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Screening of Polyphosphate Accumulating Organisms and Their Phosphorus Removal Performance

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Abstract [**Objectives**] To study the phosphorus removal performance of phosphate accumulating organisms (PAOs). [**Methods**] Activated sludge from domestic sewage treatment plant was used as the strain source, and phosphate accumulating organisms were screened by plate streaking method and dilution coating plate method. Six kinds of excellent phosphate accumulating organisms were obtained by metachromatic granule staining experiment, total phosphorus experiment and simulated sewage phosphorus removal experiment to assist the observation of bacterial morphology and experiment of phosphorus removal capacity. In addition, the influencing factors of phosphorus removal capacity (nitrogen source, trace metal ions) were analyzed. [**Results**] In the case of simulated sewage, the phosphorus removal rate of strain b was the highest, reaching 66.25%, while the phosphorus removal rate of strain e and f was about 10% lower than that of the phosphorus uptake experiment. [**Conclusions**] This study is expected to provide a theoretical reference for the gradual optimization of the screening method of phosphorus removal bacteria in domestic sewage treatment.

Key words Phosphate accumulating organisms (PAOs), Separation and screening, Biological phosphorus removal

1 Introduction

With the continuous improvement of human living standards and the rapid development of industrial production, the amount of sewage discharged is gradually increasing, which leads to serious eutrophication of water body and the growth of a large number of algae. Under anaerobic conditions, the harmful gases produced by the decomposition of organic matter and the biotoxins produced by some plankton can harm fish, and if people and animals drink them for a long time, they will be poisoned and sick. One of the most important factors causing eutrophication is that the concentration of phosphorus in water exceeds the standard. Therefore, the solution of water eutrophication has attracted much attention. Compared with traditional physical and chemical phosphorus removal methods, biological phosphorus removal technology is widely used in many sewage treatment plants because of its low energy consumption, good environmental protection, high removal efficiency and good sustainability.

The phosphate accumulating bacteria, also known as phosphate accumulating organisms (PAOs) are a kind of microorganism that can absorb phosphate excessively and plays a key role in the process of biological phosphorus removal. Phosphate accumulating organisms can use inorganic phosphorus compounds (such as phosphate) in wastewater to synthesize polyphosphate and form granular polyphosphate. These polyphosphates can accumulate and store a large amount of phosphorus in cells, thus removing phosphorus from wastewater. Yuan Ye *et al.* [1] found that under suitable conditions, the removal rate of phosphate accumulating organisms for total phosphorus reached 84.50%.

In this study, we separated and screened excellent strains from the activated sludge of sewage treatment plant, and determined the total phosphorus content of phosphate accumulating organisms and studied its phosphorus removal effect in simulated domestic sewage. Then, we discussed the morphology and phosphorus removal ability of different phosphate accumulating organisms, in order to provide a theoretical reference for the gradual optimization of the screening method of phosphate accumulating organisms in domestic sewage treatment.

2 Materials and methods

2.1 Experimental materials The activated sludge used in the experiment came from the aerobic biochemical pool of the second sewage treatment plant in Duanzhou District of Zhaoqing City. The samples were packed in wide-mouthed conical flasks and put into a biochemical incubator at 30 ℃ for static culture. The supernatant was sucked and discarded after a period of time. Finally, the bottle with less water content in the wide-mouthed conical flask was selected for the separation and purification of bacteria. Nutrient broth solid medium (LB): 5.4 g nutrient broth medium, 5.4 g agar powder, add deionized water to 300 mL, sealed with sealing film, sterilized at 121 ℃ for 20 min, cooled to 55 - 60 ℃ [2], poured plate.

Phosphorus-deficient medium: anhydrous sodium acetate 2.001 3 g, potassium sulfate 17.83 mg, magnesium sulfate 81.12 mg, disodium hydrogen phosphate dodecahydrate 46.3 mg, ammonium chloride 152.8 mg, calcium chloride 8.3 mg, trace elements

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2 mL, pH 7.2, constant volume to 1 000 mL[3], sterilized at 121 ℃ for 20 min.

Phosphorus-rich medium: anhydrous sodium acetate 2.001 3 g, calcium chloride 25.68 mg, potassium dihydrogen phosphate 25 mg, ammonium chloride 305.52 mg, magnesium sulfate 91.26 mg, trace elements 2 mL, pH 7.2, constant volume to 1 000 mL[4], sterilized at 121 ℃ for 20 min.

2.2 Screening and purification of phosphate accumulating organisms The samples were diluted to the concentrations of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}, and 0.2 mL of different concentrations of the diluent was spread on the surface of the plate, and then cultured in a biochemical incubator at 30 ℃ for 3 d. The strains in the plates with the concentrations of 10^{-3}, 10^{-4} and 10^{-5} were purified by plate streaking method to obtain single colonies with the same colony characteristics, which were put into a refrigerator at 4 ℃ for later use.

Under aseptic conditions, 6 strains of bacteria with the ability to remove phosphorus were selected after purification, and 3.85% (*V*/*V*) bacterial solution of each strain was prepared, which was inoculated into 150 mL wide-mouth conical flasks containing 50 mL of phosphorus-deficient medium. Three groups of parallel experiments were set up for each strain, which was cultured in a constant temperature shaker at 30 ℃ and 140 r/min for 24 h, and then centrifuged at 10 000 r/min for 2 min[5]. The gun head sucked the supernatant and discarded it, and the strain was washed with sterile deionized water and centrifuged again. All the bacteria were transferred to a 150 mL wide-necked conical flask containing 50 mL phosphorus-rich medium and cultured at 30 ℃ and 140 r/min for 3 d.

2.3 Metachromatic granule staining experiment Metachromatic granule staining reagent[4]: solution A: dissolved 0.15 g of toluidine blue and 0.20 g of malachite green in 2 mL of ethanol in a 100 mL brown volumetric flask, added 1 mL of ethanol, mixed well, fixed the volume with deionized water, stood for 24 h, and filtered for later use. Solution B: 50 mL brown volumetric flask, iodine 333.3 mg, potassium iodide 0.5 g, deionized water to constant volume for use.

Metachromatic granule staining and slide making: dropped normal saline on a clean glass slide, evenly mixed the bacterial moss with the normal saline to form a thin film, dried the water with an alcohol lamp, and fixed the shape. Dyed with solution A for 4 min, washed with water, and absorbed excess water; dyed with solution B for 1 min, washed with water and dried[6], and observed the results under the microscope[7].

2.4 Effect of phosphate accumulating organisms on phosphorus removal Preparation of simulated sewage: potassium nitrate 60 mg, anhydrous sodium acetate 2.001 3 g, potassium dihydrogen phosphate 25 mg, ammonium chloride 305.52 mg, magnesium sulfate 91.26 mg, calcium chloride 25.68 mg, trace elements 2 mL, deionized water to 1 000 mL volumetric flask[4], pH 7.2, sterilized at 121 ℃ for 20 min.

Three groups of parallel experiments were set up for the six strains, and the corresponding 3.85% (*V*/*V*) bacterial solution was prepared, inoculated into 150 mL wide-mouth conical flasks containing 50 mL phosphorus-deficient medium, cultured in a constant temperature shaking table at 30 ℃ and 140 r/min for 42 h, and washed twice with sterile deionized water. The corresponding bacterial sludge was put into a wide-mouth conical flask filled with simulated sewage, and cultivated in a constant temperature shaker at 30 ℃ and 140 r/min for 3 d. Ammonium molybdate spectrophotometric method (GB 11893-89) was used to determine the total phosphorus content of the simulated sewage after 3 d of cultivation with the total phosphorus content of the blank simulated sewage as the reference[8].

2.5 Determination of total phosphorus (TP) The content of total phosphorus was determined by ammonium molybdate spectrophotometry (GB 11893-89). Weighed 5 mL of uniform blank phosphorus-enriched culture medium and put it into a corresponding colorimetric tube with a stopper. Took 10 mL of different bacteria liquid of phosphorus-enriched culture after shaking, centrifuged for 2 min at 100 00 r/min, and then took 5 mL of the supernatant into the corresponding colorimetric tube with a stopper. Calibrated each colorimetric tube to 25 mL of scale line, added 4 mL of potassium persulfate solution, and digested at 120 ℃ for 30 min. After cooling, fixed the volume to 50 mL with deionized water, added 1 mL of ascorbic acid solution separately, after 30 s, removed 2 mL of molybdate solution, mixed well after each addition, and placed the colorimetric tube at room temperature for 15 min in the dark. The visible spectrophotometer was used to adjust the wavelength to 700 nm, and the absorbance was measured to determine the concentration of total phosphorus.

3 Results and analysis

3.1 Morphological observation of phosphate accumulating organisms The colonies were selected from LB medium, and six plates with the same colony characteristics were obtained by separation and purification, which were numbered a, b, c, d, e and f, respectively. Metachromatic granules are polymers of inorganic metaphosphoric acid, usually formed in phosphorus-rich environments, and have the function of storing phosphorus and energy and reducing the osmotic pressure of cells. Pan Chao *et al.*[9] found that E43, F2 and J1strains had the ability to accumulate phosphorus through metachromatic particle experiment. Zhang Hua *et al.*[8] found that the four strains all contained blue-black granules and were excellent phosphate accumulating organisms. Liu Yuchen *et al.*[10] examined the strains XC-1, XC-2 and XC-3 by metachromatic granule staining experiment, and found that the three strains all had strong phosphorus uptake capacity. Therefore, the metachromatic granule staining experiment can be used to verify whether the strain is phosphate accumulating organism. If the microorganism shows dark blue-black particles and green cells in the staining results, metachromatic particles are present[11]. The results of metachromatic granule staining experiment are shown in Fig. 1. The results showed that all the six strains con-

tained metachromatic granules and had the function of storing phosphorus.

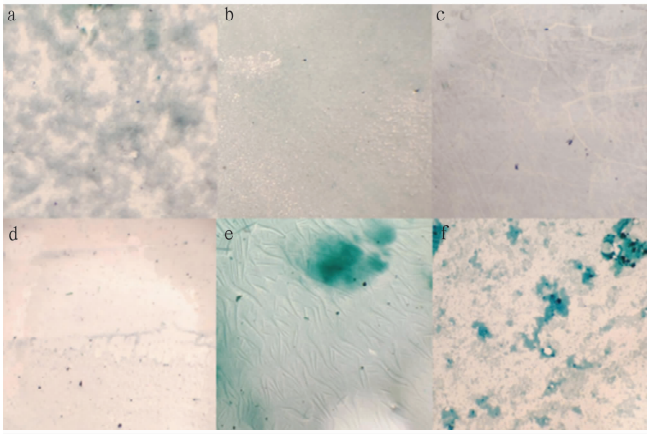

Fig. 1 Metachromatic granule staining experiment results of strains a, b, c, d, e, f

3.2 Phosphorus removal capacity of phosphate accumulating organisms It can be seen from Table 1 that the phosphorus removal rates of strains a, b, c, d, e and f are 20.83%, 19.93%, 3.05%, 8.91%, 67.11% and 34.05%, respectively. Strain e had the highest phosphorus removal capacity, while strains c and d had the lowest phosphorus removal capacity. As shown in Table 1, the phosphorus removal rates of the six strains in the total phosphorus experiment and the simulated sewage. The phosphorus removal rates of a, b, c and d were higher than that of TP, while the phosphorus removal rates of e and f were lower than that of TP.

Table 1 Removal rate of nitrogen and phosphorus by strain %

Strain number	Phosphorus removal rate of TP experiment	Phosphorus removal rate of simulated sewage
a	20.83	40.34
b	19.93	66.25
c	3.05	30.62
d	8.91	21.97
e	67.11	54.87
f	34.05	25.57

The results showed that there were some differences in the phosphorus removal rate between the total phosphorus experiment and the simulated sewage experiment. We compared the ratio and operation of the two experiments, and the difference was in the ratio of potassium nitrate.

4 Conclusions

In this study, we selected six excellent phosphate accumulating organisms (a, b, c, d, e, f) from activated sludge of domestic sewage treatment plant. The six strains of phosphate accumulating organisms had the same characteristics, contained metachromatic granules and PHB granules, and had the functions of phosphate storage and oxidative metabolism of polyphosphate. The phosphorus removal rates of the six phosphate accumulating organisms were 20.83%, 19.93%, 3.05%, 8.91%, 67.11% and 34.05%, respectively. The phosphorus removal rates of the six strains of phosphate accumulating organisms in the simulated wastewater were 40.34%, 66.25%, 30.62%, 21.97%, 54.87% and 25.57%, respectively. The phosphorus removal rates of e and f phosphate accumulating organisms were lower than that of phosphorus uptake experiment, and the phosphorus removal rates of a, b, c and d were higher than that of phosphorus uptake experiment. This study is expected to provide a theoretical reference for the gradual optimization of the screening method of phosphorus removal bacteria in domestic sewage treatment.

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