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

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Abstract

This study proposed an AO-SBR combined with iron-carbon micro-electrolysis (ICME) particles system for sewage treatment at low temperature and explored the dephosphorization mechanism and microbial community structure. The experimental results illustrated that ICME particles contributed to phosphorus removal, metabolic mechanism and microbial community structure. 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, NH_4^+-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 SBR system to 10.89% in ICME-SBR system at 10°C. Additionally, metabolic rate of PAOs was promoted, and the activities of DHA and ETS both reached the maximum value of 13.34 ug·mg^{-1}VSS·h^{-1} and 102.88 ug·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 of the performance of sewage treatment in cold area

Introduction

Nitrogen and phosphorus are important nutrient sources, however, the high concentration of nitrogen and phosphorus in sewage will lead to water eutrophication, which will not only have a great impact on people's lives but also seriously affect industrial and agricultural production. 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. Temperature is the key parameter that affects microbial activity and brings difficulties to sewage treatment. The sedimentation performance of activated sludge become worse, the adsorption capacity of activated sludge decrease, and the community structure and quality reduce. 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 couldn't 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 (Fu et al., 2010; Ruan et al., 2010), pharmaceutical wastewater (Xu et al., 2016), coking wastewater (Lv et al., 2011), landfifill leachate (Ying et al., 2012), electroplating wastewater (Zhang et al., 2014; Wang et al., 2015), and oily wastewater (Cheng et al.,2010). Micro-electrolysis technology expands new ideas for denitrification and dephosphorization in wastewater due to its advantages of cost-effective, operational simplicity and high efficiency. It has been reported (Peng et al., 2020) that ICME was introduced into the traditional anaerobic/anoxic/oxic process to strengthen the low C/N and C/P sewage treatment. The Fe/C-based denitrification and dephosphorization 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-A^2O system. Ma (MA et al., 2018) 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 strengthened activated sludge property 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 activator of enzyme activity, an appropriate amount of iron ions could promote biological reactions (Qian et al., 2018). In addition, chemical phosphorus precipitation also contributes greatly to phosphorus removal (Deng et al., 2017). 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. K.fustianos (Fytianos et al., 1998) 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 .(Susham et al., 2005; Shin et al., 2008; Buresh et al., 1976) By combining microorganisms with micro-electrolysis, [H]/H_2 and Fe^{2+} generated by micro-electricity provide electron donors for autotrophic denitrifying bacteria and achieve synchronous nitrification and denitrification.(Deng et al., 2016a; Deng et al., 2016b)) 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 (Xing et al., 2016; Deng et al., 2016c)

Herein, the new iron-carbon micro-electrolysis (ICME) particles was synthesized, coupling with the sequential batch reactor(SBR) were developed for sewage treatment at low temperature. The effect of ICME particles on the improvement of nitrogen and phosphorus removal in SBR system and the promotion of phosphorus accumulation bacteria metabolism were investigated, microbial community structures were analyzed through high-throughput detection technology.

Materials And Methods

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 (water-based polyurethane). ICME was obtained by drying in a strictly anaerobic atmosphere. The production process of ICME particle is described as follow: 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 persulfate 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 ℃ for 60-90 min under the condition of no oxygen.

ICME -SBR System

An iron-carbon micro-electrolysis coupled sequencing batch reactor (ICME-SBR) was used in the test, as illustrated in Fig. 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 was 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 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 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 solenoid valve. The experimental operation of SBR was initiated at low temperature when the pollutant removal rate and effluent concentration were stable, the reactor was considered to be started successfully.

Operational strategy

The experimental operation of ICEM-SBR was initiated by inoculating activated sludge from the secondary sedimentation tank of a northern sewage treatment plant (Shenyang, China). The experiment lasted for more than 120 days, the sludge concentration of the reaction system was about 3000 mg/L, and the temperature was controlled at 10 °C. The ICME-SBR underwent several phases: (A) Startup phase with aerobic DO 3 mg $L^{-1}$and pH 7.0 for 60 d; (B) Working phase with different ICME particle dosage (0 g Fe-C/g MLSS, 1 g Fe-C/g MLSS, 1.7 g Fe-C/g MLSS, 2.6 g Fe-C/g MLSS, 3.3 g Fe-C/g MLSS, 4 g Fe-C/g MLSS), under each dosage, a stabilization period needed, followed by ten days of continuous monitoring and the test results analyzed; (C) Working phase with different pH (6.0, 6.5, 7.0, 7.5, and 8.0), and different DO (1 mg/L, 2 mg/L, 3 mg/L, 4 mg/L) in the aerobic stage, the DO in anaerobic stage was kept below 0.2 mg/L, each experiment included stabilization period and continuous monitoring, determined the optimal operating conditions for ICME-SBR; (D) Work stage with the typical cycle, the metabolic process of polyphosphate accumulating organisms (PAOs) in the typical cycle was analyzed, the detection indicators include TP, PHB (polyhydroxybutyrate), Ploy-P (poly-phosphorus), glycogen, COD, DHA (dehydrogenase), and ETS (electron transporter); (E) Microbial community structure analysis, select 3 sludge samples for high-throughput detection and comparative analysis, named G1, G2, and G3, where

G1 was taken from the second settling tank of the sewage treatment plant, and G2 was taken from the SBR low temperature operation stable sludge, G3 was ICME-SBR sludge stable at low temperature.

Wastewater quality and analytical methods

Synthetic wastewater prepared with tap water was used in the experiment, ingredients included CH_3COONa (carbon source, 170-220 mg/L COD), KH_2PO_4 (phosphorus source, 7-11 mg/L TP), NH_4Cl (nitrogen source, 20 mg/L), additionally, other components including $MgSO_4 \cdot 7H_2O$, $CaCl_2$, $NaHCO_3$ and trace elements.

Samples from influent and effluent were collected regularly, and the concentrations of COD, TP, NH_4^+-N and TN were determined according to standard methods (State Environmental Protection Administration of China, 2002). Biomass PHB content was detected by capillary-gas chromatography (Cavaillé et al., 2013). Glycogen was calculated by the anthrone-sulfuric acid method (Zhang et al., 2015). ETS and DHA were detected by Iodonitrotetrazolium chloride (INT) (Trevors et al., 1982) and Tetraazolium red (TTC) (Nielsen et al., 1975). The following detection equipment was used: the temperature was measured in real time by an electronic water heater, the DO and pH value were measured with DO meter (DO530, sinomeasure company, China) and pH meter (PH160, sinomeasure company, China), respectively. The internal structure of micro-electrolysis particles was observed by scanning electron microscopy (S-4800, Hitachi Co. Ltd., Japan) 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 analyzed by high-throughput sequencing (Illumina NovaSeq, novogene Co. Ltd., China). DNA was extracted using CTAB method. The V3-V4 region of microbial 16S rRNA was amplified with the barcoded forward primer 341F (CCTACGGGNGGCWGCAG) and reverse primer 806R (GGACTACNNGGGTATCTAAT). The PCR was performed using a PCR instrument (Phusion8 High-Fideity PCR MaterMix with GC Buffer) and high-efficiency high-fidelity enzymes. Then, the PCR products were quantified accurately (TuSeq®DNA PCR-Free Sample Preparation Kit) and were used for high-throughput sequencing (NovaSeq6000).

Results

Characterization and properties of ICME particles

The appearance, internal morphology and metal element composition of ICME particles were shown in Fig. 2. ICME particles were granular with an average particle size of 0.8 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. The specific surface area of ICME particles was 25.40 m^2/g, and the average pore diameter and total pore volume were 4.53 nm and 0.012 cm^3/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 reactor, 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 oxidized and could form a large number of fine catalytic galvanic cells. Therefore, the physical and chemical properties of ICME particles indicated that it was suitable for biological carriers of wastewater treatment.(Deng et al., 2016a).

ICME -SBR performance under the varying influence factors

Influence of particle dosage

Fig. 3 depicted the COD, TP, NH_4^+-N, and TN removal efficiency under different ICME particle dosage. 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 low temperature environment, the sewage treatment 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. In the fourth stage, 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 stabilized, the average removal efficiency increased from 65.57% to 73.73% with increased ICME particle dosage from 0 g Fe-C/g MLSS to1.7 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 was 90.09% and 0.92 mg/L. 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 was obvious that dosing of ICME particles was effective for the reduction of nitrogen. When the dosage was enhanced from 0 g Fe-C/g MLSS to 2.6 g Fe-C/g MLSS, NH_4^+-N removal efficiency significantly increased, and the corresponding removal efficiency increased from 43.78% to 65.34%. Due to a large amount of NH_4^+-N removal, the TN removal rate has to be improved. The TN removal efficiency was reached up to averagely 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.

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 Fig. 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 pH value of 7. The removal efficiency of TP increased first and then generally decreased with 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_4^+-N and TN presented firstly increased and then decreased. The phenomenon was similar to TP removal. The removal efficiency of NH_4^+-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_4^+-N and TN was 60.08% and 70.61%, and the average effluent concentration was 6.21 mg/L and 9 mg/L. The removal rates of NH_4^+-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_4^+-N in the effluent increased slightly. In this study, the most suitable pH was 7.

Influence of DO

DO is an important control parameter in the process of biological nitrogen and phosphorus removal. Fig. 5 depicted the COD, TP and nitrogen removal efficiency under different DO in aerobic reaction. The removal efficiency of COD gradually increased and stabilized 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 mg/L-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 unfavorable 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 increase the DO was effective for the reduction of NH_4^+-N and TN, NH_4^+-N average effluent concentration decreased from 13.48 mg/L to 4.14 mg/L with increased DO from 1 mg/L 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 mg/L and 9.27 mg/L.

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 was highlighted in Fig. 6. In the anaerobic P-release phase, activated sludge poly-phosphate 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 mg/g VSS 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, and 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 mg/g VSS and 109.34 mg/g VSS at the end of 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 changes of DHA (dehydrogenase) and ETS (electron transporter) activity values in a typical cycle were shown in Fig. 7. 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, the measured DHA and ETS activity values increased by 5.31 $ug·mg^{-1}$ $VSS·h^{-1}$ and 25.97 $ug·mg^{-1}$ $VSS·h^{-1}$ by the 30 min anaerobic, which was the maximum in the anaerobic phase. The enzyme activity increased rapidly could be partly because the organic molecular particles in the simulated domestic sewage were small and easy to be absorbed and utilized by activated sludge microorganisms. At the end of the anaerobic phase, the DHA and ETS activities were 8.90 $ug·mg^{-1}$ $VSS·h^{-1}$ and 82.47 $ug·mg^{-1}$ $VSS·h^{-1}$, 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 $ug·mg^{-1}$ $VSS·h^{-1}$ and 102.88 $ug·mg^{-1}VSS·h^{-1}$, respectively, after 30min of aerobic treatment, and then showed a downward trend. The DHA and ETS gradually decreased to 6.54 $ug·mg^{-1}VSS·h^{-1}$ and 75.65 $ug·mg^{-1}$ $VSS·h^{-1}$ with substrate consumption in the stage of aerobic phosphorus absorption, respectively, it was a sharp contrast to aerobic 30 min.

Microbial community in ICME-SBR

Microbial diversity

Microbial diversity at three different stages was analyzed by high throughput sequencing. The sample diversity index abundance statistics table of the three samples under the 97% consistency threshold was shown in Table 1. The numbers of 1670, 1149, and 1116 OTUs were obtained respectively. The coverage values of all samples were over 99% indicate that this sequencing was sufficient to cover the whole microbial community.

The diversity of the sample was analyzed 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 characterize species richness and evenness(Qian et al., 2018), respectively. According to statistical analysis, the order of the Shannon index from high to low was G3, G2and G1 for the three phases. The Simpson index showed a similar trend in the three phases.

Microbial community structure in phylum and genus levels

As shown in Fig. 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, Myxococcota and Firmicutes, etc. Similar finds were observed in sewage treatment systems (Karanasios et al., 2010). 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 unidentified_Bacteria, 1.9%, 6.1% and 4.8% for Actinobacteriota, 0.4%, 0.15% and 0.16% for Acidobacteriota.

Fig. 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 (Fahrbach et al., 2006) (belonging to Rhodocyclaceae) and Dechloromonas, 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, which 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%), 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. (Freitag et al., 2006; Zhu et al., 2013; Lim et al., 2009; He et al., 2005).

Discussions

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. Same phenomenon was also presented by Zhang et al[33](Zhang et al., 2018), 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. The Fe-C particles in the sewage treatment process could improve sewage treatment efficiency as the immobilized carrier of microorganisms and remove some pollutants through the physical and chemical properties of fillers. 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. (El Samrani et al., 2004; Li et al., 2009) pH affects the growth, reproduction, and metabolism of microorganisms by affecting microbial enzyme activity, microbial cell plasma membrane charge distribution and cell permeability. When pH was low, 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 oxidized to Fe^{3+} in an oxygen environment, and phosphorus could be removed by iron phosphate precipitation (Zhang et al., 2009), 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), pH7.5 was more suitable for the growth of PAOs than pH7 (Wang et al., 2013; Lopez-Vazquez et al., 2009). Higher pH may decrease the abundance of GAOs and increase the proportion of PAOs (Qin et al., 2016). 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 the effect of pH 7 on microbial activity. Meanwhile, it showed that the micro-electrolytic chemical phosphorus removal effect at pH7 was better than that at pH7.5. At a higher pH level, the Fe^{2+} generated by the electrolytic reaction forms ferrous hydroxide with OH^{-}, which adsorbs the particles with weak negative charge in the pollutant and forms flocculates to be removed, this process was easy to form 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 (Brdjanovic et al., 1998), besides, high DO would increase the abundance of GAOs (Oehmen et al., 2007; Carvalheira et al., 2014) 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 NH_4^+-N (Zhou et al., 2018). According to the Monod equation, the specific growth rate of nitrifying bacteria is affected by the concentration of NH_4^+-N and DO. When the concentration of influent NH_4^+-N is constant, the specific growth rate increases with the increased DO.

Analysis of metabolic mechanisms of PAOs in ICME-SBR

In the study of metabolic characteristics of PAO, in the anaerobic P-release phase, PAOs hydrolyzed intracellular poly-P into orthophosphate and release it out of 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 (Acevedo et al., 2017; Mino et al., 1998). 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 (Wang et al., 2016). The removal of COD and TP in the system was realized. The activity of

DHA and ETS reflects the number of active microorganisms and the ability to metabolize organic matter. The activity of enzymes can be measured by determining the rate of product formation or substrate utilization (Tipton et al., 1992). 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 (Goel et al., 1998). The enzyme activity in aerobic stage was higher than that in anaerobic stage, which indicated that the micro-electrolysis particles had a stronger promoting effect on the microbial activity under aerobic condition.

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 irons 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 (Tikhonov et al., 2015) that 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 (Li et al., 2018) can be involved in biological phosphorus removal and denitrification processes and metabolize a variety of organic carbohydrates and proteins. In addition, Acidobacteria is related to the iron cycle and can reduce iron under anaerobic conditions. Firmicutes (Nicholson et al., 2000) 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 SBR system to 10.89% in ICME-SBR system. Candidatus_Accumulibacter was the dominant PAOs in activated sludge and belongs to the Rhodocyclus family of Proteobacteria (Crocetti et al., 2000; Freitas et al., 2005). Acinetobacter was recognized PAOs, which was first isolated by Fuhs and Chen (Xing et al., 2016) 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 (Fuhs et al., 1975). 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 (Shuai et al., 2011); Dokdonella, Hydrogenophagd (Mantri et al., 2016) and Acidovorax (Vasiliadou et al., 2009) were reportedly related to denitrification.

Conclusions

The system of ICME-SBR realized 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, NH_4^+-N and TN in the effluent were 38.58 mg/L, 0.85 mg/L, 6.24 mg/L and 9.27 mg/L, respectively. In the metabolic characteristics of PAOs, the contents of ploy-P and glycogen decreased by 83.78 mg/g VSS 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 mg/g VSS 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 $ug \cdot mg^{-1} VSS \cdot h^{-1}$ and 102.88 $ug \cdot mg^{-1} VSS \cdot h^{-1}$ 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.

Declarations

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Ethics approval and consent to participate.

Not applicable

Consent for publication

That all the authors mutually agree that it submitted to ESPR.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

The corresponding author, LW is responsible for ensuring that the descriptions are accurate and agreed by all authors. LW and GMJ conceived and designed the study. GMJ and WH performed the experiments. HYH investigated and verified research data. CYM and WYQ provided the study materials and other analysis tools. All authors read and approved the manuscript.

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Tables

Table 1 The Diversity of the Samples

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

Figures

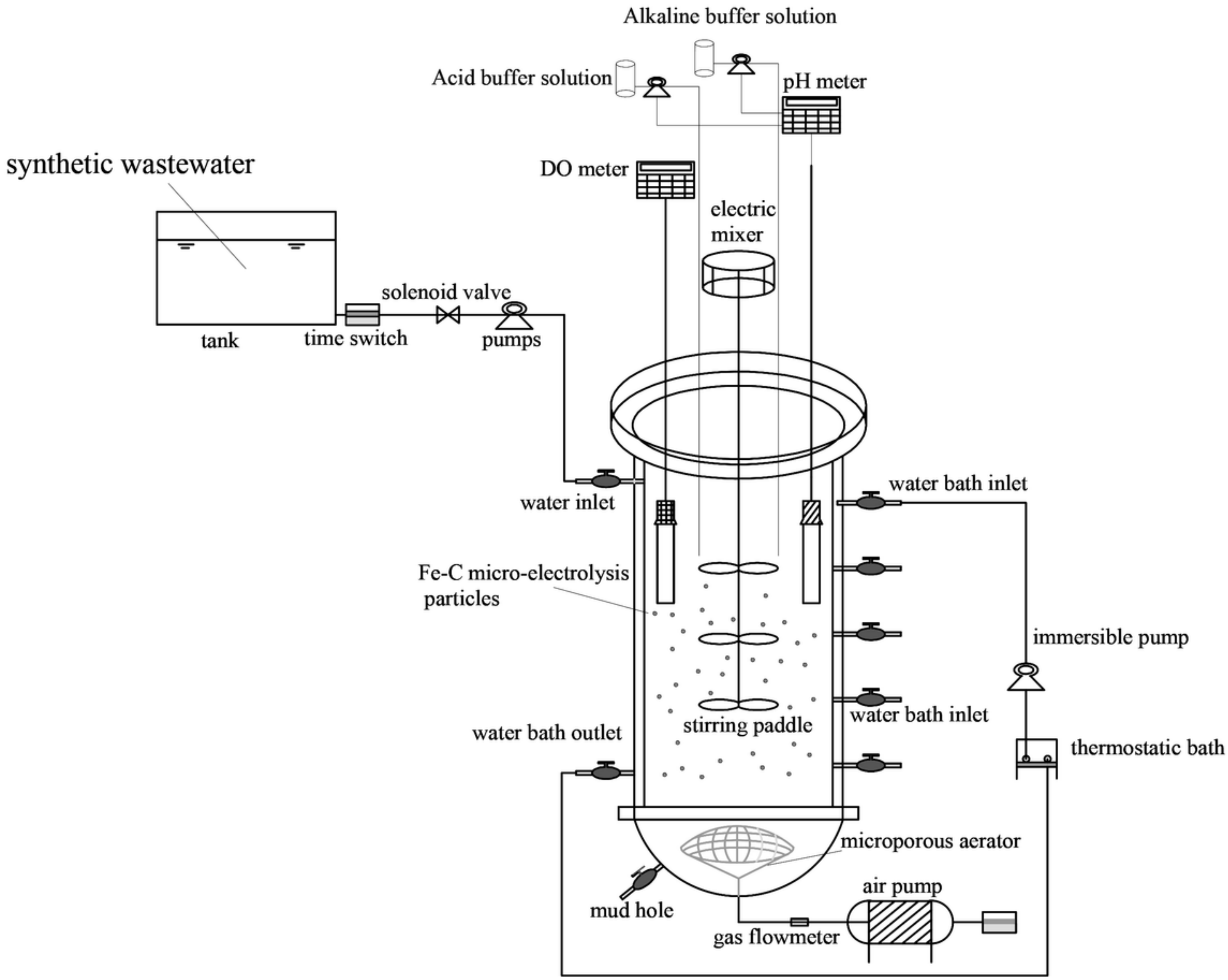

Figure 1

Schematic of the test device

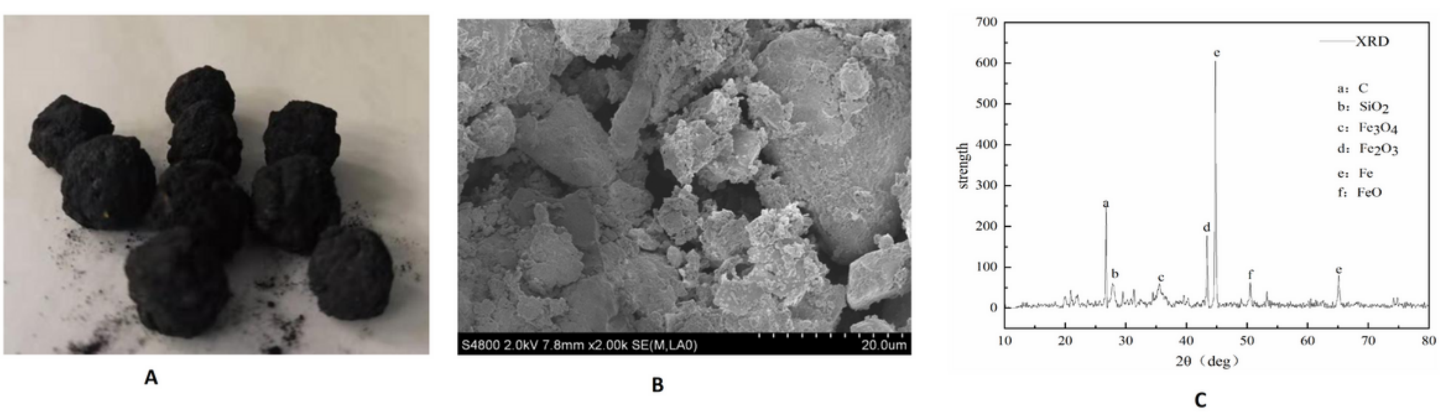

Figure 2

Internal structure and XRD characterization of ICME particles ((a)Particle appearance; (b)SEM photograph at 2000× of inner structure; (c)XRD within particles)

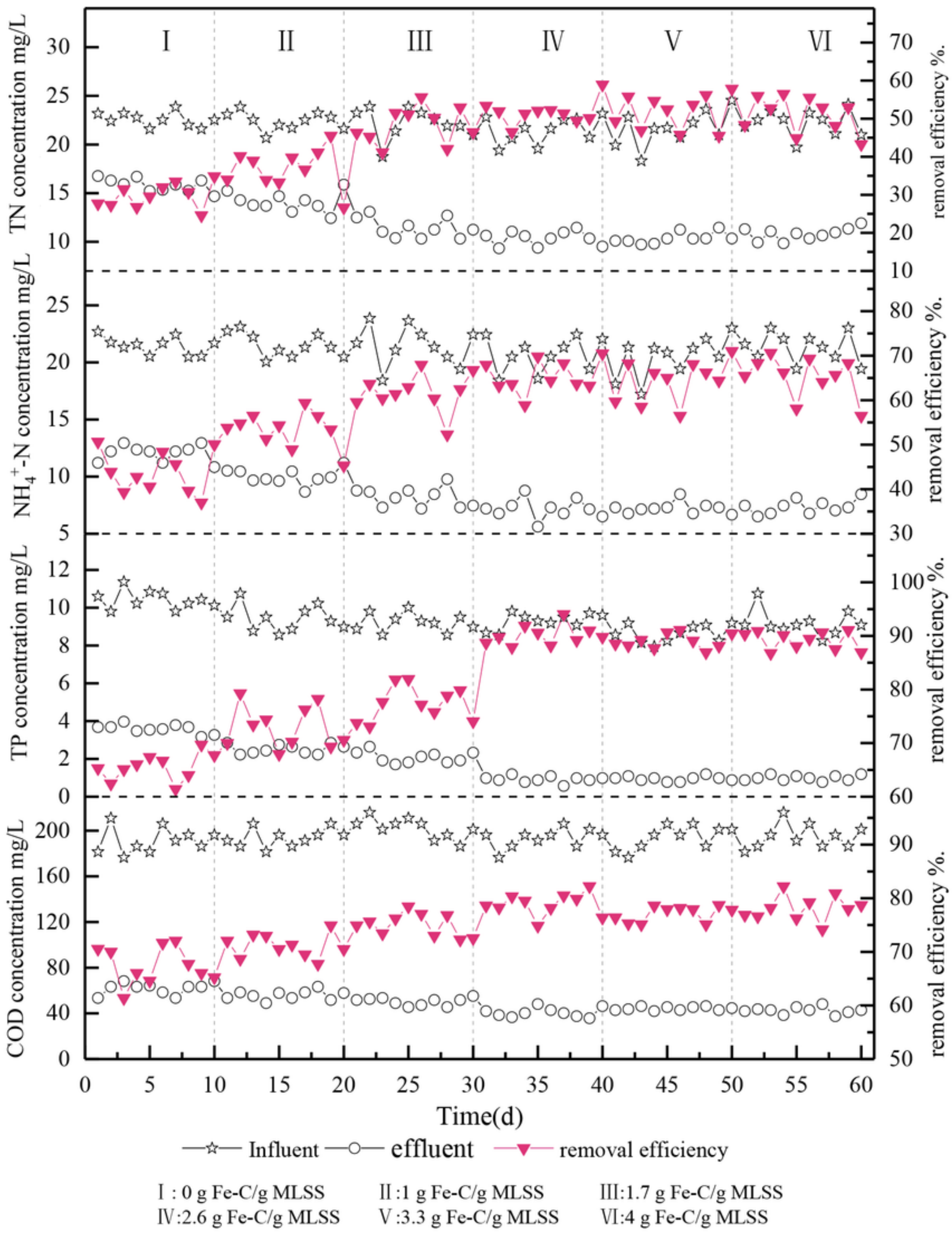

Figure 3

Effect of ICME particle dosage on wastewater treatment

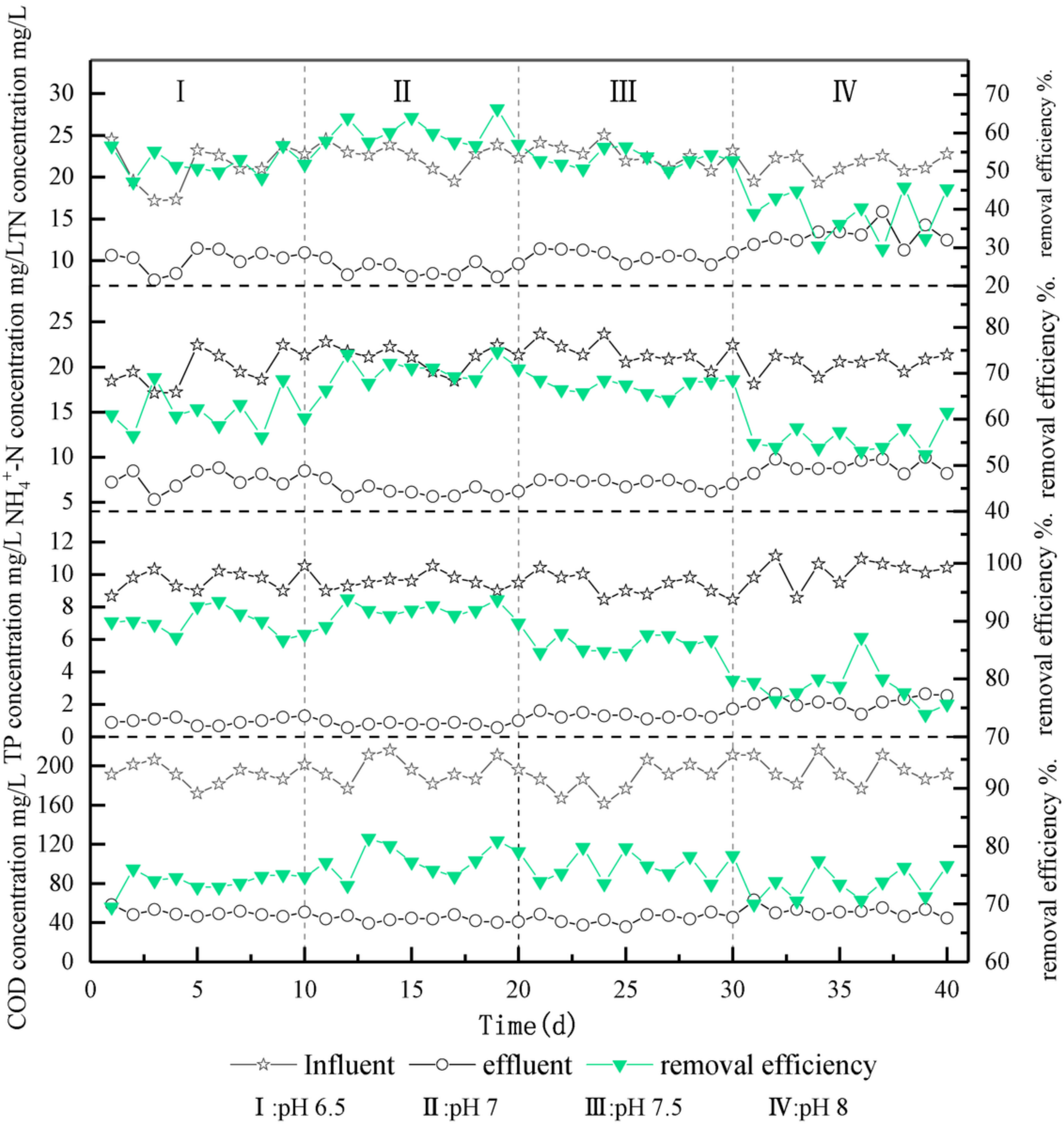

Figure 4

Effect of pH on sewage treatment

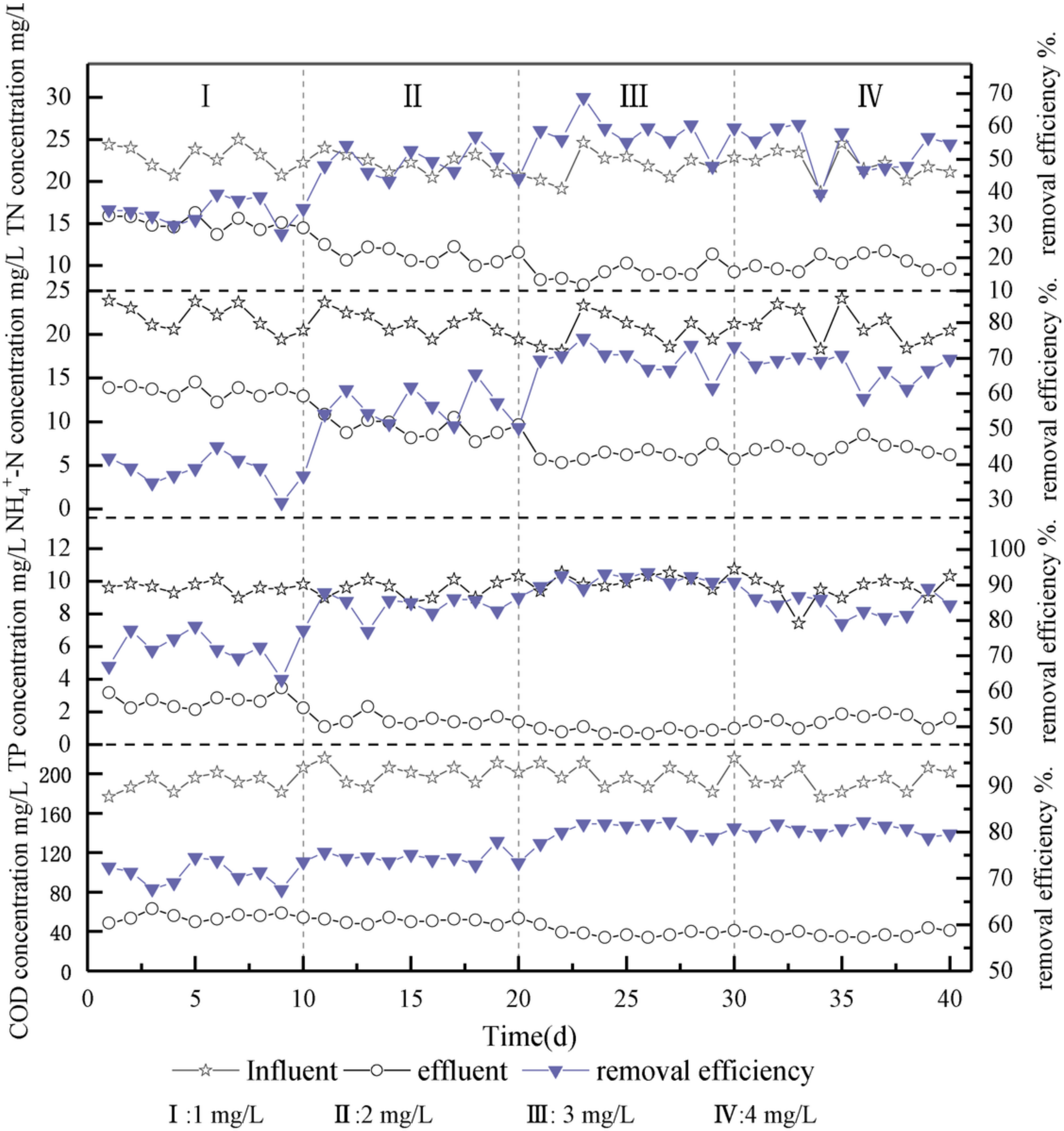

Figure 5

Effect of DO on sewage treatment

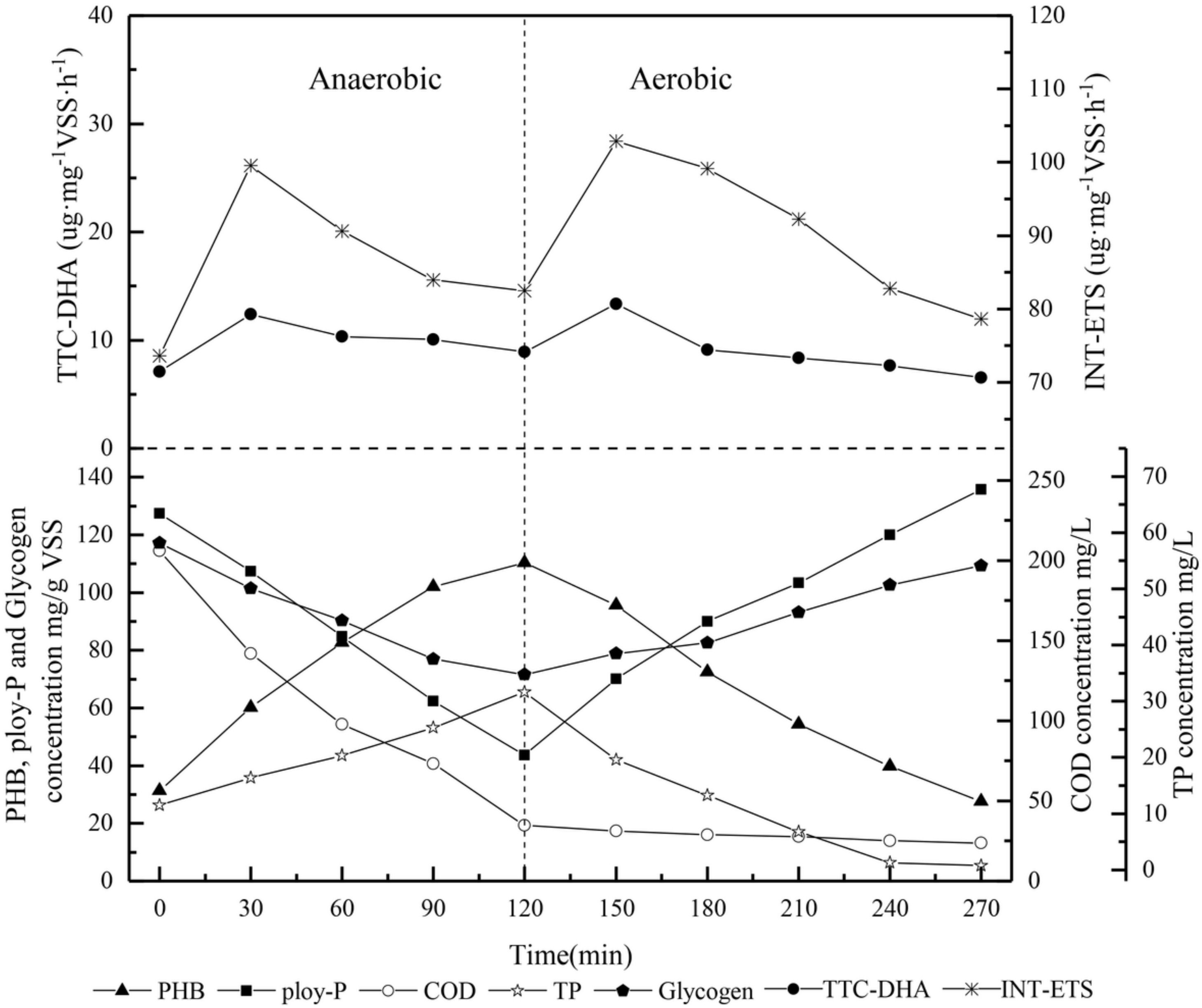

Figure 6

Variations of COD, TP, PHB, poly-P, DHA and ETS in typical cycle

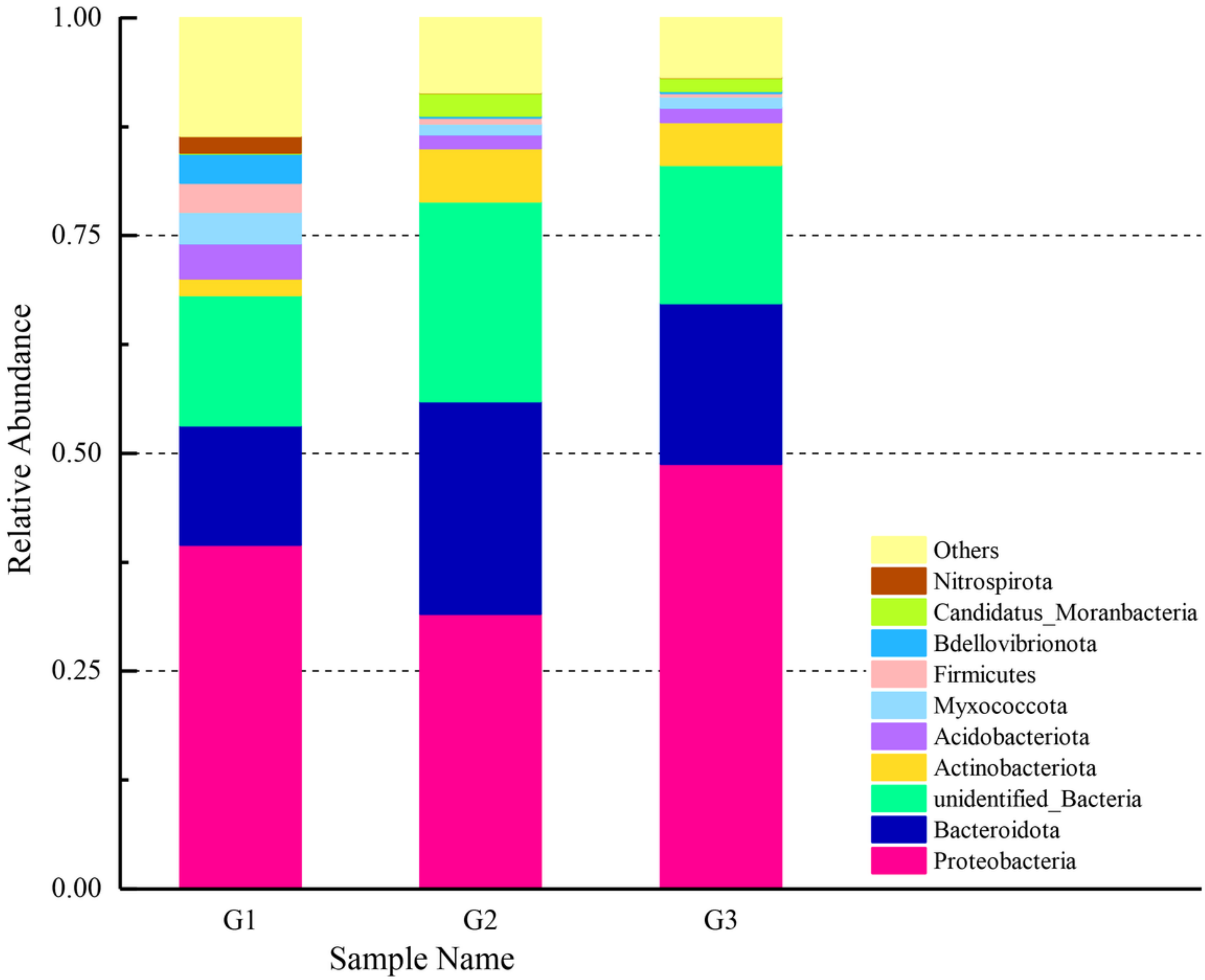

Figure 7

Column chart of relative abundance of species at phylum level

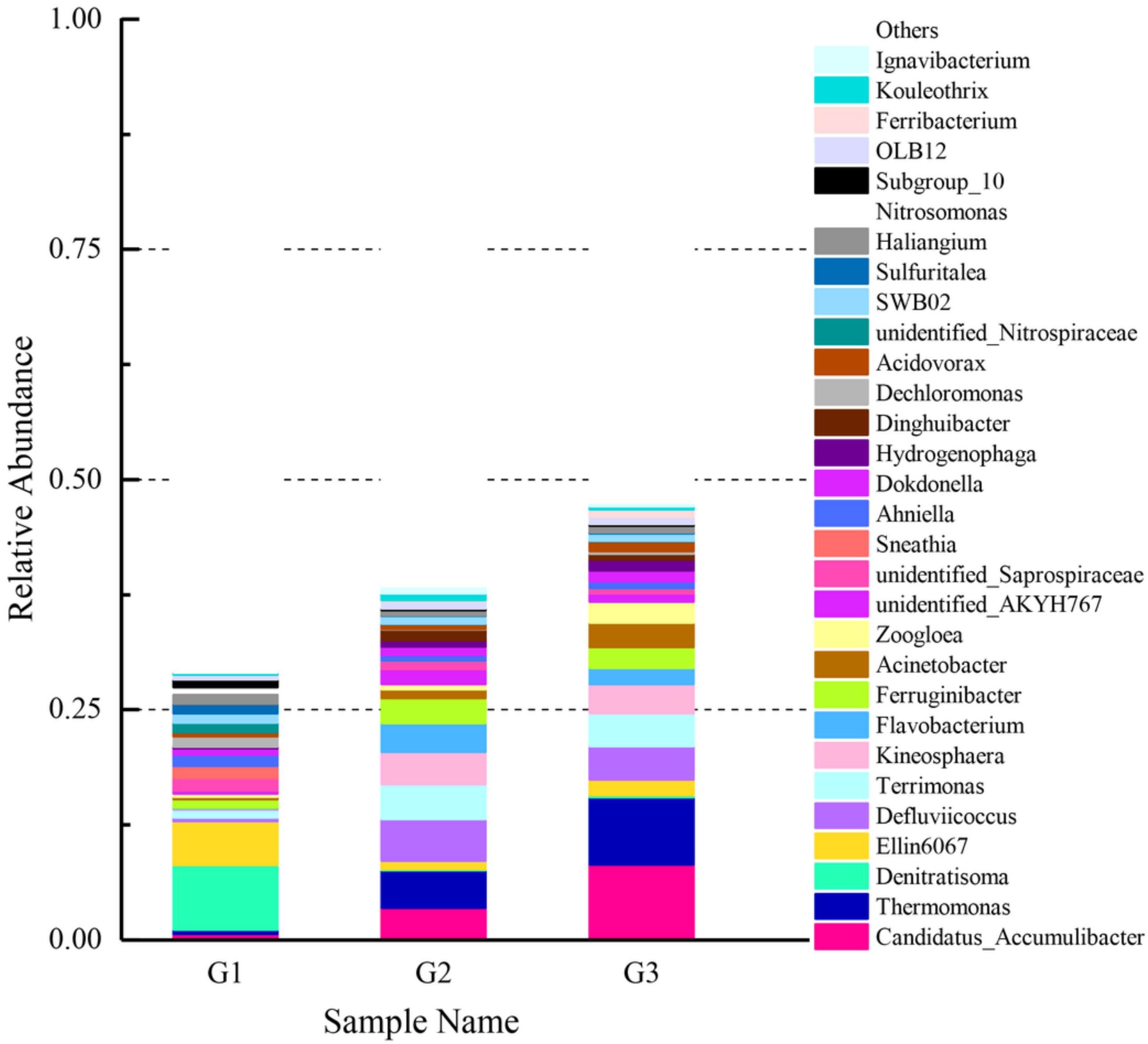

Figure 8

Column chart of relative abundance of species at the genus level

Supplementary Files

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