottom of the reactor. Finally, in the decanting stage, we discharge the treated wastewater from the reactor.

Incubation process and influent for incubation

During the incubation process, we put the seeds of bacteria into the SBR and fed the bacteria with the influent made by adding $(NH_4)_2SO_4$, $NaNO_2$, and $KHCO_3$ to DI water. We added $(NH_4)_2SO_4$ as the ammonium source and $NaNO_2$ as the nitrous acid source. Bacteria require a stabilizing pH between 6.8 to 7.5 to grow most efficiently (Jin et al., 2012), so I added $KHCO_3$ as the pH buffer to keep the pH around 7. Table A1 shows the influent we made.

Table A1. Influent concentration for incubation process.

Substrates / Days.	$(NH_4)_2SO_4$ (mM)	$NaNO_2$ (mM)	$KHCO_3$ (g)
1	80	88	3
2	90	99	3
3	100	110	4
4	110	121	4
5	120	132	4
6	140	154	4
7	160	176	4
: .			
90	160	176	4

Starting from 80 mM $(NH_4)_2SO_4$ with a rate of $(NH_4)_2SO_4$ to $NaNO_2$ of 1:1.1, we increased the concentration of $(NH_4)_2SO_4$ by 10 mM every day until it reached 160 mM. We chose the rate of $(NH_4)_2SO_4$ to $NaNO_2$ to be 1:1.1 based on our previous batch experiments. In such an environment, only bacteria that can make use of ammonia and nitrite can survive because the excessive ammonia significantly inhibits the growth of other bacteria (Sultana 2014). As the concentration of influent gradually increased day by day, bacteria other than AOB slowly died out. After 90 days of incubation, we observed that the ammonium removal rate of the reactor was stabilized and the nitrate level in the reactor was negligible. Thus, we assumed that the majority of the bacteria remaining in the reactor was AOB and we could proceed to next step.

Batch experiments

In order to determine the nitrogen removal rate, we conducted several sets of batch experiments. For all of the batch experiments, we chose 200ml Erlenmeyer flasks as the reactors. For each of the batch reactors, we put a 1 g/L VSS of the AOB into the flask, which was determined in the Batch Set #1, and filled it to 100ml with our target wastewater. Next, we put a magnetic rotor into the flask and sealed it. We then put the flask onto the BIPEE 78-1 Laboratory Magnetic Heating Stirrer to react for about 3 hours. During the 3-hour reaction period required for the reaction to complete, we opened the flask every 20 minutes to collect samples. For each sample collection, we used 3ml simsii syringes to collect about 2 ml of solution and filter it with simsii Nylon Syringe filters to remove the suspended solid. We measured the concentration of NH_4^+-N, NO_2^--N, NO_3^--N, and pH using testing reagent sets from Hach Company. We used the High Range Ammonia reagent set for NH_4^+-N, the NitriVer 3 Reagent Set for NO_2^--N, and the NitriVer X reagent set for $NO_3^- - N$. Finally, we calculated the removal rates of NH_4^+ and NO_2^- as an indicator of inhibitory effect. The formula is shown below,

$$\text{Substrate removal rate} = \frac{Initial\ Substrate\ concentration - Final\ Substrate\ Concentration}{Time * VSS}$$

Batch Set #1: determine the suitable pH and VSS of AOB for the experiment

From our incubation process outlined above, we found that the pH in the reactor must be between 6.8 and 7.5 to keep the AOB functioning properly. Also, according to the formula of partial nitrification, the AOB produces acid during this reaction. Therefore, we expected the in-reactor pH to decrease by 0.3 to 0.4 throughout the testing period. Thus, we decided to test the nitrogen removal rate for two pH concentrations; 7 and 7.5.

We also needed to determine how much bacteria we should use for each batch reactor in order to reach the lowest ammonia concentration possible within the 3-hour reaction period. To do this, we used VSS, the mass of bacteria in a certain volume of fluid, as the measurement for the biomass. In our incubator, the VSS for AOB was 1 g/L. According to our data, a higher VSS concentration will result in better ammonium removal performance only if AOB is not at a concentration that AOB have to compete for resources. Thus, in order to make sure that we are not putting excessive AOB into the reactor so that AOB start to compete for resources, we tested two concentrations of AOB, which were VSS at 1g/L and 0.5g/L. Table 1 shows the concentrations we used for this set of batch experiments.

Table 1. Setup for the first set of batch experiments to test the suitable pH. (total volume:100ml)

Batch #	pH	VSS	Initial NH_4^+-N
1	7.5	1g/L	100
2	7.5	0.5g/L	100
3	7	1g/L	100
4	7	0.5g/L	100

Batch Set #2: free ammonia inhibition on AOB

We conducted the second set of batch experiments to study the correlation between free ammonia and the ammonia removal rate of AOB. Due to the limitation on the size of the magnet heating stirrer, we could only do six batch experiments at one time. As a result, we had to separate the testing criteria into three batch groups: low, medium and high ammonia concentration. Within each group, we used synthetic, regular and Cambi wastewater to test the FA inhibition. The synthetic wastewater was the wastewater we made in the lab. The formula was the same as the influent made in the incubation process. The regular centrate was the centrate without the Cambi pretreatment process. The Cambi pretreated centrate was centrate that was treated by Cambi pretreatment process. The data from this batch experiment allowed us to find out the correlation between the change in substrate concentration over the reaction period and the FA concentration. Table 2,3,4 shows the solution we used for this set of batch experiment.

Table 2: Setup for the batch experiments for high ammonium concentration (total volume:100ml)

Batch #	VSS (g/L)	Biomass(ml)	WW	% of WW	Initial NH_4^+-N (mg/L)
1	1	33.5	Synthetic	0	1085
2	1	33.5	Synthetic	0	2170
3	1	33.5	Regular	50	1085
4	1	33.5	Regular	100	2170
5	1	33.5	Cambi	50	1085
6	1	33.5	Cambi	100	2170

Table 3: **Setup for the batch experiments for medium ammonium concentration** (total volume:100ml)

Batch #	VSS (g/L)	Biomass(ml)	WW	% of WW	Initial NH_4^+-N (mg/L)
1	1	33.5	Synthetic	0	271.25
2	1	33.5	Synthetic	0	542.5
3	1	33.5	Regular	12.5	271.25
4	1	33.5	Regular	25	542.5
5	1	33.5	Cambi	12.5	271.25
6	1	33.5	Cambi	25	542.5

Table 4: **Setup for the batch experiments for medium ammonium concentration** (total volume:100ml)

Batch #	VSS (g/L)	Biomass(ml)	WW	% of WW	Initial NH_4^+-N (mg/L)
1	1	33.5	Synthetic	0	54.25
2	1	33.5	Synthetic	0	108.5
3	1	33.5	Regular	2.5	54.25
4	1	33.5	Regular	2.5	108.5
5	1	33.5	Cambi	5	54.25
6	1	33.5	Cambi	5	108.5

Batch Set #3: Cambi inhibition on AOB

We conducted the third set of batch experiments to investigate the correlation of the percentage of Cambi centrate in the reactor and the ammonium removal rate. In this set, we only conducted experiments on regular centrate and Cambi pretreated centrate so that we could get a direct view of how the ammonium removal rate varies due to the presence of Cambi pretreated centrate. Table 5 shows the detailed information of Batch Set #3. We repeated all of the batch experiments in Table 5 by three times.

Table 5. Experimental set-up for the Batch Set #3 (total volume:100ml)

Batch #	VSS (g/L)	Biomass(ml)	WW	% of WW	Initial NH_4^+-N (mg/L)
1	1	33.5	Cambi	2.5	54.25
2	1	33.5	Cambi	5	108.5
3	1	33.5	Cambi	12.5	271.25
4	1	33.5	Cambi	25	542.5
5	1	33.5	Cambi	50	1085
6	1	33.5	Cambi	100	2170
7	1	33.5	Regular	2.5	54.25
8	1	33.5	Regular	5	108.5
9	1	33.5	Regular	12.5	271.25
10	1	33.5	Regular	25	542.5
11	1	33.5	regular	50	1085
12	1	33.5	regular	100	2170

Data analysis

Batch Set #1

For Batch Set #1, we calculated the 3-hour ammonium removal rate for each of the batch reactors using the substrate removal rate formula provided above. We then compared the ammonium removal rate of Batch No. 1 to that of Batch No. 3 and ammonium removal rate of Batch No.2 to that of Batch No. 4 to determine which pH value resulted in higher treatment efficiency. Also, we compared the ammonium removal rate of Batch No.1 to that of Batch No.2 and Batch No. 3 to Batch No.4 to determine which VSS we should use for the next step of the experiments. We chose pH =7.5 and VSS=1 g/L for our case.

Batch Set #2

For this set of batch experiment, we recorded the nitrogen removal rate in for three kinds of wastewater and compared them of the same concentration. Also, we made a graph of 3-hour nitrogen removal rate of these three centrates so that we could have a direct view of at what point that the inhibition effect of FA on AOB start to have an negative effect on ammonia removal rate.

Batch Set #3

Even though we can use the data from Batch Set #2, we decided that the minor change in the environmental condition for these experiments on different days may have a significant effect on our result. So, we did three sets of the experiments of regular centrate and Cambi Centrate and graphed them separately. In order to determine at what concentration that Cambi pretreated centrate start to have a significant negative effect on ammonium removal rate, we conducted t-tests for at concentration for Cambi and regular centrate. We calculated the concentration of free ammonia according to the following formula:

$$[FA]=[\text{total ammonium}]/(10^{(pKa-pH)}+1)$$

RESULTS

Batch experiments

Batch set #1: Determine the most suitable pH and VSS of AOB for the experiment

All four batch experiments reached equilibrium at 3-hours. We used the initial and final ammonium concentration to calculate the ammonium removal rate. Figure 1 represents the change of ammonium concentration in the reactors during the 3-hour reaction period. Table 6 represents the ammonia removal rate for the four batch experiments in Batch Set #1. Since Batch 1 reached the highest ammonium removal rate with a relatively short period, we chose pH 7.5 and VSS=1g/L as the reaction condition for the following batch experiments.

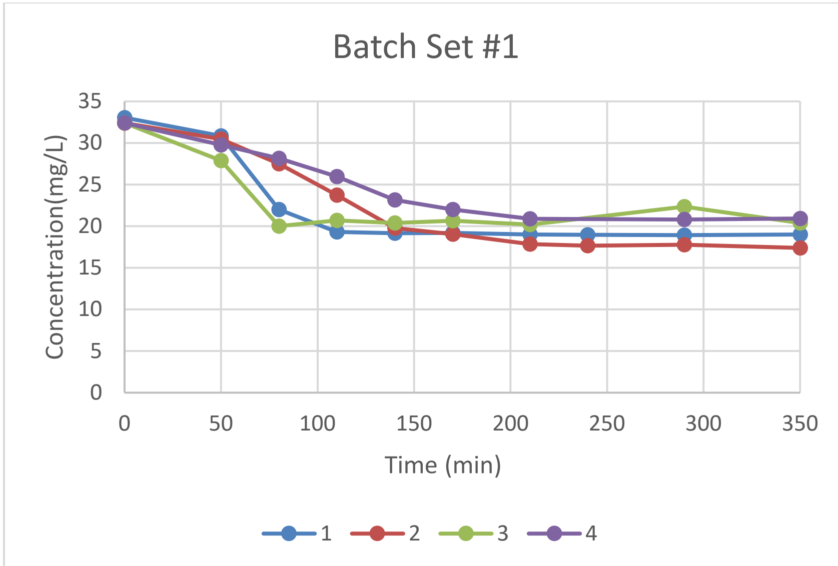

Figure 1: Concentration vs. elapsed time graph for the first set of the batch experiments

Table 6: ammonium removal rate ($\frac{mg}{min*g\ VSS}$) for Batch Set #1

Batch #	1	2	3	4
Ammonium removal rate	0.099224	0.090141	0.085798	0.065942

Batch set #2: Free ammonia inhibition on AOB

Figure 2 represents the data of the batch experiments in Table 2, which is the graph for 1085 mg/L and 2170 mg/L Initial NH_4^+-N. Batch 2,4&6 used the initial ammonium concentration of 2170 mg/L, and all of the three batch experiments went beyond their initial ammonium concentration during the 3-hour reaction period without additional ammonium input.

For Batch 1,3&5, the ammonium concentration slightly decreased. We put detailed data of ammonium removal rate for Batch 1-6 in Table 7**.**

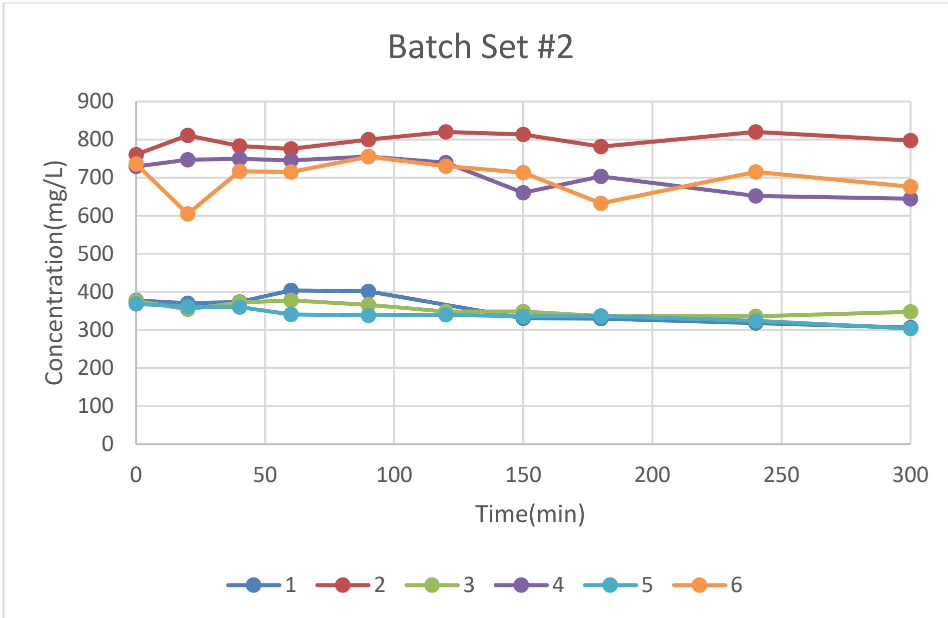

Figure 2. Concentration vs. elapsed time graph for the second set of the batch experiments (Ammonium)

Table 7 shows the ammonium removal rate for all of the batch experiment. And Figure 3 represents the ammonium removal rate vs. initial ammonium concentration graph for all experiments in Batch Set #2.

Table 7: ammonium removal rate ($\frac{mg}{min*g\ VSS}$) for Batch Set #2

Initial NH_4^+-N (mg/L) / Influents	54.25	108.5	271.25	542.5	1085	2170
Synthetic	0.035295	0.063302	0.162589	0.287455	0.238948	-0.1229
Regular	0.023989	0.037709	0.115447	0.209544	0.096441	0.282084
Cambi	0.027033	0.041563	0.117827	0.22241	0.216355	0.197375

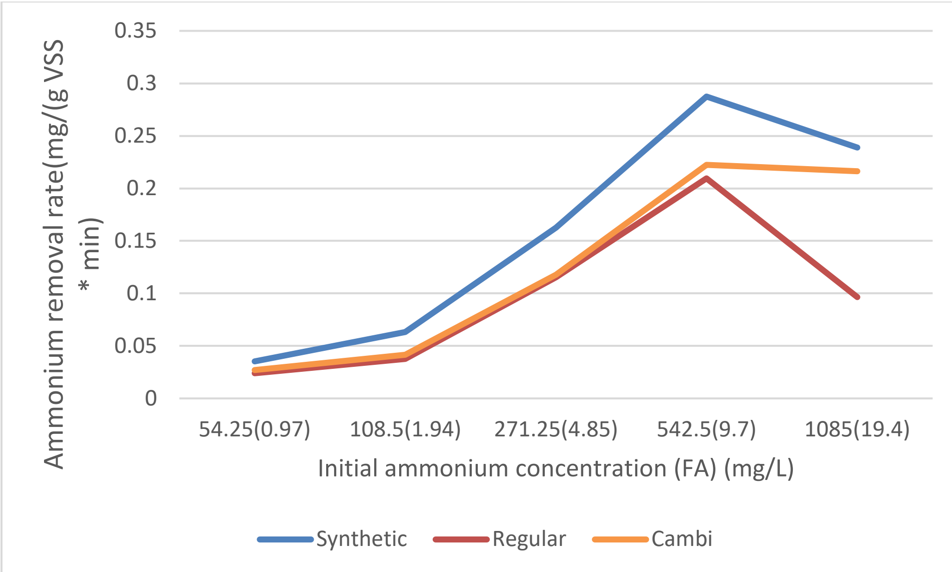

Figure 3. Ammonium removal rate vs initial ammonium concentration for Batch Set #2.

Batch Set #3: Cambi inhibition on AOB

Figure 4 shows the graph of ammonium removal rate vs. initial ammonium concentration for all the data in Batch Set #3. The red curve represented the data for regular centrate the blue curve represented the curve for Cambi pretreated Centrate. Table 8 shows the p-values for the t-test. All of them are not lower than 0.05, which means that the difference between the ammonium removal rate for Cambi centrate and regular centrate was not statistically significant.

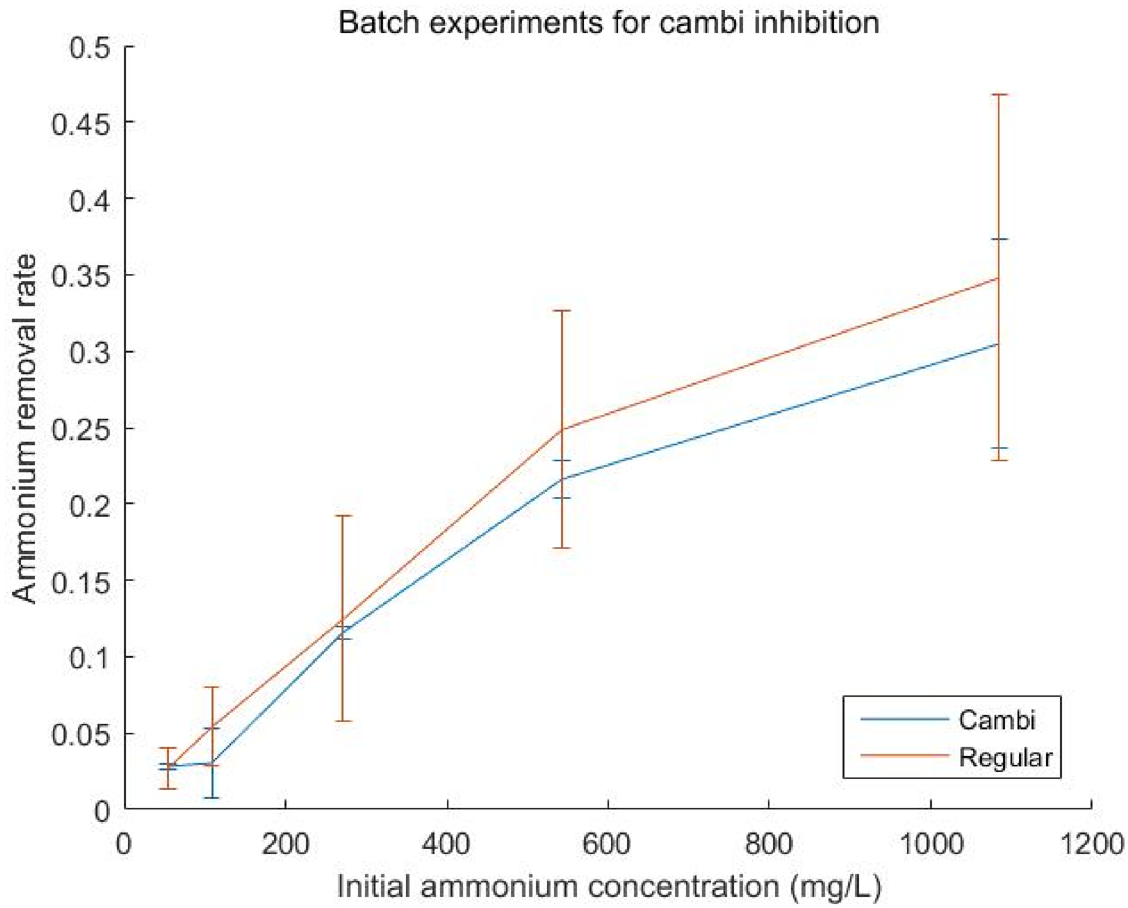

Figure 4. ammonium removal rate vs. initial ammonium concentration graph for the Batch Set #3

Table 8. P-value for all the test in Batch Set #3

Initial ammonium concentration (mg/L)	**54.25**	**108.5**	**271.25**	542.5	1085
P-value	0.7996	0.2286	0.707	0.3912	0.827

DISCUSSION

We found that high concentrations of FA have significant effects on the ammonium removal efficiency of AOB, but we didn't observe the significant inhibitory effect of Cambi pretreated centrate on AOB. In the first set of the batch, we verified that the optimal pH and VSS for our batch experiments are 7.5 and 1g/L VSS. In Batch Set #2, we found that wastewater with a high FA concentration could completely stop the AOB from consuming ammonia and converting it to nitrite at 2170mg/L $NH_4^+ - N$. More importantly, we found out that we can achieve the highest ammonium removal rate at 542.5 mg/L $NH_4^+ - N$ with a FA concentration no higher than 9.7 mg/L. After analyzing data form Batch Set #3, we verified that Cambi centrate does not have a significant inhibitory effect on ammonium removal rate of AOB according to our data. This contradicts previous research that the Cambi pretreatment process has a significant inhibitory effect on AOB activity (Figdore et al. 2011).

Batch Set #1: Determine the suitable pH and VSS

In determining the suitable reaction condition for our following experiments, we conducted the four batch experiments with different pH and VSS. The result is shown in Figure 1 and Table 6, and the setup for each curve can be found in Table 1. From Table 6, we can see that the reactors with pH 7.5 have a significantly higher ammonium removal rate compared to the reactor with pH 7 at T=140 min, which indicates that reactor with pH 7.5 has a higher treatment efficiency. Previous literature indicated that the suitable pH for AOB is 6.8 to 7.2 (Ahn 2006), which is lower than our tested concentration. One possible explanation for such a discrepancy between my result and literature could be that my reactors are too small. Since my batch reactors are only 100ml, the acid produced by conversion of ammonium to nitrite could result in a pH drop during the reaction period. As a result, initial pH should be higher than required so that it can fall to the range of 6.8 to 7.2 during the reaction period. Therefore, it is reasonable to choose pH 7.5 for the following experiments even though it is higher than indicated in the literature. However, we can only apply this assumption to the batch experiments with a reactor as small as ours. With a larger reactor or a more advanced reactor with pH control system, our assumption will not be valid.

Also, the ammonium removal rate at 140 min is higher for the reactor with 1 g/L VSS (indicated by Curve 1&3) compared to reactors with 0.5 g/L VSS (indicated by Curve 2&4). Such result indicated that using 1g/L VSS is more efficient. According to those facts, we decided that we would use pH = 7.5 and VSS =1 g/L for our batch experiments.

Batch Set #2: Free ammonia inhibition on AOB

We conducted Batch Set #2 to determine the correlation between free ammonia concentration and the ammonium removal rate. Also, we want to find the concentration of FA and ammonium at which the ammonium removal rate start to decrease. Figure 2 shows the result for Batch 2,4&6, which are batch experiments for synthetic, regular and Cambi wastewater with the 2170 mg/L initial ammonium concentration. We are not able to reach a conclusion for these three batch experiments because they unexpectedly went above the initial ammonium level during the reaction. Such result indicates that AOB cannot convert ammonia to nitrite in such a high concentration. More importantly, the results in the batch experiments with 2170 mg/L initial ammonium removal rate indicated that we cannot apply the partial nitrification process to the treatment of wastewater with a high ammonium concentration without a dilution process. We suspect that the increase in the ammonium concentration for the three reactors with 2170 mg/L initial ammonium concentration occurred due to the degradation of dead AOB killed by the excess of ammonium concentration in the reaction. The rest of the ammonium removal rate is shown in Table 7 and plotted in Figure 3.

From the Figure 3, we could see that there was only one peak for the ammonium removal rate for the concentration ranging from 0 to 1085 mg/L ammonium concentration, which verified my hypothesis that the ammonium removal rate would switch from an increase to decrease at a certain FA concentration. A previous study indicated that the effect of FA inhibition starts at an ammonium concentration of 770mg/L (Jin et al., 2012), which means that the turning point in my experiments should have been around 700 mg/L. However, the turning points, which is also the point at which we can achieve the highest ammonium removal rate, is at 542.5 mg/L $NH_4^+ - N$ with a FA concentration no higher than 9.7 mg/L. Such discrepancy could result from insufficient data points at the ammonium level near 700mg/L.

Batch Set #3 Cambi inhibition on AOB

For the final set of batch experiments, we conducted the test for Cambi Centrate and regular centrate independently to find the exact correlation between the initial ammonium concentration and ammonium removal rate. We found that even though ammonium removal rate for regular centrate was higher at all concentrations, but the t-test indicated that there was no significant difference between the average of the two set of data. None of the p-values are smaller than 0.05. Therefore, we conclude that Cambi pretreated centrate doesn't have a significant inhibitory effect on AOB. Such conclusion refuted our hypothesis that the Cambi centrate have a stronger inhibitory effect on AOB compared to regular centrate, which contradicts to the conclusion reached in Figdore et al. (2011). Therefore, further studies are necessary to verify whether Cambi pretreated centrate can inhibit the metabolism of AOB. If the conclusion of this study is verified to be the actual case, it will imply that we can apply Cambi pretreatment process into the treatment process involving the use of PN process.

We successfully finished all sets of batch experiments and came up with our result, however, there are limitations on my study design that makes it hard for me to reach a decisive conclusion. Due to the limited period of my research and insufficient lab equipment, I only did four sets of batch experiments for each wastewater so that it can meet the minimum requirement for statistical tests. Therefore, even though the scattered data indicated a trend of how AOB activity varies due to FA and Cambi concentration, the turning point and the peak point on the graph is not precise due to the lack of data points. Moreover, our research only focused on first half of the PN/Anammox process. The inhibition effect of Cambi pretreated centrate and FA on Anammox bacteria is not yet studied. Therefore, in order to verify that we can safely apply Cambi pretreatment process to the system with PN/Anammox process, its inhibitory effect on Anammox bacteria has to be investigated.

In the future, we aim to obtain more data sets for Cambi and regular centrate. With more data sets, we could specify the peak point and the turning point of ammonium removal rate for both Cambi and regular centrate. Also, we plan to specify the compound in Cambi centrate that may have an inhibitory effect on AOB. Currently, potential candidates for the causes the stronger inhibition effect are Cu, COD, alkalinity and turbidity. (Figdore et al., 2011) If the potential suspects are verified to have an inhibitory effect on AOB, we can try to further improve the treatment efficiency of Cambi pretreated process by removing these inhibitors.

This study was conducted under the sponsorship of SFPUC to determine if it is possible to adopt the Cambi pretreatment process in their southwest wastewater treatment plant. They wanted this pretreatment process because they would like to reduce the treatment pressure in the mainstream for their plant during the demanding season. In order to make sure that the installation of this pretreatment process worth its cost for SFPUC, we conducted this research to provided SFPUC with the reference on the inhibitory effect caused by Cambi centrate under difference concentration of the influent. By incorporating our data with the benefits provided by Cambi pretreatment process, SFPUC may be able to reach a conclusion on whether they will install the newly developed Cambi THP pretreatment process. Also, according to Figdore et al. (2011), dilution can be one method to effectively reduce the inhibitory effect on AOB. Therefore, our data can also be used to inform sites that already installed Cambi pretreatment process to improve the performance of their reactors. After all, Cambi-THP is still a novel technology. Further researches need to be conducted to improve its performance so that it can be widely accepted by the wastewater treatment industry.

On the other side, our batch data regarding the inhibitory effect of FA on the ammonium removal rate of AOB provided a general reference for wastewater treatment plant to modify their system so that they can maintain the optimized treatment efficiency for their partial nitrification reactors.

In conclusion, our research determined that we can achieve the highest ammonium removal rate in partial nitirfication reactor at around 542.5 mg/L $NH_4^+ - N$ with a FA concentration no higher than 9.7 mg/L. Also, our result indicated that Cambi pretreated centrate did not have a higher inhibitory effect on activities of AOB compared to that of regular centrate.

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