Factors for Promoting Polyhydroxyalkanoate (PHA) Synthesis in Bio-Nutrient-Removal and Recovery System

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Abstract. In the near future, the bio-nutrient-removal (BNR) system is no longer confined to meet the nitrogen and phosphorus removal requirement, but operated for sequestering and recovering carbon, nitrogen and phosphorus from wastewaters and generate more high-value-added by-products. Polyhydroxyalkanoate (PHA) is a metabolite of microorganisms under unfavourable growth conditions for their own energy storage and utilization. More and more researches have gradually uncovered the impacting factors on the microbial synthesis of PHA in the BNR and none-BNR system. This mini-review summarizes the impacting factors of carbon source types and dosage, nitrogen concentration and phosphorus recovery and temperature variation on the enrichment of PHA by microbial populations. Furthermore, the potential genomic reasons for promoting PHA synthesis are introduced. It provides a new perspective to stimulate the PHA synthesis by the functional microorganisms and its potential benefit for bio-nutrients removal and recovery in BNR system.

1. Introduction
In general sewage treatment plants, the lack of influent readily biodegradable chemical oxygen demand (rbCOD) will limit nitrogen and phosphorus removal efficiencies in the BNR systems. For example, the rbCOD / TN and rbCOD / TP ratios of urban sewages are confined within 4 and 16, respectively. Therefore, to achieve high efficient N and P removal and to meet the increasing stringent TN and TP discharging standard, external carbon sources are needed to compensate for the rbCOD deficiency. In the case of excessive carbon (C) and nutrient limitation, the bacteria tend to turn their metabolism into producing PHA, which is mainly consist of poly-3-hydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV) and poly-3-hydroxy-2-methylvalerate (PH2MV). As early as 1974, it has been reported that the accumulation of PHA was found in some specific microorganisms (e.g., polyphosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) in the BNR system. Some species of PAOs can transform external carbon source into PHA for simultaneous denitrification and P accumulation. Some GAOs are also capable of absorbing VFAs and converting them into PHA and perform denitrification with the stored PHAs. PHAs are also an important carbon source for N removal (intracellular carbon source based denitrification) and P removal and recovery. The effect of carbon source type as well as dosage on the microbial synthesis of PHA is important fields to be explored. Temperature is another factor also worth considering in practical applications.

2. Carbon Source Type and Dosage
The researches on carbon source types have been extensively explored. Most of the studies applied acetate or propionate as external carbon source. Ethanol can also be used as external carbon source.
in the EBPR system during long-term operation and achieve considerable phosphorus removal [1]. Many studies suggest that acetic acid is beneficial to the growth of PAO, yet some studies have shown that when acetic acid is used as a carbon source, the overproduction of polysaccharides would result in unstable phosphorus removal [2]. In the treatment of landfill leachate via endogenous denitrification (ED), using acetate as the sole carbon source is more likely to stimulate denitifiers to store PHA than using propionate. In this way, higher nitrogen removal efficiency was achieved. As the comparison, the lowest PHA amount was observed when using glucose as the carbon source [3]. The study also investigated the composite carbon sources (acetate + propionate, acetate + propionate + glucose) and achieved similar denitrification efficiencies. The type of carbon source also affected the composition of PHA. It was reported that propionate mainly induced the synthesis of PHV, whereas the use of acetate induced the synthesis of PHB, the ratio of PHB / PHV synthesized under the mixture of acetate and propionate was directly dependent on the microbial uptake rate of acetate and propionate [4]. In the moving bed biofilm reactor (MBBR) system, the feasibility of using two common carbon sources, methanol and ethanol, was evaluated on the removal of micro-pollutants from biofilm. The methanol-fed MBBR contained higher microbial abundance than the ethanol-fed MBBR, suggesting that improved biotransformation of the target compound may be associated with higher microbial abundance [5]. In terms of the production of PHA using a mixed microbial culture, the effect of substrate composition on the PHA accumulation capacity was investigated. Six organic acids, malate, citrate, lactate, acetate, propionate and butyrate were tested. Only three volatile fatty acids (VFAs) were found to cause PHA production, with the feeding of acetate and butyrate resulting in PHB production, propionate promoting the formation of PHB / PHV copolymer (51% PHV) [6]. In addition to carbohydrates and VFAs, other feeding organics, such as sugars, agricultural wastes and whey, should mainly promote the synthesis of short-chain-length PHA (scl-PHA), while the synthesis of medium-chain-length PHA (mcl-PHA) should use long carbon chain VFAs or oily waste [7]. This provides a new idea for the future carbon source selection in the BNR system. Besides these external carbon sources, the sludge generated within the system can also be recycled and reused as a carbon source. For example, primary sludge in the system after sonication was reused as an alternative carbon source and brought out high PHV containing biomass as well as ideal nitrogen and phosphorus removal [8]. Undoubtedly, such experiment would inspire an innovation of source recovery in the BNR system. The dosing mode of carbon source will also affect the PHA synthesis. In Tian et al.’s study, an intermittent dosing of carbon source was employed and it was found that phosphorus uptake was significantly increased while increasing PHA content in biomass. In the process of phosphorus recovery, excess PHA stored in the biomass facilitated subsequent simultaneous nitrification / denitrification and phosphorus removal. And this operating strategy helps to choose some special functional microbial communities [9]. Some studies also use protein as a carbon source alternative. The protein itself contains C, N, P, K, Mg and other elements to meet the needs of microbial growth, and combined the release of P with magnesium ammonium phosphate for phosphorus recovery [10]. However, protein caused the introduction of a certain amount of ammonia nitrogen, this problem has not yet been solved. The above researches provide ideas and directions of how to improve the content of PHA and how to enrich functional population in the BNR system. 3. N and P Concentration In addition to the carbon source type, concentrations of N and P will directly affect the amount and efficiency of PHA synthesis. Numerous studies have demonstrated that higher PHA levels are available at limited nitrogen or phosphorus concentrations [11]. In anaerobic-anoxic/nitrification sequencing batch reactor (A2N-SBR) with COD / P and COD / N of 20.6 and 6.9, respectively, the high abundance of DPAO was confirmed. With limited N or P, there is more PHA synthesis and an increased proportion of PHV. When the P load is maintained at a constant level, the biodiversity of the population decreases as the carbon load increases, and returns to its original level after P load declined [12]. PHA based denitrification (PBD) treating municipal wastewater in a biofilm reactor was reported to be capable of removing high nitrate concentrations (39-52 mg N / L) with lower COD requirements (COD / N = 4.6-5.3). Particulate organisms intercepted in the denitrification stage were found to have the denitrification capacity solely based on PHA [13]. At a very high C / P ratio, P is
depleted before C so that a microbial community with low PHA storage capacity but capable of rapidly accumulating P is selected. The rate of biomass uptake for a specific substrate is a linear function of the cell's P content [14].

4. Temperature Variation
Temperature ranges (35, 42 and 55 °C) were investigated using sewage sludge to produce PHA and reached the highest amount up to 0.39 gPHA / gVSS at 42 °C [15]. Short-term temperature changes (15-30°C) affect only the PHB synthesis rate but do not affect the maximum PHB storage capacity of the mixed cultures. Changes in PHB synthesis rate over long-term temperature changes are also affected by the adaption ability of the microbial culture. The temperature coefficient associated with PHA metabolism is higher than the short-term temperature change. On the other hand, the maximum growth rate of acetate-based mixed bacteria decreased with increasing temperature. At higher steady-state temperatures (30 °C), the mixed population still had a very high PHB storage (84 wt%). The rate decreased at lower temperatures (15 °C) and therefore required longer growth times [16].

5. Horizontal Gene Transfer
In recent years, apart from the researches on the production of PHA using a single strain, more researches have concentrated on the production of PHA using mixed microbial culture and the effects of related factors on PHA synthesis. In the BNR system, the ability to produce and store PHA leads to changes in the microbial community and determines the selection of dominant functional species. It is very important to explore the synthesis and storage of PHA in functional microbial communities under the changes of carbon, nitrogen and phosphorus ratio, temperature and the types and dosage of carbon source. In addition, with the development of multi-omics studies, the contribution of microbial community growth patterns and the specific horizontal translocation of PHA-encoding genes to the mutual aid microbial community would be discovered.

The ability of PHA synthesis can be obtained or enhanced through horizontal gene transfer between different species [17]. The genome analysis of Pseudomonas extremiaustralis revealing the existence of another PHB-related gene cluster, phbFPX, that has a high degree of similarity to the gene belonging to Burkholderiales and a gene cluster phaC1ZC2D that encodes mcl-PHA. All mcl-PHA genes showed high similarity to genes from Pseudomonas, and interestingly, this gene cluster also showed native insertion of seven ORFs unrelated to mcl-PHA metabolism [18]. Halomonas sp. TD01, a commercially viable halophilic strain of scl-PHA production, was found to employ PHA synthase, depolymerase, and regulators / repressor proteins all participating in the PHA metabolic pathway. They found different levels of horizontal gene transfer among the genomes of different strains [19]. The results of the research results could enlighten the improvement of the ability of PHA in the mixed microbial culture of BNR system from genomics level.

6. Conclusion and Outlook
The above three factors are the most common factors in the current studies. In addition to carbon source types and dosage, N and P content, temperature, pH, sludge retention time (SRT) and mean cell residence time (MCRT) and many other factors will also affect the ability to synthesize PHA of the microbial functional communities. The change of quantity, composition and rate of PHA synthesis under the influence of many factors should be taken into account, especially the harsh environment such as low-temperature environment which often encounter in practical applications. Furthermore, selecting the economical carbon source for application accompanied with the P accumulating and recovery is one of the future research directions. More deeply, the availability of genomic information of the microbial community in the BNR system not only provides a theoretical basis for genetic modification of microorganisms, but also provides an in-depth approach on selection and enrichment of the functional population in the BNR system, on improving the nitrogen and phosphorus removal efficiency of the reactor removal efficiency and on recovering N, P, carbon sources and other high-valued by-products simultaneously.

7. References
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