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APPLICATION OF PARTIAL NITRITATION/ANAMMOX PROCESS FOR TREATMENT OF WASTEWATER WITH HIGH SALINITY

Xin Zhang

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Department of Land and Water Resources Engineering

Royal Institute of Technology (KTH)

SE-100 44 STOCKHOLM, Sweden

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SUMMARY IN SWEDISH

Kombinationen av partiell nitritation och anaeroba ammonium oxidation (Anammox) är en resurseffektiv sätt att ta bort kväve från avloppsvattnet. I denna avhandling analysen gjordes för att undersöka hur salthalten i avloppsvattnet påverkar processen.

Två strategier av salthalt ökning testades i två reaktorer. De Anammox och nitritation bakterier arbetat i reaktorerna under övervakning. De fysiska, kemiska parametrar och aktiviteten hos bakterierna i reaktorerna var övervakas.

Från resultaten av de olika parametrarna i reaktorerna kan det räknat ut att strategin i reaktor 1 är bättre än i reaktor 2. Betingelsen av reaktor 1 var mer stabil och den kan arbeta mer effektivt. För reaktor 2 var reaktorn alltid vid kanten av kollaps, och de experiment var tvungna att ändras på grund av den dåliga bakterier aktivitet och reningsgrad.

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ABSTRACT

The combination of partial nitritation and anaerobic ammonium oxidation (Anammox) is a composting way to remove the nitrogen in the wastewater. In this article the analysis was made to investigate how the salinity in the wastewater affects the process. Two strategies of salt concentration increase were tested in two reactors. The physical, chemical parameters and the activity of the bacteria in the reactors were monitored. The results of two strategies were compared and the reactor with less salt in each period showed higher bacteria activities and efficiency. Finally the outlook for the future research was made.

Key words: Nitrogen Removal; Partial Nitritation; Anammox; SAA; OUR.

1. INTRODUCTION

1.1. Background

Nitrogen is a common element and one of the main components parts of the environment, the element nitrogen was discovered as a separable component of air, constituting 78.08% by volume of Earth's atmosphere. Also nitrogen can be found in all living organisms, it typically makes up around 4% of the dry weight of plant matter, and around 3% of the weight of the human body, as well as a large component of animal waste. The nitrogen cycle in figure 1 shows the movement of the nitrogen in or out of the biosphere and the different compounds formed.

Nowadays nitrogen compounds are discharged in the water cycle because of human activity, which lead to the eutrophicaiton. The nitrite is deleterious for all the livings in the water, which will indirectly threaten the human health. So it is always discussed about how to remove the nitrogen from the water efficiently.

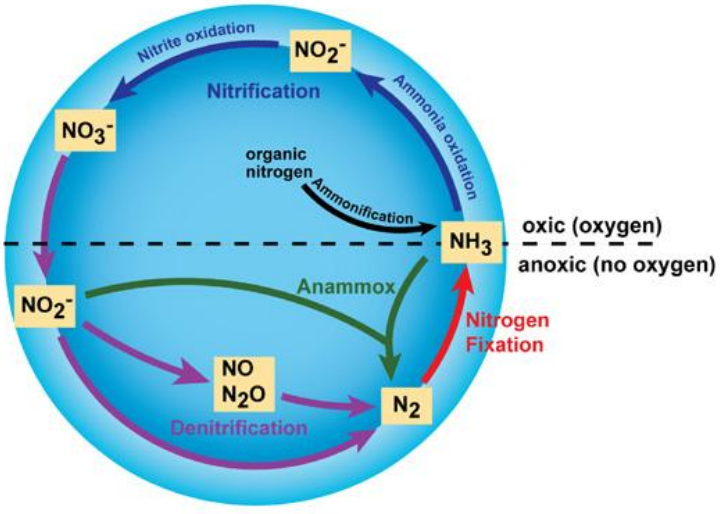


Fig. 1 Nitrogen cycle Processes (Bernhard, 2010).

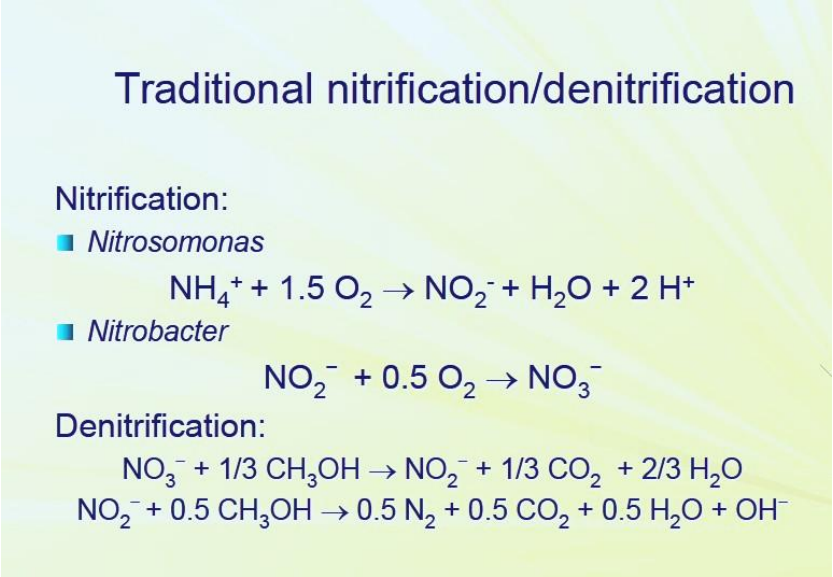


Fig. 2 Reaction of nitrification/denitrification.

1.2. Conventional ways of nitrogen removal

Nowadays as the developing of the technology, there are many ways to remove the nitrogen from the waste water. All these ways can be divided into three kinds: physical, chemical and biological ways. Comparing with the physical and chemical ways, the biological ways of nitrogen removal are the most promising and widely-used methods. The biological treatment costs less, with low energy and carbon source required, which are very appropriate for the nitrogen removal in practical operation.

The nitrogen in the water is generally in the form of ammonium or organic nitrogen, and the traditional way of removal is biological nitrification/denitrification (Fig. 2). Recently the conventional biological nitrogen removal process is more and more popular and applied by modern WWTPs. This method is especially effective in dealing of the waste water from anaerobic digester effluents, landfill leachate, industry wastewater containing high concentrations of nitrogen, etc.(Wiesmann, 1994). In this thesis a new composting method is discussed: Anaerobic ammonium oxidation (Anammox) combined with partial nitritation

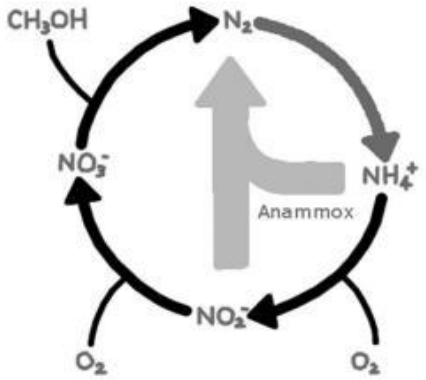
1.3. Introduction of Partial nitritation/Anammox Process

The Anammox process was found 20 years ago (Mulder et al, 1995) but was predicted to be real 30 years ago (Broda, 1977). The principle of the process is that the Anammox bacteria can consume ammonium and nitrite under anaerobic condition and create nitrogen gas. (Schmid et al, 2005; Mulder et al, 1995) The reaction equation is as follows:

$$NH_4^+ + 1.3NO_2^- + 0.066HCO_3^- + 0.13H^+ = 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2H_2O$$

Figure 3 (Sustainable Sanitation and Water Management, 2011) is a flow chart showing the processes of Anammox reaction. In practical application, the Anammox process is always combined with partial nitritation so that the ammonium can be oxidized into nitrite first and then takes part in the reaction of Anammox. The whole system is autotrophic. According to Ahn (2006), 60% less oxygen will be consumed by the partial nitritation/Anammox process comparing with the traditional process, and no organic carbon source required. What's more, 85% of the operational costs will be saved. The cost of the

Fig. 3 The Anammox reaction flow chart.



autotrophic nitrogen removal process is 1 Euro per kg N removed, which means 1~3 Euro per kg N removed can be saved. (Van Dongen et al, 2001)

1.4. Physical parameters affecting the Anammox process performance

1.4.1. *PH*

PH is the main factor which affects the equilibrium of the ammonium/ammonia and nitrite/nitrous acid. In partial nitritation/Anammox process, performance efficiency could be inhibited by free ammonia when PH is above 8 and nitrous acid when PH is below 7.5. So the PH should always be 7.5 ~ 8, which is the appropriate PH value for the reactions. The PH is important for the running of the reactors. (Van de Graaf et al, 1996)

1.4.2. *Dissolved Oxygen*

Dissolved oxygen (DO) is an important factor which will affect the stability of the bacteria. As was known in the Partial nitritation process, the oxygen is aquired to oxidize the ammonium and produce nitrite for the Anammox process. But the Anammox bacteria are anaerobic and the oxygen will cause the inhibition. So a balance value should be evaluated to make sure that all the dissolved oxygen is used for the nitrite production and will not affect the Anammox bacteria. Also the inhibition of DO is reversible (Strous et al, 1997; Schmid et al, 2007), which means the DO value can be controlled.

1.4.3. *Temperature*

The bacteria needs appropriate temperature to grow. So keeping the temperature stable is necessary. The temperature will also affect some other parameters such as DO. What's more, the speed of the chemical reaction and the equilibrium will change if the temperature varies.

1.4.4. *Hydraulic retention time (HRT)*

The HRT is a measure of the average time that a soluble compound remains in a constructed bioreactor. The value of HRT is inversely proportional to the flow rate of inflow and outflow. Generally optimum HRT is determined by many factors. The HRT value changes along with the changes of the activity of the bacteria. The lack of ammonium will lose the real efficiency of the removal, while too much ammonium can cause the inhibition. In addition, the HRT can also be decreased to wash away the inhibitors sometimes.

2. INFLUENCE OF SALINITY ON PARTIAL NITRITATION/ANAMMOX PROCESS

The investigation has already started before by Glass and Silverstein, 1999; Campos et al, 2002; Vredenbregt et al, 1997. Although the combination of partial nitritation and Anammox is accepted as an effective and promising method of nitrogen removal, there are still some problems to explore. The salinity can be a main factor which affects the Anammox bacteria. In modern times, more and more waste water with high salinity is produced from fish canning industry, landfill leachate, leather industry, etc. (Dapena-Mora et al, 2006) Especially in the fish canning industry, the use of the sea water during the manufacturing processes will produce huge amounts of high salinity waste water which needs to be handled.

According to Kartal et al (2006), the activity of freshwater Anammox will change if they are exposed in the salt water. So it is important to know how the bacteria react and how much the removal efficiency will change if the salinity increases. Dalsgaard et al (2003) and Kuypers (2003) represented that the Anammox bacteria was found in marine sediments in the sea, which guarantee that the bacteria can work in saline environment. The anammox bacteria are also proved to have the ability to adapt to some exterior chemicals (Thamdrup and Dalsgaard, 2002; Toh et al, 2002; Waki et al, 2007).Liu et al (2008) revealed that without the adaptation the inhibition of ammonium oxidation to nitrite happened at the level of 15 g/L NaCl, and it is proved by Liu et al (2009) that the Anammox bacteria can work at salinity 30 g/L after the adaptation in an aerobic reactor. The same experiments of adaptation which were made by Windy et al (2005) and Kartal et al (2006) were proved to be successful.

3. AIM OF THESIS

The aim of the thesis is to investigate how the salinity affects the Anammox bacteria. A pilot study was built up to explore the adaptation to the high salinity in partial nitritation/Anammox process. Two strategies of the stepwise salt concentration increase will be analyzed. The reaction of the bacteria and the efficiency of the nitrogen removal are under monitoring.

4. MATERIAL AND METHODS

4.1. Introduction of the Hammarby Sjöstadsverket

Hammarby Sjöstadsverk is a leading research facility in the field of wastewater treatment and environmental technology in Sweden. It was built up by Stockholm Water AB when the town quarter Hammarby Sjöstadsverk was under expansion, and the facility was open officially in October 2003. The facility is set up for long-term national and international research programs/projects and consultancy, testing and development for the industry and other partners. Nowadays it cooperates with many research institute, WWTP and industry. The aim of the facility is to contribute to improve the existing technologies as well as explore the new methods. Since January 1, 2008 all research at the plant is performed by a consortium consisting of IVL and KTH (Hammarby Sjöstadsverk, 2011).

Now in Hammarby Sjöstadsverk, several projects are running, including the Anammox Technology: nitrogen reduction. This thesis is part of the

Anammox project, and the experimental part of the thesis is performed at Hammarby Sjöstadsverk.

4.2. The experimental design

Two reactors of partial nitritation/Anammox process were built up, in which the different adaptation strategies were analyzed. The Anaerobic digestion reject water (supernatant) mixed with NaCl were used as the inflow. The reactors ran 2 weeks first after the start to make the bacteria stable. Because the sudden increase of the salt will reduce the activity of the bacteria (Kincannon et al, 2008; Fernandez et al, 2008), the salt was increased stepwise into the inflow. The salinity increased every period, and each period lasted for two weeks. For reactor 1, the salinities for all the periods are 5, 10, 15, 20 g/L; For reactor 2, the salinity for all the periods are 2.5, 5, 7.5, 10 g/L. In the end the salinity in reactor 1 should be 20 g/L and the salinity in reactor 2 should be 10 g/L (Fux et al, 2002).

4.3. Description of the laboratory scale pilot plant reactors

First, two biofilm reactors of 10L using hydrophilic net type acryl fiber biomass carrier (Fig. 4) was built up. For every reactor 4 L of biofilm carriers originating from full-scale partial nitritation/Anammox reactors which are in operation at Himmerfjärden WWTP was used. Then put 8L 1:1 diluted supernatant (4 L water + 4 L supernatant) in each reactor.

Next step was to adjust the condition of the environment. Because the biofilms are very heavy and sink at the bottom of the reactor, the mixer should be put in to the reactor and adjusted to the appropriate rotating speed. In this experiment a two-blade mechanical stirrer was used. The mixer should make all the biofilm carriers suspending in the water, but the speed cannot be too high to avoid ruining sludge on the biofilm. The heater was also needed. The temperature in the reactor should always be stable and appropriate. Temperature was maintained constant by means of aquarium heater, and was set at about 26 ℃. The oxygen supply is needed by the partial nitritation process. The value is adjusted at 1.5 ~ 2 g/L. The HRT was set almost 4 days, i.e. the speed of the inflow was set at about 1.4 ml/min. The pure supernatant was used as the inflow. At last the reactors needed to be covered to avoid the evaporation which may affect the results of the experiments.

Usually the bacteria need two weeks to adapt the new environment. So the further operation of the experiments should be done at least 2 weeks after the start. (Fig. 5)

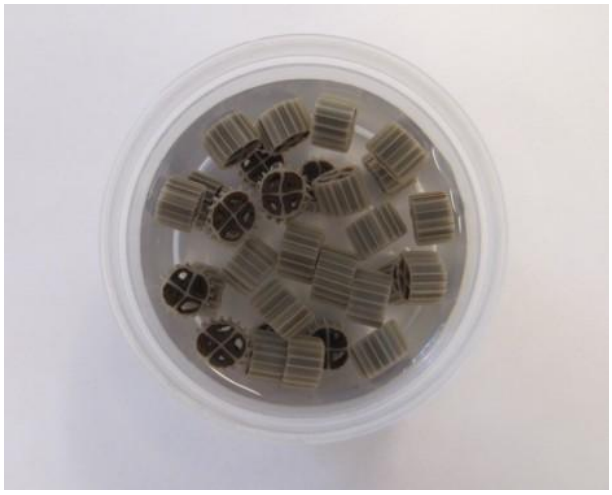


Fig. 4 Biofilm used to build the reactor.

Fig. 5 Reactors of the whole experiment.

4.4. Measurement of Physical Parameters

4.4.1. *PH*

The PH value was measured with the PH meter WTW pH 330 (WTW Laboratory products and On-Line Instrumentation, 2011).

4.4.2. *Conductivity*

The instrument for measuring the conductivity and salinity is WTW Cond 330i (WTW Laboratory products and On-Line Instrumentation, 2011). The salinity value is also used for the measurements of DO.

4.4.3. *DO*

The DO meter HACH HQ 30d (HACH, 2011) is used for the measurement. Automatic salinity correction was used for minimizing the error.

4.4.4. *Flow rate*

Graduated cylinder and timer are used in this measurement. When measuring the flow rate, check how much supernatant can be achieved within 3 minutes, and then calculation can be done to get the value of the flow rate.

4.5. Measurement of Chemical Analysis

In the chemical analysis, the parameters measured are ammonium in the inflow and out flow, nitrite in the outflow and nitrate in the outflow.

All the liquid samples are needed to be filtrated before the test. The instruments for the filtration are as figure 6, and the methods are the same as the normal suction filtration. The filtrated liquid is used for the experiments afterwards.

The main instrument for the chemical analysis is HACH LANGE XION 500 spectrophotometer (HACN LANGE, 2011). And the different cuvettes (Fig. 7) are used for each chemical to be measured. When doing the analysis, first the filtrated sample was diluted to reckon

Fig. 6 The filtration in chemical analysis.

that the concentration of the target chemical is within the range of the measurements. Then follow the instructions on the box of the cuvettes to mix the sample with the solution in the cuvettes. After that, the spectrophotometer can start the measurement and obtain the value on the display.

The ammonium of the inflow was measured once per week. For the outflow, ammonium, nitrite and nitrate should be measured twice per week. If the condition of the reactors is not stable, chemical analysis have to be done to check if the reactor is back to normal or not.

4.6. Specific Anammox Activity

The Specific Anammox Activity (SAA) is a practical way to determine the activity of the Anammox bacteria by detecting the slope of the curve presenting the pressure of the nitrogen gas produced along time. As was mentioned before, the Anammox bacteria should be kept in the stable environment of 25 ℃ during the whole experiment in order to get the best performance. PH is also an important factor for the SAA test. In this test the SAA Buffer are used to provide the appropriate PH

Fig. 7 Cuvettes in chemical analysis.

environment. The buffer should provide the PH between 7.7 ~ 7.8 which is the best condition for the Anammox bacteria, and nitrogen element can not be induced or else the results would be interfered. The buffer was made of 0.14 g/L KH_2PO_4 and 0.75 g/L K_2HPO_4.

After all the preparation the SAA test can be started. Here are the steps: First, make the buffer with appropriate salinity for the test. Second, Pick the rings out from the reactors and wash them with buffer. The aim of this step is to wash away the suspended solids and dissolved chemicals which are stuck on the rings. It is important that the rings can never expose in the air. Third, take out the bottles with working space of 25 ml. In this test 2 or 3 bottles are needed for each reactor. Add 15 rings in each bottle, and then fill the bottle with buffer to the mark of 25 ml. Next, weigh each bottle to make sure the volume of the buffer is accurate. Calculations of how much weight should the content be inside the bottle are made according to different salinity of buffer and Table 1 shows the relationship between the salinity and the weight. If not, add more buffer or remove the buffer in the bottle to adjust. Fourth, close the bottle with cover. On each cover there is a hole with membrane to prevent the gas inside the bottle from getting out. On the other hand the syringe needle can get through membrane to add chemicals. Free volume of bottle was flushed with nitrogen gas during 5 min to remove oxygen from the free volume of bottle and dissolved oxygen from the liquid. Fifth, after finishing the nitrogen replacement put all the bottles in the water bath under 25 ℃ for 7 minutes to heat up the bottle and provide the appropriate temperature for Anammox bacteria to work. Then use syringe needle to prick the membrane to make the pressure the same inside and outside. Then quickly add 0.5 ml NH_4Cl (70 mg/L) and 0.5 ml $NaNO_2$ (70 mg/L) in each bottle. Then measure the pressure inside the bottle with pressure meter and use it as the original pressure when time is 0. Finally, measure the pressure inside the bottle every 20 or 30 minutes. (Fig. 8) In each SAA test we have to have at least 5 values of pressure, for example the pressure value at 0, 30, 60, 90,120 min.

The curve showing the relationship between the pressure in the bottle and the time can be obtained and then the slope of the curve can be used to calculate the results of the SAA. The normal SAA test should be done twice a week and the SAA of full salinity was also applied in the experimental part. Normally the SAA test was done twice per week to check the activity of the bacteria.

4.7. **Oxygen Uptake Rate**

Oxygen uptake rate (OUR) is a laboratory test which used to analyze metabolic activity of organisms in aquatic systems. The rate at which the microbes consum oxygen is an indicator of the biological activity of the system and is called the oxygen uptake rate. (Oxygen uptake test, 2011) The aim of this test is to monitor the performance of the bacteria of

Table 1. The relationship between the salinity and weight inside the bottle.

Salinity (g/L)	Density of buffer (g/L)	Weight inside the bottle (g)
0	1,0	24
5	1,005	24,0
10	1,01	24,17
15	1,015	24,26
20	1,02	24,34
25	1,025	24,43
30	1,03	24,51

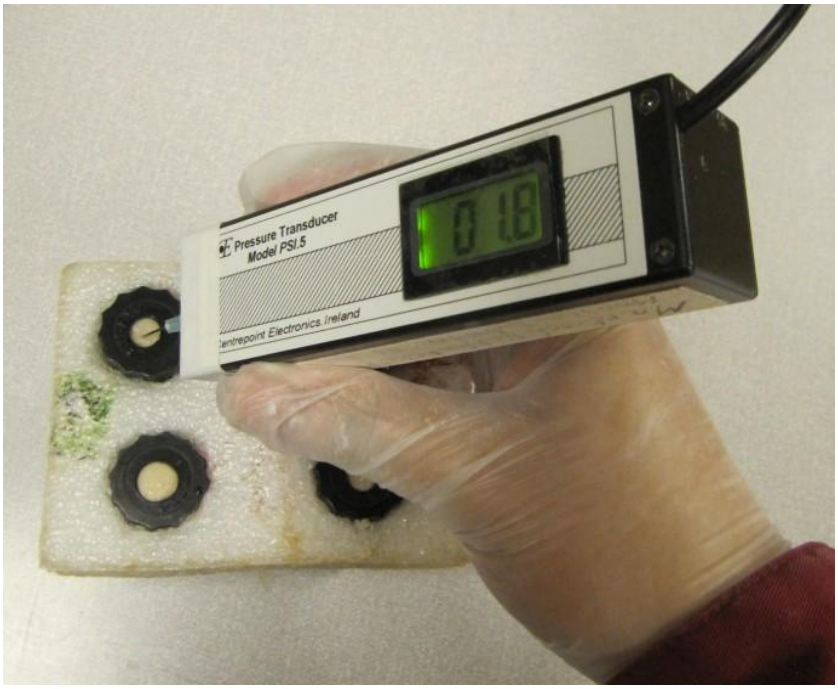


Fig. 8 Measuring the pressure in SAA test.

nitritation. The results are given as a series of DO measurements taken on a sample over a period of time.

Before every test DO meter was calibrated according to instrument user manual, and then the DO value can be measured. A 3-head glass bottle (total volume 1.7 L) with 1:10 diluted supernatant was used as the working place for the biofilms. The mixing was running during the whole test with constant temperature of 25 ℃. The DO meter was connected with computer so that the specific software "Testo" can write down the DO value and mV value along the time. Usually the software can record all the values during 15 minutes. (Fig. 9)

When the data was collected, drew a plot of DO along time, and then drew a best straight line which went through all the dots. The slop can be used to calculate the results. Normally the OUR test should be done once per week.

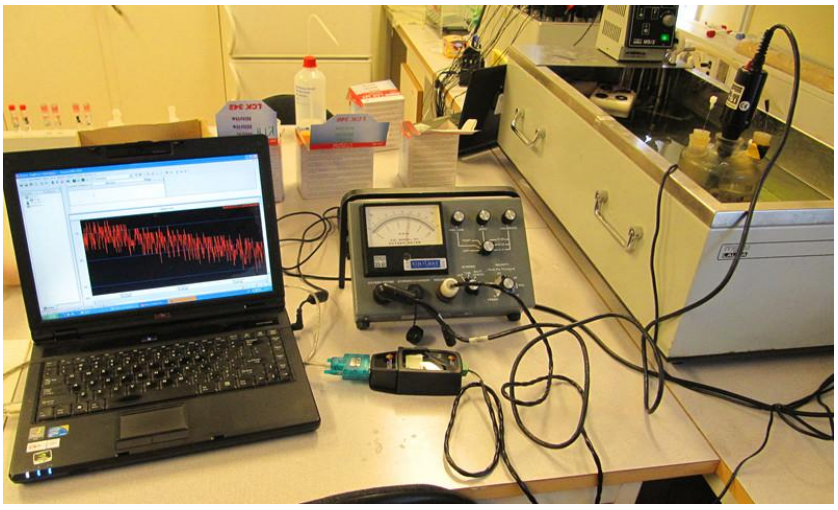


Fig. 9 The OUR test.

5. RESULTS AND DISCUSSION

5.1. Results and discussion in reactor 1

The reactors were operated as planed before the salinity was 10 g/L in reactor 1. But when the salinity was increased to 10 mg/L, the reactor is really unstable so this period was extended to 3 weeks. Also after the salinity increased to 15 g/L, the condition of reactor 1 was always precarious and the SAA results of R1 is almost 0 which means the serious inhibition. So the salinity in reactor 1 stayed at 15 g/L until the end of the test.

The different graphs are present to illustrate the raw data of the experiments. And the comments and discussion part are under each graph. The vertical black lines on the graphs divided the different periods with different salinity. These periods are 0, 5, 10, 15 g/L.

5.1.1. *Physical Parameters in R1*

The results were presented as curve diagram. (Fig. 10) Because the high amount of ammonium was consumed, which lead to a decrease of the PH value. On the graph it can be figured out that in the first period (salinity = 0 g/L) the PH value is jumping at first and then back to normal in the end of the period. The PH value keeps suitable until the end of the period 2 when the salinity is almost 10 g/L. After that the value is jumping till the end, and the jumping amplitude is very big when salinity is 15 g/L.

Because in the practical experiments, it's hard to always keep a specific value of PH. So it's impossible to keep the value of ideal PH value all the time. In this experiment, the PH value between 7 and 8 is available for the work of Anammox bacteria. From the results it can be seen that during the first period (salinity 0), the PH is unstable and exceed the edge of 7 or 8. This is because the Anammox bacteria were put into a new environment at the beginning of the experiment and they need time to get used to it. So the instability is acceptable. After the salinity 10 g/L, the PH is really unstable because the increase of the salt (5 g/L) in a sudden is really a big amount, the bacteria cannot stand. So that's why the inflow was stopped during the periods of salinity 10 g/L and salinity 15 g/L. Although the actions were taken, the bacteria were still unstable and cannot get back to normal.

According to the temperature and the conductivity in R1 (Fig. 11 & 12), the temperature is stable, and the conductivity in the outflow increases gradually along the stepwise increase of the conductivity in the inflow. In this experiment, the temperature is adjusted by the heater, and the

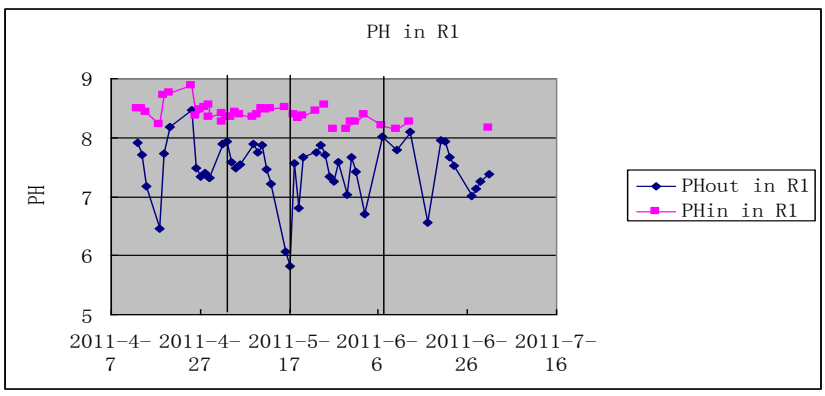


Fig. 10 Results of PH in reactor 1.

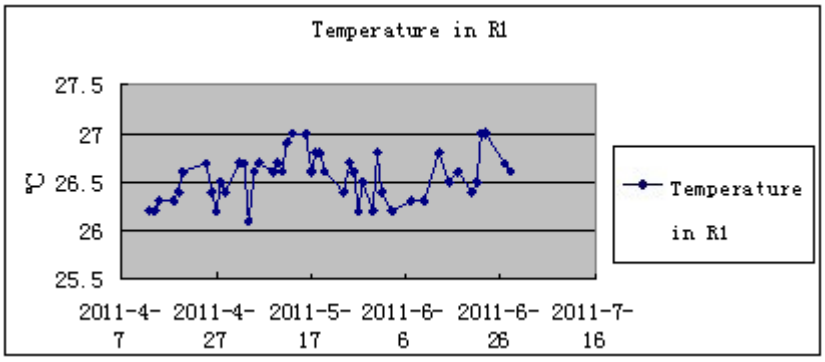


Fig. 11 The results of temperature in R1.

conductivity is mostly decided by the salt in the water. So these two parameters are the same as expected. The missing dots on the line of inflow are the time when inflow was stopped. The reason of stopping the inflow is as mentioned before in the last part the reactor was very fragile and cannot work at all.

The DO in R1 (Fig. 13) is unstable in each period when the salinity increases. When salinity is 0 g/L, the DO is decreasing gradually. But when salinity is 5, 10 and 15 g/L, the DO value always jump to a very high value in middle or the end of each period.

In the first period (salinity =0), the bacteria need some time to adapt so the DO was adjusting all the time. And after the salinity increased, the activity of the bacteria was inhibited. The oxygen demand was less than before. But the aeration was still supplying oxygen in original rate. So the DO in the reactor jumped to a high level. When this happened, the operation the handle this was to decrease the aeration rate. That's why the situations are the same in period of salinity 5, 10 and 15 g/L.

The results of the HRT (Fig. 14) are illustrated that the curve is stable before the salinity 10 g/L. After 10 g/L the HRT increase once more than before. The HRT is in contrast to the flow rate of the inflow. The results show that before the salinity 10 g/L, the reactors were running well with high efficiency of removal. So the flow rate is faster to provide more ammonium and wash away the nitrate. But after the salinity 10 g/L, the reactors started to be unstable, so the operation of increasing the HRT were taken. Because the efficiency is not as good as before, the flow rate of inflow should be decreased to prevent from the inhibition of the ammonium. There are also some days during which the inflow stops to stabilize the reactor when the condition of the reactors is not well with high salinity. These days are marked in the Appendix.

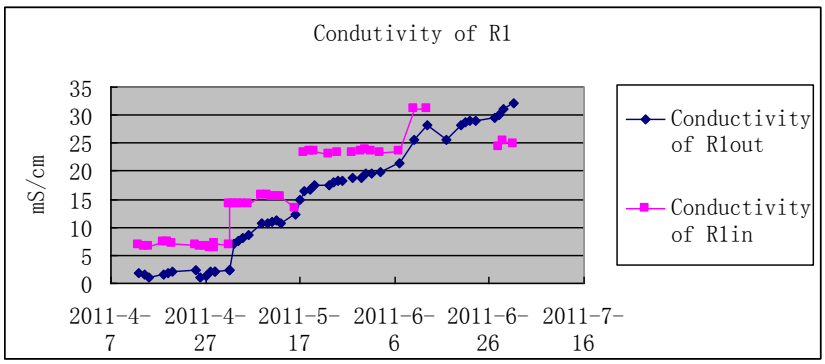


Fig. 12 The results of conductivity in R1.

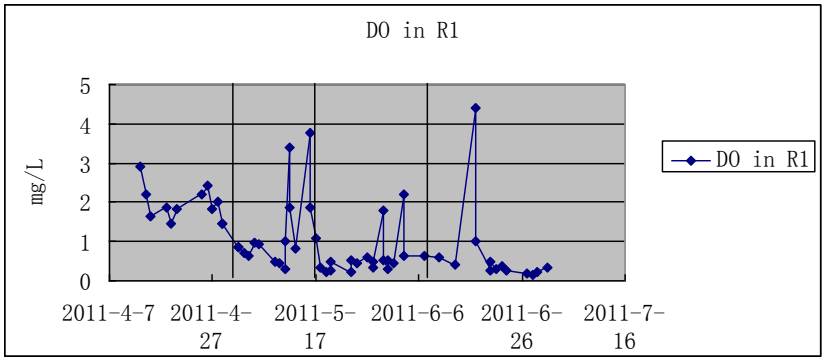


Fig. 13 The DO in R1.

5.1.2. *Chemical Analysis*

According to the principles mentioned before, the ammonium, nitrite and nitrate are the key compounds in this analysis. The efficiency of the nitrogen removal can be judged by the amount of the ammonium removed. The normal value of the concentration of ammonium is 50 ~ 120 mg/L. If the concentration is more than 50 mg/L, the inhibition will happen and lower the activity of the Anammox bacteria. But if the concentration is more than 120 mg/L, it means that the Annammox bacteria cannot get enough substrate for the reaction, and the efficiency is less than the real efficiency.

The figure 15 shows the results of ammonium, nitrite and nitrate in R1 during the whole experimental period. For the ammonium, the curve of inflow is constant and stable, but the curve of the outflow is wavy, especially during the period of salinity 0 g/L and salinity 15 g/L.

The curve of inflow stopped in the middle of salinity 10 g/L. because after that the reactor was unstable. Many operations and measures were taken, including stopping the inflow, diluting the inflow in different ratio, etc. So the inflow value is skipped in this part. For the outflow, the instability in period 1 is because that the bacteria didn't get used to the new environment and the efficiency of the bacteria is unstable. The concentration also increased after the salinity 15 g/L. The reason is that the activity of the bacteria very low. The ammonium in the reactors cannot be consumed and accumulated more and more. The curve in the nitrite is going on well until the middle of the period of salinity 10 g/L. The curve of nitrate shows that the concentration is unstable in salinity 0 g/L and very high after salinity 5 g/L and then back down to very low after salinity 10 g/L. These two curves show the condition of the reactor. The nitrite was as expected before the salinity 10 g/L. But when the amount of the ammonium increased further more, the activity of

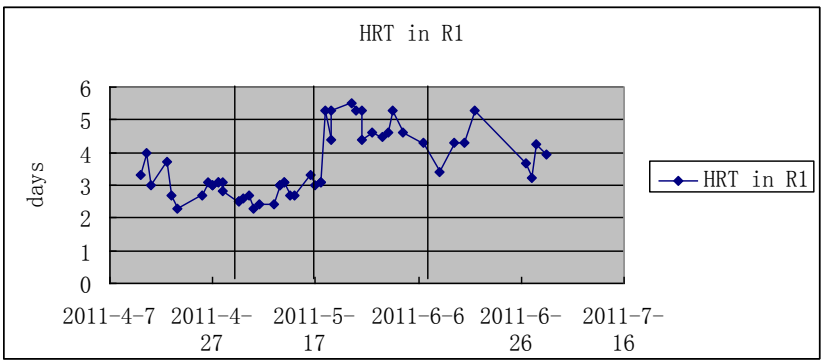


Fig. 14 The results of HRT in R1.

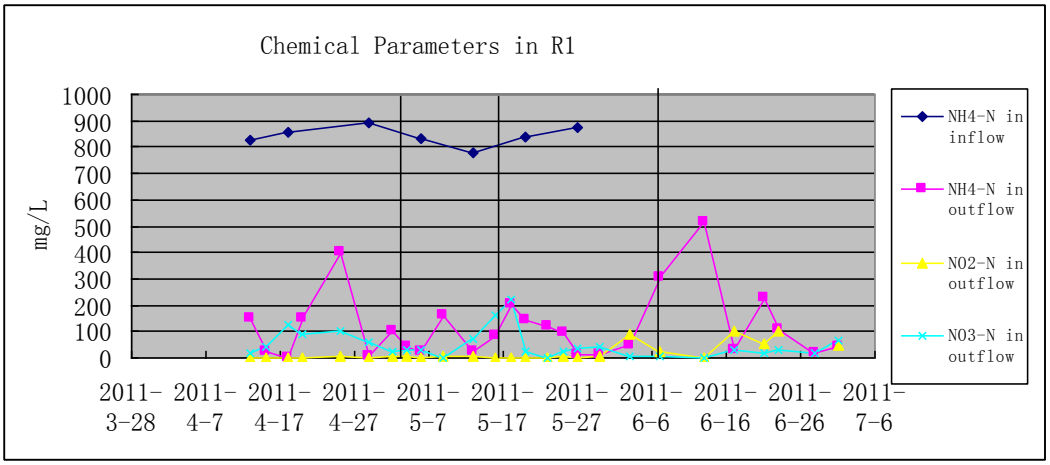


Fig. 15 The ammonium, nitrite and nitrate in R1.

Annamox bacteria is very low. The nitrite produced was accumulated because it can not either be used for the reaction or be washed away along the outflow. The nitrate is high when salinity reached almost 10 g/L. The DO was very high for some time which leaded to the high concentration of the nitrate. And at salinity 15 g/L the concentration is very low because the low activity of the bacteria. The inhibition of the salinity decreases the activity of the bacteria a lot.

5.1.3. *SAA in R1*

In the graph of normal SAA (Fig. 16) it can be figured out that as the salinity increasing, the value of the SAA decreases. Each time when the reactor steps into a new period, the SAA values fall significantly in a sudden. The SAA values recover a little in periods of salinity 5 g/L and 10 g/L, but no recovery in salinity 15 g/L.

Now the activity of the Anammox bacteria can be analyzed according to the results. In the first period when salinity was 0 g/L, the bacteria were working under the appropriate environment without any inhibitors. The average value of the activity was about 1.5 g N / d·m^2, which can be regarded as the 100% of the activity in R1. In the second period when salinity was 5 g/L, the value fell in a sudden to almost 1 g N / d·m^2 because of the inhibition of the salt. Although the bacteria adapted the

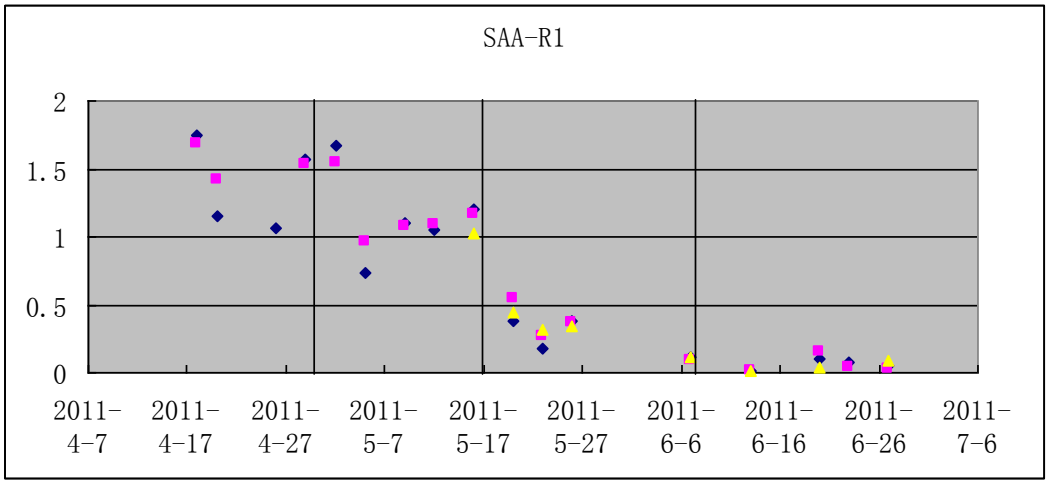


Fig. 16 The results of SAA in R1.

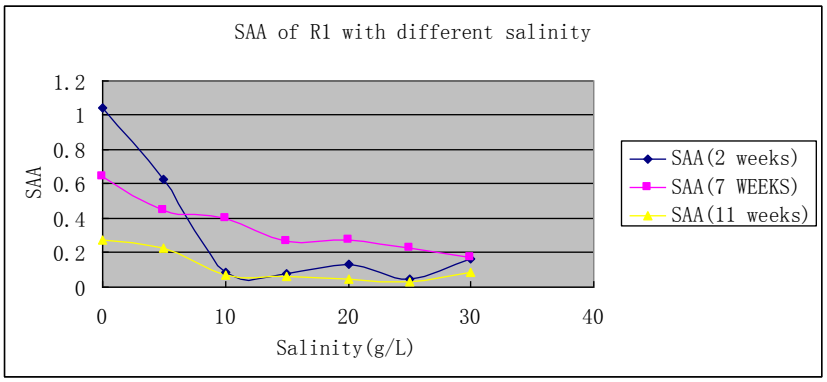


Fig. 17 The SAA of R1 with different salinity.

new saline environment a little, still the activity didn't recover a lot. In this period, the activity was only about 70% ~ 80% comparing with the SAA with no salt. In the third period when salinity was 10 g/L, the SAA fell to about 0.3 ~ 0.5 g N / d·m^2 with no sign of the recovery. This meant that the bacteria cannot adapt anymore. But the bacteria can still work, even though the activity was only 20% ~ 30%. In the last period with salinity 15 g/L, the inhibition of the salt was so serious that the activity of the bacteria was almost 0 and there was no sign of recovery. The amount of the salt was too much for the Anammox bacteria which they cannot stand.

Besides the normal SAA mentioned before, 3 times of SAA with full salinity was applied 2 weeks, 7 weeks and 11 weeks from the start. The SAA with full salinity analyze the activity of Anammox bacteria in different saline environment at the same time. The average value of the SAA is used here to present the results (Fig. 17) and figure 18 shows how much activity left comparing with the fresh water SAA which is regarded as 100%.

The time of these three SAA test were at the end of salinity 0 g/L (SAA after 2 weeks), at the end of salinity 10 g/L (SAA after 7 weeks) and at the end of salinity 15 g/L (SAA after 11 weeks).

The activity of the Anammox bacteria under different salinity can be discussed now. After 2 weeks the bacteria in the reactor were stable and the activity now was highest. But the bacteria had never been lived in the saline environment before, so the ability of resisting the salt was very weak. So the SAA at salinity 0 g/L was the highest. When the salinity increased, the SAA decreased in a sudden. It even reached almost 0

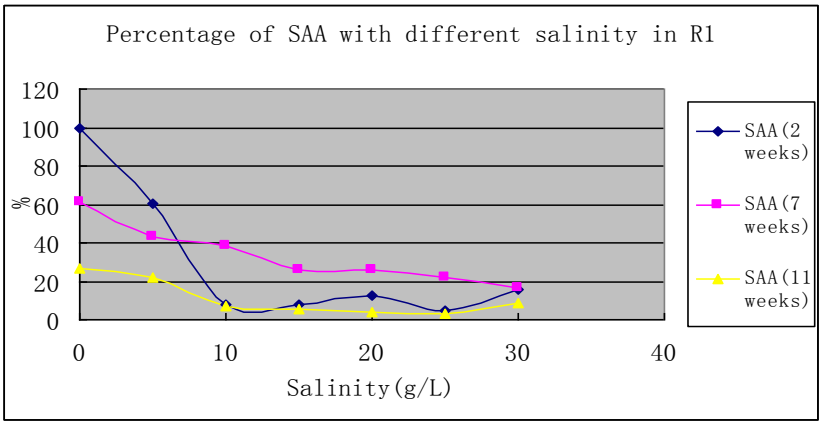


Fig. 18 Percentage of SAA with different salinity in R1.

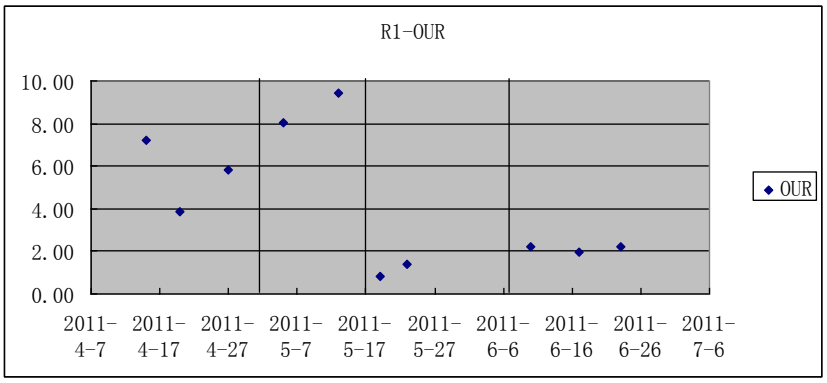


Fig. 19 The results of OUR in R1.

when the salinity is more than 10 g/L. After 7 weeks, the bacteria were in the saline environment for some time and the activity at salinity 0 g/L decreased 40%. But after 5 weeks of the adaptation, the Anammox bacteria had the ability of resisting the salt. The bacteria showed active even when the salinity was 30 g/L. Although the activity was not very high, but at least the results showed the effect of the adaptation After 11 weeks, the activity of the bacteria was very low even at the start. This can be explained that the bacteria were suffering serious inhibition and they cannot work under this condition. The salt amount is beyond the ability of the adaptation.

A short summary of the SAA test can be made that the scenario of reactor 1 is hard for the bacteria to adapt. 5 g/L more salts is hard to adapt for the bacteria to stand. And the activity was very low even before the salinity reaches 10 g/L. After that the bacteria are inhibited seriously and show nearly no activity.

5.1.4. *OUR in R1*

The OUR value (Fig. 19) is high when the salinity is 0 g/L and even higher in the period of salinity 5 g/L. But when the salinity increases to 10 g/L, the activity drops down significantly and almost doesn't recover till the end.

The change of the dots on the graph can be used to describe the situation in the reactor. In the first period of salinity 0 g/L, the bacteria were in the process of get used to the new environment, so it's acceptable that some instabilities happened in this period. Then two weeks later, the bacteria got to the best state. In the second period of salinity 5 g/L, the adaptation was in process successfully, and the situation is very good. According to the graph, the activity of the bacteria

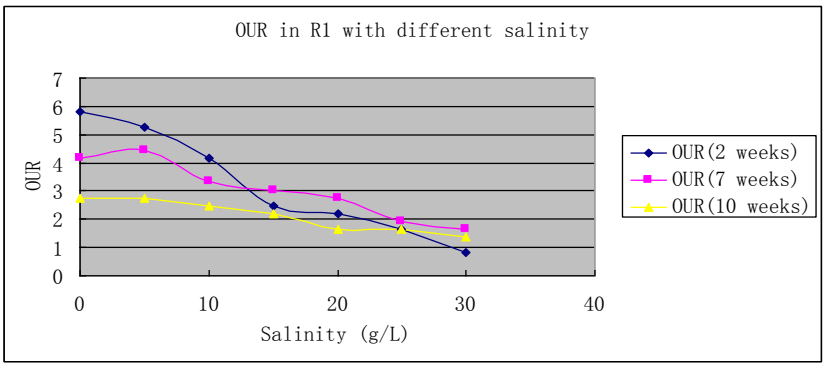


Fig. 20 The OUR with full salinity in R1.

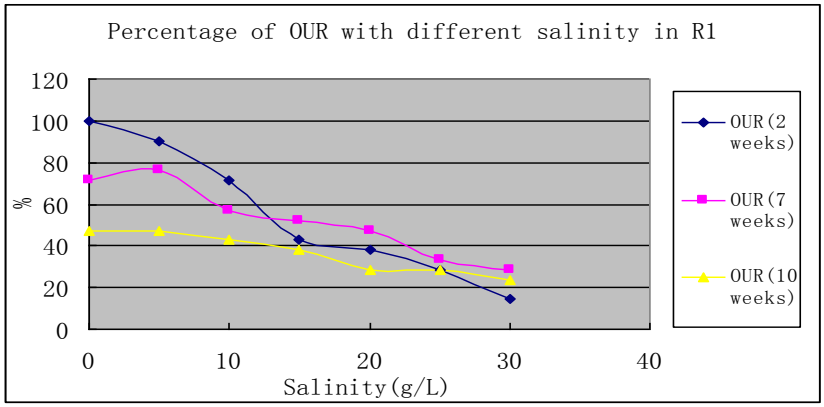


Fig. 21 Percentage of OUR with different salinity in R1.

even got higher in this period. But in the period of salinity 10 g/L, the serious inhibition happened and the activity was influenced with a sudden drop, with only 10% activity left. The reason for this is that the salinity is too high and the adaptation time is too short so the bacteria cannot stand working under this environment. And the inhibition kept going on until the end of the experiment although the activity recovered to 20% comparing to that in salinity 0 g/L.

Three times of OUR tests with full salinity were still applied in this experiment. The time of these 3 OUR tests were at the end of salinity 0 g/L (OUR after 2 weeks), at the end of salinity 10 g/L (OUR after 7 weeks) and at the middle of salinity 15 g/L (OUR after 11 weeks). Figure 20 shows the results, at the same time figure 21 shows the percentage of OUR comparing with the fresh water OUR. These 3 curves are all going down gradually. The OUR value are almost the same after the salinity reaches 10 g/L.

The condition of the adaptation can be analyzed by this result. Comparing these three curves and it can be found that after the adaptation, the activity of the bacteria was lower than that before the adaptation. Take the condition of salinity 0 g/L for example. After 2 weeks before the salinity increase, the OUR value was almost 6 g O_2/m^2d. This activity was achieved under the most appropriate environment, which can be regarded as the best activity of the bacteria. But after 7 weeks and 10 weeks, the activities of the bacteria at 0 g/L were about 4 g O_2/m^2d and 3 g O_2/m^2d, only performing about 70% and 50% comparing with the best activity. Then as the salinity increases, the activities of the bacteria are all going down gradually. The effect of the adaptation can be figured out when salinity is more than 15 g/L. When the salinity was more than 15 g/L, the bacteria which adapted for 7 weeks showed activity at 50% of the best activity even if the salinity was increasing to 20 g/L. These bacteria had the ability of resisting the saline environment. But for the adaptation of 2 weeks, the increasing of the salinity almost leaded to a sudden decrease of the activity. The activity was very low when the salinity reached 15 g/L. The situation for the bacteria of 10 weeks adaptation was even worse. The bacteria didn't show the low activity during the whole experiment. This is because the saline environment really causes the inhibition of the bacteria, and they cannot show high activity even from the start.

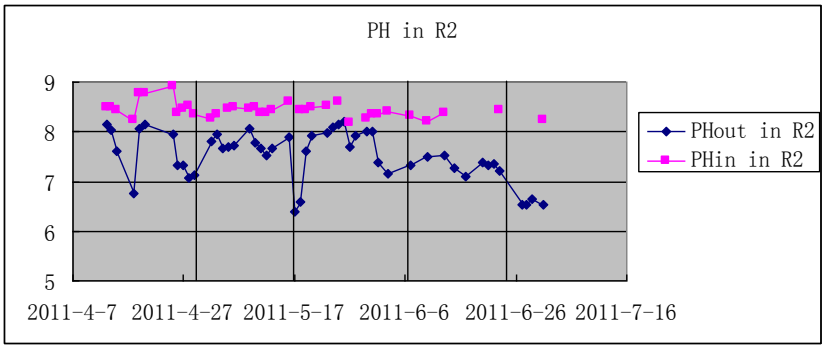


Fig. 22 The results of PH in R2.

5.2. Results and discussion in reactor 2

The vertical black lines on the graphs divided the different periods with different salinity. These periods are 0, 2.5, 5, 7.5 and 10 g/L.

5.2.1. *Physical Parameters in R1*

The figure 22 shows the results of the PH in reactor 2. The dots on the graph are really stable except for two dots at the beginning of the period of salinity 5 g.L.

The condition of the R2 is better than R1 according to the graph. In the first period of salinity 0, the instability is acceptable because the bacteria need time to get used to the new environment. Then as the salinity increases, the PH value are all within the appropriate limits, which means that the condition in R2 is always good and the adaptation is going well. The two dots which exceed the limit at the beginning of salinity 5 g/L means that this salinity is a little hard for the bacteria to afford and lead to the instability for a while. But the bacteria adapt this new saline environment quickly and the PH values all get back to normal. But when salinity increased to 10 g/L, the inhibition happened and the PH values were unstable again. The bacteria obviously cannot get use to this environment, which leaded to the bad condition of the reactor.

The figure 23 & 24 showed the temperature and the conductivity in R2. These two graphs shows that the condition of the temperature and conductivity were all as expected. The temperature is important as mentioned before, and the stepwise increasing of the conductivity in the inflow makes the conductivity in the outflow increasing gradually. It can be figured out that these two parameters also provide good condition for the bacteria and reaction.

The results of the DO are presented as figure 25. In the salinity 0 g/L, the DO was not very stable. Then the values were stable in salinity 2.5, 5

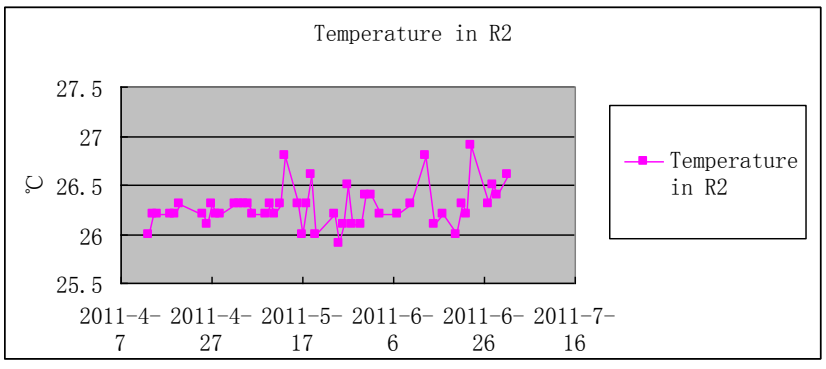


Fig. 23 The temperature in R2.

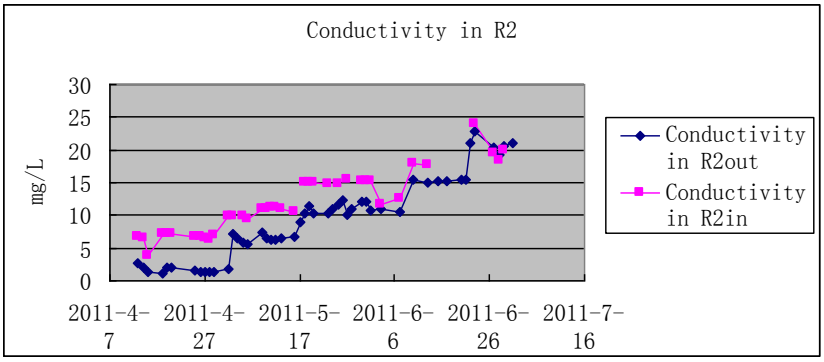


Fig. 24 The conductivity in R2.

and 7.5 g/L although there are some unusual dots. And much high DO appear when the salinity reached 10 g/L.

The curve of DO showed the state of the R2 in the whole experiments. The instability of the bacteria caused the jumping of the DO in the period salinity 0 g/L. After that the adaptation started. Although some instability happened, but at last the bacteria get over it and adapt the saline environment. When salinity increased to 5 g/L, the activity of the bacteria was inhibited and the efficiency of the work was not as well as before. The demand of the oxygen was not as much as before, which lead to the decrease of the DO. But still the reactor was stable. The unusual dots were caused when the operation was taken to adjust the aeration. Because the aeration rate were set and didn't change, so sometimes the DO acted unusual if the activity of the bacteria changed. In the final period of salinity 10 g/L, the serious inhibition happened, which meant that the activity decreased a lot, so that the extra oxygen are dissolved into the water.

The HRT which was set in R2 is as the figure 26. The curve shows that the HRT are stable before and after the salinity 5 g/L. The HRT after the salinity 5 g/L is once more that that before the salinity 5 g/L

As was mentioned before, HRT is in contrast to the flow rate of the inflow. The results show that before the salinity 5 g/L, the reactors were under good condition. The efficiency of the bacteria were very high, which the high flow rate was set to provide the enough substrate for the reaction as well as wash away the produced nitrate to avoid the inhibition. But after the salinity 5 g/L, the instability happened in the reactor. So the operation was taken to slow down the flow rate. The bacteria in this condition cannot consume so much ammonium and too much ammonium will lead to the inhibition. Also there were some actions taken in order to stabilize the reactor, and sometimes the action were not

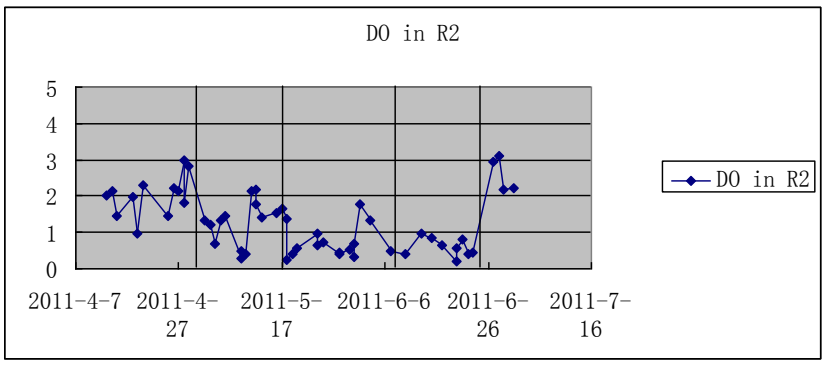


Fig. 25 The results of DO in R2.

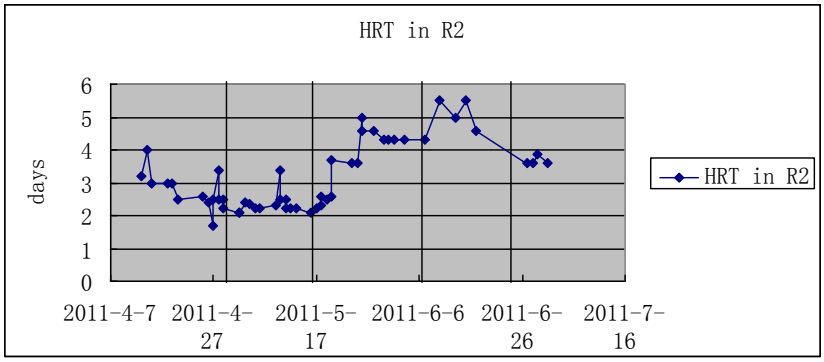


Fig. 26 The results of HRT in R2.

always the same, which may affect the HRT. The details of theses actions were marked and can be found in the Appendix.

5.2.2. *Chemical Analysis in R2*

The analysis was focusing on the concentration of ammonium in inflow and out flow, the nitrite and nitrate in the out flow. (Fig. 27) For the ammonium, the curve of the inflow shows the concentration of the ammonium in the supernatant, which is constant and stable. For the out flow, the value are mostly within the range 0~200 mg/L, except for several dots exceeding the limit.

It can be figured out that the removal of ammonium in the R2 went very well during the experiment. The concentration of the ammonium was appropriate nearly during the whole project. As was mentioned before, the concentration of the ammonium in the outflow cannot be too high or too low. According to the graph, the ammonium concentration was just as what was expected before.

This also means that the adaptation in R2 went very well and successfully, and the bacteria in the reactor were very stable. The dots which exceed the limits were shown in salinities 0, 5, and 10 g/L. The instability of salinity 0 g/L was explained before. In salinity 5 g/L, this salinity is high enough to disturb the bacteria. But after the long time of adaptation, the bacteria can get over and keep on working under the saline environment. So the ammonium concentration rises for a wile and then got back to normal. In salinity 10 g/L, this salinity is a little too much for the bacteria even if the adaptation lasted for a long time. The bacteria needed more time to handle this.

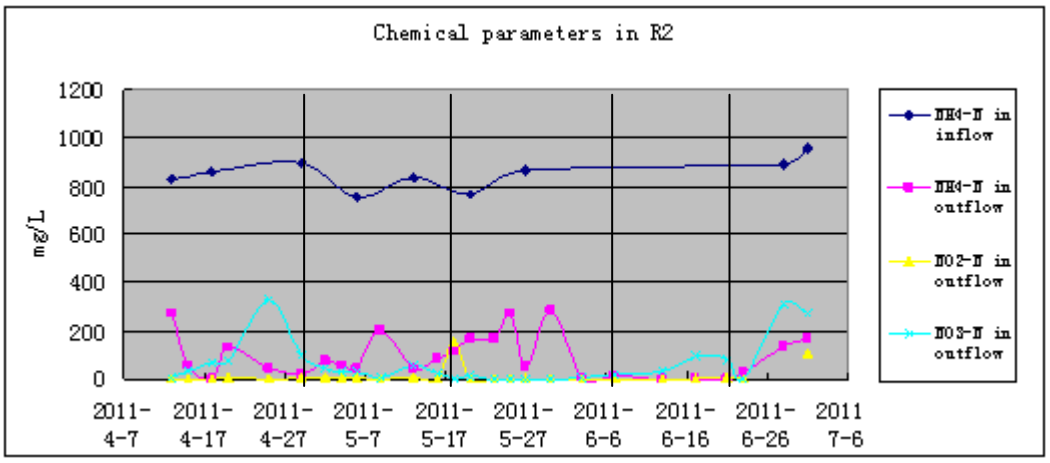


Fig. 27 The ammonium, nitrite and nitrate from inflow and outflow in R2.

For the curve of nitrite, the concentration is always close to 0 except for two dots. For the curve of nitrate, the concentration is stable and within in the range in the most of time. But in the first and last period, there are three dots which are with extremely high value.

From the nitrite and nitrate value, it can be known that the concentration of nitrite and nitrate in the R2 were always acted as what was expected. The nitrite, which was the intermediate product, should always be at low concentration. The concentration under 100 mg/L for the nitrate was also normal. This means that the reactions in the R2 were stable during the whole project. And the adaptation of the bacteria went very well, too. About the two unusual dots in nitrite graph and three unusual dots in nitrate dots, they are supposed to be the error in the test, because the values of these dots are extremely high and not like others. Even the instability might happen in the adaptation process, the values of these dots are so high that cannot be used to analyze the condition of the reactor. The reason of causing these dots is explored in the chapter of Uncertainty.

5.2.3. *SAA in R2*

In the graph of SAA (Fig. 28), the values start to decrease at salinity 5 g/L. When the salinity reaches 10 g/L, the SAA values still have the sign to rise.

Now the analysis of the activity of Anammox bacteria can be made. In the period of salinity 0 g/L, the Anammox bacteria took two weeks to reach the stability. At this time the SAA reached the best value at about 1.3 g N / d·m2. In the next period of salinity 2.5 g/L, the activity didn't decrease, but increased a bit. This can be explained by Dapena-Mora et al (2010) that the small increase of salinity to 3 g NaCl/L increases SAA where further increase leads to decrease of SAA. This is also the reason why the activity decreased in the third period when salinity was 5 g/L. In this period the SAA was below 1 g N / d·m2, which was only 70% of the best bacteria activity. In the next period of salinity 7.5, the activity was 40% of the best activity. This is because of the inhibition of the salt. But the adaptation had the effect, which was the main reason why the SAA didn't keep on decreasing when salinity was 10 g/L.

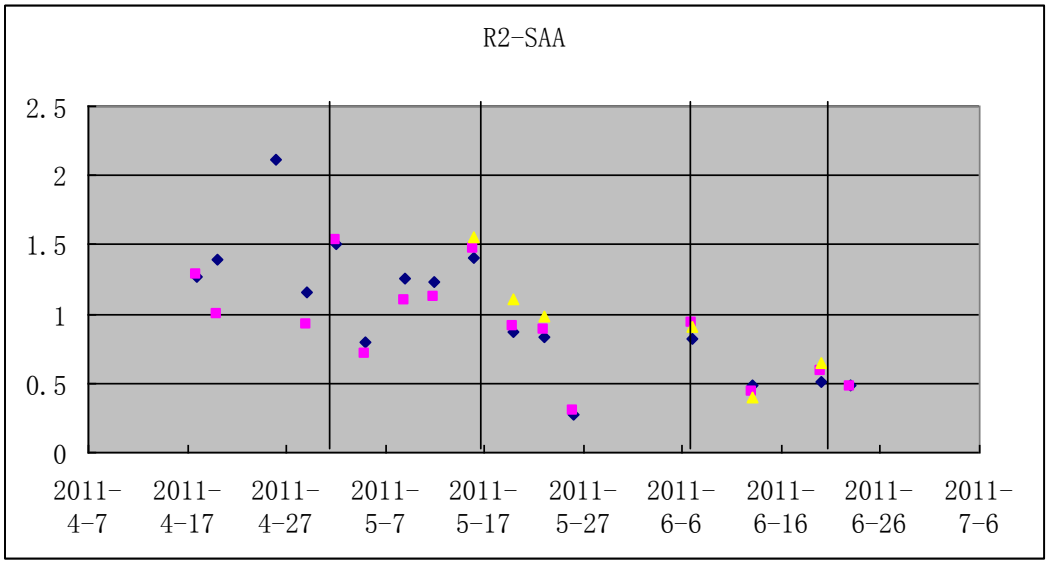


Fig. 28 The results of SAA in R2.

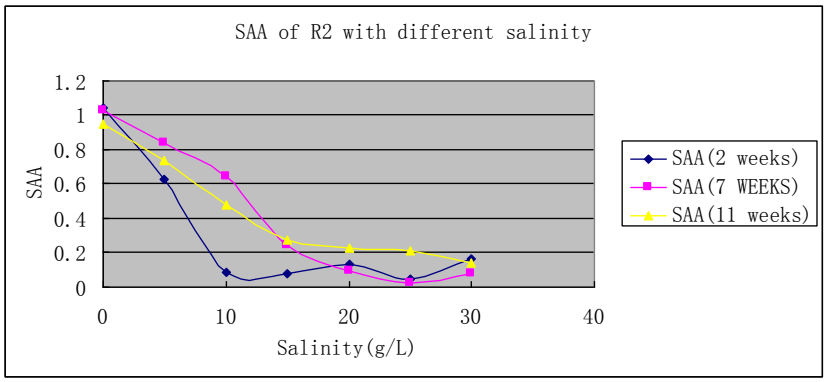


Fig. 29 The results of SAA with different salinity in R2.

Three times of SAA with full salinity was applied as well, still 2 weeks, 7 weeks and 11 weeks from the start. The average value of the SAA is used here to present the results and the percentage comparing with the fresh water SAA. (Fig. 29 and 30)

The time of these three SAA test were at the end of salinity 0 g/L (SAA after 2 weeks), at the end of salinity 5 g/L (SAA after 7 weeks) and at the beginning of salinity 10 g/L (SAA after 11 weeks).

Now the results can be discussed. First it can be found that the SAA values in three curves are almost the same when salinity is 0 g/L. This means the adaptation in this scenario will hardly decrease the best activity of the Anammox bacteria. After the two weeks the bacteria didn't contact any saline environment, so the ability of resisting the salt was very weak. The activity was almost 0 when the salinity was after 10 g/L. After 7 weeks, the ability of resisting the saline environment was much higher. The bacteria were still very active when the salinity was 5 and 10 g/L. The activity reached 0 when the salinity was more than 20, which proved that the adaptation was very successful. After 11 weeks, the acitivity was a little lower than 7 weeks. This is because that the salinity in the reactor now rose to 10 g/L. This amount was very high for the bacteria and inhibition happened. Some bacteria may be killed by the salt which leaded to the decrease of the salinity.

A small summary can be made as well here in R2. This scenario of adaptation can be regarded as successful. The bacteria can show activity even the salinity reached 10 g/L. The reactor was running well with little problem.

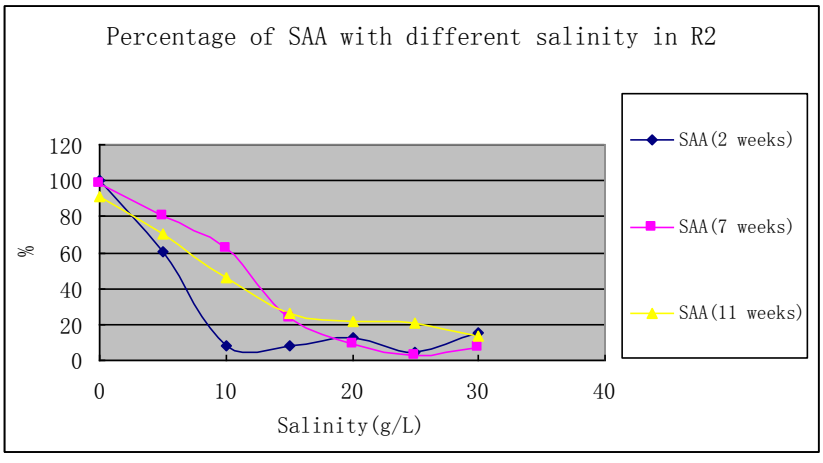


Fig. 30 Percentage of SAA with different salinity in R2.

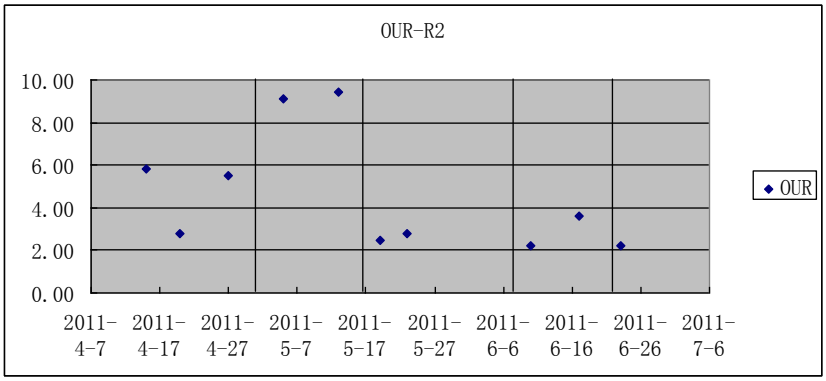


Fig. 31 The results of normal OUR in R2.

5.2.4. *OUR in R2*

The results of normal OUR can be illustrated as figure 31. The OUR values are as expected in salinity 0 g/L, and even higher in salinity 5 g/L. But after salinity 10 g/L, the OUR values decrease in a sudden to a very low value.

Now the discussion of the OUR in R2 can be made. When the salinity increased to 2.5 g/L, the activity of the bacteria increased. The situation is that the activity will get higher with small amount of salt, just the same as SAA. But when the salinity increased to 5 g/L, the inhibition happened. The activity decreased to only 30% comparing with the highest activity. Even though the activity recovered a little after the adaptation for some time, the activities were still very low. In order to analyze the bacteria's ability of resisting salt, three times of OUR test with full salinity were applied for R2. The time of these 3 OUR tests were at the end of salinity 0 g/L (OUR after 2 weeks), at the end of salinity 10 g/L (OUR after 7 weeks) and at the middle of salinity 15 g/L (OUR after 11 weeks). The results and percentages are shown in figure 32 and 33.

Now the activity of the bacteria can be analyzed according to the curves in the graph. The activity of the bacteria after the adaptation was lower than that before the adaptation. This is because the salt causes the inhibition and some bacteria cannot get over this inhibition in a short time. Take salinity 0 g/L for example, the OUR values are 6, 4 and 3.5 g O_2/m^2d, which means 100%, 70% and 60% of the activity. Then bacteria's activity can be analyzed resisting the salt. For the OUR which was done 2 weeks after the start, the ability of resisting salt is low because the adaptation didn't start and the bacteria cannot get used to

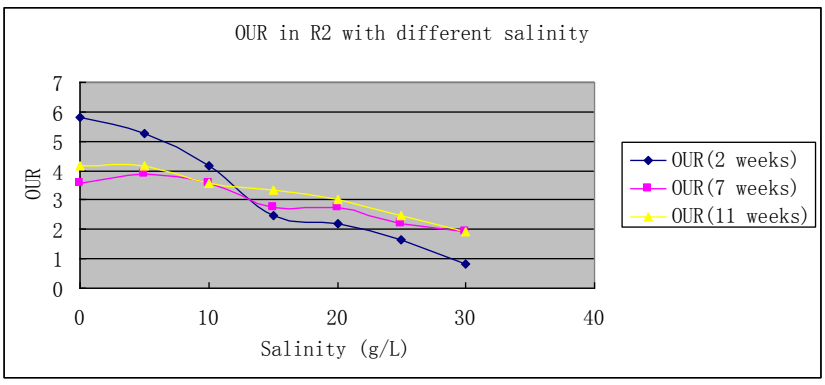


Fig. 32 The OUR test with full salinity in R2.

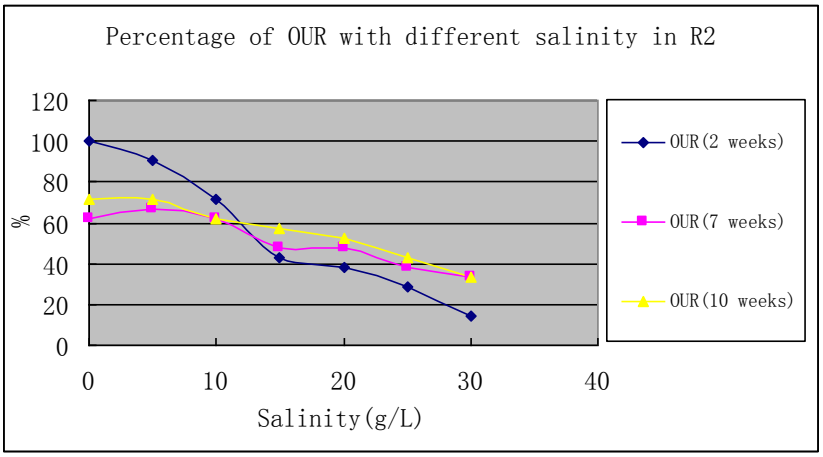


Fig. 33 Percentage of OUR with different salinity in R2.

this sudden change. But for OUR tests of 7 weeks and 11 weeks after the start, they show activity during the whole salinity range. This means that the adaptation was successful.

5.3. Comparison of the reactors and two strategies

In the previous part the discussion of two reactors are made separately. Now the comparison can be made to check the effect of the adaptation. In each reactor the period of salinity 0 ~ 10 g/L are chosen.

An overall comparison can be made now. First, the comparison is about the conditions in both reactors. For reactor 1, the bacteria are really unstable because they have to adapt big amount of salinity in short time. The inhibition effect in this reactor is serious. This can be known by the instability of the physical parameters in reactor 1, for example the DO limitation. (Camops et al, 2002) The PH and DO values are always jumping during the whole project, which shows the bad condition of reactor 1. Comparing with reactor 1, the performance of reactor 2 is much better. The increase of salt amount was not so much, and the reactor took twice as much time as reactor 1 to reach the same salinity. The amount of the salt was affordable for the bacteria, and enough time was given to get over this saline environment. The PH and DO value are more stable than reactor 1. What's more, the reactor 1 was really fragile during the whole environment, and it was always at the edge of the collapse after the salinity was more than 5 g/L. Under this condition many operations and measures were taken to make it stable and get back to normal. But for reactor 2 the situation was always good, only with some extra adjustment. And this stability lasted until the salinity 10 g/L. So comparing with reactor 1, the strategy in reactor 2 is better for the bacteria to adapt, with fewer problems to handle. Second, the concentration of the ammonium, nitrite and nitrate can be compared according to the chemical analysis. It can be found that the concentrations of these key chemicals in reactor 1 are fluctuant and not as stable as reactor 2. In order to keep the efficiency of the removal at 90%, many adjustments and operations were taken for reactor 1, and still the instability happened all the time. But for reactor 2, it is easy to keep the reactions in the reactor. Third, the change of the activity of the bacteria can be analyzed according to the SAA and OUR tests. In the SAA test, the activity of bacteria in reactor 1 was a little better than reactor 2 during the period salinity 5 ~ 10 g/L. But according to the full salinity SAA test, the bacteria in R1 were suffering serious inhibition and the value of the best activity of the bacteria decreased, which didn't

happen in reactor 2. What's more, reactor 2 is better than reactor 1 in the ability of resisting salt after the adaptation.

Also some specific problems can be compared. For the activity drop, it can be achieved from the results of SAA and OUR test that the activity in reactor 2 drops slower than reactor 1. At the same time, the losses of activity are more in reactor 1 than that in reactor 2. The full salinity SAA and OUR tests represent that in reactor 2 the bacteria still keep more than 90% activity in SAA and 60% activity in OUR tests. But the losses are much more in reactor 1. The efficiency of the removal in reactor 2 is higher than reactor 2 because of the high activity of the bacteria.

5.4. Uncertainty under experimental work

Systematic error

In the SAA test, the pressure meter is very sensitive. So each time before the test the calibration should be made. But no one can guarantee that the meter is 100% accurate. And also the sensor of the pressure meter is a tiny syringe needle which is very fragile and easy to be stuck. All these will affect the accuracy of the final results. What's more, when the salinity increases, the Anammox bacteria are not stable and the pressure in the bottle will have only little change because of the low bacteria activity, and this little change can hardly be detected by the pressure meter. So we can often get strange results, which obviously affect the judgments of the bacteria performance.

Influence of the operation

When the reactors are running, they should be taking care of all the time. But since all the operations are done by hands, sometimes it's not so accurate. For example, the aeration instrument is really hard to control; the really good DO value today may turn to a bad value tomorrow, and it's really hard to adjust. These changing of physical parameters will obviously affect the final results.

Different time period

In our experiment, the reactors are biological systems, and many unexpected things would happen for these livings. Because the R1 is really fragile at salinity 10 g/L, the period of this salinity step is extended in order to make the microbes survive. But in this condition the adaptation time for each period is different, which may affect the final results.

6. CONCLUSIONS

The strategy of reactor 1 is hard for the bacteria to work, and many measures should be taken to keep the reactor running. Still many problems were met after the salinity 5 g/L. The activity and the reactions are always unstable during the whole project. The activity drop much when the bacteria expose in the saline environment, with low nitrogen removal efficiency. For reactor 2, the strategy is much better. The bacteria can show high activity and even can afford the saline environment of salinity 10 g/L. The reactor was running under good condition without many adjustments. The activity of the bacteria can keep high when exposed in the salt water, with low decrease of the removal efficiency.

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HACH: http://www.hach.com/.

APENDIX I

Table 1A Pyisical parameters of reactor 1.

Date	pH	O_2	Temp		Conductivity		Loading		Comments
	in [-]	R1 [-]	R1 [mg/l]	R1 [^oC]	in [mS/cm]	R1 [mS/cm]	ml/min	HRT[d]	
2011-4-13	8.49	7.91	2.91	23.5	6.76	1.8	1.67	3.3	No inflow for a while
2011-4-14	8.48	7.7	2.19	24	6.56	1.66	1.4	4	
2011-4-15	8.43	7.17	1.65	26.2	3.91	1.01	1.85	3	
2011-4-18	8.23	6.45	1.87	26.2	7.27	1.44	1.5	3.7	Add new inflow
2011-4-19	8.71	7.73	1.44	26.3	7.24	1.85	2.05	2.7	
2011-4-20	8.76	8.17	1.82	26.3	7.1	2.2	2.4	2.3	
2011-4-25	8.87	8.47	2.2	26.4	6.8	2.46	2.05	2.7	
2011-4-26	8.37	7.48	2.44	26.6	6.64	1.07	1.8	3.1	Stop inflow for 1 day
2011-4-27	8.47	7.34	1.81	26.7	6.55	1.28	1.9	3	
2011-4-28	8.51	7.4	2	26.4	6.34	2.14	1.78	3.1	
2011-4-29	8.55	7.32	1.46	26.2	6.18	2		3.1	
2011-4-29	8.34	7.32	1.46		7.05	2	1,97→ 2,17	2.8	
2011-5-2	8.41	7.89	0.87	26.5	6.68	2.23	2.25	2.5	Start add salt （5g/L）
2011-5-2	8.27	7.89	0.87		14.22	2.23		2.5	New inflow with salt
2011-5-3	8.34	7.93	0.7	26.4	14.12	6.99	2.13	2.6	
2011-5-4	8.34	7.58	0.65	26.7	14.12	7.46	2.07→ 2.23	2.7	
2011-5-5	8.42	7.48	0.96	26.7	14.22	8.08	2.4	2.3	
2011-5-6	8.39	7.54	0.94	26.1	14.13	8.56	2.3	2.4	
2011-5-9	8.34	7.89	0.47	26.6	15.63	10.83	2.35	2.4	
2011-5-10	8.38	7.75	0.44	26.7	15.57	10.72	1.87	3	
2011-5-11	8.48	7.88	0.31 →1.0	26.6	15.44	11.04	1.77	3.1	
2011-5-12	8.46	7.46	3.38 →1.87	26.7	15.4	11.16	2.05	2.7	
2011-5-13	8.49	7.21	0.82	26.6	15.32	10.77	2	2.7	
2011-5-16	8.51	6.06	3.77 →1.87	26.9	13.44	12.17	1.6	3.3	
2011-5-17		5.83	1.09	27		14.88	1.9	3	
2011-5-18	8.38	7.57	0.33	27	23.2	16.43	1.8	3.1	
2011-5-19	8.32	6.8	0.24	26.6	23.4	16.65	1.05	5.3	Change outflow into 1:10 supernatent

2011-5-20	8.36	7.67	0.26 →0.5	26.8	23.4	17.39	1.25→ 1	4.4→5 .3	Electricity stopped in weekends
2011-5-23	8.44	7.74			23.1	17.5			
2011-5-24		7.87	0.23 →0.5 3	26.8		17.96	0.9	5.5	
2011-5-25	8.55	7.7	0.45	26.6	23.2	18.17	1	5.3	
2011-5-26		7.33		26.4		18.18	1.1	5.3	
2011-5-27	8.57				23.7				
	8.14	7.25	0.6	26.7	23.2	18.38	1.07→ 1.25	5.3→4 .4	
2011-5-28		7.58	0.35 →0.4 8	26.6		18.8	1.2	4.6	
2011-5-30	8.14	7.04	1.79 →0.5 2	26.2	23.6	18.92	1.25	4.5	
2011-5-31	8.26	7.66	0.3→ 0.52	26.5	23.7	19.6	1.2	4.6	
2011-6-1	8.26	7.43	0.44	26.2	23.6	19.54	1.1	5.3	
2011-6-3	8.39	6.71	2.22 →0.6 2	26.8	23.3	19.9	1.2	4.6	
2011-6-7	8.19	8.02	0.64	26.4	23.4	21.3	1.3	4.3	
2011-6-10	8.13	7.8	0.58	26.2	31	25.7	1.5	3.4	
2011-6-13	8.27	8.1	0.41	26.3	31	28.2	1.3	4.3	
2011-6-15									Change the outflow to 1:5 supernatant and Stop the inflow
2011-6-17		6.55	4.4→ 1	26.8		25.6	1	5.3	Start the inflow
2011-6-20		7.95	0.25 →0.4 9	26.5		28.2	0.8→1 .2		
2011-6-21		7.93	0.31	26.6		28.8	0		Stop the inflow
2011-6-22		7.67	0.39	26.4		29.1	0		
2011-6-23		7.52	0.27	26.5		28.9	0		
2011-6-27		7.02	0.19	27	24.3	29.5	1.5	3.67	
2011-6-28		7.14	0.15	27	25.3	30.1	1.7	3.24	
2011-6-29		7.25	0.22	26.7	24.7	31.1	1.3	4.239	
2011-7-1	8.15	7.37	0.35	26.6		32	1.4	3.94	

Table 2A Pyisical parameters of reactor 2.

Date	pH		O_2	Temp	Conductivity		Loading		Comments
	in [-]	R2 [-]	R2 [mg/l]	R2 [^oC]	in [mS/cm]	R2 [mS/cm]	ml/min	HRT[d]	
2011-4-13	8.49	8.14	2	26	6.76	2.6	1.75	3.2	
2011-4-14	8.48	8.03	2.15	26.2	6.56	2.09	1.4	4	No inflow for sometime
2011-4-15	8.43	7.61	1.45	26.2	3.91	1.33	1.85	3	
2011-4-18	8.23	6.75	1.99	26.2	7.27	1.21	1.85	3	Add new inflow
2011-4-19	8.78	8.06	0.98	26.2	7.22	1.92	1.83	3	
2011-4-20	8.76	8.16	2.29	26.3	7.1	2	2.2	2.5	
2011-4-25	8.91	7.94	1.46	26.2	6.64	1.48	2.15	2.6	
2011-4-26	8.37	7.32	2.21	26.1	6.64	1.33	2.3	2.4	
2011-4-27	8.47	7.32	2.12	26.3	6.55	1.38	3.2→2.2	1.7→2.5	
2011-4-28	8.51	7.07	3.0→1.8	26.2	6.34	1.4	1.65→2.22	3.4→2.5	
2011-4-29	8.55	7.13	2.84	26.2	6.18	1.42			
	8.34				7.05		2,2→2,53	2.5→2.2	
2011-5-2	8.41	7.82	1.34	26.3	6.68	1.87	2.65	2.1	Start to add salt (2.5g/L)
	8.26				9.86				New inflow with salt
2011-5-3	8.36	7.94	1.2	26.3	9.9	7.24	2.33	2.4	
2011-5-4		7.66	0.7	26.3		6.54	2.37	2.34	
2011-5-5	8.47	7.69	1.32	26.3	9.76	5.93	2.55	2.2	
2011-5-6	8.48	7.72	1.46	26.2	9.49	5.64	2.6	2.2	
2011-5-9	8.45	8.05	0.27→0.5	26.2	11.08	7.32	2.45	2.3	
2011-5-10	8.5	7.79	0.39	26.3	10.93	6.52	1.67→2.1	3.4→2.5	
2011-5-11	8.37	7.67	2.15	26.2	11.21	6.21	2.27→2.57	2.5→2.2	
2011-5-12	8.37	7.52	2.16→1.79	26.3	11.16	6.24	2.55	2.2	
2011-5-13	8.44	7.67	1.43	26.8	11.04	6.45	2.5	2.2	
2011-5-16	8.6	7.9	1.53	26.3	10.43	6.77	2.7	2.1	
2011-5-17		6.39	1.66	26		8.97	2.5	2.2	
2011-5-18	8.43	6.58	1.38→0.25	26.3	15.09	10.41	2.47→2	2.3→2.6	
2011-5-19	8.44	7.6	0.41	26.6	15	11.33	2.2	2.5	Change outflow into 1:10 supernatent
2011-5-20	8.5	7.92	0.57	26	14.92	10.27	2.0→1.5	2.6→3.7	
2011-5-23	8.53	7.98			14.7	10.38			Electricity stopped in

									weekends
2011-5-24		8.08	0.95 →0.6 4	26.2		10.96	1.5	3.6	
2011-5-25	8.59	8.16	0.72	25.9	14.74	11.66	1.5	3.6	
2011-5-26		8.2		26.1		12.27	1.1	5	Start pumping salt water to R2, v=10ml/min
2011-5-27	8.58				14.68				
	8.19	7.69	0.45	26.5	15.5	10.12	1.17	4.6	
2011-5-28		7.93	0.42	26.1		10.88	1.2	4.6	
2011-5-30	8.25	8	0.53	26.1	15.3	12.07	1.3	4.3	Stop inflow in R2
2011-5-31	8.34	8.02	0.32 →0.7	26.4	15.27	11.99	0		
2011-6-1	8.34	7.37	1.76	26.4	15.2	10.71	0		Change inflow of R2 to 1:1 supernatent
2011-6-3	8.41	7.17	1.34	26.2	11.6	10.88	2.2	2.5	
2011-6-7	8.31	7.33	0.47	26.2	12.6	10.6	2.1→1. 2		
2011-6-10	8.21	7.49	0.4	26.3	18	15.4	1	5.5	
2011-6-13	8.39	7.52	0.98	26.8	17.7	14.98	1.1	5	
2011-6-15		7.26	0.83	26.1		15.2	1	5.5	
2011-6-17		7.09	0.64	26.2		15.33	1.2→1. 5	4.6→ 3.6	
2011-6-20		7.39	0.19 →0.5 8	26		15.37	1.6		
2011-6-21		7.34	0.8	26.3		15.49	1.6		
2011-6-22		7.35	0.41	26.2		21.1	1.6		
2011-6-23	8.44	7.22	0.45	26.9	24	22.9	1.5		
2011-6-27		6.52	2.94	26.3	19.44	20.3	1.5	3.6	
2011-6-28		6.52	3.11	26.5	18.3	19.2	1.5	3.6	
2011-6-29		6.64	2.18	26.4	19.96	20.5	1.4	3.86	
2011-7-1	8.22	6.53	2.2	26.6		21	1.5	3.6	

APENDIX II

Table 3A Chemical parameters of reactor 1.

Date	NH_4-N [mg/l]		NO_2-N [mg/l]	NO_3-N [mg/l]	Comments
	in	out	out	out	
2011-4-13	827.5	151	0.976	16.8	No inflow for a while
2011-4-15		21.2	1.31	41.5	
2011-4-18	857.5	<2	<2	123	Add new inflow
2011-4-20		149	2.23	88.5	
2011-4-25		400	3.03	102	
2011-4-26					Stop inflow for 1 day
2011-4-29	895	8.8(<20)	1.63	62.5	
2011-5-2		103	4.87	23	Start add salt （5g/L）
2011-5-2					New inflow with salt
2011-5-4		40.8	4.74	27.5	
2011-5-6	835	23	2.71	27.65	
2011-5-9		159	3.2	12.1<25	
2011-5-13	777.5	25.7	4.01	72	
2011-5-16		82	2.94	164	
2011-5-18		206	1.42	219	
2011-5-19					Change outflow into 1:10 supernatent
2011-5-20	837.5	141	0.366	23.25	Electricity stopped in weekends
2011-5-23		117	0.293	1.4	
2011-5-25		92.9	4.43	21	
2011-5-27	872.5	9.35	1.17	35.9	
2011-5-30		10.1	4.2	43.35	
2011-6-3		45.4	90.1	7.81	
2011-6-7		305	22.1	7.8	
2011-6-13		516	1.43	0.265	
2011-6-15					Change the outflow to 1:5 supernatant and Stop the inflow
2011-6-17		27.1	100.2	27.2	Start the inflow
2011-6-21		228	55.6	15.05	Stop the inflow
2011-6-23		109	104	29.15	
2011-6-28		20.6		19.5	
2011-7-1		41.6	49.9	67.5	

Table 4A Chemical parameters of reactor 2.

Date	NH_4-N [mg/l]		NO_2-N [mg/l]	NO_3-N [mg/l]	Comments
	in	out	out	out	
2011-4-13	827.5	274	0.935	5.8	
2011-4-14					No inflow for sometime
2011-4-15		53	2.17	29.55	
2011-4-18	857.5	<2	<2	66.5	Add new inflow
2011-4-20		130	4	76.5	
2011-4-25		40.6	2.94	325	
	895	17.4	3.48	99	
2011-5-2		75.9	4.4	40.6	Start to add salt (2.5g/L)
					new inflow with salt
2011-5-4		52.8	5.16	32.1	
2011-5-6	757.5	42.4	3.39	31.95	
2011-5-9		203	2.83	5.55	
2011-5-13	835	42.9	3.87	57.5	
2011-5-16		87.2	2.14	26.75	
2011-5-18		115	153.5	-	
2011-5-19					Change outflow into 1:10 supernatent
2011-5-20	767.5	170	0.366	13.85	
2011-5-23		166	0.464	0	Electricity stopped in weekends
2011-5-25		275	0.36	0	
2011-5-26					Start pumping salt water to R2, v=10ml/min
2011-5-27	867.5	48.5	0.43	0.5	
2011-5-30		284	0.95	0	stop inflow in R2
2011-6-1					Change inflow of R2 to 1:1 supernatent
2011-6-3		6.01	1.68	4.46	
2011-6-7		15.4	1.37	20.45	
2011-6-13		2.7	1.49	32	
2011-6-17		2.24	1.47	90.5	
2011-6-21		1.9	3.36	81.5	
2011-6-23		24.3	2.1	30。25	
2011-6-28	892.5	134		306.5	
2011-7-1	957.5	169	108	270	

APENDIX III

Table 5A Normal SAA for reactor 1.

R1					
Date	Salinity （g/L）	bottle 1	bottle 2	bottle 3	Average
2011-4-18	0	1.7408	1.6839		1.7123
2011-4-20	0	1.1546	1.421		1.2878
2011-4-26	0	1.0693			1.0693
2011-4-29	0	1.5633	1.5312		1.5473
2011-5-2	0	1.6662	1.5418		1.604
2011-5-5	5	0.7357	0.9665		0.8511
2011-5-9	5	1.0988	1.0812		1.09
2011-5-12	5	1.0444	1.0871		1.0658
2011-5-16	5	1.2043	1.1617	1.0231	1.1297
2011-5-20	10	0.3756	0.5435	0.4453	0.4548
2011-5-23	10	0.1812	0.2629	0.3167	0.2536
2011-5-26	10	0.3757	0.365	0.3367	0.359133333
2011-6-7	10	0.109454	0.089475	0.10851	0.102479422
2011-6-13	15	0.01421	0.017763	0.01066	0.014210335
2011-6-20	15	0.106578	0.156314	0.03553	0.099472344
2011-6-23	15	0.078157	0.042631		0.0603939
2011-6-27	15	0.049736	0.021316	0.09196	0.054337167

Table 6A SAA of full salinity in reactor 1.

Salinity (g/L)	2 weeks	7 weeks	11 weeks
0	1.04132	0.640757	0.275325
5	0.62967	0.44918	0.225589
10	0.08551	0.396779	0.073589
15	0.08205	0.270282	0.060394
20	0.13111	0.272645	0.044407
25	0.04568	0.230992	0.030197
30	0.16325	0.172334	0.087038

Table 7A Normal SAA for reactor 2.

R2					
Date	Salinity （g/L）	bottle 1	bottle 2	bottle 3	Average
2011-4-18	0	1.2683	1.2825		1.2754
2011-4-20	0	1.3926	0.9957		1.1942
2011-4-26	0	2.1138			2.1138
2011-4-29	0	1.1578	0.9249		1.0413
2011-5-2	0	1.5027	1.5347		1.5187
2011-5-5	2.5	0.8002	0.7046		0.7524
2011-5-9	2.5	1.2597	1.0967		1.1782
2011-5-12	2.5	1.2256	1.1191		1.1724
2011-5-16	2.5	1.3997	1.4708	1.5525	1.4743
2011-5-20	5	0.8658	0.9108	1.1081	0.9616
2011-5-23	5	0.8277	0.8846	0.9876	0.8999
2011-5-26	5	0.269	0.3		0.2845
2011-6-7	5	0.826746	0.935788	0.90683	0.889788744
2011-6-13	7.5	0.483151	0.429863	0.39434	0.435783604
2011-6-20	7.5	0.512587	0.586684	0.64454	0.581270366
2011-6-23	10	0.490257	0.472494		0.4813751
2011-6-27	10	-	-	-	-

Table 8A SAA of full salinity in reactor 2.

Salinity (g/L)	2 weeks	7 weeks	11 weeks
0	1.04132	1.024893	0.945241
5	0.62967	0.836289	0.733355
10	0.08551	0.645727	0.481375
15	0.08205	0.245128	0.273041
20	0.13111	0.094905	0.229142
25	0.04568	0.027236	0.212648
30	0.16325	0.077311	0.143118

APENDIX IV

Table 9A Normal OUR for reactor 1.

Date	OUR		
	Salinity (g NaCl/l)	average, (mg/L O2 rem)/sec	g O2 / m2 .d
2011-4-15	0	-0.0026	7.19
2011-4-20	0	-0.0014	3.87
2011-4-27	0	-0.0021	5.81
2011-5-5	5	-0.0029	8.02
2011-5-13	5	-0.0034	9.4
2011-5-19	10	-0.0003	0.83
2011-5-23	10	-0.0005	1.38
2011-6-10	15	-0.0008	2.21
2011-6-17	15	-0.0007	1.94
2011-6-23	15	-0.0008	2.21

Table 10A OUR of full salinity in reactor 1.

Salinty	2 weeks	7 weeks	10 weeks
0	5.81	4.15	2.76
5	5.25	4.42	2.76
10	4.15	3.32	2.49
15	2.49	3.04	2.21
20	2.21	2.76	1.66
25	1.66	1.94	1.66
30	0.83	1.66	1.38

Table 11A Normal OUR for reactor 2.

Date	OUR		
	Salinity (g NaCl/l)	average, (mg/L O2 rem)/sec	g O2 / m2 .d
2011-4-15	0	-0.0021	5.81
2011-4-20	0	-0.001	2.76
2011-4-27	0	-0.002	5.53
2011-5-5	2.5	-0.0033	9.12
2011-5-13	2.5	-0.004	9.4
2011-5-19	5	-0.0009	2.49
2011-5-23	5	-0.001	2.76
2011-6-10	7.5	-0.0016	2.21
2011-6-17	7.5	-0.0013	3.59
2011-6-23	10	-0.0013	2.21

Table 12A OUR of full salinity in reactor 2.

Salinty	2 weeks	7 weeks	10 weeks
0	5.81	3.59	4.15
5	5.25	3.87	4.15
10	4.15	3.59	3.59
15	2.49	2.76	3.32
20	2.21	2.76	3.04
25	1.66	2.21	2.49
30	0.83	1.94	1.94