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Original Research

Effects of matrix types on formation and transformation of energy-accumulating substances in enhanced biological phosphorus removal (EBPR)

D. Li[1], Z. Fang[2*], X. Long[2], R. Tang[2], S. Di[2]

[1] Department of Oil Application & Management Engineering Logistical Engineering University, Chongqing, China
[2] Department of National Defense Architectural Planning & Environmental Engineering, Logistical Engineering University, Chongqing, China

Abstract: Enhanced biological phosphorus removal (EBPR) has been widely used in wastewater treatment. In this study, a laboratory investigation of activated sludge in A/O-SBR reactor was conducted to probe the effects of the matrix types on EBPR polyphosphate, intracellular polysaccharide, polyhydroxyalkanoates (PHA) formation and transformation. There is a decrease in anaerobic condition and an increase in aerobic condition for the intracellular glycogen of sodium propionate matrix and sodium acetate matrix. While the intracellular glycogen of glucose matrix shows a decreasing tendency in both anaerobic and aerobic reaction process. Sodium acetate matrix is beneficial to the formation of polyhydroxybutyrate (PHB), but the content of PHB is relatively small. PHB and poly-3-hydroxyvalerate (PHV) contents in PHA are quite similar in both anaerobic and aerobic reactions with a PHB/PHV ratio of 0.83-1.45. The synthesis of PHV and PHB is mainly in the initial anaerobic stage (0 h - 1 h). Glucose matrix is helpful to the formation of PHV. The content of polymphosphorus shows an increasing tendency in both anaerobic and aerobic stages, suggesting that glucose matrix acclimation of the reactor favors the formation of polymphosphorus.

Key words: Enhanced biological phosphorus removal, polyphosphate, intracellular polysaccharide, PHA.

Introduction

In recent years, eutrophication of water body has attracted more and more people's attention around the world, and the removal of nutrients in wastewater has become a hot topic of researchers (1-12). Among the present techniques, enhanced biological phosphorus removal (EBPR) has been widely applied in the removal of phosphorus in wastewater (13-22). The first process of EBPR need acclimate activated sludge to allow the activated sludge in alternate aerobic and anaerobic conditions through control of the reactor to enrich polyphosphate bacteria, and use biodegradable organic carbon (such as acetate and propionate) in anaerobic conditions (6-11,14,15,18,22). Polyphosphate bacteria can absorb biologically degradated organic carbon under anaerobic condition, and intracellularly store organic carbon in the form of polyhydroxyalkanoates (PHA) (6,8,13). The required capabilities of PHA storage were provided by polyphosphate and/or glycogen decomposition, and glycogen decomposition offered reducing agent simultaneously (7-11). Under aerobic conditions, the phosphorus accumulating organisms grew itself by stored PHA under anaerobic condition, the phosphorus in the liquid phase was absorbed and the intracellular glycogen was supplied. Microorganisms and extracellular polymers became rich in phosphorus when the aerobic condition was ended, and the removal of phosphorus from wastewater can be achieved by the removal of activated sludge (17,19,21,22).

The process of EBPR includes conversion and energy transfer and utilization between various polymers (polyphosphate, PHA and glycogen). Therefore, it is necessary to investigate the use and transfer of bacterial cells and extracellular biopolymeric materials during EBPR process to optimize the phosphorus removal function of system.

Materials and Methods

EBPR activated sludge culture and reactor control

The experiment was conducted with activated sludge in A/O-SBR reactor in laboratory. The effective volume of the reactor is 15 L, and the temperature was controlled at 20±2 ^{o}C. SBR activated sludge inoculation from JIGUANSHI sewage treatment plant in Chongqing city. Two cycle operations of the reactor were run per day, each cycle included anaerobic operation for 4 h, aerobic operation for 7 h, settlement for 50 min and draining and idling for 10 min. Instantaneous water supply was used for artificial water distribution, mud was removed before 2 min of the end of aerobic phase to maintain an SRT of 20 days. The gas flow rate is regulated by a stepless speed regulator, and the DO concentration of the reactor was controlled at 3.0-5.0 mg/L before the end of the aerobic reaction. The pH value of the reactor is weakly basic. The artificial simulated domestic sewerage was used as the inlet water of reactor, the substrates in three reactors were sodium acetate, propionate and glucose, respectively with COD:N:P of 100:5:2.

EPS extraction

Extraction and separation of EPS and bacterial cells were performed using ultrasonic cation exchange resin. A certain volume of sludge mixture was taken for centrifuge at 4050×g for 15 min, the supernatant was remo-

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*** Corresponding author:** Zhendong Fang, Department of National Defence Architectural Planning & Environmental Engineering, Logistic Engineering University, College Town, Shapingba district, Chongqing, China 401311. Email: zhendong_fang@126.com

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ved. The sludge samples from centrifugal sedimentation were suspended and placed in ice water bath for dispersing by ultrasonic method. 001×7 type cation exchange resin intermittent water was extracted, the dispersed sludge samples were mixed with the resin at adding weight of 80 g/gVSS, the mixture in ice water bath was placed in agglutination mixer for ion exchange reaction. After agitation, resin particles were filtered using nylon mesh filter with a diameter of 250 μm to obtain a mixed solution of EPS and sludge particles. After centrifugal separation of the mixed solution for two times, EPS was obtained from the supernatant, and the precipitate was regarded as bacterial cells.

Analysis of carbohydrates

Anthrone method, as a colorimetric determination, can be used for the measurement of polysaccharide concentration in sample. In this study, glucose was used as a standard substance. Polysaccharide and anthrone reacted under acidic conditions to produce a color of blue and green. 1 mL of sample was mixed with 2 mL of anthrone reagent and diluted in sulfuric acid, the mixed solution was placed in water bath at 100 ^{o}C for 14 min until the reaction was complete. After the solution was cooled down to room temperature, the absorbance of sample was measured via a Mettler Toledo UV5Bio spectrophotometer at a wavelength of 625 nm.

PHA determination

Freeze-dried sludge sample (0.015 g) was weighed and placed in a hash digestion tube, followed by adding chloroform (2 mL), acidified methanol (2 mL) which was sulfuric acid containing 100 mg/L sodium benzoate with a volume ratio of 4%. The sample was digested at 105 ^{o}C for 6 h. After cooling, distilled water was added, the sample was violently stirred for homogeneous mixing. After stand of the solution for 1 h, the lower organic phase was absorbed to the chromatographic analysis bottle for the analysis and measurement of sample.

Gas chromatography (GC-2010 Plus High-end GC, Shimadzu) was used for analysis of PHA that contains polyhydroxybutyrate (PHB) and poly-3-hydroxyvalerate (PHV) in this study. The conditions of gas chromatographic instrument were described hereafter. The gas chromatography is equipped with DB-5 chromatographic column (30 m in length, 0.25 mm in internal diameter, 0.25 μm in the thickness of the film), the split ratio is 10:1, nitrogen is used as the carrier gas with a flowing rate of 40 mL/min, hydrogen and air are used as the auxiliary gas with flowing rates of 40 mL/min and 400 mL/min, respectively. The working temperature of the FID detector is 280 ^{o}C, and the inlet temperature is 250 ^{o}C. A increasing temperature program was used, first at 70 ^{o}C for 2 min then increased to 150 ^{o}C at a heating rate of 25 ^{o}C/min and the temperature was hold for 1 min, then increased to 300 ^{o}C at a heating rate of 25 ^{o}C/min for post run of 2 min. An automatic sampling mode was used, 3 μL of sample was injected each time.

In the experiments, poly(3-hydroxybutyrate-*co*-3-hydroxyl valeric acid) purchased from sigma Aldrich (cat No:80181-31-1, a PHV content of 8 %) was used as the standard substance of PHB and PHV.

Extraction and determination of polyphosphate

Freeze-dried sample (around 0.015 g) was weighed for extraction of phosphate contents in different forms using a modified STS method. In STS method, the phosphates in sample were divided into positive phosphate (PO43--P), low molecular weight polyphosphate (LMW poly-P, which is referred to as oligomeric phosphate), phospholipid (Lipid-P), DNA phosphorus (DNA-p), high molecular weight polyphosphate (poly-P HMW, which is referred to as polymeric phosphate), protein phosphorus and residual phosphorus (Protein+Residue-P). After STS extraction to obtain different components, the phosphorus determination was pre-treated by closed Reflux Digestion, followed by anti-molybdenum and anti-antimony spectrophotometric measurement. The various phosphate contents in EPS were represented by the difference of various contents between phosphates in sludge and bacterial cells.

Results and Discussion

Effect of matrix type on intracellular glycogen transformation

There are differences presented in polysaccharide content of bacterial cells cultured by different substrate types. They exhibit different changing trends, as shown in Figure 1. The run time less than 4.5 h represents anaerobic stage and the run time larger than 4.5 h is aerobic stage. It can be seen that there is a decrease in anaerobic condition and an increase in aerobic condition for the intracellular glycogen of sodium propionate matrix and sodium acetate matrix. However, the intracellular glycogen of glucose matrix displayed a decreasing tendency in both anaerobic and aerobic reaction process. The intracellular glycogen contents of all three matrix showed rapid decrease in initial anaerobic reaction process. The anaerobic reduction of sodium propionate matrix was 28.19 mg/gVSS, the decreasing amount of glycogen in initial anaerobic stage was 14.88 mg/gVSS, accounted for 52.79 % of the total reduction, the increasing amount of glycogen in initial aerobic stage was 43.94 mg/gVSS. The glycogen reduction of sodium acetate matrix was 29.34 mg/gVSS, the decreasing amount of glycogen in

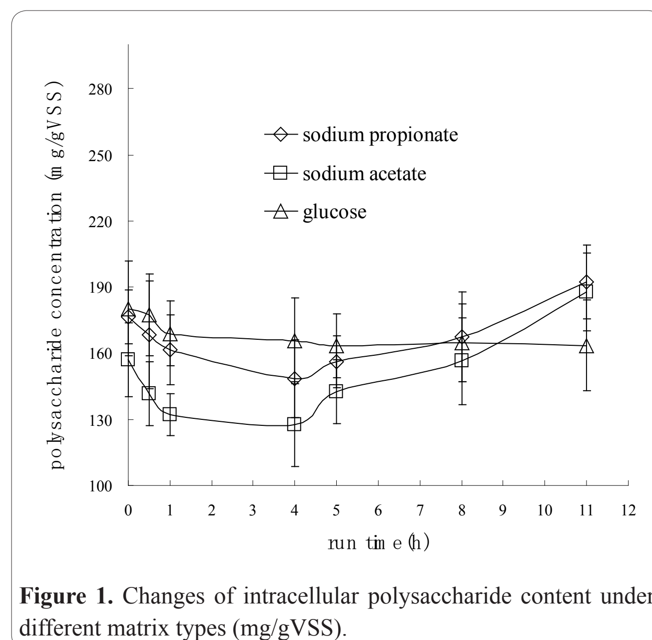

Figure 1. Changes of intracellular polysaccharide content under different matrix types (mg/gVSS).

initial anaerobic stage was 25.09 mg/gVSS, accounted for 85.50 % of the total reduction, aerobic increased amount was 59.89 mg/gVSS. The decreased anaerobic amount of glucose was 14.63 mg/gVSS, the glycogen reduction in initial anaerobic stage of was 11.37 mg/gVSS, accounted for 77.73% of the total reduction, the glycogen content was relatively stable during the aerobic stage.

Effects of matrix types on the formation and transformation of PHA

Figure 2 shows the changes of PHA composition and concentration in acetate matrix reactor. The reactor with sodium acetate as substrate, the contents of PHB and PHV in PHA are 9.35-97.65 mg/gVSS and 5.08-25.26 mg/gVSS, respectively in the reactor with acetate matrix. During the process of anaerobic and aerobic reactions, PHB content was significantly greater than PHV content, PHB content accounts for 64.80%-81.31% of PHA, and a PHB/PHV ratio is 1.84-4.35. This result indicates that sodium acetate matrix is beneficial to the formation of PHB, while the content of PHB is relatively small.

The change in the concentrations of PHA composition during the whole reaction period of sodium propionate matrix reactor was shown in Figure 3. Figure 3 indicates that the contents of PHB and PHV in the sodium propionate reactor are 3.03-58.65 mg/gVSS

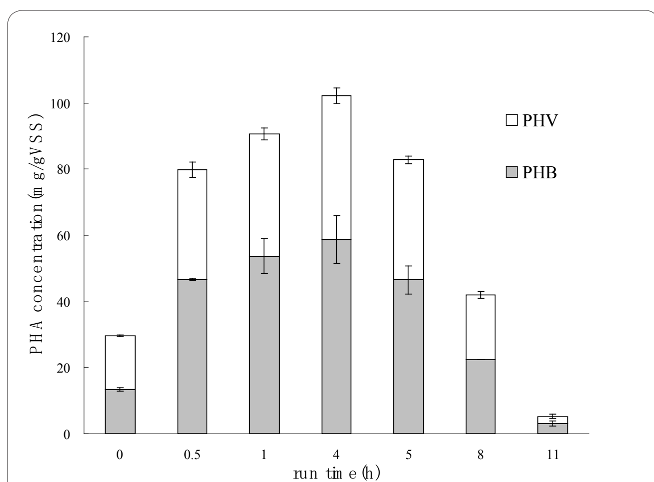

Figure 2. Change in concentrations of PHA composition in sodium acetate matrix reactor (mg/gVSS).

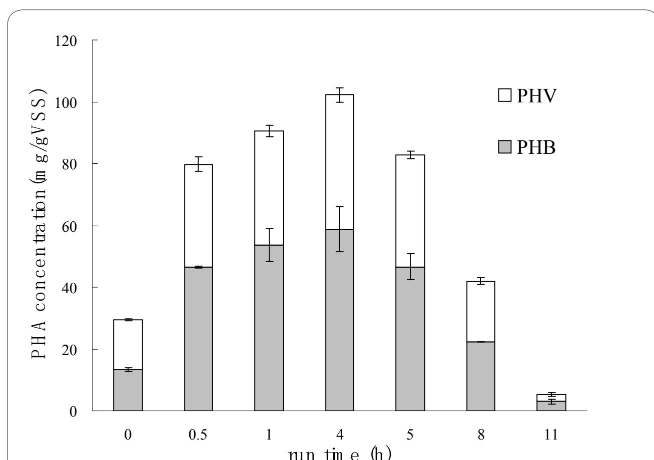

Figure 3. Change in concentrations of PHA composition in sodium propionate matrix reactor (mg/gVSS).

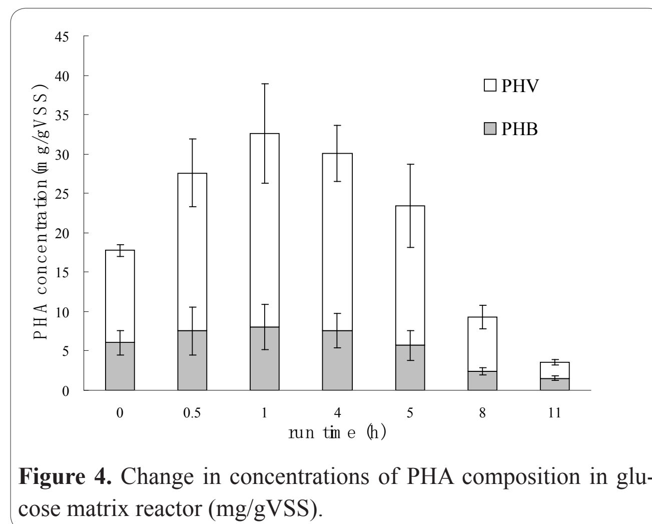

Figure 4. Change in concentrations of PHA composition in glucose matrix reactor (mg/gVSS).

and 2.24-43.58 mg/gVSS, respectively. PHB and PHV contents in PHA were quite similar in both anaerobic and aerobic reactions, a ratio of PHB/PHV is 0.83-1.45. The synthesis of PHV and PHB was mainly in the initial anaerobic stage (0 h - 1 h), and then slowly increased. Microorganisms consumed PHA in aerobic phase, the degradation amounts of PHB and PHV were 43.51 mg/gVSS and 34.02 mg/gVSS, respectively.

Figure 4 shows that the glucose matrix is helpful to the formation of PHV, PHV content was significantly greater than PHB content in anaerobic and aerobic reactions, PHV content accounts for 57.48%-75.66% of PHA, a ratio of PHB/PHV is 0.32~0.74.

Effect of matrix type on formation and transformation of polyphosphate

In the process of anaerobic and aerobic reactions, the contents of phosphate, oligomeric phosphate and polyphosphate of the bacterial cells in the reactor with sodium propionate matrix are 1.29-1.85 mg/gVSS, 1.65-2.37 mg/gVSS and 1.89-2.93 mg/gVSS, respectively. All three contents are quite close. The content of polyphosphorus exhibited an increasing tendency in anaerobic stage, as well as in the early aerobic stage (4-8 h), while gradually decreased in the middle and late aerobic stage (8-11 h). The contents of phosphate, oligomeric phosphate and polyphosphate of the bacterial cells in the reactor with sodium acetate matrix are 2.03-3.20 mg/gVSS, 0.90-3.43 mg/gVSS and 2.01-2.90 mg/gVSS, respectively. All three contents are also quite close, but there is a large range for the content of polyphosphorus, indicating that polyphosphate formation and degradation processes in the reactor are very obvious. The contents of phosphate, oligomeric phosphate and polyphosphate of the bacterial cells in the reactor with glucose matrix are 1.70-2.68 mg/gVSS, 2.99-5.53 mg/gVSS and 5.01-6.75 mg/gVSS, respectively. It can be seen that polyphosphorus dominates the content in three components, its content reaches above 70%. Meanwhile, the content of polymphosphorus shows an increasing tendency in both anaerobic and aerobic stages, suggesting that glucose matrix acclimation of the reactor favors the formation of polymphosphorus, which store the materials with high energy for the bacterial cells on the one hand, also allow the activated sludge absorb a large amount of phosphorus to strengthen the removal of phosphorus from wastewater on the other hand.

The degradation of both intracellular polysaccharide and polyphosphate were able to provide energy source for polyphosphate bacteria to absorb organic compounds under anaerobic conditions, but the phenomenon of polyphosphate formation and degradation was different from that of the intracellular polysaccharide. The energy needed by polyphosphate bacteria first came from degradation of the polyphosphate under anaerobic conditions when concentration of intracellular polysaccharide remained constant, and polyphosphorus presented a high rate of degradation. While the degradation rate of polyphosphate was significantly reduced when excessive degradation of glycogen occurred within bacterial cells. The reactor with glucose matrix was more dependent on the degradation of intracellular polysaccharides and more polyphosphate was formed comparing the other two types of matrix.

The activated sludge of sodium acetate matrix mainly synthesized PHB, which accounted for about 64.80%-81.31% of PHA. The contents of PHB and PHV synthesized by the activated sludge of sodium propionate matrix were similar. The activated sludge of glucose matrix mainly synthesized PHV, which accounted for approximately 57.48%-75.66% of PHA.

Acknowledgements

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