



# Nutrient Removal from Municipal Wastewater in a Pilot-scale Plant with A<sup>2</sup>/O Process Operated at Low SRT by Ex-situ Bioaugmentation

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## ABSTRACT

Enhancing nutrient removal from municipal wastewater by bioaugmentation was investigated in a pilot plant operated in A<sup>2</sup>/O process. Reject water from sludge treatment was used as a feed for ex-situ cultivation of nitrifier-contained activated sludge in a new process named RAO for bioaugmentation. The experimental results showed that ex-situ bioaugmentation was very effective for enhancing nutrient removal. Ammonium and phosphorous concentrations in the effluent in mainstream could be reduced to less than 1mg/L at a HRT of 10h and SRT of 8d. FISH test proved that nitrifier community of activated sludge in side-stream was identical to that in mainstream, which resulted in little nitrification loss during bioaugmentation.

*Keywords:* Municipal wastewater; nutrient removal; activated sludge; reject water; bioaugmentation

## 1. INTRODUCTION

Removal of nutrient substances such as nitrogen and phosphorous in municipal wastewater has been long emphasized due to their negative effects on receiving water body. Up to today, the most widely used process in practise is A<sup>2</sup>/O (Anaerobic/Anoxic/Oxic) in which municipal wastewater flows sequentially through partitioned zones, in which phosphate, nitrogen and organic substances are removed. In the process, bacteria with different functions, such as phosphate accumulation organism (PAO), Nitrifier and Denitrifier, coexist in a single sludge. Due to different growth environment requirements, optimization of the process is of great impor-

tance. For PAO, low operating SRT (Sludge Retention Time) is favoured because the final amount of phosphorous removal is proportional to the amount of biological phosphorous accumulating bacteria wasted. Also, PAO could successfully compete with glycogen accumulation organism (GAO, bacteria resulting in deterioration of biological phosphorous removal process) only at short sludge retention times (Whang and Park, 2006). However, high operating SRT is preferred for nitrifier because of its slow growth rate. Therefore, how to balance operating SRT in the process is crucial for high efficiency for both bacterial groups.

Nitrification capability in the mainstream can be enhanced by Bioaugmenting nitrifier cultivated in a side-stream with reject water, a by-product in sludge treatment in wastewater

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treatment plant (WWTP). Several processes such as BABE (Salem et al, 2004), ScanDeNi (Rosen and Huijbregsen, 2003) and InNItri (Kos, 1998) are reported and used effectively in relieving stresses in winter or in situation overloading. However, no research is reported in simultaneous enhancement of nitrogen and phosphorous removal under low SRT operation.

In this paper, a new process named RAO (Reverse Anoxic/Oxic) for nitrifier cultivation is introduced. The results for nitrifier bioaugmentation to enhance nutrient removal in a pilot-scale WWTP operated at low SRT are presented and evaluated.

## 2. TECHNICAL CONSIDERATION

In WWTP with bioaugmentation, two ecological systems exist. One is the activated sludge cultivated by wastewater to be treated in the mainstream; and the other is the activated sludge cultivated by reject water in side-stream. In order to maintain augmented-nitrifier and keep it growing in the main stream, nitrifying bacteria composition in two microbial communities should match each other, including microbial genera and distribution. Therefore, the culture in the side-stream for bioaugmentation should have the same ecology in the mainstream otherwise the effectiveness of bioaugmentation will be reduced. *Nitrosomonas Europea* of ammonia oxidizing bacteria (AOB) and *Nitrobacter* of nitrite oxidizing bacteria (NOB) are demonstrated with the characteristics of faster growth rate and low substrate affinity. It is expected these two genera would grow and sustain in the system under low SRT.

Although microbial ecology in the mainstream is almost identical to that in the side-stream cultivated by in situ bioaugmentation as BABE process, the kinetic enhancement of nitrification is suspicious due to very low nitrifier concentration in the side-stream. High nitrifier concentration in the activated sludge in side-stream will be expected in ex-situ bioaugmentation since separated sludge is used.

A new process named RAO (Reverse Anoxic/Oxic) for culture development using reject water is developed in which anoxic and aerobic reactors are arranged reversely so that ammonium concentration in the reactor can be maintained at low level. High nitrifier concentration and activity will be obtained and an identical microbial ecology for nitrifier with mainstream system will be expected simultaneously.

## 3. MATERIALS AND METHODS

### 3.1 Pilot plant

A pilot plant was built in 4<sup>th</sup> Municipal Wastewater Treatment Plant in Xi'an, China. The process employed in the pilot plant is shown in Figure 1. A<sup>2</sup>/O is used for main stream treatment and Aerobic/Anoxic for side stream (reject water) treatment. Wasted sludge in side stream in which nitrifier is enriched is pumped back to the main stream for bioaugmentation.

The total net volume for A<sup>2</sup>/O is 3.6 m<sup>3</sup> in which the volume ratio of anaerobic/anoxic/aerobic is 1/2/6 and that for RAO is 0.25 m<sup>3</sup> in which the volume ratio of anoxic/aerobic is 1/3. The flow rate in the main stream is 0.36 m<sup>3</sup>/h which is equivalent to a hydraulic retention time (HRT) of 10h, and that in side stream is 0.0072 m<sup>3</sup>/h (2% of flow rate in the main stream), which equals to a HRT of 34h. Dissolved oxygen (DO) concentration in aeration tank was controlled between 2-3 mg/L automatically.

### 3.2 Wastewater

Wastewater used in the main stream and reject water in the side stream are the same as those in full scale plant. Their characteristics are shown in Table 1.

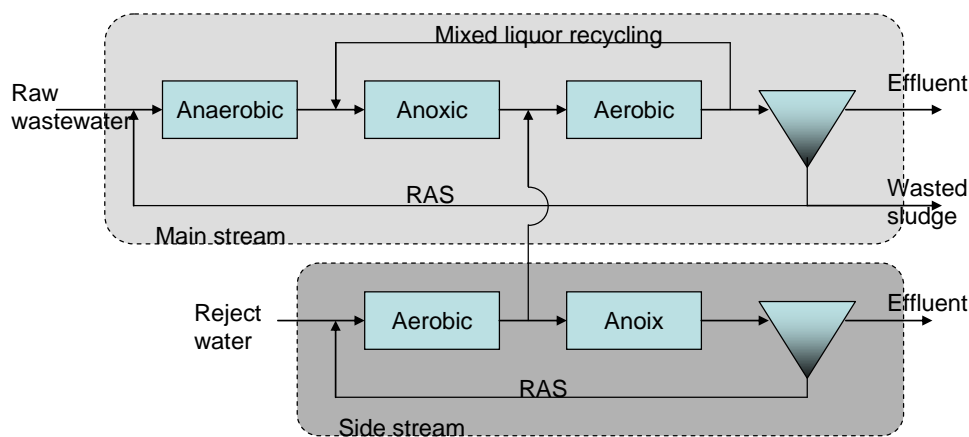
### 3.3 Analytical methods

Nitrate, nitrite and phosphate were analysed simultaneously by ion chromatography. Ammonia, COD, SS and VSS were conducted follow-

ing Standard Methods (APHA, 1998).

For fluorescence in situ hybridization (FISH) test, samples were pre-treated with 4% paraformaldehyde for fixing and ultrasonic (20 kHz, five minutes) in order to break up large flocs

prior to hybridization. In situ hybridizations of cells were performed with fluorescently labeled rRNA-targeted oligonucleotide probes (Table 2), which was provided by Microsynth Co. (Augct, China).



**Figure 1** Experimental system employed in the pilot plant

**Table 1** Characteristics of municipal wastewater and reject water used in the experiment

| Components           | SS<br>(mg/L) | NH <sub>3</sub> -N<br>(mg/L) | TKN<br>(mg/L) | TCOD<br>(mg/L) | SCOD<br>(mg/L) | PO <sub>4</sub> <sup>3-</sup> -P<br>(mg/L) |
|----------------------|--------------|------------------------------|---------------|----------------|----------------|--|
| Municipal wastewater | 140-280      | 26-49                        | 34-62         | 220-580        | 80-210         | 1.8-8.4                                    |
| Reject water         | 212-606      | 120-480                      | 140-620       | 288-989        | 147-670        | 13.2-40                                    |

**Table 2** Probes used for FISH and the corresponding hybridization conditions

| Probe    | Specificity                           | conc <sup>a</sup> | References                |
|----------|---------------------------------------|-------------------|---------------------------|
| NSO1225  | Ammonia oxidizing beta-proteobacteria | 35                | Mobarry et al. (1996)     |
| Nsv443   | Nitroso-spira, -lobus, -vibrio        | 30                | Mobarry et al. (1996)     |
| Nmv      | Nitroso-coccus                        | 35                | Pommerening et al. (1996) |
| Ntspa662 | Nitrospira                            | 35                | Daims et al. (1999)       |
| NIT3     | Nitrobacter                           | 40                | Wagner et al. (1996)      |
| Ntcoc206 | Nitrococcus mobilis                   | 10                | Juretschko, (2000)        |
| Ntspn693 | Nitrospina gracilis                   | 20                | Juretschko, (2000)        |

<sup>a</sup>Concentrations presented as percentage of formamide in hybridization buffer

## 4. RESULT AND DISCUSSION

### 4.1 Main stream reactor performance

The pilot plant has run for more than 1 year. High removal efficiency was achieved for both nitrogen and phosphorous even when SRT was

controlled as low as 8d.

Change of ammonium concentrations in influent and effluent is shown in Figure 2. The pilot plant was seeded with sludge from the A<sup>2</sup>/O reactor in WWTP, which was operated in a long SRT of 15d. The seed sludge had a very high nitrification capability. Therefore, nitrifica-

tion is very good at the start-up even SRT was controlled in 8d. With operation, nitrification activity deteriorated gradually, which resulted in an increase of ammonium in the effluent. Ammonium in the effluent was nearly equal to that in the influent on day 125. Nitrification activity was lost completely.

After bioaugmentation, nitrification in the main stream recovered gradually. Ammonium in the effluent decreased gradually. On day 215 (after 90 days of bioaugmentation), ammonium in the effluent was less than 10 mg/L. Bioaugmentation is very effective for enhancing nitrification in activated sludge process for municipal wastewater treatment in low SRT.

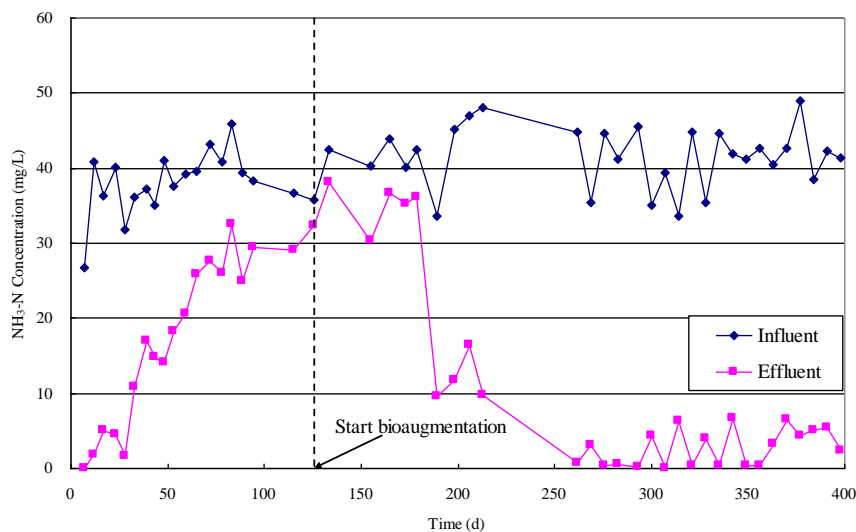
Change of phosphate concentrations in influent and effluent is shown in Figure 3. At the beginning, the system had a very low phosphate removal capability, which resulted in a high phosphate in the effluent. With operation, phosphate removal capability was built up gradually, which owned to low SRT. Before bioaugmentation, phosphate concentration in the effluent reached less than 1 mg/L. After bioaugmentation, no significant effect was observed on phosphate removal. Phosphate concentration in the effluent was kept at low levels even though

phosphate concentration in the feed increased from 3-4 to 4-7 mg/L. However, at the same time, phosphorous concentration in the WWTP (15 days of SRT) was more than 1 mg/L. This result clearly showed that short SRT favours phosphorous removal in biological nutrient process.

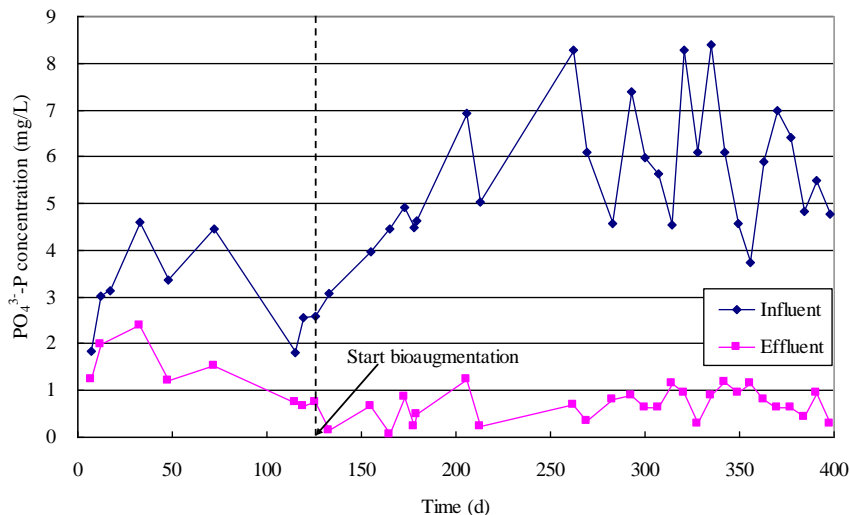
#### 4.2 Profiles of SCOD, NH<sub>3</sub>-N, NO<sub>3</sub>-N and PO<sub>4</sub>-P

Profiles of SCOD, NH<sub>3</sub>-N, NO<sub>3</sub>-N and PO<sub>4</sub>-P along with different units in the main stream are shown in Figure 4. Significant phosphate is released accompanying with SCOD uptake in anaerobic stage and taken up in the following anoxic and aerobic stages, which means PAOs are enriched and performed well in the reactors. Denitrifying PAO also presented in the sludge.

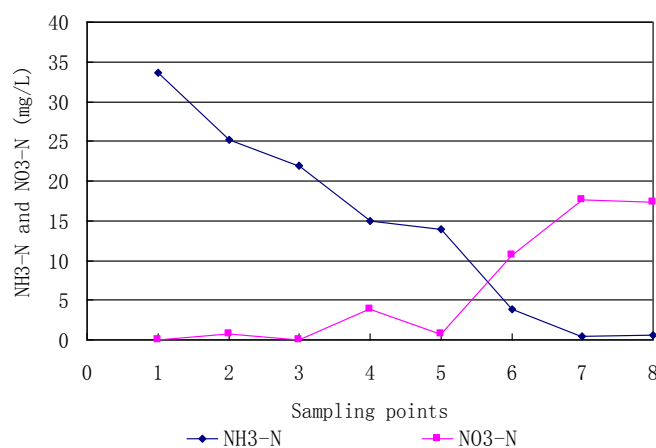
Three compartments were employed in the aerobic stage. No nitrification was observed in the first compartment even significant oxygen uptake was detected, which suggested that oxygen was utilised by heterotrophic bacteria for COD oxidation and PAO for PHB respiration. When COD in the liquor is consumed, Nitrification takes place (compartment 2 and 3).



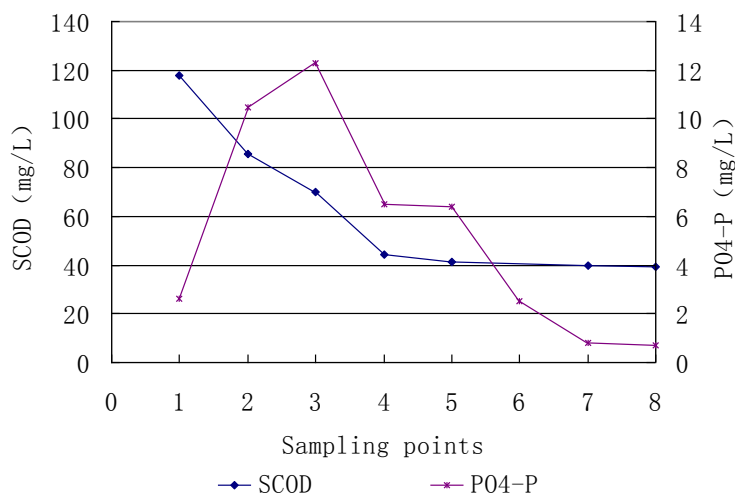
**Figure 2** Change of nitrogen profiles before and after bioaugmentation



**Figure 3** Change of phosphorous profiles before and after bioaugmentation



(a) NH<sub>3</sub>-N and NO<sub>3</sub>-N



(b) SCOD and PO<sub>4</sub>-P

**Figure 4** Profiles of SCOD, NH<sub>3</sub>-N, NO<sub>3</sub>-N and PO<sub>4</sub>-P along with different units in the main stream (1-Influent; 2-Anaerobic 1; 3-Anaerobic 2; 4-Anoxic; 5-Aerobic 1; 6- Aerobic 2; 7- Aerobic 3; 8-Effluent)

### 4.3 Side stream reactor performance

Change of ammonium and ammonium utilization rate (AUR) and nitrite utilization rate (NUR) in side stream reactor is shown in Figures 5 and 6. After 53 days operation, influent ammonium concentration increased from 120 to nearly 400 mg/L and ammonium loading rate (Ns) increased from 0.96 to 6.69 mg/g VSS/h correspondingly. However, effluent ammonium was kept under 10 mg/L, which means that very good nitrification performance was achieved. In fact, AUR and NUR in the sludge increased dramatically from 2.07 mg NH<sub>3</sub>-N/g VSS/h and 1.78 mg NO<sub>2</sub>-N/g VSS/h to 8.91 mg NH<sub>3</sub>-N/g VSS/h and 8.62 mg NO<sub>2</sub>-N/g VSS/h respectively. After that, AUR was kept unchangeable and NUR increased continuously to as high as 12.86 mg NO<sub>2</sub>-N/g VSS/h. The observed nitrifier production rate could reach 0.088 mg /mgN. The results suggest that reject water from sludge treatment can be used to cultivate nitrifier for bioaugmentation to enhance nitrification in the

mean stream effectively.

### 4.4 FISH test results

Before augmentation, nitrifier concentration in the sludge was very low due to low SRT, which resulted in low nitrification rate in the sludge and high ammonium concentration in the effluent. After bioaugmentation, nitrifier concentration in the sludge increased significantly. AOB number increased from  $5.13 \times 10^6$  cell/mL to  $3.72 \times 10^7$  cell/mL and NOB number increased even more, from  $2.75 \times 10^5$  cell/mL to  $1.66 \times 10^6$  cell/mL. Change of nitrifier concentration suggested that nitrifier added from side stream maintained and grow well in the system. This suggestion can be certified clearly by microbial genera distribution in Figure 7. *Nitrisomonas* was the dominated AOB in both sludge in main stream and *Nitrobacter* was the dominated inside stream NOB. Reverse Anoxic/Oxic process showed very effective nitrifier enrichment under high ammonium concentration.

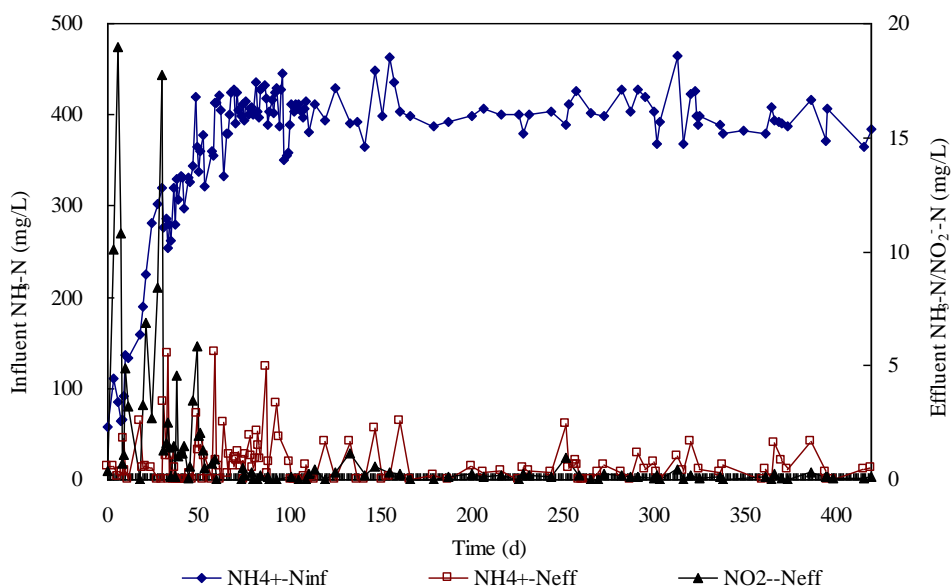
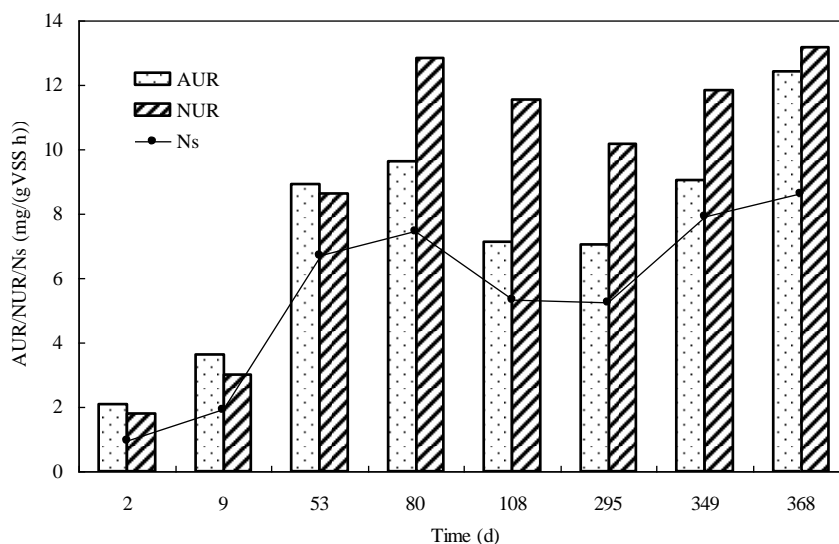
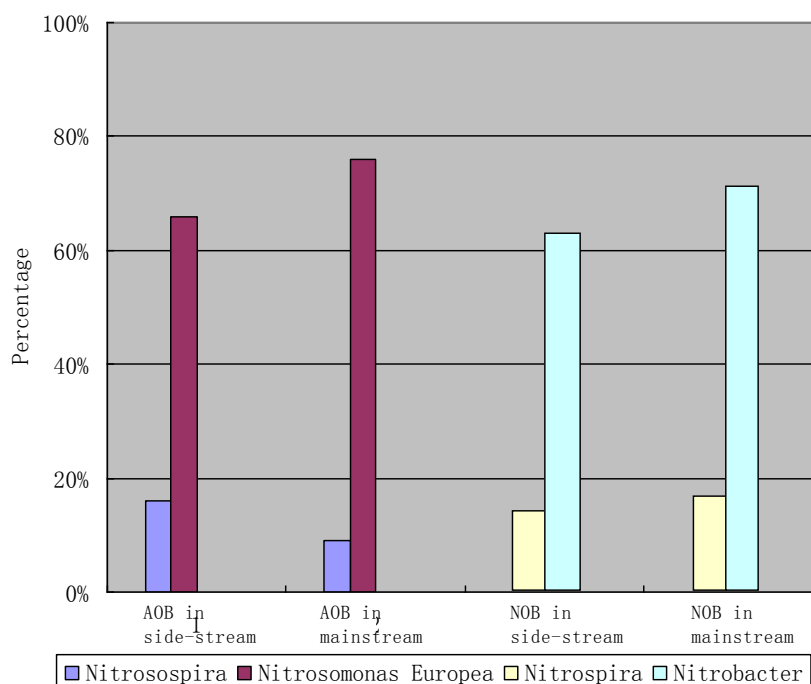


Figure 5 Change of ammonium in side stream reactor



**Figure 6** Change of AUR and NUR in side stream reactor



**Figure 7** Comparison of Nitrifier genera in mainstream and side-stream

## CONCLUSIONS

High nitrification can be maintained in low SRT in the mainstream through bioaugmentation of nitrifier cultivated from side-stream with reject water, which guarantees high phosphorous removal at the same time. Nitrifier community in side-stream operated in RAO process is identical to that in main stream so that effective bio-

augmentation can be achieved. Ex-situ bioaugmentation is an effective way to enhance nutrient removal from municipal wastewater in WWTP.

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