Effect of carbon source type on phosphorus removal efficiency of MBBR and characteristics of fluorescent substances in water

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Abstract. In this paper, Moving Bed Bioreactor (MBBR) reactor was used as the research object to explore the effects of carbon source type on phosphorus removal performance of MBBR system and the changes of microbial metabolic characteristics in typical cycles. The results showed that the absorption and release of phosphorus by sodium propionate and starch were obvious. The accumulation and degradation of PHA and glycogen were observed in the three reactors, and PHA was prior to glycogen decomposition. When sodium acetate was used as carbon source, glycogen decreased during the stationary phase, and the other two did not change significantly. The intensity of fluorescent substances varies greatly with the type of carbon source. The fluorescent substances in the effluent are mainly tyrosine-like and protein-like substances, fulvic acid-like substances and humic acid-like substances. Therefore, the suitable carbon source type should be selected according to the microbial characteristics in the water treatment process to improve the treatment efficiency.

1. Introduction
Carbon source type will affect the community structure competition between polyphosphate-accumulating bacteria and polysaccharide-accumulating bacteria and the regulation of key enzyme activities [1,2]. When the polysaccharide bacteria are anaerobic, the synthesis of Polyhydroxyalkanoates (PHAs) by organic matter in water does not release phosphorus. Once the polysaccharide bacteria occupy the dominant system, the phosphorus removal efficiency will be significantly reduced. Carbon source type also affect the activity of key enzymes in the removal process of microbial-driven phosphate. Therefore, the phosphorus removal efficiency is greatly affected by the type of organic carbon source. Many scholars have studied the phosphorus removal efficiency of different types and different proportions of combined carbon source. Han Yun [3] carried out pilot-scale experiments on phosphorus removal by oxidation process using sodium acetate and glucose as two different types of carbon source, and found that the release / absorption rate of glucose as carbon source was less than that of sodium acetate under the same conditions. Dacheng Luo [4] found that starch was supplied as a sole carbon source the phosphorus removal was a little different, that a new metabolic pathway can occur even without anaerobic phosphorus release. Chen [5] compared the effects of different acetic acid / propionic acid ratios on biological phosphorus removal in the system with A/O treatment process as the research object, and found that the sewage with high propionic acid content had better phosphorus removal effect.
in the long-term operation.

Therefore, MBBR (Moving Bed Biofilm Reactor) is taken as the research object, which is a typical representative of the intermittent biofilm method. It is very simple in process operation and operation cycle, and saves the occupied area, whose treatment efficiency is high for sewage treatment which amount of water is small. In this study, three MBBR reactors were used. During the reaction, three types of carbon source (sodium acetate, sodium propionate and starch) were used respectively. The effects of carbon source on the phosphorus removal performance of the MBBR system and the changes of microbial metabolic characteristics in the typical cycle were explored, so as to provide scientific support for further understanding of the MBBR system.

2. Materials and Methods

2.1. Experimental apparatus

The MBBR device is shown in Figure 1. The reactor is an organic transparent glass cylinder with a rectangular glass cover outside, in which the height is 700 mm, the inner diameter is 250 mm, and the effective volume is about 24.5 L. The aeration device is arranged at the bottom center of the water tank, and the aeration system is composed of air compressor, control valve, air supply pipeline, gas flowmeter, pressure stabilizing cavity, vacuum pressure gauge and microporous aeration plate (the aeration hole spacing is 10 mm and the aperture is 0.75 mm). During aeration, tiny bubbles are discharged through aeration micropores to ensure that the filler fully contacts with the water body and has sufficient dissolved oxygen in the reactor. The reactor runs two cycles every day, each cycle is 12h, the operation time of each stage are: aeration for 10h, stop aeration for 1.5h, water exchange for 0.5h, aeration quantity for 150 ~ 200L / h. The polyurethane sponge with a filling rate of 30% was used as the suspension filler in the experiment. The structure was a loose and porous square block of 10 mm × 10 mm × 10 mm. The specific surface area was up to 3 400 m²/m³, and the porosity was greater than 95%. The filler had good hydrophilicity, fast film-forming, high biological adhesion and wear resistance.

![Figure 1. Schematic diagram of the test device.](image)

1. Air compressor 2. Air supply pipeline 3. gas flow meter 4. Dissolved oxygen monitor 5. water inlet 6. Polyurethane sponge filler 7. Vacuum pressure gauge 8. Outlet 9. Aeration plate and aeration pinhole 10. Stabilizing cavity

2.2. Experimental influent water quality

The experimental influent water quality refers to the water quality of typical urban domestic sewage in China to prepare water samples [6]. Glucose, ammonium chloride and potassium dihydrogen phosphate were used as carbon source, nitrogen source and phosphorus source, respectively. Sodium bicarbonate and trace elements were added to ensure the normal growth and reproduction of microorganisms. The concentration of influent water in start-up stage and stable operation stage is shown in Table 1, and the
concentration of other nutrients and trace elements is shown in Table 2.

Table 1. Influent composition table of each stage of the reactor.

| Operation phase   | Concentration settings of influent components                                                                 |
|-------------------|-------------------------------------------------------------------------------------------------------------|
| Film hanging start| COD: 400±10 mg/L, NH₄⁺-N: 20±3.8 mg/L, TP: 4±0.5 mg/L                                                      |
| Stable operation  | COD: 400±13.6 mg/L, NH₄⁺-N: 42±2.2 mg/L, TP: 5.5±0.6 mg/L                                                 |

Table 2. Concentration table of other nutrients and trace elements.

| Component       | Concentration (mg/L) | Component       | Concentration (mg/L) |
|-----------------|----------------------|-----------------|----------------------|
| CaCl₂           | 10.5                 | FeCl₃           | 0.9                  |
| MgSO₄·7H₂O      | 90                   | KI              | 0.18                 |
| NaHCO₃          | 110                  | MnCl₂·4 H₂O     | 0.06                 |
| MnCl₂·4 H₂O     | 0.12                 | CuSO₄·5 H₂O     | 0.03                 |
| CuSO₄·5H₂O      | 0.03                 | ZnSO₄·7H₂O      | 0.12                 |
|                 |                      | FeSO₄           | 0.6                  |
|                 |                      | EDTA            | 10                   |

Table 3. Conventional indicators and detection methods.

| Test items | Analysis items                                      |
|------------|-----------------------------------------------------|
| CODcr      | HJ/T399-2007 Rapid digestion spectrophotometry      |
| NH₄⁺-N     | HJ535-2009 Nano reagent spectrophotometry            |
| TP         | GB11893-89 Ammonium molybdate spectrophotometry      |
| PO₄³⁻      |                                                     |
| DO         |                                                     |
Results and discussion

During the metabolism of P-accumulating microorganisms, glycogen and PHA as the intracellular energy storage substances play a very important role, and their consumption and circulation process reflect the metabolic characteristics of P-accumulating microorganisms [2,11]. By comparing the changes of COD, TP, glycogen and PHA in the typical cycle of three phosphorus removal reactors, the possible mechanism of the difference in phosphorus removal performance of the system with different carbon sources was further discussed. After the phosphorus absorption in the liquid phase reached a stable state after the change of influent carbon source in the reactor, the contents of COD, TP, glycogen and PHA in the liquid phase of the reactor under different carbon sources were detected.

3.1. Effect of carbon source type on water quality parameters in reactor

As shown in Figure 2, TP in the reactor with sodium acetate as carbon source decreased from 5.08 mg·L⁻¹ at the initial stage to 3.05 mg·L⁻¹ at 10 h, with a decrease of 2.03 mg·L⁻¹, and 0.37 mg·L⁻¹ phosphorus was released into the liquid phase at the end of the standing period. At the same time, most of the carbon sources were consumed within 2 h after the start of aeration, and the COD concentration remained stable from 406.5 mg·L⁻¹ to 38.6 mg·L⁻¹ after 6 h. The contents of PHA and glycogen in the reactor were significantly accumulated. In the early stage of aerobic, the mass fraction of PHA was synthesized from 23.58 mg/g to 49.08 mg/g, which reached the peak value before glycogen synthesis and decreased to the level before aeration at about 4 h. When PHA concentration began to decline, glycogen continued to accumulate, and reached the maximum value (149.22 mg/g) at about 4 h, and then gradually decreased to 70.37 mg/g at the end of the stationary phase. This is because the energy required for microbial growth and uptake of phosphate in the liquid phase after external carbon source consumption is mainly derived from the decomposition of energy storage substances.

Different from sodium acetate, when sodium propionate was used as the sole carbon source of the reactor, the TP concentration in the system decreased rapidly and the phosphorus release was more obvious. The lowest TP concentration at the 10th hour after aerobic treatment was 1.13 mg·L⁻¹, and the variation of phosphorus release at the static stage was 0.61 mg·L⁻¹, which was higher than that when sodium acetate was used as the carbon source and reached the equilibrium point of phosphorus absorption and release later. In addition, when the carbon source was sodium propionate, the accumulation of PHA in the microorganism was higher than that in the reaction system with sodium acetate as carbon source (25.50 mg/g in sodium acetate system and 32.76 mg/g in sodium propionate system), but the accumulation of glycogen was just the opposite (66.56 mg/g in sodium acetate system and 45.31 mg/g in sodium propionate system), which was consistent with the conclusion obtained by Changke [12] in SBR reactor.

In the typical cycle of MBBR with starch as carbon source, the TP treatment effect was between sodium acetate and sodium propionate. TP concentration reached the minimum of 2.14 mg·L⁻¹ at 8 h, and decreased by 57.9 %. The COD concentration was 253.31 mg·L⁻¹, which was 81.89 mg·L⁻¹ more than that of the reactor with sodium acetate. The PHA reached the peak of 51.73 mg/g at 4 h, and the glycogen content was less than that of the other two reactors.

In summary, the phosphorus uptake and release of sodium propionate and starch were obvious in one reaction cycle under three types of carbon source. The accumulation and degradation of PHA and
glycogen were observed in the three reactors, and PHA was prior to glycogen decomposition. When sodium acetate was used as carbon source, glycogen decreased in the stationary phase, and the other two did not change significantly. When sodium acetate is the main carbon source, the polymer synthesized by PAO is mainly PHB, while when sodium propionate is the main carbon source, the polymer synthesized by PAO is mainly PHV. Starch needs to be transformed into small molecular organic matter before being absorbed by PAO [4]. The intracellular energy storage substance PHA is most easily used by microorganisms under aerobic conditions, so its decomposition is prior to glycogen. When glycogen and PHA levels drop to the initial level, polyphosphate begins to decompose, and TP concentration in wastewater increases.

![Diagram](image1)

**Figure 2.** COD, TP, PHA, glycogen change diagram during typical cycle of MBBR reactor.

3.2. *Effect of carbon source type on DOM in MBBR effluent*

After microbial treatment, the effluent of MBBR reactor contains a certain amount of polymers with fluorescent functional groups. In order to further understand the influence of different types of carbon source on the fluorescent organic compounds in the effluent of the reactor, three-dimensional fluorescence spectroscopy (3DEEM) was used for water quality analysis. It can not only qualitatively analyze the pollutants in water, but also quantitatively analyze the content of specific dissolved organic compounds [13,14].

In this paper, the composition and content of soluble fluorescent substances were analyzed by parallel factor analysis. 3DEEM data were divided and processed by Matlab data analysis software, and the fluorescent components and positions were determined by Origin software after plotting. As shown in Figure 3, three components were determined as follows: component 1 (Ex / Em) - C1-A (245 / 420), C1-B (330 / 420). Component 2 (Ex / Em) - C2-A (260 / 476), C2-B (370 / 470). Component 3 (Ex / Em) -
C3-A (265 / 290). The comparison of the three-dimensional fluorescence standard analysis showed that C1-A was fulvic acid substance, C1-B, C2-A and C2-B were fulvic acid-like substances and humic acid-like substances, respectively, and C3-A was tyrosine-like and protein-like substances. It can be seen from Figure 4 that when the influent carbon sources were sodium acetate, sodium propionate and starch, the fluorescence intensity of C1 and C2 changed little, and the fluorescence intensity of C3 was sodium acetate > starch > sodium propionate.

**Figure 3.** Fluorescence components and their positions in reactor effluent under different types of carbon source.

Humic acid and fulvic acid in domestic sewage are mainly derived from microbial metabolites, and the content is relatively stable in the biological treatment stage. Tyrosine-like and protein-like substances have good biodegradability, and can be absorbed by microorganisms in the aerobic stage. The content of protein-like substances has a good correlation with COD. The lower the COD concentration in the effluent of the reactor, the lower the content of protein-like substances.

**Figure 4.** Fluorescence component intensity diagram of MBBR effluent under different types of carbon source.

4. Conclusions
This study investigated the effects of carbon source type on phosphorus removal performance of MBBR system and the changes of microbial metabolic characteristics in typical cycles. Based on these findings,
the conclusions follow:

(1) When sodium propionate was used as carbon source, microorganisms had higher utilization efficiency, better sewage treatment effect, and faster degradation of total phosphorus in water, but had no obvious effect on COD removal in wastewater. (2) Carbon source type is the main reason for the difference of fluorescent organic matter in the effluent of the reactor. Among the three carbon sources, sodium acetate had the greatest influence on the content of tyrosine and protein (C3) in the effluent.

Acknowledgments

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