Enrichment and Cultivation of Denitrifying Phosphorus Removing Bacteria

Jinlan Lian [1, a], Xing Jin [2,b*]

[1] School of Resources and Environmental Engineering,Jilin Institute of Chemical Technology , China

[2] School of Chemical and Pharmaceutical Engineering,Jilin Institute of Chemical Technology, China

[a] lianjinlan@sina.com, [b] jinxing70@sina.com

Keywords: SBR; Enrichment; Cultivation; DPB

Abstract. The denitrifying phosphorus removal bacteria (DPB) were enrichment using SBR reactor and two stages of training method under the low carbon source with nitrate as electron acceptor. The system was operated under the condition of anaerobic-aerobic-precipitation-discharge for 10 cycles in the first stage, and the condition of anaerobic-aerobic-anoxic-aerobic -precipitation-discharge were used for 40 cycles in the second stage. Results showed removal rate of PO_4^{3-}-P , NH_4^+-N and COD was 95% ,80% and 90% when the effluent concentration was below 0.4mg/L, 3mg/L and 20mg/L respectively, which indicated that enrichment and cultivation of DPB in a set of SBR was feasible.

Introduction

Denitrifying phosphorus removing bacteria (DPB) can denitrify nitrogen and remove phosphorus at the same time in anoxia condition, using oxygen, nitrite and nitrate as electron acceptor[1]. The character of DPB can successfully solve the carbon source problem and competition problem of various bacterium genus in the process of denitrification and dephosphorization. The carbon source has two functions what can effectively solve the contradiction of sludge age, carbon source and nitration and phosphorus removal, release and absorption; furthermore, it has the advantage of saving the amount of aeration and reducing the production of sludge[2-3].

In this study the backflow sludge in the A/O process were used as the sludge sample which collected from an urban sewage treatment plant in a certain city. SBR reactor were used and took the nitrate as electron acceptor to enrichment culture DPB with a low carbon source and investigated the performance of synchronal denitrification and dephosphorization.

Experiment

Equipment and procedure

The SBR reactor in time sequence were used in this experiment as the equipment of DPB cultivation . It is with a diameter of 30cm, a height of 40cm, a volume of 25L and an escape pipe which can discharge sludge in the bottom of the reactor. The instantly influent water were used, and the amount of influent water in one cycle were 19~20L. In the anaerobic and anoxia condition, the mechanical stirrer were used to keep the sludge suspended and uniform, and the temperature is controlled at 30℃. In the anoxia stage, the potassium nitrate solution with different concentration was added according to the need, which would be used as the electron acceptor needed in the reaction.

The project and method of analysis

Waste water samples after centrifuge was analyzed by the state standard method . The analysis project and method were shown in Table 1.

Table 1. The project and method of analysis

project	method
COD_{cr}	Potassium dichromate method
NH_4^+-N	Na 's reagent spectrophotometric method
NO_3^--N	Thymol Spectrophotometry
NO_2^--N	N - (1 - naphthyl) B two amine photometric method
PO_4^{3-}-P	Molybdenum antimony anti - spectrophotometric method
MLSS	Gravimetric method

The main reagent

Sludge culture fluid were prepared by artificial simulation of low carbon city sewage, and added ethanol, NH_4Cl and KH_2PO_4 to it for obtaining a certain concentration of COD,NH_4^+-N and PO_4^{3-}-P. And added CaO 0.014g/L; KI 0.030g/L; $MgCl_2$ 0.040g/L; $ZnSO_4$ 0.050g/L; $CuSO_4$ 0.016g/L; $CoCl_2$ 0.005g/L; $AlCl_3$ 0.010 g/L; $FeSO_4$ 0.050g/L; EDTA 0.001g/L; $SnCl_2$ 0.001g/L; $NaNO_3$ 0.040g/L to the water. The water quality were shown in Table 2.

Table 2. The quality of raw water

Parameter	COD(mg/L)	NH_4^+-N(mg/L)	PO_4^{3-}-P(mg/L)	PH
value	150	50	10	7-8

Enrichment and cultivation of DPB

This study took the backflow sludge in the A/O process as the experiment sludge which was collected from an urban sewage treatment plant in a certain city, and the cultivation and domestication of DPB were divided into two stages. The first stage used the operation mode of anaerobic/anoxia and had 10 operation cycles. The second stage used the operation mode of anaerobic/aerobic/anoxia/aerobic and had 40 operation cycles. Gradually increase the anaerobic and lack of oxygen during the operation of time , gradually reduce the aerobic time. The details were shown in Table 3, the SBR operation mode.

Table 3. The SBR operation mode

operation stage	Cycles	Time (h)			
		anaerobic	anaerobic	anaerobic	anaerobic
First stage	10	3	3	-	-
Second stage	40	2.5～3	1.5～0	1.5～4	0.5～0

The first stage is the cultivation and domestication of PAO_S, uses the operation mode of anaerobic 3h/aerobic 3h/precipitation 0.5h/drainage 0.5h and has 10 operation cycles. The details were shown in Table 3, the SBR operation mode. In the cultivation course of this stage, the DO of anaerobic stage and aerobic stage are 0.03-0.2mg/L and 2-4mg/L, respectively. The influent COD, PO_4^{3-}-P and MLSS are controlled at about 150mg/L, 10mg/L and 3000mg/L, respectively. The concentration of PO_4^{3-}-P in every period were shown in Fig.2.

The second stage is the enrichment of DPB phosphorus removal bacteria. Under the operation mode of anaerobic/aerobic/anoxia/aerobic/precipitation/drainage, gradually reduce the aerobic operation time while extend the anoxic and anaerobic operation time and increase the amount of nitrates added. It has 40 operation cycles and the details were shown in Table 3, SBR operation mode. The DO concentration of the anaerobic stage, aerobic stage and anoxia stage were 0.03-0.2mg/L, 2-4mg/L and 0.2-0.5 mg/L, respectively. The influent COD, PO_4^{3-}-P and MLSS concentration were maintained at

about 150mg/L, 10mg/L and 3000mg/L, respectively. The amount of NO_3^--N and anhydrous sodium sulfite concentration added were 40mg/L and 5mg/L, respectively.

The conventional enrichment method of DPB phosphorus removal bacteria were A/A/O operation mode. This method were beneficial to the sufficient absorption of phosphorus in the aerobic stage. However, in the course of the experiment, it was found that if the rbCOD in the anoxia stage was too high, the denitrifying process occurred easily. So this experiment used the opposite process of the conventional A/A/O operation mode, namely, A/O/A operation mode. It effectively ensured the beneficial condition of denitrifying process, but it was found in the course of experiment that this method had a great influence on the phosphorus removal process in the next cycle. In order to solve the problem, we increased the short time aeration after the anoxia stage,the aerobic run. It not only effectively absorbed the surplus phosphorus, but also improved the settling performance of sludge.

Results and discussion

Analysis of the enrichment and cultivation of DPB

Figure 1 shows the change of PO_4^{3-}-P concentration in the first stage. From Figure 1 , we can see that the concentration of effluent PO_4^{3-}-P reached 0.4 mg/L with removal of 95.8%, and the COD removal were found to be more than 90%. after the cultivation and domestication in the first stage. Because the seed sludge was collected from the backflow sludge in the A/O process, there was a rich content of PAOs in it. Trained after 10 cycles, PAOs can operate steadily in the system. With the seed sludge adapted to the new living environment, taken 7 cycles, effluent TP can achieve below 1mg/L. The aerobic absorption of phosphorus content is twice as anaerobic phosphorus release. It can consumed 10 mg COD when released 1 mg P, which is closed to the “7-10 mg acetate is about to remove 1 mg P” pointed out by Wentze.

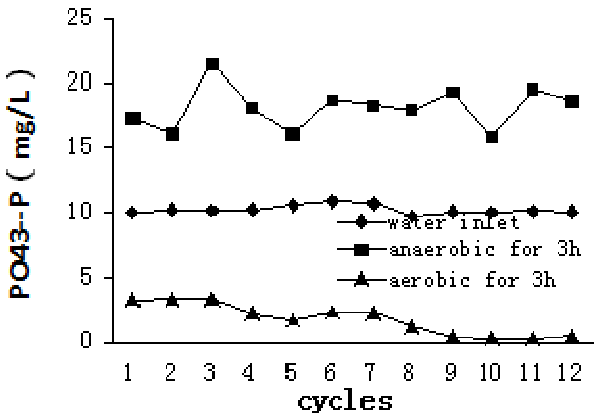

Fig.1. The change of PO_4^{3-}-P concentration in the first stage

Figure 2 shows the $PO4^{3-}$-P removal efficiency changes. As shown in Figure 2, PO_4^{3-}-P concentration reduced obviously, and the $PO4^{3}$—P removal efficiency can retain more than 95%, after the enrichment of DPB in the second stage(running 40 cycles). What’s more, NO_3^-N and NO_2^-N can’t be tested out in the wastewater, which shows that we can achieve the removal of nitrogen and phosphorus synchronously, finish the cultivation and domestication of DPB[4].

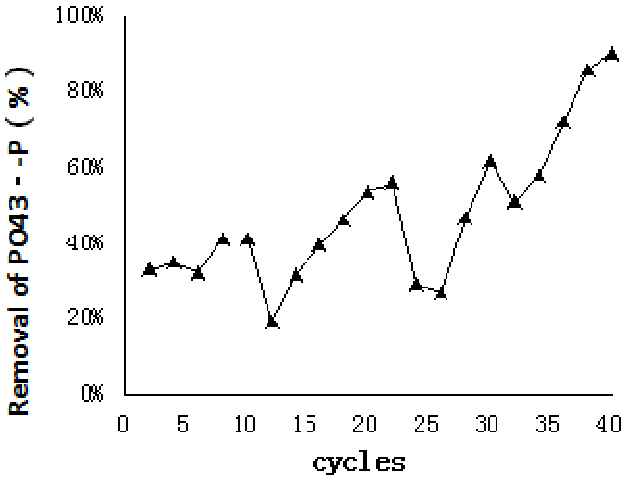

Fig.2. The removal of PO_4^{3-}-P in the second stage

The determination of DPB

The content of DPB in sludge were tested in the study. DPB rate in the whole PAOs was calculated according to the way that calculate the ratio of anoxic absorption rate of phosphorus and aerobic absorption rate of phosphorus, pointed by Wachtmeister[5]. Sludge fully released phosphorus was taken 1000 ml, tested out that its MLSS was 3000mg/L. Divide it in two halves equally, one absorbed phosphorus in the aerobic mode(DO 2 mg/L), another in the anaerobic mode(DO 0.11mg/L, adding 50mg/L NO_3^-N one time), then we can get the DPB content of 94.9%.

3. Stable running denitrifying denitrification and phosphorus removal performance of the system

DPB enriched effectively after the cultivation and domestication, and the system operated steadily. The system operated in anaerobic stage for 3 h and in anoxic stage for 4 h. Monitor the change of PO_4^{3-}-P、COD and NO_3^-N concentration, the result were shown in figure 3.

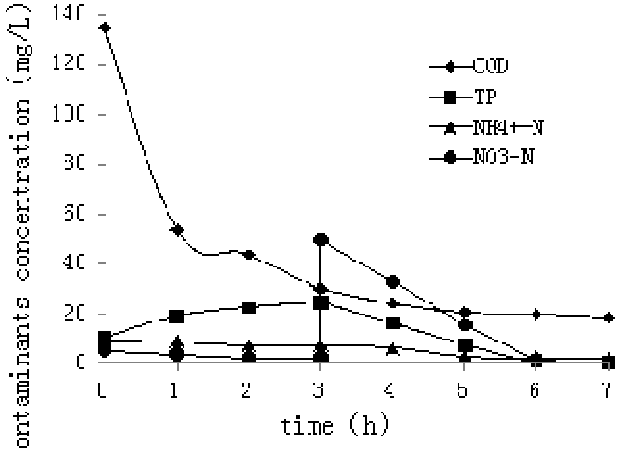

Fig.3. The removal of contaminants by a stable operation system

In anaerobic stage, COD concentration shows a rapidly declining trend, with the increase of PO_4^{3-}-P concentration, which shows a obvious phenomenon of release phosphorus. In the end of the anaerobic stage, the concentration was increased to 24.56mg/L. However, in the anoxic stage, the downward trend of COD concentration slowed down, while the PO_4^{3-}-P concentration dropped rapidly, which shows the phenomenon of Denitrifying phosphorus absorbing. After the anoxic mode(4 h), the PO_4^{3-}-P concentration and the NO_3^-N concentration were decreased to 0.38mg/L and

0.022mg/L, respectively. In the system it can reach as high as 96% phosphorus removal efficiency and 99% nitrogen removal efficiency. From the character of Denitrifying phosphorus removal, there are mount of Denitrifying phosphorus removing bacteria in the reactive system, which can use NO_3^-N as electron acceptor, to absorb phosphorus.DPB has become the advantage bacterials in SBR system, owing to the cultivation and domestication.

Conclusion

1.After the cultivation and domestication in the two stages, DPB were enriched rapidly, what content can reach 94.9%.

2. After the treatment of wastewater what has a low carbon source using steadily operating DPB, the removal rate of PO_4^{3-}-P , NH_4^+-N and COD was 94% ,78% and 86%respectively when the effluent concentration was below 0.4mg/L, 3mg/L and 20mg/L respectively, which indicated that enrichment and cultivation of denitrifying phosphorus removing bacteria in a set of SBR was feasible.

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