Metabolism of polyphosphate-accumulating organisms(PAOs) in biological phosphorus removal process under P-limiting and low temperature conditions

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Abstract: Lower temperature or polyphosphate limitation is the favorable condition to enrich polyphosphate-accumulating organisms (PAOs) or glycogen accumulating organisms (GAOs) in biological phosphorus removal process. In this study, a sludge highly enriched in PAOs was used to investigate the metabolic pathways and carbon transformation in batch tests under the two conditions for elucidating the contradiction. The results showed that the PAOs are able to take up acetate and store it as polyhydroxyalkanoates (PHA) in anaerobic phase although the poly-P pool is emptied through washing with negligible P release. The stoichiometric ratios of the PHB and PHV production and glycogen degradation to the acetate uptake combined with the Nile blue staining indicated that the PAOs possess the anaerobic metabolism of GAOs using glycogen as the sole energy source, but no transformation of PAOs to GAOs is observed. After reconverting to the normal operation, phosphorus uptake and release are recovered the same as the parent reactor.

Key words: PAOs; GAOs; P-limiting; low temperature

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Phosphorus (P) is a key nutrient that stimulates the growth of algae and other photosynthetic microorganisms, and must be removed from wastewater to avoid eutrophication in aquatic systems [1]. Biological phosphorus removal promotes the removal of P from wastewater, and can be relatively easy and economical implemented by recirculating polyphosphate-accumulating organisms (PAOs) through anaerobic and aerobic conditions. In the anaerobic stage, PAOs store the VFA present in the influent as poly-β-hydroxyalkanoates (PHA) from the energy generated by polyphosphate hydrolysis, while the reducing power is from the glycogen hydrolysis [2]. However, glycogen is also the sole or primary source of energy for anaerobic VFA uptake by glycogen accumulating organisms (GAOs) which do not perform phosphorus release and uptake but compete with PAO_S for substrates [3-4].

Diverse studies have been undertaken aiming at getting a better understanding about the influence of different environmental and operating conditions on the PAOs-GAOs competition. The effects of temperature, types of carbon sources, pH and influent COD/P ratio have been observed to play an important role on the competition between PAOs and GAOs [5-7]. Most of the studies with the lower temperature is favor to PAOs and P-limiting provide advantage to GAOs [5,8-9]. However, the metabolism employed by PAOs in the conditions of P-limiting and low temperature has not been addressed since the contradiction. In the present study, a lab-scale SBR with highly enriched PAOs was used to investigate the metabolic pathways and carbon transformation under P-limiting at low temperature conditions, also the recovery of P release and uptake was took into account.

1 Materials and Methods

1.1 SBR Setup and Batch Test

A PAOs culture was enriched in a lab-scale sequencing batch reactor (SBR). This SBR, hereafter referred to parent reactor, had a working volume of 4.0 L. The SBR was operated at 20 ℃ in cycles of 6 h (90 min anaerobic, 210 min aerobic, 45 min settling phase and 10 min decanting). At the beginning of each cycle, 2.0 L of synthetic medium was fed to the parent reactor over a period of 5 min, 400 mL of mixed liquor were removed on a daily basis (100 mL per cycle) from SBR, resulting in a solids retention time (SRT) of approximately 10 d. At the end of the settling period 1.9 L of supernatant was pumped out from the reactors, resulting in a hydraulic retention time (HRT) of 12 h. The reactors were constantly mixed at 350 r/min except during settling and decant phases. The inoculation sludge came from the A^2/O reactor in the lab which

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was in stable status more than 1 a. A batch reactor with an effective volume of 1.0 L was employed for the batch test. The reactor was controlled at 10 ℃ in the refrigerator ahead of 1 h before the batch test. At the beginning of the test, 400 mL of enriched PAOs sludge was manually transferred from the parent SBR. The test consisted of five anaerobic-aerobic cycles with various purposes. In cycles 1 and 2, surplus acetate was supplied in the anaerobic phase to simulate the release of PO_4^{3-}-P (500 mg·L^{-1} and 300 mg·L^{-1}). After the extended phase of anaerobic P release, the sludge was washed to deplete the poly-P content in the bacterial cells. In cycles 3 and 4, the anaerobic and aerobic metabolism of PAOs in the absence of poly-P pool was studied with negligible phosphate release. Cycles 4 and 5 were aimed to investigate the recovery of phosphorus release and uptake by PAOs after the reintroduction of phosphorus to the sludge by adding 100 and 50 mg/L in 20 ℃. Each cycle consisted of a 2 h anaerobic phase, a manual settling, decanting and washing phase, and a 3 h aerobic phase. At the end of each anaerobic period, the biomass was allowed to settle and the supernatant was decanted. The biomass was then washed twice with the effluent from the parent SBR to remove the phosphate released during the anaerobic period.

1.2 Raw Water and Analysis Method

The synthetic media was used as the raw water in the SBR and batch test. For the parent SBR, the synthetic media contained sodium acetate as the sole carbon source (0.815 g·L^{-1}, COD = 400 mg·L^{-1} = 12.5 mmol-C·L^{-1}), and the NH_4Cl and KH_2PO_4 was used as nitrogen and phosphorus source with the concentration of 0.057 g·L^{-1} and 0.0658 g·L^{-1} (NH_4^+-N = 15 mg·L^{-1}, PO_4^{3-}-P = 15 mg·L^{-1} = 0.48 mmol-P·L^{-1}. The trace metals present in the media were prepared as described by Smolders [10]. As for the batch test, the media was the same as that in parent SBR except for the phosphorus concentration.

The conventional water quality index such as COD, NH_4^+-N, NO_3^{-1}-N, PO_4^{3-}-P and MLSS, MLVSS was measured according to the references [11]. Glycogen was determined with the method of Zeng [12]. PHA analysis and staining was performed using the method of Chen [13] and Oehmen [14] respectively.

2 Results and Discussion

2.1 Performance of SBR

After the inoculation of the seed sludge, the parent SBR was continuously operated for more than two months before the batch test. The biomass activity reached steady state and the SBR working in a steady state with excellent EBPR performance during the test. Fig. 1 shows a representative cycle of the SBR. It can be seen that with the sole carbon source of sodium acetate, the enriched PAOs phenotype is clearly displayed. During the anaerobic period, acetate is fully taken up accompanied by P release, PHA storage and glycogen consumption. Under the subsequent aerobic phase, P uptake, PHA utilization, and glycogen replenishment are observed. At the end of the aerobic phase, the MLSS and MLVSS are 3011 and 2117 mg·L^{-1} respectively, resulting in an average MLVSS/MLSS ratio of 0.70. The low ration indicates high inorganic matter storage, such as poly-P, which suggests that PAOs are the dominant microorganisms. The stoichiometry of the anaerobic P-release/C-uptake is 0.41 mol-P/mol-C, which shows a typical pattern of enriched PAOs system according to the study of Smolder [10].

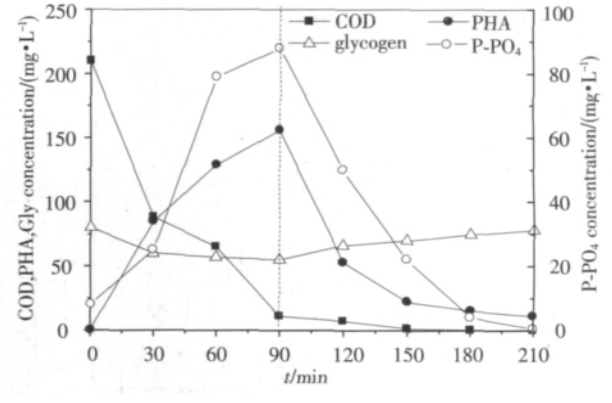


Fig. 1 Carbon, phosphorus, PHA and glycogen transformation during a representative cycle in the parent SBR

2.2 Performance of Batch Test

One of the typical cycle of the substrates and phosphate profiles in the batch tests are shown in Fig. 2. In the first anaerobic period, 333 mg·L^{-1} of COD is taken up with a simultaneous release of PO_4^{3-}-P of 152 mg·L^{-1}. The release of P to uptake of acetate uptake ratio is 0.47 mol-P/mol-C, which is comparable to that of the parent SBR. In the subsequent aerobic period, the poly-P pool can not be regenerated as the washing period. In the second cycle, uptake of COD is 205 mg·L^{-1}, which is accompanied by a phosphate release of only 13 mg·L^{-1}. This gives rise to only a P release to acetate uptake ratio of 0.065 mol-P/mol-C, which is considerably lower than that observed in cycle 1 and the parent reactor. The low P release indicates that the poly-P pool in PAOs cells is suppressed obviously. In Cycles 3 and 4, it is further confirmed that the release of P is less than 3 mg·L^{-1}. However, during both cycle 3 and cycle 4, the 100 mg·L^{-1} of COD added is completely taken up, indicating that alternative energy sources are used for the acetate uptake.

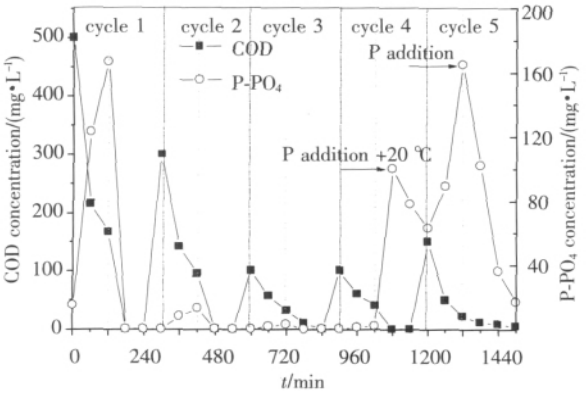


Fig. 2 Representative profile of carbon and phosphorus transformation in batch test

The profiles of acetate, glycogen, PHB and PHV measured during cycle 4 are shown in Fig. 3.

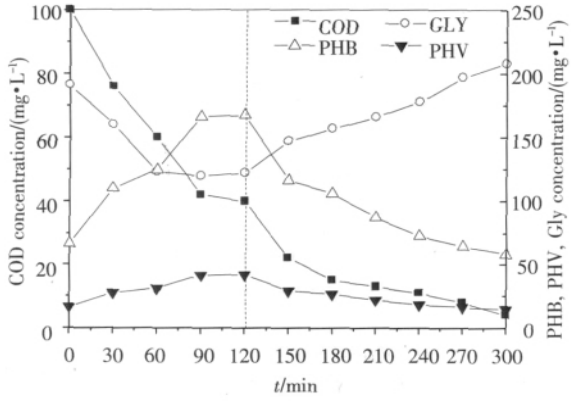
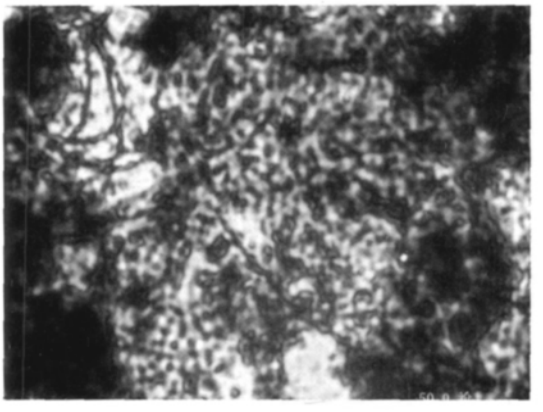
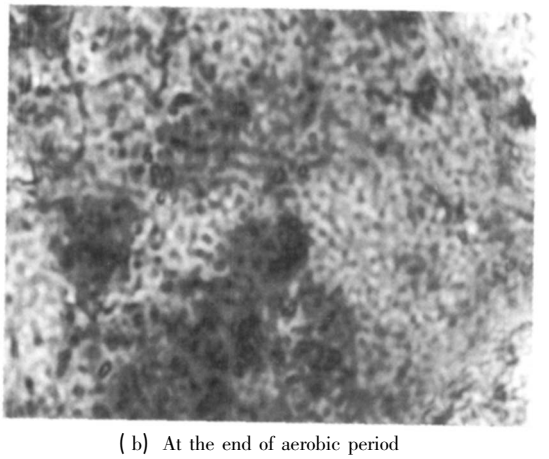


Fig. 3 Representative profile of carbon, PHB, PHV and glycogen transformation in cycle 4

It shows that the anaerobic acetate uptake is accompanied by negligible P release and the consumption of glycogen. Also the production of PHV and degradation of glycogen is much higher than those previously reported in the literatures with enriched PAOs cultures. These characteristics suggest that under the low temperature and P-limiting conditions the biomass behaves more like GAOs than PAOs. However, the acetate uptake rates of 0.068 mol-C/mole-C biomass · h^{-1} suggests that PAOs must have strongly contributed to the uptake of acetate. Assuming that the acetate uptake is accomplished by 20% GAOs, then a specific acetate uptake rate of 0.31 mol-C/mole-C biomass · h^{-1} is attained which is almost twice of the maximum acetate uptake rate by GAOs reported to date [12]. Fig. 4 shows the staining images with Nile blue, in which black represents PHA and amethyst represents cytoplasm. The samples are taken in cycle 3 at the end of anaerobic and aerobic phases, respectively. There is a clear difference between the PHA staining from biomass at different phase. At the end of the aerobic period the cells contained much less PHA which is consistent with the results of Fig. 3. The results presented in Fig. 4 combined with that in Fig. 3 directly show that the PAOs participate in the anaerobic and aerobic carbon transformation but display GAOs phenotype in the absence of intracellular poly-P under low temperature, also the small amount of PHV production may have been due to the use of TCA cycle or the modified succinate—propionate pathway.



(a) At the end of anaerobic period



(b) At the end of aerobic period

Fig. 4 Image of Nile-blue-stained PHA

2.3 Recovery of P Uptake and P Release and the Metabolic Pathways of PAOs

In the aerobic period of cycle 4100 mg · L^{-1} PO_4^{3-}-P are added to test the recovery of P uptake after the P-limiting disturbance. It can be seen that the uptake of phosphate occurs immediately after the phosphate addition. There is 37 mg · L^{-1} PO_4^{3-}-P uptake by the biomass but the uptake ceased at the end of this cycle. One possible reason can be that on the conditions of P-limiting and low temperature, the enriched PAOs acclimatize to use glycogen as the primary energy source which gives a superiority of glycogen production to poly-P tool when PHA is getting to lower concentration. This amount of phosphate taken up is released completely to the bulk in the following anaerobic period

in cycle 5, where acetate is added. In the aerobic period of cycle 5, the phosphate released anaerobically is completely taken up, and phosphate uptake continues after another 60 mg · L^{-1} PO_4^{3-}-P is added. The results show that phosphorus uptake and release recover rapidly after the disturbance.

3 Conclusions

Under poly-P-limited and low temperature conditions, PAOs behave as GAOs with respect to the carbon transformation and the reaction stoichiometry. The PAOs can take up acetate under anaerobic conditions and store as PHA based on glycolysis of glycogen. After rehabilitating the normal operation condition, the phosphorus uptake and release recover rapidly.

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