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# High Effective Aerobic Denitrification Strain and its Performance in Treating Nitrogen Wastewater

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## ABSTRACT

Through the screening, DNA extraction and polymerase chain reaction (PCR) amplification methods, an aerobic denitrification strain was obtained. The strain was preliminarily identified to be *Paracoccus*. Its optimum growth conditions were pH 7.5, 30°C, and 160 r/min. The strain's degradation ratio decreased with the increase of the nitrate concentration. The nitrogen wastewater treatment experiment (conventional activated sludge flask experiment) showed that the total nitrogen (TN) removal efficiency was 80% in aerobic denitrification strain system and 50% in the activated sludge treatment system, which indicated that the aerobic denitrification played a main role in removing the TN.

## INTRODUCTION

It is well known that nitrogen is one of the main factors causing eutrophication of water body (Djambazov et al. 2015). In recent years, wastewater drainage and rural non-point source pollution caused the deterioration of environment, which enlarged nitrogen wastewater discharge. The wastewater biological denitrification process is based on anaerobic-aerobic processes such as the anaerobic-aerobic (A/O), anaerobic-anoxic-aerobic (A2/O) and sequence bioreactor reactor (SBR) process. But these processes had some common problems: (1) infrastructure investment; (2) high operating cost; (3) lager oxygen supply (Zhu et al. 2008). Aerobic denitrification strains such as *Paracoccus pantotropha*, *Pseudomonas* sp., etc. were investigated for their effective nitrogen removal (Zhao et al. 2010, Hung et al. 2005). It seemed a good way to solve the nitrogen removal.

In order to get more knowledge of performance of aerobic denitrification strains, especially their application in nitrogen wastewater, one aerobic denitrification strain was screened from activated sludge. At the same time, the growth factors and the performance in nitrogen biodegradation of the strain were studied. Then the performance of the strain was studied in treating nitrogen wastewater by comparison of the same amount of activated sludge.

## MATERIALS AND METHODS

**Microorganisms origin:** The sample came from the activated sludge in aeration tank of one coking wastewater treatment plant in Shaanxi, China.

**Culture medium:** Culture medium was referred to Zhou (2006).

a. Enriched culture medium (EM): Beef extract 1.0 g/L, $KNO_3$ 1.0 g/L and peptone 5.0 g/L.

b. Selective culture medium (SM): Glucose 2.7 g/L, sodium acetate 0.5 g/L, $NaNO_2$ 1.0 g/L, $KNO_3$ 2.0 g/L, pH 7.0-7.5.

**Strain separation:** Every 10 mL origin sample was inoculated in 240 mL EM culture medium in the shake flask, and cultured on the rotary shaker with intermittent aeration at 30°C and 160 r/min for 3 days. From the growth measurement, ten of the good growth strains were selected according to their absorbance. And then these strain samples were inoculated in SM culture medium with continuous aeration at 30°C and 160 r/min for 1 day. Ultraviolet spectrophotometric method was used to determine the content of TN before and after vaccination. Then the strain of the best nitrogen removal efficiency was chosen.

**Strains identification**: Strain morphological, physiological and biochemical characteristics were determined according to Zhou (2015). DNA of the strain was extracted and amplified by polymerase chain reaction (PCR) (Xia et al. 2005), and the PCR product was sequenced by a biotechnology company.

**Preservation:** The strains were preserved on the SE

medium at 4°C.

**Growth increment:** The growth increment of the strain was represented by the OD value at 500 nm wavelength.

**Instruments:** Table high-speed freezing centrifuge, PCR instrument, electrophoresis instrument, ultraviolet-visible spectrometer, constant temperature oscillated instrument, biochemical incubator, scan electronic microscope (SEM).

**Analytical items and methods:** $COD_{Cr}$, TN and $NH_4^+$-N, were analysed according to Chinese NEPA standard methods (N.E.P.A. Chinese 1997).

**Wastewater treatment experiment:** Beaker experiment was operated in the laboratory. That is, a beaker (effective volume is 1 L) was used to simulate the aeration tank, 2 g/L of strain was put into the beaker, and then the wastewater (Table 1) was added. The equal amount of activated sludge (from the wastewater treatment plant) was also put into another beaker with the same wastewater for comparison. The two reaction beakers were run in the form of sequencing batch reactor for 50 days. The samples were measured once every day.

## RESULTS AND DISCUSSION

### Strains Screening and Identification

An aerobic denitrification strain (TN removal efficiency was best) was screened by the above procedure. It has ball shaped cell and Gram negative. Fig. 1 shows the SEM photo.

The strain was basically identified to be *Paracoccus.* (Compared with the NCBI, in Table 2)

### Optimum Growth Conditions

**The influence of pH**: Different pH values (from 4 to 10) in the DM (strain was inoculated) were given to determine the best growth conditions. At the condition of 30°C and 160 r/min for 3 days, the $OD_{500}$ was measured. And it could be seen in Fig. 2 that the strain grew well at pH range of 7-8. And there was a drop from 8 to 9. Alkaline condition was not suitable for bacterial growth. For the safety of the strain, the pH value was 7.0-8.0.

**The influence of temperature:** In fact, temperature was very important to the growth of the strain. Usually higher temperature will lead to higher growth. At the condition of pH 7.0 and 160 r/min for 1 day, the strain was inoculated in DM

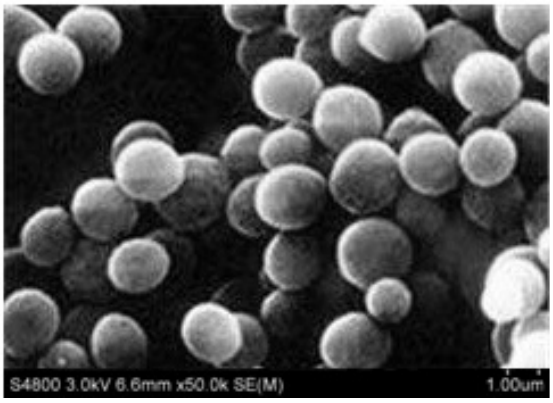



Fig. 1: SEM photo of the strain.

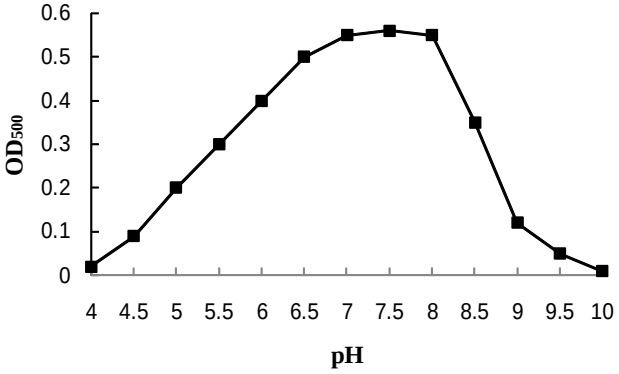



Fig. 2: The influence of pH value.

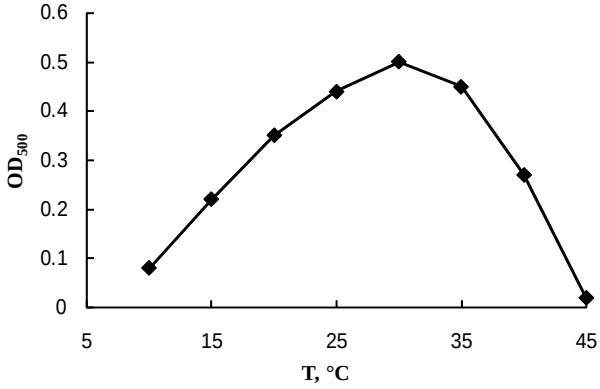



Fig. 3: The influence of temperature.

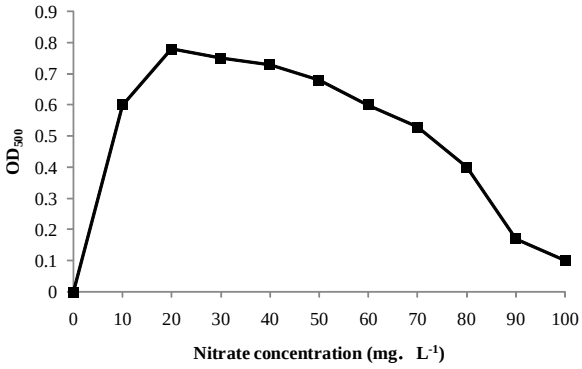
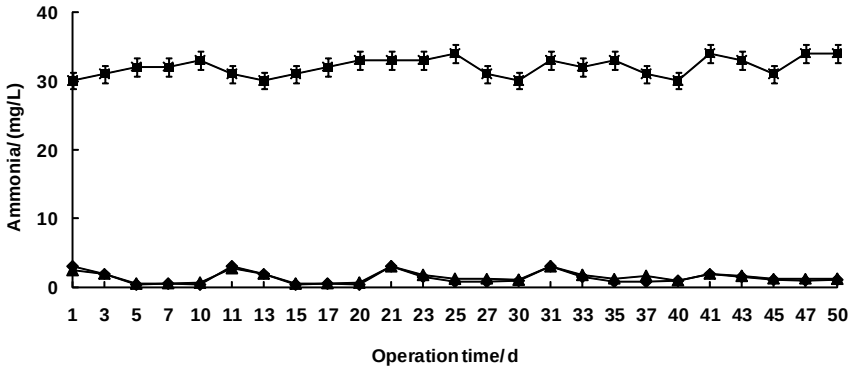
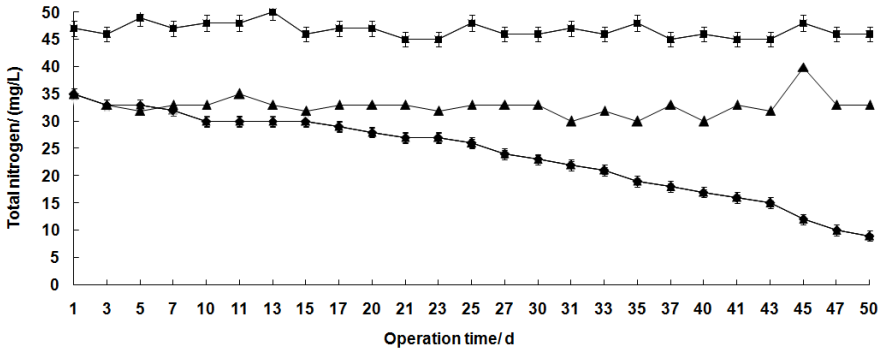



Fig. 4: The influence of nitrate concentration.




■Influent ◆aerobic denitrification stain treatment system ▲activated sludge treatment system

Fig. 5: Variations of ammonia.




■Influent ◆aerobic denitrification stain treatment system ▲activated sludge treatment system

Fig. 6: Variations of total nitrogen.

in a temperature range (from 10°C to 40°C). From Fig. 3, the strain's $OD_{500}$ was good at 25-35°C, which meant that the strain grew well at this temperature range. The best temperature could be 30°C.

**The influence of nitrate concentration:** The strain was inoculated at the same amount in the culture medium with different nitrate concentration for 24 hours' oscillating culture at 30°C; the rotation speed was 160 r/min. The results shown in Fig. 4 indicated that in the starting stage, the strain grew well with the increase of nitrate concentration. The $OD_{500}$ of strain was the highest at 20 mg/L of ammonia concentration. Then $OD_{500}$ of strain decreased because of the nitrate endurance to the strain. At 100 mg/L ammonia concentration, the $OD_{500}$ of strain was lower than ever. So we could get the conclusion that the strain had a good tolerance or decomposition to nitrate.

## Performance in Treating Nitrogen Wastewater

In order to get more knowledge of the strain and its application in nitrogen wastewater, its performance in treating nitrogen wastewater was studied (Table 1). As a comparison, the same amount of activated sludge from MWTP was used to treat the nitrogen wastewater.

**Ammonia removal**: As shown in Fig. 5, the ammonia removals were both high in treating wastewater (removal ratio were both over 95%), which indicated that the aerobic denitrification strain and the activated sludge could remove the ammonia. The main reason is that there are a lot of ammonia-oxidizing bacteria (AOB) and the nitrite-oxidizing bacteria (NOB) in the activated sludge, which could change the ammonia to nitrate nitrogen (de Silva et al. 1998). As a result, the aerobic denitrification strain and the activated sludge could get the same removal ratio of ammonia.

**TN removal**: No doubt, the aerobic denitrification strain benefits the total nitrogen removal. For the activated sludge, the total nitrogen removal is only 50%, and it is not the similar experience in the ammonia removal, indicating that the activated sludge does not have good

Table 1: Nitrogen wastewater characteristics.

| Item | Total Nitrogen (mg/L) | Ammonia (mg/L) | Nitrate (mg/L) | Nitrite (mg/L) | $COD_{Cr}$ (mg/L) | pH |
|---|---|---|---|---|---|---|
| Influent | 45-50 | 20-45 | 2-20 | 0-3 | 270 | 6.9-7.2 |

Table 2: Sequences length and closest phylogenetic affiliation of the strain.

| Strains | Sequences length | Phylogenetic relationship | |
|---|---|---|---|
| | | Species | Similarity |
| 1 | 190 | *Paracoccus* sp. | 193/195(98.9%) |

ability to remove nitrogen.

From Fig. 6 we can see that in the strain treatment system 60%, 74.5%, 79%, 80% and 80% TN removal was achieved with the time. The results indicated that the aerobic denitrification bacteria played an important role in denitrification, and the TN removal had increased with the strain's adoption to the wastewater.

## CONCLUSIONS

1. A kind of high denitrification ability aerobic denitrification strain was screened, which was preliminarily identified to be *Paracoccus.* The optimum growth conditions were pH 7.0-7.5, 30°C and 160 r/min.

2. When the ammonia concentration was above 600 mg/L, the strain's degradation ratio decreased with the increase of the ammonia concentration. The strain had a good tolerance to ammonia.

3. The total nitrogen removal efficiency was high to 80% in aerobic denitrification strain treatment to the nitrogen wastewater. So it was a highly effective nitrogen degradation strain and had better application prospects in nitrogen wastewater treatment.

## ACKNOWLEDGMENTS

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