

The Recovery and Synthesis of Polyhydroxyalkanoates in the Process of Nitrogen and Phosphorus Removal in Low-Carbon Sewage Based on pH and Nitrogen and Phosphorus Restriction Under Anaerobic-Oxygen Limited Process

Kequan Zhang

Guangzhou University

Qian Fang (✉ gz_fq@126.com)

Guangzhou University <https://orcid.org/0000-0001-8672-8200>

Yihan Xie

Guangzhou University

Yujia Chen

Guangzhou University

Tong Wei

Guangzhou University

Yanyu Xiao

Guangzhou University

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Abstract

Polyhydroxyalkanoate (PHA) is a new type of bio-polyester which is expected to replace traditional petroleum-based plastics. It is also a critical transformation hub of carbon source in nitrogen and phosphorus removal in sewage. Based on the anaerobic-oxygen limited process, the experiment took organic solid waste fermentation liquid as carbon source control hub and realized PHA synthesis and recovery in denitrification and phosphorus removal from low carbon sewage the SBR reactor by regulating pH value and nitrogen and phosphorus restriction.

The experimental results showed that when the ratio of C/N and C/P was 150, the content of PHA accounted for 50.39% and 36.07 of the dry cell weight, respectively. Besides, it was found that increasing the C/N ratio was beneficial to increasing the proportion of PHV in PHA. This study proved the feasibility of using an anaerobic-oxygen limited process to recover PHA in nitrogen and phosphorus removal from low-carbon sewage, which saves gas and reduces energy consumption. At the same time, it also provides some help for the follow-up study of low-carbon urban sewage nitrogen and phosphorus removal coupled with resource recovery of PHA to guide the water industry economy to develop in a circular and sustainable direction

Highlights

- PHA synthesis and recovery in the process of denitrification and phosphorus removal from low carbon sewage.
- The pH value of weak alkalinity plays a good role in promoting PHA production under the anaerobic - oxygen limiting process.
- The highest content of PHA accounted for 50.39% of the cell dry weight was obtained when the C / N ratio was 150.
- Increasing the C/N ratio was beneficial to increasing the proportion of PHV in PHA.

1. Introduction

With the increasing demand for plastics in the market, some studies have pointed out that as of 2014, global plastic production reached 311 million mg[1]. In 2010, the amount of plastic waste produced by 192 coastal countries entering the ocean had compared 4.8 to 12.7 million tons. Suppose the waste management infrastructure is not improved in time. In that case, the amount of non-biodegradable plastic waste flowing into the ocean is expected to increase by order of magnitude by 2025[2]. Therefore, to solve the increasingly severe environmental pollution problem, it is highly urgent to replace the non-degradable traditional petroleum-based plastics with biodegradable biopolymers.

Polyhydroxyalkanoate is a kind of biopolymer accumulated by various microorganisms under unbalanced growth conditions[3]. This type of biological polyester can be processed into thermoplastic materials, which have physical and chemical properties similar to traditional plastics (P.P. and LDPE), and

due to its unique biodegradability and renewability, it can effectively alleviate the accumulation of plastic waste in the environment[4]. Therefore, the production and application of PHA have attracted much attention.

At present, the most common technology for industrial production of PHA is mainly to use natural pure strains to ferment and cultivate. However, this method requires a strict aseptic environment and high-quality carbon source substrate, which increases the complexity and cost of the operation process[5]. And some studies have pointed out that the operating cost of the PHA production process with pure culture is 5-10 times that of traditional petroleum-based plastics[6]. It has primarily hindered the widespread application of PHA in the market. To make PHA better adapt to market competition, as an effective alternative, mixed microbial culture (MMC) can effectively utilize a large number of cheap waste substrates without sterilization. This diverse culture method dramatically reduces the use rate of expensive carbon sources, so it has high environmental and economic value[3,7]. Related reports indicate that the residual sludge and food waste from sewage treatment plants are suitable substrates for PHA production after pretreatment of two common VFA-rich liquids[6]. Therefore, more and more attention has been paid to the production of PHA by MMC. Because it not only reduces the cost of the production process but also integrates the biological treatment of wastewater and organic waste and effectively promotes the coordinated development of resource recycling and circular economy. At present, sewage treatment plants in northern China use substances produced by anaerobic fermentation of waste sludge (VFA) as the precursor substrate for the recovery of PHA, thereby achieving the goal of reducing the cost of raw materials for the synthesis of PHA[8]. It realized efficient PHA production in the sludge fermentation broth mainly containing valerate by using the MMC method[9]. However, in the south of China, due to the low carbon source of raw water in its sewage treatment plants, the primary sedimentation tank, and sludge anaerobic digestion tank are usually not set up, so it is impossible to use the sludge anaerobic fermentation liquid in its process as the carbon source for PHA synthesis. Therefore, it is impossible to use the sludge anaerobic fermentation liquid in its own process as the carbon source for PHA synthesis. In addition, a certain amount of carbon source needs to be consumed in the process of nitrogen and phosphorus removal, therefore, it is not realistic to use sludge fermentation broth as carbon source to synthesize PHA as in northern China. Besides, other studies have shown that low-concentration wastewater will also have a certain limitation on nitrogen and phosphorus removal[10]. Therefore, it is more challenging to simultaneously recover and synthesize PHA in the process of nitrogen and phosphorus removal in low carbon areas. [11][12] found that PHA can be used as an important transformation hub for carbon sources in the process of nitrogen and phosphorus removal in sewage, and studied the simultaneous nitrification and denitrification phosphorus removal (SNDPR) process based on PHB as the driving force of carbon sources, which also confirmed that hypoxia was a necessary condition for the occurrence of SNDPR. This process not only effectively solves the problem of traditional nitrogen and phosphorus removal process in the treatment of urban sewage with low carbon source, but also provides a new idea and approach for the development of low carbon sewage nitrogen and phosphorus removal process. On this basis, Fang et al studied the use of actual solid waste fermentation broth as an external carbon source and used the activated sludge mixed flora to synthesize

PHA under oxygen-limited conditions, effectively confirming the feasibility of low oxygen synthesis of PHA[13]. This process has a tremendous energy-saving effect and fits the actual situation of low-carbon sewage in southern China. It also skillfully uses food and sludge waste, playing the dual role of treating waste with waste and turning waste into treasure.

Besides, it was found from numerous studies on PHA synthesis that changes in process parameters could achieve a higher biomass enrichment effect, which significantly improved the ability of PHA synthesis by using activated sludge mixed microorganisms. The metabolic process of PHA is usually closely related to some parameter changes, for instance, the nutrient ratio C/P ratio, C/N ratio and pH value of external environmental factors required for microbial growth. At present, scholars have different regulations on the optimal pH value of PHA synthesis. Most studies believe that the neutral, alkaline environment is most suitable for activated sludge accumulation of PHA. It was found that in the range of pH greater than or equal to 7, the accumulation efficiency of PHA was significantly higher than that of acidic conditions[14]. When the pH value is 8.8-9.2, PHA has the best all-around production performance. The study found that the pH of 8 or 9 was an appropriate environment for PHA accumulation, and the yield of PHA was the highest when pH was 7.5- 8.5[15,16]. It was found that the content of PHA synthesized by activated sludge reached the maximum when the pH value in the reaction process was not controlled[17].

In addition to the influence factor of pH, nutrient sources (mainly phosphorus (P) and nitrogen (N)) are also crucial parameters during the synthesis process[18]. It was found that when the concentration of nitrogen and phosphorus was limited, the metabolism of PHA was extensively promoted. And in most studies, it was found that the effect of nitrogen limitation on the overall performance of PHA synthesis from activated sludge was greater than that of phosphorus limitation. It was reported that the accumulation of PHA was higher in N-restricted conditions than in P-limited states (51% and 42%, respectively)[14]. Similar to the results, it was also found that the PHA content under the N-restricted condition was higher than that under the P-restricted condition (59% and 37%, respectively)[19]. However, some related studies have found that limiting phosphorus is more conducive to accumulating PHA in activated sludge than limiting nitrogen. The PHA content obtained under the P-restricted condition was higher than that under the N-restricted condition (54% and 42%, respectively)[20]. The maximum accumulation of PHA could reach 51% of dry weight under the conditions of limited oxygen and nutrient elements and restricted phosphorus source[18]. In addition, a few studies found that limiting nitrogen and phosphorus concentration had different effects on PHA monomer content. In determining nitrogen and phosphorus, increasing the respective ratio has a good impact on PHB synthesis but has little impact on PHV[19].

At this stage, most of the researches on the PHA synthesis process at DOMestic and abroad are focused on the anaerobic-aerobic, complete aerobic process, and microaerobic process. There is almost no research on the synthesis of PHA under the condition of oxygen restriction. This study combines the characteristics of low-carbon sewage and discusses the effect of different pH values on the all-around performance of PHA production based on the anaerobic-oxygen limited process; it is also proposed that

the total amount of PHA synthesized by activated sludge and the proportion of PHV in monomer can be effectively increased by increasing C/N ratio under weak alkaline pH value, which effectively proves the feasibility of PHA synthesis under anaerobic-oxygen limited process. This study provides a new idea and approach for low carbon municipal sewage treatment plants to move from the single nature of "COD removal and nitrogen and phosphorus removal" to the comprehensive "recovering energy and resources in sewage."

2. Materials And Methods

2.1 Experimental device

In this study, a cylindrical SBR reactor made of two Plexiglas was used with a total volume of 10 L and an adequate volume of 7.5 L. The temperature of the reactor is controlled at 25°C, and a timer controls the reaction time. The peristaltic pump is utilized to feed and drain water, and the rotor flowmeter controls the aeration rate. Besides, the top of SBR is equipped with a stirring paddle to ensure the reactor's uniform reaction mixing. The outer wall is equipped with a sampling port, and the bottom is fitted with a sludge discharge port. At the same time, online detection devices for pH and DO. are provided for monitoring. The anaerobic-oxygen limited process was utilized to run the reactor for three cycles a day. Each cycle consists of the following phases: influent 10 min, anaerobic reaction two h, oxygen limiting reaction three h, precipitation 25 min, drainage 5 min, and the rest of the time was static.

2.2 Experimental sludge

The sludge used in the test was taken from the excess sludge in the Guangzhou Lijiao sewage treatment plant's secondary sedimentation tank. The operation process was an improved A/O process. The primary characteristics of sludge are shown in the table below.

Table 1 The characteristics of the sludge.

Parameters	MLSS(mg/L)	SV(%)	SVI(mg/L)	pH
numeric value	12829	97	756	7.56

2.3 Experimental wate

Artificial water was used as the influent, anhydrous sodium acetate as carbon source, NH₄Cl as nitrogen source, KH₂PO₄ as phosphorus source, MgSO₄·7H₂O, FeSO₄·7H₂O and CaCl₂ as trace elements, and NaHCO₃ as pH regulator. The pH value of water distribution should be kept at about 7. The components of trace elements in water distribution are shown in Table 2.

Table 2 The ingredient of trace elements.

Matter	Concentration(g/L)	Matter	Concentration(g/L)
MgSO ₄ ·7H ₂ O	0.12	FeSO ₄	0.05
CaCl ₂	0.03	KI	0.1
MnCl ₄ ·7H ₂ O	0.12		

2.4 Experimental design

Based on the anaerobic-oxygen limited process, low carbon wastewater was designed to achieve nitrogen and phosphorus removal, and the synthesis of PHA was promoted by regulating two influencing factors. The specific experimental design is as follows.

2.4.1 Operation of SBR system by an anaerobic-oxygen limited process

The sludge from the secondary sedimentation tank of the sewage treatment plant was inoculated into the SBR reactor, and an anaerobic-oxygen limited process operated the system. Due to the low carbon source in urban sewage in this area, the influent COD starts from 200 mg/L and increases at the speed of 100 mg/L · d until the maximum COD concentration of influent is 1,000 mg/L. The proportion of nitrogen and phosphorus increased slightly under the balanced nutrition ratio (C: N: P = 100:5:1), and the influent was prepared according to C: N: P = 100:6:2. The reactor's initial MLSS was controlled to be about 3800 ~ 4,500 mg/L, and the DO_i concentration in the oxygen limiting section was controlled below 2 mg/L. Water samples and sludge samples were collected after the system was running for three days, eight days, and eleven. The concentrations of acetic acid, PHA, and released phosphate (TP) were determined. PHA synthesis and phosphorus removal by activated sludge under anaerobic-oxygen limited process were compared and analyzed.

2.4.2 Effect of pH value on the synthesis of PHA

To evaluate the capacity of activated sludge to synthesize PHA under anaerobic and oxygen-limited conditions, batch experiments were carried out under different pH conditions: 5.0, 6.0, 7.0, and weakly alkaline pH (due to the artificial configuration of sodium acetate, without controlling pH, the reactor was in a weak alkaline environment with fluctuation of 7.5-8.5). In the experiment, 10% dilute sulfuric acid, and 10% sodium hydroxide solution were used as pH adjustment solutions, and the pH was strictly controlled within the preset value of 0.2. The MLSS in the reactor was controlled at 4,500-5,000 mg/L, and the rotameter adjusted the DO_i in the reactor to maintain the DO_i value below 2 mg/L under oxygen limiting condition.

2.4.3 Effect of C / N and C / P ratio on PHA synthesis by activated sludge

Two groups of nutrient ratio restriction tests with different nitrogen and phosphorus concentrations were designed to evaluate activated sludge's PHA synthesis capacity under these two other nutrient conditions. As in the previous experiments, the D.O. of the oxygen limiting stage was maintained below 2 mg/L. First of all, a P-limiting investigation was carried out in a one #SBR reactor. The influent COD was increased to 1,500 mg/L, other nutrient ratios except phosphorus were kept unchanged, and the influent carbon-nitrogen balance was maintained at 20:1. The synthesis capacity of PHA was investigated when the C / P ratio was 100, 150, 200, 250, and 500. The N-limiting experiment was carried out in a two # SBR reactor similar to phosphorus limitation. The PHA synthesis was investigated when the C / N ratio was 20, 60, 100, 120, and 150.

Besides, the effects of different nutrient ratios on the synthesis of PHA monomers were further explored. The change rules of PHA monomer components under two conditions of phosphorus and nitrogen limitation were investigated when the maximum amount of PHA was synthesized to establish a method to control PHA monomer's proportion according to the actual production requirements.

2.5. Analytical methods

Routine analysis items of the water samples were analyzed following the standards specified in "water and wastewater detection and analysis methods" (4th Edition)[21]. See Table 3 for details.

Table 3 Analytical items and methods for wastewater quality.

Analysis project	Test method
COD	Potassium dichromate digestion method
T.P.	Molybdenum antimony Spectrophotometry
DO □pH	On line monitoring of wtw340i instrument in Germany
NH4 ⁺ -N	Nessler's Reagent Spectrophotometry
MLSS	Filter paperweight method
SV%	100 ml cylinder method

The determination method and instrument conditions of test PHA: Gas chromatography was used to detect PHA, and Agilent 7980A-5975C GC-MS was used. The specific operation method is as follows: put the appropriate amount of sludge powder after centrifugation and freeze-drying into the heat-resistant tube, add 2 ml chloroform, 2 ml acidified methanol solution with a volume fraction of 10%, and 2 ml methanol benzoate solution in turn. After sealing, water bath at 100°C for four h, cool to room temperature, add 2 ml demonized water, shake for 10 min, and then stand for stratification. The lower organic phase was filtered with 0.45 um filter membranes and added into the injection bottle for

chromatographic analysis. Agilent 7980A-5975C GC-MS was used for the experiment. The chromatographic conditions were as follows: the injection port temperature was 230°C, the chromatographic column type was DB⁻¹, 30 m × 0.25 mm × 0.25 μm. The heating program: after holding at 100°C for 2 min, the temperature was raised to 160°C at the speed of 15°C / min for 2 min, and the mass scanning range of MS was 20-550 da. Finally, the standard internal method was used to calculate the content of PHA: the mass of PHB and PHV in the normal sample of PHA under different mass gradients was taken as the Y-axis, and the ratio of PHB peak area and PHV peak area to benzoic acid peak area was taken as X-axis to draw the standard curves of PHB and PHV respectively. The contents of PHA and PHA were calculated, respectively. To avoid the error caused by the test operation, each sample is detected more than twice by GC-MS to improve test data accuracy.

2.6 Calculations

The relevant calculation for the determination of PHA included in this test is shown in the following formula:

$$\text{PHA content: \%PHA} = g_{\text{PHA}} \cdot g^{-1} \cdot \text{VSS} \cdot 100 \quad (1)$$

$$\text{substrate consumption: } \Delta S = S_1 - S_0 \text{ (Cmmol/L)} \quad (2)$$

$$\text{PHA produced: } \Delta \text{PHA} = \text{PHA}_1 - \text{PHA}_0 \text{ (Cmmol /L);} \quad (3)$$

$$\text{PHA yield: } Y_{\text{PHA/S}} = \Delta \text{PHA} / \Delta S \text{ (Cmmol PHA Cmmol/S)} \quad (4)$$

$$\text{response time: } \Delta t = t_1 - t_0 \text{ (h)} \quad (5)$$

$$\text{PHA production rate: } R_{\text{PHA}} = \Delta \text{PHA} / \Delta t \text{ (Cmmol/h)} \quad (6)$$

$$\text{substrate consumption rate: } R_S = \Delta S / \Delta t \text{ (Cmmol/h)} \quad (7)$$

In the above formula, t_1 and t_0 are the time and initial time to reach the maximum concentration of PHA, S_1 and S_0 (Cmmol/L) are the substrate concentrations at the maximum synthesis and the beginning, respectively; PHA_1 and PHA_0 (Cmmol/L) are the PHA concentrations at the maximum and the beginning, respectively.

3. Results And Discussion

3.1 Phosphorus removal and PHA synthesis in an anaerobic-oxygen limited process

An anaerobic-oxygen limited process started the SBR reactor. The variation of phosphate concentration and PHA content was shown in Fig. 1 after three days, eight days, and eleven operation days.

It can be seen from Fig. 1 that with the increase of working days, the phosphorus release amount in the anaerobic stage showed a continuous upward trend. After 11 days of operation under an anaerobic-oxygen limited process, the concentration of phosphate in water increased from 8.5 mg/L to 77.45 mg/L in the first half-hour of anaerobic treatment, and the total amount of TP released at the end of the anaerobic process reached the maximum (85.4 mg/L). The abundant PHA synthetic bacteria cause this in the activated sludge of municipal wastewater treatment plant, among which PAOs is one of the common synthetic bacteria. Based on the principle of enhanced biological phosphorus removal from wastewater, under anaerobic condition[22], the polyphosphate accumulating bacteria can rapidly decompose the phosphorus accumulating in the cells, release a large amount of phosphate, and the concentration of phosphorus in the reactor rises quickly; in the oxygen limiting stage, the polyphosphate accumulating bacteria take molecular oxygen as electron acceptor and absorb excessive phosphorus from water to form polyphosphate. As shown in Figure 1, phosphorus in the system is almost completely absorbed at the end of oxygen limiting. It can be observed in Fig.1 that the variation of PHA content in activated sludge is highly correlated with the phosphorus release and absorption curve. With the increase of working days, the system's phosphorus removal effect is better, and PHA synthesis also increases. This is due to PAOs under anaerobic conditions. Many organic nutrients in wastewater are absorbed using the energy in glycogen and the hydrolysis of polyphosphate in cells. The external carbon source volatile organic acids (VFA) are converted into intracellular energy storage substances, thus accumulating PHA. In the oxygen limiting stage, PAOs absorb excessive phosphate from wastewater as energy storage and use free oxygen as an electron acceptor to oxidize the internal carbon source stored under anaerobic conditions. Therefore, PHA was slowly consumed. After 11 days, PHA content synthesized by activated sludge reached 20.19% of dry sludge weight. This shows that activated sludge has a high activity after anaerobic oxygen limiting operation. When the influent containing carbon source and nutrient solution enters the system, it can quickly absorb external carbon source and decompose phosphorus accumulation in cells, effectively remove phosphorus and complete a certain amount of PHA synthesis.

In conclusion, after 11 days of stable operation, the system can recover and synthesize a certain PHA amount while effectively removing phosphorus.

3.2 Influence of pH on the accumulation and synthesis of PHA

After the above 11 days of anaerobic-oxygen-limiting operation, PHA content was measured at the end of the anaerobic period to account for 20.19% of the sludge's dry weight. Based on this, the change law of COD and PHA synthesis under the conditions of weak alkaline pH (7.5-8.5), pH=7, pH=6, pH=5 is further examined, and the results are shown in Fig. 2.

As can be seen from Fig.2, when entering the oxygen-limiting stage, the substrate is consumed rapidly. At this point, the COD degradation curve shows a clear downward trend, the microorganism is in a highly active state, and the substrate's utilization efficiency is also higher. After the oxygen-limit of 1h, the PHA content reached the highest value, accounting for 28.22% of the sludge's dry weight. Both the synthesis of PHA and the degradation of COD are affected when pH is checked. As shown in Fig.2, the residual part

of COD in the oxygen-limiting end-stage system is not degraded, which directly affects PHA synthesis. It can be seen that the content of PHA has also been reduced, and at the time of anaerobic 1.5 h reached the highest value, accounting for 28.17% of the dry weight of sludge, under the condition of pH-6, the utilization rate of the system substrate is significantly inhibited, PHA synthesis has become irregular. Under the condition of pH-5, COD has been almost degraded, the amount of PHA is decreasing, and the system has gradually lost the synthesis ability of PHA.

The synthesis of PHA at different pH values is analyzed dynamically, and the results are shown in Table 4.

Table 4 Kinetic parameters of PHA production under different pH.

nitrogen limiting						
pH	%PHA	Δ PHA (Cmmol/L)	Δ S (Cmmol/L)	$Y_{\text{PHA/S}}$	r_{PHA}	r_{S}
5	19.31	9.47	NA	NA	4.73	NA
6	21.13	4.74	6.39	0.74	2.37	3.19
7	28.17	21.10	25.83	0.82	7.03	8.61
no control [7.5-8.5]	28.22	17.89	26.04	0.69	5.96	8.68
phosphorus limiting						
pH	%PHA	Δ PHA (Cmmol/L)	Δ S (Cmmol/L)	$Y_{\text{PHA/S}}$	r_{PHA}	r_{S}
5	13.52	-2.68	-1.46	-1.84	-1.34	-0.73
6	18.39	-0.40	-8.54	-0.05	-0.20	-4.27
7	23.97	14.60	14.79	0.99	9.73	9.86
no control [7.5-8.5]	25.81	30.69	27.08	1.13	20.46	18.06

Δ S: substrate consumption (Cmmol/L); Δ PHA: PHA produced during the assay (Cmmol/L); %PHA: PHA content in biomass(%gPHA*g⁻¹CDW);

r_{S} : substrate consumption rate (Cmmol/h); r_{PHA} : PHA production rate (Cmmol/h); $Y_{\text{PHA/S}}$: PHA to substrate stoichiometric yield (CmmolPHA/Cmmol S).

The main kinetic and stoichiometric parameters of PHA formation are shown in Tab 4. Under the condition of nitrogen limitation, when pH is not controlled, it is the most favorable for the total production of PHA. Considering the accumulation of PHA in this stage, it reached the highest value under several conditions, accounting for 28.22% of the cell dry weight. The substrate consumption rates of this stage also got the maximum value, which was 8.68 Cmmol S / L / h, which indicated that microorganisms were absorbing the carbon source to synthesize PHA. Besides, the PHA productivity r_{PHA} reached 5.96 Cmmol PHA / L / h, and $Y_{\text{PHA/S}}$ were also high, going 0.69 Cmmol PHA Cmmol/S. Under the condition of phosphorus limitation, when the pH was not controlled, the substrate consumption rate was the highest ($r_s = 18.06$ Cmmol S / L / h), the production rate of PHA was the highest ($r_{\text{PHA}} = 20.46$ Cmmol PHA / L / h), the maximum accumulation of PHA was 25.81% of the cell dry weight, and the storage capacity of PHA was 1.13 Cmmol PHA Cmmol/S, which indicated that carbon source was being used for the accumulation of PHA. The results also showed that the formation and substrate conversion of PHA was inhibited in the acidic pH range of less than 7. The overall production performance of PHA could be improved by controlling the pH in weak alkaline conditions. Consistent with the previous report of other authors, the substrate's polymer yield is higher than different pH values (0.33 Cmmol PHA Cmmol/S) at pH 8.8-9.2, indicating that carbon source is being used for PHA accumulation[14].

The above experimental results show that pH affects the metabolic process of MMC, so it has a significant influence on the synthesis. According to the experiment's kinetic analysis and component results, the accumulation capacity of PHA is the strongest under the weak alkaline condition without controlling the pH value. The synthesis amount tends to decrease with the decrease of pH value. This may be due to the following reasons

(1) The effect of pH on enzyme activity is the main reason for decreasing PHA synthesis ability at low pH. As the water was developed with sodium acetate as a carbon source, it was weakly alkaline. The strain of PHA synthesis in activated sludge adapted to this condition had better adaptability to alkaline conditions. When the pH was adjusted to be acidic, the enzyme activity might be affected. The enzyme is the fundamental factor of a biochemical reaction. There are a lot of amino acid side chain groups in its molecule. The action of the enzyme is mainly through the dissociation of these side-chain groups. With different pH, the dissociation state of these side-chain groups will be other. The proportion of specific ionic groups with catalytic activity in the total amount of enzyme will be different, so the enzyme's catalytic ability will be different[23].

(2) The electronegativity of bacteria changed. The lower pH has reached the electric point of some bacteria, and the surface charge of bacteria has changed from negative to positive. Some extracellular secretions and active substances adsorbed on the surface of bacteria dissociate into the solution, leading to the unstable COD change. The bacteria can not absorb the carbon source matrix to synthesize PHA, so the synthesis ability is reduced. As shown in[24], when the acid pH values were 4.0, 5.5, and 6.5, PHA accumulation was the lowest, and no apparent PHA accumulation phenomenon was observed. This phenomenon can be explained as follows: under low pH value, acetate is still in the form of undissociated, and this undissociated acetic acid will rapidly diffuse into bacterial cells, and then it dissociates and exerts an

intracellular proton load, thus reducing the intracellular pH value. This decrease in internal pH may result in a reduction of PHA production.

3.3 Influence of nutrient ratio on PHA synthesis by activated sludge

The ratio of C / N and C / P is a significant factor affecting PHA content synthesized by activated sludge. Under the condition of limiting nutrient ratio, the content of PHA synthesized by microorganisms is higher. In this section, pH = 7.5 is controlled under anaerobic oxygen limiting process because previous results (Section 3.2) have shown that weak alkaline conditions without pH control under this process are most conducive to PHA production. The second group of tests was conducted by gradually reducing nitrogen and phosphorus concentration in the influent water. Variation of PHA content in a running cycle is shown in Fig. 3.

It can be seen from Fig.3 that in the process of limiting phosphorus source, the content of PHA showed a rising trend in the anaerobic stage but gradually decreased in the oxygen limiting stage. Since in the anaerobic phase, microorganisms use the energy released from polyphosphate esters to absorb organic substrates (VFAs) and store them in the internal carbon source (PHA). The oxygen limiting section consumes the inner storage material PHA when the external carbon source is insufficient, so the synthesis trend is shown in the figure. It can be seen from Fig.3 that when the C: P ratio increases from the initial 100 to 150, the PHA content presents a significant upward trend and reaches the highest value at the end of anaerobic treatment, at this time, the content accounts for 36.07% of the dry sludge weight; if the C: P ratio continues to increase, the content of PHA is gradually decreased. When the C: P ratio increased to 200, 250, and 500, the content of PHA did not fall immediately at the initial stage of oxygen limitation but continued to increase. The following analysis is made on this situation. Due to the continuous reduction of phosphorus source in the system, the absorption and utilization of carbon source by activated sludge are hindered, and part of carbon source will remain at the end of the anaerobic process. Microorganisms make extensive use of organic matter, which can accumulate intracellular storage material PHA and synthesize polyphosphate ester and glycogen and other energy storage materials at the same time. Therefore, a large amount of oxygen needs to be consumed. At the initial stage of oxygen limitation, the reactor's actual dissolved oxygen concentration is always deficient, which is actually in micro oxygen or low oxygen state. Some studies have demonstrated that the micro oxygen state is more beneficial to PHA accumulation[25]. Therefore, microorganisms use some of the capacity provided by dissolved oxygen to convert the residual organic matter into PHA, resulting in the phenomenon of PHA synthesis after the start of the oxygen limiting stage. The results showed that limiting phosphorus concentration significantly affected PHA accumulation by activated sludge mixed bacteria. However, excessive phosphorus limitation will lead to the deterioration of sludge settling performance, increased flocculent sludge viscosity, and decreased synthesis. With the increase of the limiting ratio, the extracellular substances with high water content were secreted by the microbial micelles, which caused the non-filamentous sludge bulking to hinder the further improvement of PHA synthesis[26].

Similar to the phosphorus limit test, it can be seen from the figure (b) that the content of PHA synthesized by activated sludge keeps increasing when the C: N ratio increases from 20 to 150. When the C: N ratio increased from 20 to 150, PHA reached the maximum at 0.5 h of oxygen limitation, which accounted for 50.39% of the cell dry weight. It have shown that under the condition of low nutrient concentration, microorganisms will be stimulated to enter the metabolic pathway of PHA accumulation rather than their biomass growth[20]. Under the condition of limited nutrients, the biosynthesis process of cellular materials is also inhibited to a certain extent, and Acetyl CoA, the intermediate product of metabolism, is transformed from the material needed to flow to the TCA cycle to form synthetic bacteria into the PHA synthesis pathway. The experimental results are similar to those obtained by[23], which indicates that excessive limitation of nitrogen concentration will lead to the proliferation of filamentous bacteria, resulting in poor sludge settleability and sludge bulking, thus reducing the PHA content. The results showed that no matter which nutrient concentration was limited, the PHA synthesis capacity of activated sludge was more substantial than that of the initial acclimation stage, which helped improve the synthesis capacity of PHA.

Besides, combined with this paper's study, it was found that the maximum synthesis of PHA under nitrogen limitation (50.39%) was higher than that under phosphorus limitation (36.07%), indicating that nitrogen limitation was more conducive to the accumulation of PHA than phosphorus limitation. As reported in[14,19], higher PHA content was obtained under N restriction than p restriction. (51% and 59% respectively). However, excessive nitrogen and phosphorus limitation will result in sludge bulking. It is an important problem to control sludge bulking while PHA production is high.

3.4 Influence of N-limitation and P-limitation on monomer components

At present,a report founds PHA synthesis from activated sludge is mainly short-chain PHA, and the main PHA monomers are 3-hydroxybutyric acid (PHB) and 3-hydroxyvaleric acid (PHV)[27]. According to the monomer structure or content, the properties can vary from hard to soft to elastic. For example, PHB is a homopolymer with poor mechanical properties, easy pyrolysis, poor solvent resistance, and high crystallinity, which makes the material hard and brittle, which is not conducive to processing and production, and limits its commercial application; In contrast, the homopolymer PHV has good physical flexibility and can be widely used as a good processing material. To adjust and control PHA with different performance to meet additional production requirements, the monomer composition of PHA reached the maximum synthesis under five different nutrient ratios (C / N ratio of 20, 60, 100, 120, 150; C / P ratio of 100, 150, 200, 250, 500) was further analyzed, and the influence of different nutrient ratio on the monomer composition of PHA was evaluated. The results are shown in Fig.4.

Fig.4 compares and analyzes the proportion of PHB and PHV under the limitation of two nutrient sources. With the decrease of phosphorus concentration, the content of PHB and PHV showed no obvious change rule when the synthesis of PHA was the highest. Since the composition of PHA is mainly determined by the type of substrate, acetic acid is used as a carbon source in the experiment, so PHB is the main product of PHA synthesized by activated sludge. When the C / P ratio was 100, PHB accounted for

67.86%. When the C / P ratio was 150, 200, 250 and 500, the proportion of PHB / PHA was 66.01%, 66.49%, 66.70% and 66.19%, respectively. The ratio of PHV / PHA was 32.14%, 33.99%, 33.51%, 33.3% and 33.81%, respectively. Under the condition of nitrogen limitation, it can be seen from the figure that the decrease of nitrogen concentration has an observable promoting effect on the accumulation of PHV when the C / N ratio increases from 20 to 150, the proportion of PHV / PHA is continually increasing, from 33.79% to 49.14%. However, the proportion of PHB / PHA decreased gradually with the C / N ratio increase. When the C / N ratio was 20, 60, 100, 120 and 150, the proportion of PHB / PHA was 66.21%, 65.42%, 60.59%, 53.81% and 50.86%, respectively.

The results showed that PHB was the principal monomer for cell synthesis, no matter the nitrogen or phosphorus limitation. Increasing the C / N ratio is beneficial to increase the proportion of PHV in PHA, but with the increase of the C / P ratio, the change of PHB and PHV is not apparent. Therefore, the final product of PHA was realized, which also provided the possibility of controlling the composition of PHA product by regulating the substrate composition in the future.

4. Conclusion

- (1) Start the reactor with an anaerobic-oxygen limited process, after three days, eight days, and 11 days of operation, the system has a good effect of phosphorus removal, accompanied by a certain amount of PHA synthesis phenomenon;
- (2) The weak alkaline pH value is the suitable condition for the synthesis of PHA by an anaerobic-oxygen limited process. When the pH value is 6.0 and 5.0, the synthesis rate of PHA is significantly reduced, and even the system loses the ability to synthesize PHA;
- (3) Under the condition of limiting nutrient ratio, higher PHA content can be obtained. Limiting nitrogen can promote the synthesis of PHA by activated sludge more than limiting phosphorus. When the ratio of C / P and C / N were both 150, the production of PHA was the highest, accounting for 36% and 50.39% of the dry sludge weight, respectively. However, excessive restriction of nutrient concentration will lead to sludge bulking. Nitrogen limitation causes filamentous bacteria bulking, while phosphorus limitation leads to viscous water bulking. Regardless of limiting the source of phosphorus or nitrogen, cells are more inclined to synthesize PHB. Besides, increasing C / N ratio can effectively improve the proportion of PHV in PHA monomer synthesized by activated sludge.

Declarations

Ethical Approval

Not applicable

Consent to Participate

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Consent to Publish

We undersigned declare that this manuscript entitled “The recovery and synthesis of polyhydroxyalkanoates in the process of nitrogen and phosphorus removal in low-carbon sewage based on pH and nitrogen and phosphorus restriction under anaerobic-oxygen limited process” is original, has not been published before and is not currently being considered for publication elsewhere.

Authors Contributions

Kequan Zhang. (Master student) conducted the experiment and finished this manuscript. Qian Fang. (Professor) designed the whole experiment and provided laboratory equipment. Yihan Xie (Master student) participated across the entire investigation. Tong Wei (Master student) and Yujia Chen (Master student) and Yanyu Xiao (Master student) assist in completing data measurement.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

The data sets supporting the results of this article are included within the article.

Author Statement

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose, and manuscript is approved by all authors for publication.

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Figures

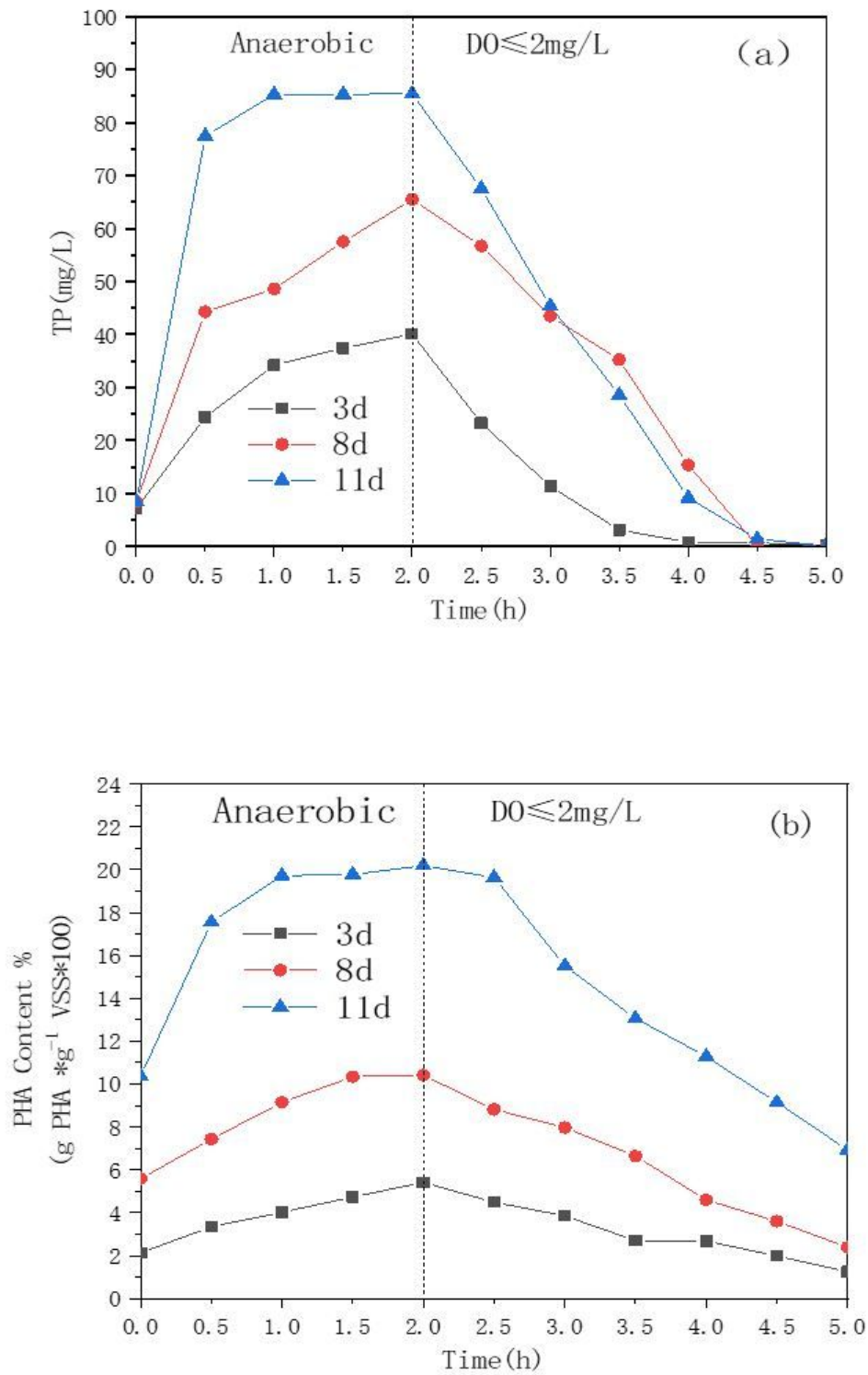


Figure 1

The change rule of dissolved phosphate and PHA content in different days under an anaerobic-oxygen limited process

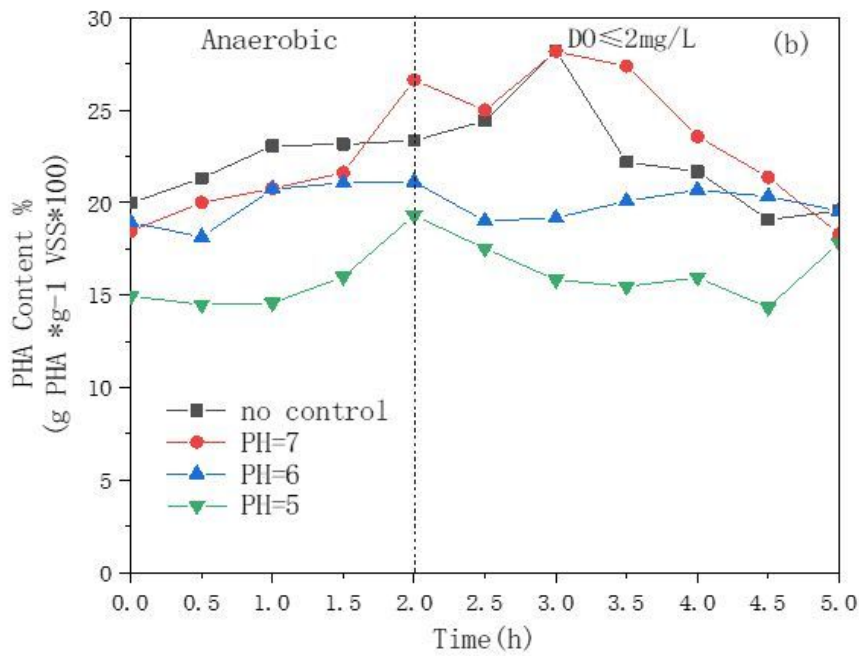
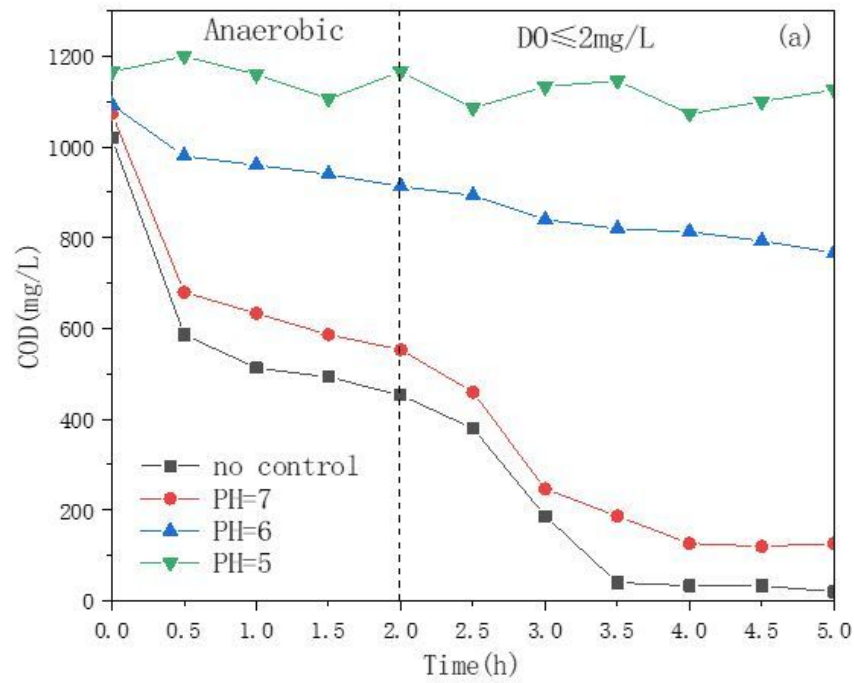


Figure 2

Effect of different pH values on PHA production (a) Degradation of substrates at different pH values under N limitation (b) Effect of pH on PHA production under N limitation

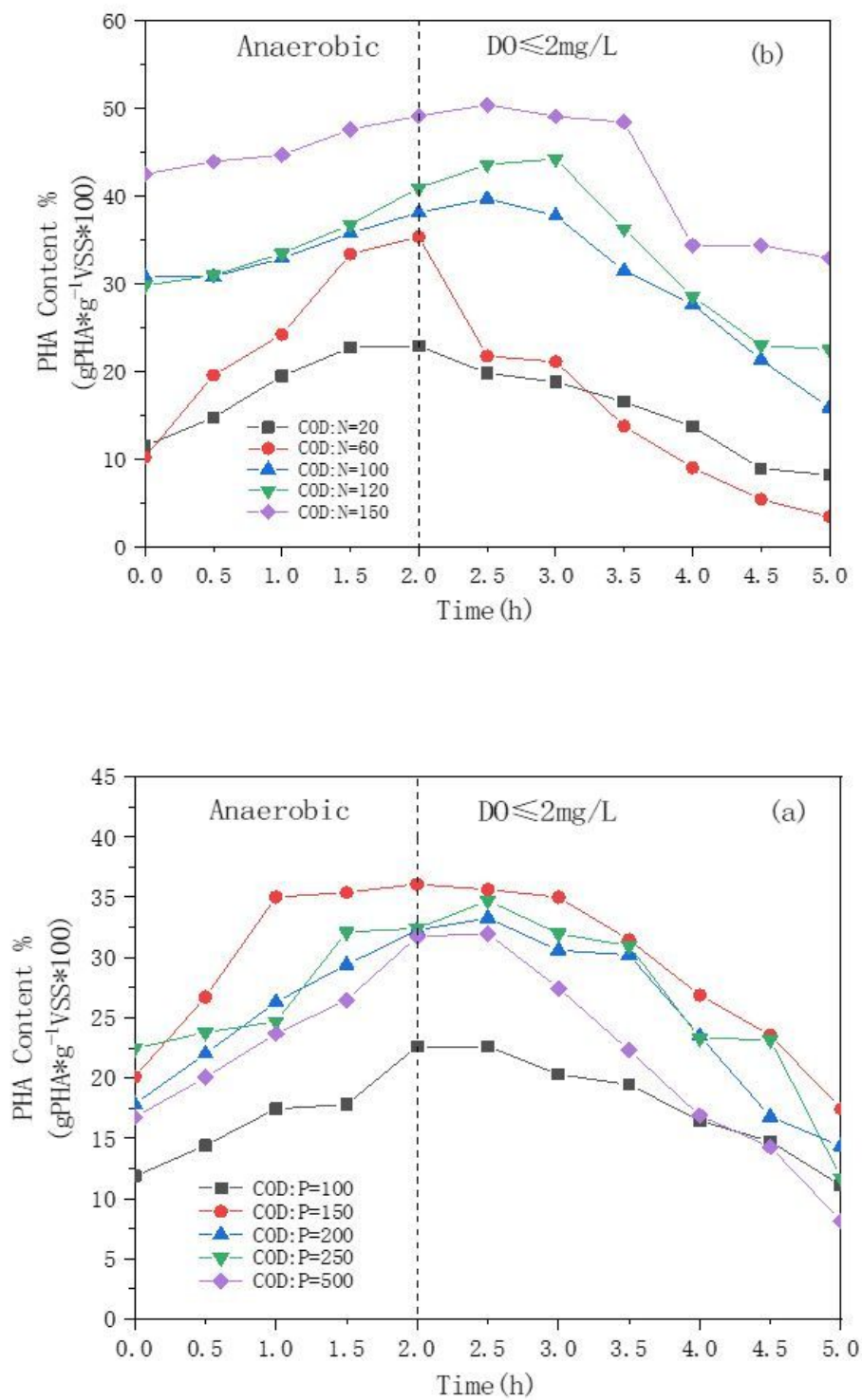


Figure 3

(a) Changes of PHA content under different c/p ratios in one operation cycle. (b) Changes of PHA content under different C/N ratios in one operation cycle.

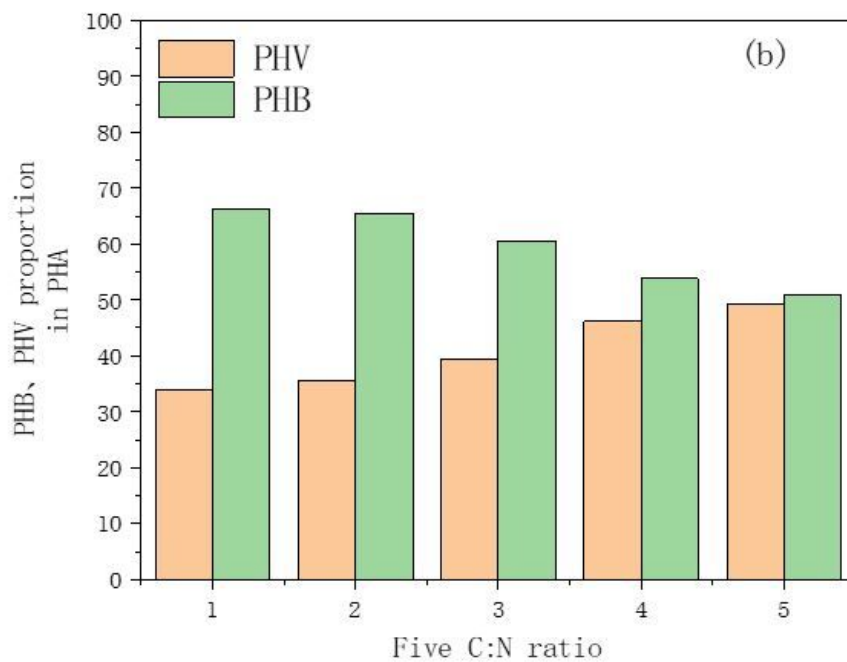
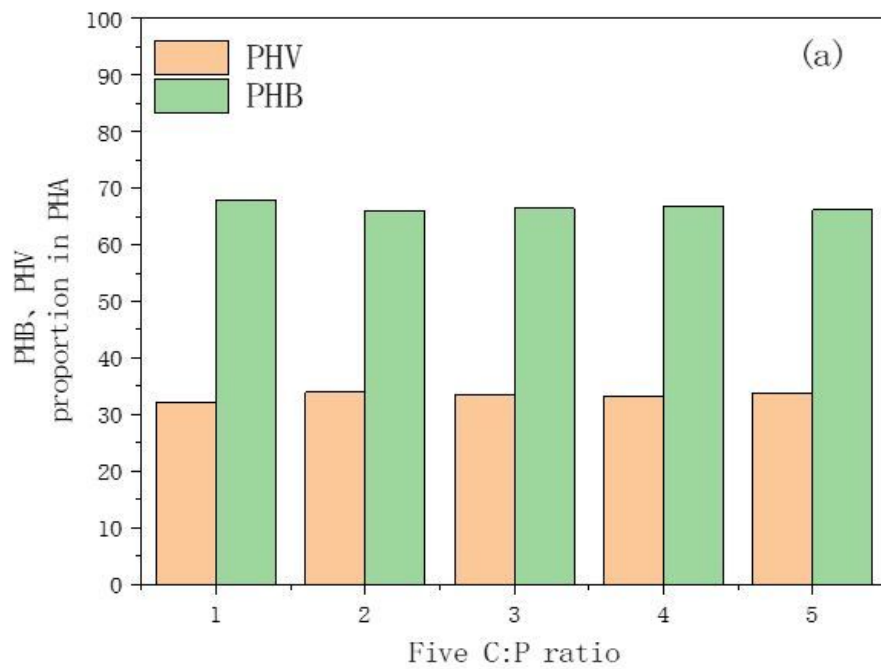


Figure 4

Changes in PHB–PHV proportion in PHA. The picture above is the proportion of PHB–PHV in PHA at different C/P ratios (100–150–200–250–500); the following diagram is the proportion of PHB–PHV in PHA at different C/N ratios (20–60–100–120–150).

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