Enhanced biological phosphorus removal in low-temperature sewage with iron-carbon SBR system

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ABSTRACT
This study proposed an AO-SBR (Anaerobic Aerobic Sequencing Batch Reactor) combined with iron-carbon micro-electrolysis (ICME) particles system for sewage treatment at low temperature and explored the dephosphorisation mechanism and microbial community structure. The experimental results illustrated that ICME particles contributed to phosphorus removal, metabolic mechanism of poly-phosphorus accumulating organism (PAO) and microbial community structure in the AO-SBR system. The optimal treatment effect was achieved under the conditions of pH 7, DO 3.0 mg/L and particle dosage of 2.6 g Fe-C/g MLSS, and the removal rates of COD, TP, NH4+-N and TN reached 80.56%, 91.46%, 69.42% and 57.57%. The proportion of phosphorus accumulating organisms (PAOs) increased from 4.54% in the SBR system to 10.89% in the ICME-SBR system at 10°C. Additionally, the metabolic rate of PAOs was promoted, and the activities of DHA and ETS both reached the maximum value of 13.34 and 102.88 μg·mg−1·VSS·h−1. These results suggest that the ICME particles could improve the performance of activated sludge under low-temperature conditions. This technology provides a new way for upgrading the performance of sewage treatment in the cold area.

1. Introduction

To prevent the eutrophication of natural water bodies and maintain good water quality, limits are set to the emissions of nutrients for treated wastewater that is returned to the nature. Nitrogen and phosphorus are important nutrient sources, so far, compared with physical–chemical treatments, biological nitrogen and phosphorus removal are being more effective, relatively inexpensive, environmental and widely used in sewage treatment. Among them, for high concentration sewage, it is more suitable to adopt microbial anaerobic treatment first, and then improve it through aerobic microorganisms. The operation cost is low and the treatment effect is more effective [1–3]. Some studies have used Anaerobic Fluidised Bed Reactor/Aerobic Moving Bed Bio Reactor (AFBR/MMBR) combination to treat currant wastewater at 35°C and achieved good treatment results.

Temperature is the key parameter that affects microbial activity. The temperature is lower than 10°C, which brings difficulties to sewage treatment, the sedimentation performance of activated sludge become worse, the adsorption capacity of activated sludge decrease and the community structure becomes simple [4]. According to this problem, most wastewater treatment plants usually adopt the chemical agent, reducing the pollution load, increasing the return flow of sludge and the residence time of sewage to ensure the effluent standard of sewage at low temperature. These measures could not always meet the requirements of sewage treatment and would increase the project investment and operation cost; therefore, it is necessary to explore a more effective method to
improve the poor performances of the traditional process at low temperature. Iron carbon micro-electrolysis (ICME) is a kind of technology, which mainly uses the principle of electrochemical corrosion and a series of synergistic effects such as flocculation, precipitation and adsorption to remove pollutants. In the past few years, the method has been used for various wastewater treatment, including dyeing wastewater [5,6] pharmaceutical wastewater [7] coking wastewater [8], landfill leachate [9], electroplating wastewater [10] and oily wastewater [11]. Micro-electrolysis technology expands new ideas for denitrification and dephosphorisation in wastewater due to its advantages of cost-effective, operational simplicity and high efficiency. It has been reported [12] that ICME was introduced into the traditional anaerobic/anoxic/oxic process to strengthen the low C/N and C/P ratios sewage treatment. The Fe/C-based denitrification and dephosphatisation processes contributed 63.1% of the TN removal and 75.3% of the TP removal, respectively, and the bacteria for nitrogen and phosphorus removal were found dominant in the developed Fe/C-A2O system. Ma [13] has also reported that the integration of micro-electrolysis with biological reactor (MEBR) performed high removal efficiency of COD, the synergistic effect in this integrated process not only improved the properties of activated sludge and improved microbial community diversity but also relieved the passivation of Fe-C filler effectively, thereby maintaining process stability. Iron is a mineral nutrient in microorganisms and an essential substance for the growth of microorganisms. It is a redox carrier, the cofactor for some enzymes and a component of the electron transport chain. As cell components and activators of enzyme activity, an appropriate amount of iron ions could promote biological reactions [14]. In addition, chemical phosphorus precipitation also contributes greatly to phosphorus removal [15]. The chemical reaction between phosphate and iron ions in wastewater is very complex, which will form a large number of products, including complexes, polymers and precipitates. Fytianos [16] proposed a chemical precipitation model for removing phosphorus from sewage by iron, which includes 15 chemical reactions and 4 solid phases. Meanwhile, zero-valent iron can also be used to reduce nitrate [17,18]. By combining microorganisms with micro-electrolysis, [H]/H2 and Fe2+ generated by micro-electricity provide electron donors for autotrophic denitrifying bacteria and achieve synchronous nitrification and denitrification [19,20].

The integrated technology of micro-electrolysis and biotechnology has a great future for wastewater treatment. However, several problems have been encountered with the mentioned studies on traditional ICME, including the blockage and deactivation of electrode materials, which leads to frequent material replacement [21,22]. Meanwhile, bentonite was selected as the adhesive for microelectrolytic particles in many studies and occupies a higher proportion, making the ineffective components in microelectrolytic particles increased. In this study, waterborne polyurethane is selected as the adhesive. It is a polymer with three-dimensional mesh structure. It can absorb water and keep the original structure from being dissolved, stabilise the microelectrolysis particle structure, prolong the service life and is suitable for microbial enrichment, and adding a metal catalyst to effectively solve the problem of particle passivation.

Herein, in the present work, the new iron-carbon micro-electrolysis (ICME) particles were synthesised, coupling with the sequencing batch reactor (SBR) were developed for sewage treatment at 10°C low temperature. The objectives are (1) to develop novel ICME particles for treating low-temperature wastewater, (2) to investigate the effect of ICME particles on the improvement of nitrogen and phosphorus removal in SBR system and the promotion of phosphorus accumulation bacteria metabolism and (3) to analyse the microbial community structures through high-throughput detection technology. More importantly, this work not only proposed novel ICME particles as an efficient low-temperature sewage treatment material but also provided a value-added reuse approach for scrap iron in line with the concept of resource recycling and environmental sustainability.

2. Materials and methods

2.1. Preparation of ICME particles

ICME contained raw materials with sponge iron (65% mass), activated carbon (22% mass), catalyst A (5% mass of Cu), composite catalyst B (3% mass of Co, Mn, Ti and Ni), pore-forming agent X (2.5% mass of ammonium carbonate), additive Y (2.5% mass of cement) and adhesive (waterborne polyurethane). ICME was obtained by drying in a strictly anaerobic atmosphere. The production process of ICME particle is described as follows: Firstly, sponge iron was soaked in 10% sodium hydroxide solution for 120 min to remove oil stains on the surface, soaked in dilute hydrochloric acid for 60 min to remove oxides on the surface and then washed to neutral with ultra-pure water and dried. Next, the raw materials were mixed and well stirred according to different mass proportions. 0.24% N, N-methylene-bisacrylamide and 1.5% potassium sulfate were added by mass percentage to the
aqueous polyurethane with 10% solid content, which was mixed with the mixture and stirred evenly. The mixture pelleted 5–10 mm in diameter. Finally, the prepared particles were put into a vacuum drying oven, and the particles upper surface was covered with activated carbon powder, which was dried at 100°C for 60–90 min under the condition of no oxygen.

### 2.2. ICME-SBR system

An iron-carbon micro-electrolysis coupled sequencing batch reactor (ICME-SBR) was used in the test, as illustrated in Figure 1. The main body of the reactor was double-layer plexiglass with a height of 85 cm, an inner diameter of 14 cm and a working volume of 12 L. Water sampling taps were set on the side of the reactor. Sludge sampling openings were set at the bottom of the reactor. Microporous aeration plate was set in the reactor bottom to connect with air compressor for aeration and DO online monitor to ensure a stable DO environment in the system, and the electric mixer was set on the top to mix sludge and wastewater. The outer layer connected with the water bath device for the water bath cycle, the device temperature was maintained by a thermostatic bath. The pH in the reactor was adjusted using 0.01 mol/L HCl or 0.01 mol/L NaOH and controlled in real-time by using a pH detection probe, acid pump and alkali pump. The reactor was operated by anaerobic/aerobic. The SBR ran with four 6 h-cycles per day, each cycle consisted of instantaneous feeding, 120 min anaerobic reaction, 120 min aerobic reaction, 30 min settling, 15 min decanting and idling, the whole process was controlled by a time controller and running cycle were consistent with the SBR operation cycle. The device temperature was main-
and X-ray diffraction XRD (D8 Advance, Bruker Co. Ltd., Germany). The BET surface area was quantified by a fully automatic specific surface area (TriStar II 3020, Micromeritics Co. Ltd., USA).

Microbial community structure was analysed by high-throughput sequencing (Illumina NovaSeq,Novogene Co. Ltd., China). DNA was extracted using the CTAB method. The V3–V4 region of microbial 16S rRNA was amplified.

**Figure 1.** Schematic of the test device.

**Figure 2.** Internal structure and XRD characterisation of ICME particles ((a) particle appearance; (b) SEM photograph at 2000x of inner structure; (c) XRD within particles).
with the barcoded forward primer 341F (CCTACGGGNGGCWGGCAG) and reverse primer 806R (GGACTACNNGGGTATCTAAT). The PCR was performed with phusion8 high-fidelity PCR matrimix with GC buffer and high fidelity enzyme from New England Biolabs to ensure amplification efficiency and accuracy. Then, the PCR products were quantified accurately (TuSeq®DNA PCR-Free Sample Preparation Kit) and were used for high-throughput sequencing (NovaSeq6000).

2.5. Statistical analysis

All experiments were conducted in duplicate, the daily results were obtained by averaging the values of repeated measurements. And the results were expressed as mean ± standard deviation. Data were analysed by one-way ANOVA to estimate the significance of the results and $p < .05$ was statistically significant.

3. Results

3.1. Characterisation and properties of ICME particles

The appearance, internal morphology and metal element composition of ICME particles were shown in Figure 2. ICME particles were granular with a particle size of 0.8 ± 0.04 cm. Under the joint action of binder and pore-forming agent, a distinct porous structure was formed, which can provide a place for microorganisms to attach, so that microorganisms are not easy to lose and maintain high microbial biomass in the reactor. The specific surface area of ICME particles was 25.40 ± 0.12 m²/g, the average pore diameter is 4.53 ± 0.13 nm and the total pore volume is 0.012 ± 0.026 cm³/g, respectively, which have good resistance to plate binding. In addition, the higher strength of ICME particles could withstand a larger load. It was not easy to be broken and worn in the reactor and has high stability, which reduced the physical loss in the water treatment process.

The XRD results showed the main substances in ICME particles include Fe and C, which were the components of the electrode. Fe was rarely oxidised and could form a large number of fine catalytic galvanic cells. ICME particles are suitable as biological carriers for wastewater treatment [19].

3.2. ICME-SBR performance under the varying influence factors

3.2.1. Influence of particle dosage

Figure 3 depicted the COD, TP, NH₄⁺-N and TN removal efficiency under different ICME particle dosages with pH 7.0 ± 0.2 and DO of 2 ± 0.5 mg/L in the aerobic stage. The results showed that with the addition of Fe-C particles in a low-temperature environment, the removal rate of all water quality indexes was improved obviously, particularly phosphorus. Under the influence of a low-temperature environment, the synthetic wastewater of SBR no addition Fe-C particle was poor, and all water quality parameters cannot meet the discharge standards. With the addition of dosage, the removal rate of COD increased gradually. When the dosage was 2.6 g Fe-C/g MLSS, the average COD concentration of the effluent was reduced to 40.79 mg/L, and the average removal rate was increased by 11.34%. The TP removal efficiency increased firstly and then stabilised, the average removal efficiency increased from 65.57% to 73.73% with increased ICME particle dosage from 0 to 1.7 g Fe-C/g MLSS. The optimal treatment effect of TP was achieved when the particle dosage was 2.6 g Fe-C/g MLSS, the corresponding average removal efficiency and effluent concentration were 90.09% and 0.92 mg/L. The same phenomenon was also presented by Zhang et al. [27], who had improved the biological aerated filter by configuring ICME particles to improve the removal efficiency of COD and TP, and the removal efficiency of COD and TP have reached 95% and 80%, respectively. Continue to increase the dosage of Fe-C particles, the removal rate has not been improved, and there is a risk of increasing sludge production. It could be seen from the removal efficiency that microorganisms play a major role in the reaction system, and the addition of ICME particles plays a promoting role.

It was obvious that dosing of ICME particles was effective for the reduction of nitrogen. When the dosage was enhanced from 0 to 2.6 g Fe-C/g MLSS, NH₄⁺-N removal efficiency significantly increased, and the corresponding removal efficiency increased from 43.78% to 65.34%. NH₄⁺-N was transformed into nitrate and/or nitrite, and the anoxic corner in the system made it continue to undergo denitrification and converted into nitrogen. The TN removal efficiency was reached up to an average of 51.72% at the dosage of 2.6 g Fe-C/g MLSS. Consequently, consideration of the treatment effect and economic factors, the dosage of 2.6 g Fe-C/g MLSS, was selected as the optimal dosage for subsequent tests.

3.2.2. Influence of pH

Under the condition of ICME particle dosage of 2.6 g Fe-C/g MLSS, the influence of pH on wastewater treatment of ICME-SBR system was shown in Figure 4. The removal efficiency of COD and TP both maintained at a relatively higher level. In the pH range of this test, pH value has little effect on COD removal, with the corresponding
average removal efficiency and concentration of 77.74% and 43.26 mg/L at a pH value of 7. The removal efficiency of TP increased first and then generally decreased when the pH value increased and the highest reduction of which was observed at pH 7 accounting for 91.67%. At a pH value of 8, the TP removal efficiency can still reach 78.66%.

The effect of pH on the removal effect of NH\textsubscript{4}\textsuperscript{+}-N and TN presented firstly increased and then decreased. The phenomenon was similar to TP removal. The removal efficiency of NH\textsubscript{4}\textsuperscript{+}-N and TN was 61.41% and 52.07% at pH 6.5, respectively. The removal efficiency was the highest when the pH was 7, the average removal rate of NH\textsubscript{4}\textsuperscript{+}-N and TN was 60.08% and 70.61%, and the average effluent concentration was 6.21 and 9 mg/L. The removal rates of NH\textsubscript{4}\textsuperscript{+}-N and TN decreased by 8.97% and 8.01%, respectively, as pH increased from 7 to 8. When the pH was increased to 8, the activity of nitrifying bacteria and the micro-electrolysis reaction were both affected, the concentration of NH\textsubscript{4}\textsuperscript{+}-N in the effluent increased slightly. In this study, the most suitable pH was 7.

3.2.3. Influence of DO
DO is an important control parameter in the process of biological nitrogen and phosphorus removal. Figure 5
depicted the COD, TP and nitrogen removal efficiency under different DO in aerobic reaction. The removal efficiency of COD gradually increased and stabilised with the increase of DO. Because the COD in domestic sewage was low and biodegradable, the effect of DO on COD removal was not obvious, compared with TP. When the DO was 3 mg/L, the average COD concentration of effluent was 38.58 mg/L. TP removal efficiency initially increased and then steadily decreased with the increased DO. For the investigated DO range of 1–3 mg/L, TP average removal efficiency was found to increase from 73.86% to 91.47% with increased DO. The too low DO was unfavourable for the PAOs aerobic phosphorus uptake. In this study, the TP average removal efficiency apparently decreased to 84.10% in the condition of DO was 4.

It was significant that an increase in the DO was effective for the reduction of NH$_4^+$-N and TN. NH$_4^+$-N average effluent concentration decreased from 13.48 to 4.14 mg/L with increased DO from 1 to 4 mg/L. The removal efficiency of NH$_4^+$-N and TN showed a similar tendency and the highest reduction of which was obtained at DO 4 mg/L, and the corresponding removal efficiency was 80.29% and 61.28%. Under the condition of DO was 3, the average removal efficiency of NH$_4^+$-N and TN was 69.41% and 57.56%, the average effluent concentration was 6.24 and 9.27 mg/L.

### 3.2.4. Metabolic characteristics of PAOs in ICME-SBR

The variation of COD, phosphorus, glycogen, polyhydroxyalkanoates (PHB) and poly-phosphate (ploy-P)
concentration in stable anaerobic–aerobic ICME-SBR system at a ICME particle dose of 2.6 g Fe-C/g MLSS, DO of 3 mg/L, pH7.0 was highlighted in Figure 6. In the anaerobic P-release phase, activated sludge polyphosphate and glycogen content showed a downward trend, and the PHB showed an upward trend. The corresponding decomposition at anaerobic for 2 h was 83.78 and 45.53 mg/g VSS, PHB production was 78.93 mg/g VSS. At the end of the anaerobic P-release, as the organic carbon source decreased, phosphorus content increase gradually in sewage. COD concentration reduced to 34.87 mg/L, the anaerobic stage played the most important role in the removal of COD, the same conclusion was obtained in Jalil’s study [1]. TP concentration reached 31.63 mg/L, which was 2.76 times the initial TP concentration. In the aerobic P-uptake phase, the poly-P and glycogen in the sludge gradually accumulated to 135.78 and 109.34 mg/g VSS at the end of the reaction. Concurrently, there was a steady decrease in the curves of PHB and TP, and the effluent TP concentration was 0.77 mg/L, PHB decomposition was 82.74 mg/g VSS. Besides, the curve of COD concentration decreased continuously in the aerobic stage, the effluent COD concentration at the end of the reaction was 23.92 mg/L.

The degradation of nitrogen, phosphorus and organic matter in sewage by activated sludge was accomplished primarily through the redox system of microbial enzymes, the activity of DHA (dehydrogenase) and ETS (electron transporter) reflects the number of active
microorganisms and the ability to metabolise organic matter. The changes of DHA and ETS activity values in a typical cycle were shown in Figure 5. At low temperatures, the addition of ICME particles kept the microbial enzyme activity values at a relatively high level. The value of DHA and ETS showed a similar tendency in the typical cycle, consistent with the study by Trevors [28]. The measured DHA and ETS activity values increased by 5.31 and 25.97 μg·mg⁻¹ VSS·h⁻¹ by the 30 min anaerobic, which was the maximum in the anaerobic phase. The enzyme activity that increased rapidly could be partly because the organic molecular particles in the simulated domestic sewage were small and easy to be absorbed and utilised by activated sludge microorganisms. At the end of the anaerobic phase, the DHA and ETS activities were 8.90 and 82.47 μg·mg⁻¹ VSS·h⁻¹, respectively. In the aerobic stage, microorganisms could quickly adapt to the aerobic environment, and the activities of DHA and ETS both reached the maximum value of 13.34 and 102.88 μg·mg⁻¹ VSS·h⁻¹, respectively, after 30 min of aerobic treatment, and then showed a downward trend. The DHA and ETS gradually decreased to 6.54 and 75.65 μg·mg⁻¹ VSS·h⁻¹ with substrate consumption in the stage of aerobic phosphorus absorption, respectively, it was a sharp contrast to aerobic 30 min.

3.3. Microbial community in ICME-SBR

3.3.1. Microbial diversity
Microbial diversity was analysed at three different stages by high-throughput sequencing. The sample diversity index abundance statistics table of the three samples was shown in Table 1. It contains 1670, 1149 and 1116 OTUs, respectively. The coverage values of all samples were over 99% indicating that this
sequencing was sufficient to cover the whole microbial community.

The diversity of the sample was analysed using OTUs, Chao1, ACE, Shannon and Simpson indices. The Chao1 and ACE indices could reflect the total number of species in the system. The Chao1 and ACE indexes of the three samples were G1 > G3 > G2. Under the influence of low temperature, the total number of species in G2 and G3 samples decreased compared with G1 sample, and the total number of microorganisms increased after adding ICME particles. Shannon and Simpson indices were used to characterise species richness and evenness [14], respectively. According to statistical analysis, the order of the Shannon index from high to low was G3, G2 and G1 for the three phases. The Simpson index showed a similar trend in the three phases.

### 3.3.2. Microbial community structure in phylum and genus levels

As shown in Figure 7, the difference of microbial communities between G1, G2 and G3 was revealed at the phylum level. The dominant microbial populations were similar at the phylum level across the three samples, including the Proteobacteria, Bacteroidota, Actinobacteriota, Acidobacteriota, Myxococota and Firmicutes, etc. Similar finds were observed in sewage treatment systems [29]. Proteobacteria was predominant and accounted for 39.6%, 31.6% and 48.8%, respectively, which suggested Proteobacteria played a dominant role in phosphorus removal in G1, G2 and G3. The percentages of other phyla in G1, G2 and G3 were 13.7%, 24.4% and 18.47% for Bacteroidota, 14.95%, 22.91% and 15.89% for Actinobacteriota, and 14.95%, 22.91% and 15.89% for unidentified_Bacteria, respectively, which suggested Proteobacteria played a dominant role in phosphorus removal in G1, G2 and G3.

Figure 8 showed the relative abundances of the dominant genera in each sample, they also present the differences in microbial communities among the samples. In G1, the dominant genera mainly included denitrifying bacteria and nitrifying bacteria. Denitrifying bacteria such as Denitratisoma [30] (belonging to Rhodocyclaceae) and Dechloromononas, nitrifying bacteria such as unidentified_Nitrospiraceae. In the sludge of ICME-SBR, the dominant genus was Candidatus_Accumulibacter, which accounts for 8.19% of G3 bacteria, by contrast, the proportion of genus Candidatus_Accumulibacter in G2 was 3.54% and G1 was 0.6%. This percentage suggests that Candidatus_Accumulibacter proliferated rapidly and played a key role in the ICME-SBR. The second dominant bacteria genus in ICME-SBR sludge was Thermonas, comprising 7.33% in G3. Other genera exhibited increased population in G3 included Acinetobacter (2.69%), Zoogloea (2.23%), Dokdonella (1.19%), Hydrogenophaga (1.17%) and Acidovorax (1.09%). Other genera that account for more in G3 included Defluviicoccus (3.64%), Terrimonas (3.55%), Kineosphaera (3.18%), Ferruginibacter (2.25%), Flavobacterium (1.77%) and Ellin6067 (1.69%), some of these genera were reportedly related to denitrification and organic degradation [27–34].

### 4. Discussions

#### 4.1. Effect of the influence factors on the ICME-SBR performance

The addition of ICME particles has improved the treatment effect of low-temperature sewage. Iron ions could increase the permeability of cell membranes, accelerate the absorption of nutrients and promote the progress of wastewater treatment. Microorganisms play a leading role as the main component of the system during sewage treatment, and ICME particles could improve sewage treatment efficiency as the immobilised carrier of microorganisms [27]. The efficient removal of TP was attributed not only to the fact that ICME particles could promote microbial metabolism but also to the chemical phosphorus precipitation caused by iron ions. Iron was widely used as a coagulant in phosphorus removal [35,36]. In addition, the adsorption properties of the microelectrolysis particles were also used in the Deng Shihai study [15].

pH affects the growth, reproduction and metabolism of microorganisms by affecting microbial enzyme activity, microbial cell plasma membrane charge distribution and cell permeability. When the pH value is lower than 6.5 [37], microbial growth was affected by the slowing down of metabolic activity. However, a lower pH value was more conducive to the progress of the micro-electrolysis reaction, the amount of Fe$^{2+}$ generated during ICME was much higher at lower pH conditions, the Fe$^{2+}$ produced by the micro-electrolysis was oxidised to Fe$^{3+}$ in an oxygen environment and phosphorus could be removed by iron phosphate precipitation [38], this ensures a high TP removal efficiency at a lower pH value. In this study, it was found that the synergistic effect of pH 7 microorganism and micro-electrolysis on TP treatment was the best. It was previously reported that at low temperature (10°C), pH 7.5 was more suitable for the growth of PAOs than pH 7 [39,40]. Higher pH may decrease the abundance of GAOSs and increase the proportion of PAOs [41]. Earlier, neutral to slightly alkaline has been shown to be more conducive to microbial nitrogen removal [42,43]. In Mandel [42] study, the pH of the SBR reactor...
was controlled between 7.4 and 8.3, and a good removal efficiency was achieved. In this test, the treatment effect of pH 7 was higher than pH 7.5, mainly because the promoting effect of micro-electrolysis on microorganisms was greater than that of the effect of pH 7 on microbial activity. Meanwhile, it showed that the micro-electrolytic chemical phosphorus removal effect at pH 7 was better than that at pH 7.5. At a higher pH level, the Fe²⁺ generated by the electrolytic reaction forms ferrous hydroxide with OH⁻, which adsorbs the particles with a weak negative charge in the pollutant and forms floculates to be removed, this process was easy to form a blockage to prevent the reaction.

The experiments performed with different DO revealed that the potential difference of micro-electrolysis reaction would be increased in aerobic condition, and the friction of bubbles could help remove the passivated film on the surface of micro-electrolysis particles, which was helpful to improve the effect of sewage treatment. However, the removal rate of TP is reduced when the dissolved oxygen is too high. Due to the gradual depletion of PHB and saturation of the biomass by poly-P, which would make the subsequent release of phosphorus insufficient, and poor phosphorus removal [44], besides, high DO would increase the abundance of GAOs [45,46] and the consumption of iron carbon micro-electrolytic particles. On the contrary, the higher DO in a certain range is beneficial to the degradation of NH₄⁺-N [47]. According to the Monod equation, the specific growth rate of nitrifying bacteria is affected by the concentration of NH₄⁺-N and DO. When the concentration of influent NH₄⁺-N is constant, the specific growth rate increases with the increased DO.

**4.2. Analysis of metabolic mechanisms of PAOs in ICME-SBR**

In the study of metabolic characteristics of PAO, in the anaerobic P-release phase, PAOs hydrolysed intracellular poly-P into orthophosphate and release it out of the cell, the energy generated is used to absorb volatile fatty acids from sewage and store internal carbon in the form of PHB. Glycogen is also converted to PHB and stored in intracellular [48,49]. In the aerobic P-uptake phase, PAOs excessively absorbed soluble orthophosphate stored intracellular poly-p, which used oxygen as electron acceptor and intracellular polymer PHB stored in the anaerobic stage as carbon source and energy source [50,37]. ICME particles were used as microbial carriers, and microorganisms were attached to their surfaces to effectively prevent microbial loss. After cleaning and drying, the loss of the system is 44 g/year. The operation cost of ICME-SBR system construction is 0.96 yuan/t, treatment effect can meet the Grade I A standard, The efficient removal of COD and TP in the system was realised. The activity of enzymes can be measured by determining the rate of product formation or substrate utilisation [51]. In this system, the enzyme activity in the anaerobic stage was mainly related to the anaerobic P-release of PAOs and organic matter degradation, while the enzyme activity in the aerobic stage is mainly related to the aerobic P-uptake of PAOs. In this experiment, the change of enzyme activity in the anaerobic and aerobic stages was different from the result of Gore’s study that the enzyme activity in the anaerobic stage was relatively high [52]. The enzyme activity in the aerobic stage was higher than that in the anaerobic stage, which indicated that the micro-electrolysis particles had a stronger promoting effect on the microbial activity under aerobic condition.

### 4.3. Microbial community analysis

The large Shannon value indicated high community diversity and a relatively high Sampson index indicated that the evenness of each species was better. The results showed that the species richness and evenness G1 was the highest and G3 was the lowest. It can be seen that the species diversity of sludge samples in the sewage treatment plant was large and evenly distributed. Species diversity continued to decline after the addition of ICME particles, this may have been because the iron enhanced the relative abundance of some microbes leading to a decline in overall diversity and the gradual concentration of community structure.

At the class level, the dominant microbes were Proteobacteria, studies have shown that [53] most of the bacteria with nitrogen and phosphorus removal and denitrifying phosphorus removal functions belong to Proteobacteria, and a small part belongs to Bacteroidota. Bacteroidetes [54] can be involved in biological

| Sample name | observed_species (OTU) | Shannon | Simpson | Chao1 | ACE | Goods_coverage |
|-------------|------------------------|---------|---------|-------|-----|----------------|
| G1          | 1670                   | 8490    | 0.99    | 1879.35 | 1733.409 | 0.997 |
| G2          | 1149                   | 7770    | 0.987   | 1195.172 | 1195.596 | 0.998 |
| G3          | 1116                   | 7587    | 0.986   | 1212.623 | 1210.088 | 0.997 |
phosphorus removal and denitrification processes and metabolise a variety of organic carbohydrates and proteins. Firmicutes [55] also occupy a certain proportion in the system and have a great contribution to the removal of nitrogen in wastewater and the maintenance of system stability. At the genus level, the proportion of PAOs increased from 4.54% in the SBR system to 10.89% in the ICME-SBR system. Candidatus_Accumulibacter

Figure 7. Column chart of relative abundance of species at the phylum level.

Figure 8. Column chart of relative abundance of species at the genus level.
was the dominant PAOs in activated sludge and belongs to the Rhodocyclus family of Proteobacteria [56,57]. Acinetobacter was recognised PAOs, which was first isolated by Fuhs and Chen [21] from activated sludge with high phosphorus removal performance on a specific medium. Thermonas could remove nitrogen and phosphorus at low temperatures. Similar findings were also observed in Wei’s study [58]. There were other bacteria related to nitrogen removal in the system, Terrimonas belongs to sphingobacterium of bacterioidea, a kind of nitrite oxidising bacteria. Nitromonas, a kind of ammonia oxidising bacteria [33], Dokdonella belongs to Proteobacteria, which is also a common denitrification bacteria in sewage treatment. It has nitrification and denitrification and could effectively promote nitrogen transformation. Ferruginibacter has electrochemical activity and could degrade some organic substances [59]. Haliangium mainly degrades organic matter through anaerobic fermentation. In other bacteria with more than 1% relative abundance, Zoogloea is a gram-negative bacteria, obligate aerobic and chemo-heterotrophic, mostly clustered in a shared bacterial micelle. Zoogloea’s abundant extracellular bacteria micelles can also remove phosphorus in large amounts by adsorption. At the same time, Zoogloea also plays an important role in the denitrification process of sewage treatment plants [60]; Hydrogenophagd [61] and Acidovorax [62] were reportedly related to denitrification.

5. Conclusions
The system of ICME-SBR realised the efficient removal of N and P in low-temperature wastewater. When the conditions of pH 7, DO 3.0 mg/L and particle dosage of 2.6 g Fe-C/g MLSS, the concentrations of COD, TP, NH₄⁺-N and TN in the effluent were 38.58, 0.85, 6.24 and 9.27 mg/L, respectively. In the metabolic characteristics of PAOs, the contents of ploy-P and glycogen decreased by 83.78 and 45.53 mg/g VSS and PHB increased by 78.93 mg/g VSS at the anaerobic end. At the aerobic end, phosphorus accumulating and glycogen accumulated to 135.78 and 109.34 mg/g VSS, and PHB decomposed to 82.74 mg/g VSS. Meanwhile, DHA and ETS reached the maximum value of 13.34 and 102.88 μg·mg⁻¹·VSS·h⁻¹ in 30 min of aerobic. The addition of ICME particles increased the abundance of proteobacteria in the reaction system and led to the increase of the typical PAOs Candidatus_Accumulibacter from 3.54% in G2 to 8.19% in G3.

Disclosure statement
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Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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