



Study on the Effect of Iron-Carbon Micro-electrolysis Process on the Removal of Nitrogen and Phosphorus from Rural Domestic Wastewater with Low Carbon to Nitrogen Ratio

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Abstract To study the removal of nitrogen and phosphorus from low C/N ratio rural domestic sewage by Fe–C mixed fillers, in this study, a laboratory-scale iron-carbon microelectronics system (IC-ME) and an activated carbon system (AC) were established to purify rural domestic sewage with a C/N ratio of 1.9–4.4. The results show that the removal rates of NO_3^- -N, total nitrogen (TN), and total phosphorus (TP) of the IC-ME system are 89.25%, 80.64%, and 92.2%, respectively. During the hydraulic retention time (HRT) of 36 h, when the C/N ratio is 1.9. They are much higher than those of the AC system (NO_3^- -N: 31.09%; TN: 64.15%; TP: 26.34%). All the indicators reached the first class B standard of “Pollutant Discharge Standard of Urban Sewage Treatment Plant” (GB18918-2002) and the first-level discharge standard of Guangxi’s “Water Pollutants Discharge

Standard for Rural Domestic Sewage Treatment Facilities” (DB45/2413–2021). Micro-electrolysis can provide electrons for denitrification, further facilitating the process. In addition, the effective phosphorus removal is caused primarily by the corrosion of the iron anodes, which produces Fe^{2+} and Fe^{3+} ions. These ions then react with PO_4^{3-} to form phosphate precipitates, and at the same time, create $\text{Fe}(\text{OH})_3/\text{Fe}(\text{OH})_2$ colloids with OH^- in the water, which can adsorb and flocculate organic phosphorus and PO_4^{3-} . Based on high-throughput sequencing studies, the microbial abundance of *Bacteroidetes*, *Chloroflexi*, and *Firmicutes* is much higher in the IC-ME system than in the AC system. Overall, the IC-ME process provides a new strategy for treating domestic wastewater in rural areas with low C/N ratios.

Keywords Removal of nitrogen and phosphorus · Low C/N ratio rural domestic sewage · Iron-carbon microelectronics system · Denitrification

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1 Introduction

There is a universal problem of nitrogen and phosphorus pollution in the world, mainly from river sewage, sewage irrigation, landfill leachate, and excessive use of chemical fertilizers in agriculture. It can cause environmental problems, such as aquatic death and the eutrophication of water bodies. In addition,

increased nitrogen concentrations in drinking water are likely to cause health problems such as bladder cancer, blue baby syndrome, and ovarian cancer (Su et al., 2017; Xin et al., 2021). The rural areas of Guangxi in China are mainly hilly and mountainous landforms, with scattered villages and low population density. There are some problems in sewage treatment, such as small discharge, scattered discharge points, and few collection and treatment facilities. The BOD/TN of Guangxi rural domestic sewage is about 2:1–5:1, which belongs to the typical low C/N ratio sewage. Rural domestic sewage is an important source of water pollution. How to minimize the discharge of nitrogen and phosphorus from domestic wastewater and how to remediate and clean nitrogen and phosphorus-contaminated water bodies has become a critical environmental issue (Fu et al., 2009; Zeng et al., 2004). An urgent scientific issue is the creation of a system for the treatment of domestic wastewater in rural areas with a low C/N ratio.

Recently, a constructed wetland has been considered as a potential domestic sewage treatment technology due to its low cost, ease of operation, and maintenance requirements (Fan et al., 2013). Nitrogen in constructed wetlands is removed through ammonification, nitrification, and denitrification by microorganisms and adsorption, filtration, and precipitation by plants (Jia et al., 2018; Wu et al., 2014). In the nitrification–denitrification process, the amount of dissolved oxygen determines how much nitrification may occur, whereas the availability of carbon sources determines how much denitrification can occur. Since constructed wetlands have low levels of dissolved oxygen, aerobic microorganisms cannot grow and reproduce, which significantly impacts their ability to denitrify nitrogen. Additionally, the low C/N ratio of domestic wastewater generally and the absence of its carbon source prevent wetlands from properly denitrifying, which has a significant negative impact on the denitrification effect of wetlands. In wetlands, phosphorus is removed by a combination of the actions of aquatic plants, substrates, and microbes, with the substrate playing a predominant role in phosphorus adsorption and precipitation (Wu et al., 2011). When the substrate adsorption site is saturated, the removal rate will decrease. Brix and Arias (2005) showed that the removal effect of constructed wetlands on phosphorus was very limited, and the traditional substrate could not remove phosphorus for a long time. In addition, He et al. (He et al., 2017) constructed an aerobic granular simultaneous nitrification, denitrification, and phosphorus

removal (SNDPR) system and investigated the effect of C/P ratio on organic carbon and nutrient removal using Illumina MiSeq pyrophosphate sequencing technology. It was found that the C/P ratio had a great effect on the TP removal rate in the system, and the lower the C/P ratio, the higher the concentration of TP in the effluent. This shows that carbon source plays an important role in pollutant removal in wastewater treatment, and low C/N ratio and low C/P ratio in wastewater treatment is still a difficult problem to solve.

Iron-carbon micro-electrolysis (IC-ME) has attracted extensive attention because of its advantages, such as simple operation, low cost, good synergy, and easy to be combined with other technologies. Numerous studies have shown that this system has been applied to many types of wastewaters, especially some industrial and urban domestic wastewater (Zhang et al., 2018), but few researchers have applied iron-carbon micro-electrolysis to rural domestic wastewater, which generally has a low C/N ratio. The contact between iron filings (anode) and activated carbon (AC) (cathode) forms a large number of microscopic primary cells, which generate electron donors through the electro-coupling corrosion of a large number of iron sacrificial micro-scale anodes, solving the problem of insufficient organic carbon in the denitrification process. IC-ME can continuously provide H as an electron donor and then form active hydrogen [H], a more powerful reducing substance than the electron donor. In addition, iron hydroxide, formed by the oxidation and precipitation of Fe(II), removes pollutants by adsorption, co-precipitation, and flocculation (Wang et al., 2009), and large amounts of iron ions lead to the precipitation of iron phosphate, resulting in phosphorus removal. Liu et al. (Jian et al., 2020) combined iron-carbon micro-electrolysis with denitrifying bacteria (*Zoogloea*) to build an iron-carbon micro-electrolysis bioreactor. For low C/N ratio (C/N=2) wastewater, it was able to effectively remove nitrate from wastewater at an HRT of 2.0 h. The maximum removal rate of TN was up to 90.10%, with no accumulation of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and denitrification products of N_2 . Wang et al. (Wang et al., 2022) used internal micro-electrolysis-Fenton-recovery ferrite (IM-Fenton-RF) to treat simulated electroplating wastewater containing chelated copper at room temperature. The IM-Fenton-RF system was able to remove 99.9% of copper at a reflux ratio=0.37, Fe^{2+} concentration=9.20 g/L, and pH of 10.18. This shows that the combination of IM-Fenton and RF can

effectively treat wastewater containing chelated copper and has great potential for industrial applications.

In conclusion, Fe–C micro-electrolysis is frequently used to denitrify and remove phosphorus from urban domestic sewage and industrial wastewater, while few studies have applied the technique to rural domestic sewage with small discharge volumes, irregular discharges, and a lack of resources and management personnel. Therefore, the objectives of this study are as follows: (1) to study the removal performance of Fe–C micro-electrolytic matrix for nitrogen and phosphorus in rural domestic sewage with different low C/N ratios; (2) to explore the mechanism of Fe–C micro-electrolysis for nitrogen and phosphorus removal by analyzing the microbial community structure in Fe–C micro-electrolytic system.

2 Materials and Methods

2.1 Experimental Device

The reactor is depicted in Fig. 1. The experimental device's main component is a plexiglass column with a working volume of 7.06 L (diameter = 15 cm; height = 40 cm). The outlet is 5 cm from the bottom of the column. Two experimental systems were established in this study: the activated carbon system AC (filled with coconut shell activated carbon) and the IC-ME system (filled with a uniform mixture of activated carbon and iron powder with iron to carbon mass ratio of 1:5). Iron chips 20–40 mesh, the main component is Fe monomer (90%), and activated carbon 5–8 mesh, both purchased from Gongyi Aolan Water Purification Material Co. Under the

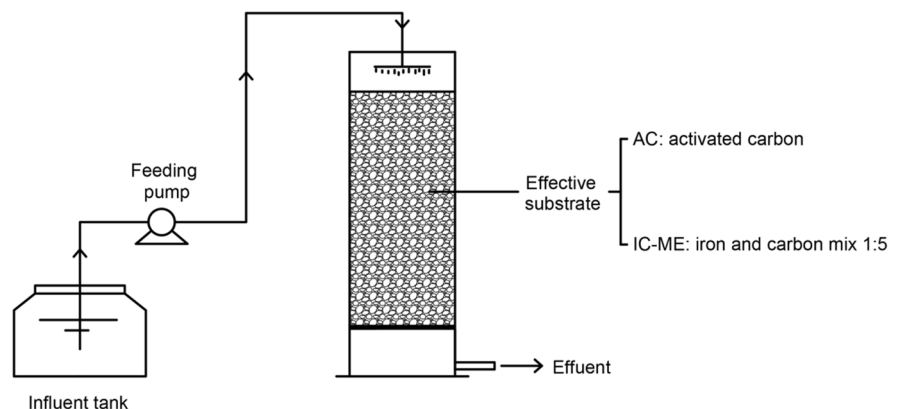
condition of pH 7, the zeta potential of iron filings was 13.76 mV, which was positively charged; the zeta potential of activated carbon was -23.32 mV, which was negatively charged.

2.2 Synthetic Wastewater and System Operation

We used glucose as the carbon source, KNO_3 and NH_4Cl as a nitrogen sources, and KH_2PO_4 as a phosphorus source to simulate domestic sewage. The concentrations of the components were ammonia nitrogen ($\text{NH}_4^+\text{-N}$): 30 ± 5 mg/L nitrate nitrogen ($\text{NO}_3^-\text{-N}$): 10 ± 2 mg/L, and total phosphorus (TP): 4 ± 0.2 mg/L. The reactor was started by a natural membrane hanging in the case of no inoculant sludge, and the water was fed from the upper end of the device through a peristaltic pump (BT100-IF, Longer, China), and the water was discharged from the outlet at the bottom of the reactor after contacting the substrate. Continuous operation for 60 days until nitrogen and phosphorus removal performance is stable. Inlet and outlet water samples are collected every 2–3 days to test and analyze the changes in the concentration of each pollutant during the reactor operation.

To investigate the effectiveness of IC-ME in denitrifying rural domestic wastewater with different C/N ratios, different doses of glucose were added to the influent water. The influent COD concentration ranged from 93.78 to 193.79 mg/L, resulting in a C/N ratio of 4.4, 2.7, and 1.9. Influent (2.5 L) was manually dosed into each system to measure various water quality indicators, and samples were taken every 3 h. At the end of the reaction, water is expelled from the outlet pipe at the bottom of the reactor. The hydraulic retention time (HRT) was 36 h.

Fig. 1 Schematic diagram of the reactor



2.3 Sample Collection and Analysis Methods

Concentrations of TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and $\text{NO}_2^-\text{-N}$ were determined using a UV-vis spectrophotometer (UV-VIS DRS, Shimadzu International Trading Co., LTD.) according to standard methods (APHA, 2005). $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$ were determined by alkaline potassium persulfate UV spectrophotometry, Nannenberg reagent spectrophotometry, N-(1-naphthyl)-ethylenediamine dihydrochloride spectrophotometry, and UV spectrophotometry, respectively. The COD level was determined using a potassium dichromate titration with a microwave digestion instrument (Shaoguan Taihong Medical Co., LTD.). The pH of incoming and outgoing water was measured with a portable pH meter (PHS-3E). The dissolved oxygen of the water was measured with a dissolved oxygen meter. Zeta potential was measured by a Brookhaven (NanoBrook Omni 1817203S).

At the end of the experiment, a number of matrix samples were taken from the upper, middle and lower parts of the two reactors and mixed uniformly to investigate the microbial community in the filler. All samples were stored at $-20\text{ }^\circ\text{C}$, and genomic DNA was extracted according to the assay requirements. Miseq applications were sequenced for the 16S V3-V4 region and the bacterial primers were 338F (50-ACTCCTACGGGAGGCAGCA-30) and 806R (50-GGACTACCAGGGTATCTAAT-30). The sequencing was performed on the Illumina MiSeq platform (Megie Bio, Shanghai, China). Sequencing data were spliced, quality controlled, and de-jointed to obtain the optimized sequence. OTU clustering was performed based on the optimized sequence to obtain the OTU abundance table for subsequent Biological information analysis.

3 Results and Discussion

3.1 The Start-up of the Reactor

3.1.1 Nitrogen Removal Performance of the Reactor During Start-up

The AC and IC-ME reaction systems operated naturally for 61 days when the influence C/N ratio was 3:1, and no sludge was inoculated. Figure 2a shows that the TN removal rate was only 47.54% after 2 months of continuous operation in the AC reaction system. This can be attributed to the $\text{NH}_4^+\text{-N}$ adsorption by the AC. Some $\text{NH}_4^+\text{-N}$ were converted

to $\text{NO}_3^-\text{-N}$ or $\text{NO}_2^-\text{-N}$, resulting in the accumulation of $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$. Figure 2b shows that COD, $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$ are removed by the physical and chemical actions of the iron-carbon carrier 11 days before the operation of the IC-ME reaction system. Meanwhile, part of $\text{NO}_3^-\text{-N}$ is reduced to N_2 , $\text{NH}_4^+\text{-N}$, or $\text{NO}_2^-\text{-N}$ by iron-carbon micro-electrolysis. During this period, COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and TN concentrations decreased to 55.12, 19.95, 3.12, and 28.18 mg/L, respectively, and the removal rates reached 54.49%, 33.81%, 71.11%, and 31.17%, respectively. An increase in $\text{NH}_4^+\text{-N}$ effluent concentrations was found between 11 and 17 days, but COD and $\text{NO}_3^-\text{-N}$ concentrations remained consistently lower, showing that the physicochemical action of the iron-carbon material was very limited in removing $\text{NH}_4^+\text{-N}$ and gradually reached saturation during the continuous operation. On the 18th day, the effluent $\text{NH}_4^+\text{-N}$ was significantly reduced, indicating that there were microorganisms in the IC-ME system, and each effluent index demonstrated a slow downward trend. On the 49th day, the effluent index of the IC-ME reaction system remained essentially steady, indicating that the biofilm in the reactor had reached a mature state by then. It has a certain ability to degrade and transform the substrate. At this time, the COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and TN concentrations in the effluent decreased to 15.01, 6.89, 1.9, and 10.16 mg/L, respectively, and the removal rates were 87.24%, 77.55%, 81.07%, and 75.07%, respectively. All indicators meet the first-level B standard of "Discharge Standard of Pollutants from Urban Sewage Treatment Plants" (GB18918-2002) and the first-level discharge standard of Guangxi, "Emission Standards for Water Pollutants from Rural Domestic Sewage Treatment Facilities" (DB45/2413-2021). There was a slight nitrite accumulation during the start-up process, but the $\text{NO}_2^-\text{-N}$ concentration was always lower than 1.0 mg/L in the later stage. In conclusion, adding iron filings based on AC can improve TN removal efficiency and reduce the accumulation of $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$.

3.1.2 Phosphorus Removal Performance of the Reactor During the Start-up

The COD and TP removal performance of AC and IC-ME reaction systems are shown in Fig. 2c-d. The good removal of TP is mainly attributed to

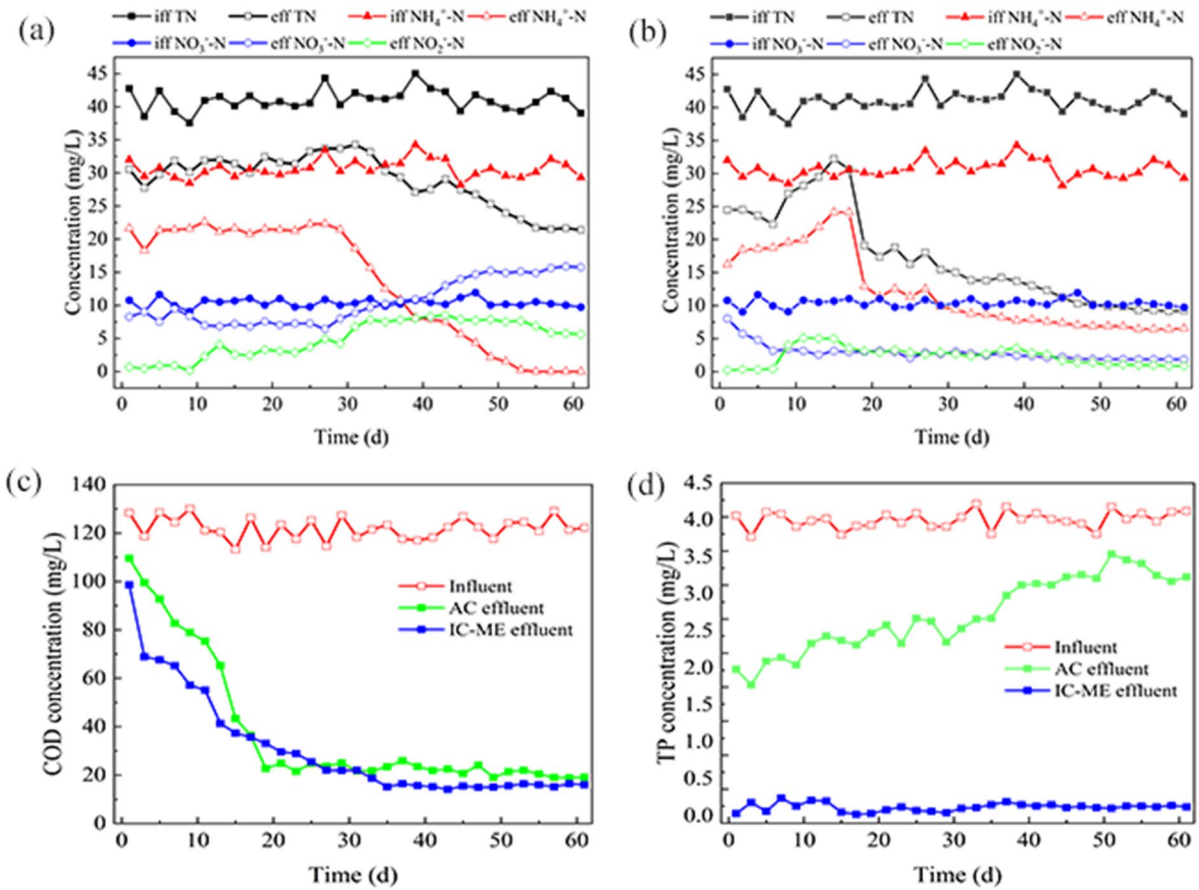


Fig. 2 **a** Nitrogen removal performance of AC reaction system; **b** Nitrogen removal performance of IC-ME reaction system; **c** COD removal performance of AC and IC-ME reaction systems; **d** Phosphorus removal performance of AC and IC-ME reaction systems

the action of Fe. In the adsorption experiment, the saturated adsorption capacity of Fe for TP was 1.6 mg/g, much higher than that of AC (0.17 mg/g) (Fig. S1 and Table S1). With the extension of the operation cycle, the adsorption of TP by AC gradually reaches saturation, so the TP effluent concentration rises slowly in the later stage. For the IC-ME reaction system, the TP removal rate always indicates a high and stable state, where the removal rate can reach more than 95%. As shown in Fig. S2, a large amount of Fe ions were released in the first 3 days of start-up and the concentration of Fe in the IC-ME system was high (8.62–8.90 mg/L), and as time increased, Fe ions in the effluent were consumed for nutrient removal (Ji et al., 2022) and its level became lower and lower until it dropped to 0 mg/L on the 45th day. This indicates that most of the iron ions are oxidized to iron oxide or iron

hydroxide, which enables a more efficient removal of phosphorus. In the two reaction systems, COD showed a consistent decreasing trend (Fig. 2c). Before 19 days, with the increase of the reaction days, the degradation rate of COD continued to increase. After 21 days, the effluent concentration of the IC-ME system still slowly decreased until it reached a stable state after 35 days. Eventually, the effluent concentration of COD in AC and IC-ME systems was 19.1 and 16 mg/L, respectively, and the removal rates were above 80%.

In general, the phosphorus removal performance of the IC-ME system is much better than that of the AC system. Huang et al. (Huang et al., 2020) likewise drew the relevant conclusion that the micro-electrolytic wetland (CW-Fe) system composed of Fe⁰-C filler can effectively enhance the nitrogen and phosphorus removal ability of ordinary

CW. The ability of activated carbon to remove TP depends primarily on its adsorption capacity, but the finite adsorption capacity of activated carbon leads to unsatisfactory removal of TP. Due to the dissolution of Fe^{2+} and Fe^{3+} in the IC-ME system, many colloids of ferric hydroxide and ferrous hydroxide are formed, thus achieving the adsorption effect of phosphorus. In addition, sufficient Fe^{2+} and Fe^{3+} lead to iron phosphate precipitation (Ys et al., 2019). Both the TP and COD effluent from the IC-ME system meets the first-level B standard of “Discharge Standard of Pollutants from Urban Sewage Treatment Plants” (GB18918-2002) and the first-level discharge standard of Guangxi, “Emission Standards for Water Pollutants from Rural Domestic Sewage Treatment Facilities” (DB45/2413–2021).

3.2 Research on Nitrogen Removal

3.2.1 Nitrogen Removal Effect of IC-ME on Different C/N Ratio Sewage

The NO_3^- -N, NO_2^- -N, and NH_4^+ -N in the two reactors were further monitored to reveal the denitrification process in the IC-ME system. Table 1 shows the variation of contaminant concentrations in the influent and effluent water throughout the experimental period. Sufficient HRT can ensure the full contact between pollutants and iron-carbon filler, and improve the removal effect of pollutants in the system. As shown in Fig. 3a, with the extension of HRT, the TN removal effect of IC-ME system on low C/N

ratio sewage gradually improves, and the TN concentration change trend of effluent water from systems with different C/N ratios water quality is roughly the same. As the C/N ratio decreases, the TN removal rate of the IC-ME system changes little. At C/N ratios of 2.7 and 4.4, the TN removal rate at 36 h was 84.43% and 84.24%. With a C/N ratio of 1.9, the TN removal rate in the IC-ME system is 80.62%, which is 16.47% higher than in the AC system. It can be observed that the C/N ratio in the range of 1.9 to 4.4 does not affect the nitrogen removal performance of the IC-ME system. Even at a low C/N ratios, IC-ME can still maintain good nitrogen removal performance. When the influent TN concentration is 40 ± 0.63 mg/L, the effluent TN concentration in the system is 8.33 ± 0.15 , 6.57 ± 0.46 , and 5.31 ± 0.14 mg/L, respectively, when C/N=1.9, 2.7, and 4.4. All of them satisfy the first-level A standard (15 mg/L) of “Discharge Standard of Pollutants from Urban Sewage Treatment Plants” (GB18918-2002) and the first-level discharge standard (20 mg/L) of Guangxi, “Emission Standards for Water Pollutants from Rural Domestic Sewage Treatment Facilities” (DB45/2413–2021), indicating that IC-ME has a good effect on nitrogen removal of low C/N ratio sewage. Si et al. (Si et al., 2020) introduced s- Fe^0 into the constructed wetland to form the constructed wetland-sponge iron coupling system (CW- Fe^0 system). Under different influences NO_3^- -N concentration (10–30 mg/L), COD/N ratio of 5, and HRT of 6 h, the nitrate removal efficiency increased by 16–76%. The addition of s- Fe^0 enhances the removal of nitrate by CW.

Table 1 Changes of each index concentration of inlet and outlet water

	Influent (mg/L)	Effluent (m g/L)					
		C/N=4.4		C/N=2.7		C/N=1.9	
		AC	IC-ME	AC	IC-ME	AC	IC-ME
DO	8.47 ± 0.10	0.24	0.23	0.22	0.34	0.08	0.16
ORP	155.33 ± 1.63	23	-12	87	-12	22	15
COD	93.78–193.79	20.19 ± 3.26	22.78 ± 3.44	25.3 ± 3.36	22.16 ± 4.99	19.8 ± 2.43	17.58 ± 3.44
NH_4^+ -N	30 ± 5.00	0	5.19 ± 0.98	0	6.47 ± 0.28	7.58 ± 0.02	6.81 ± 1.67
NO_3^- -N	10 ± 2.00	5.26 ± 0.03	0	6.08 ± 0.11	0	7.55 ± 0.04	1.18 ± 0.03
NO_2^- -N	0	0.19 ± 0.01	0.12 ± 0.02	0.27 ± 0.01	0.1 ± 0.01	0.28 ± 0.02	0.35 ± 0.30
TN	40 ± 7.00	5.45 ± 0.41	5.31 ± 0.14	6.35 ± 1.02	6.57 ± 0.46	15.41 ± 0.71	8.33 ± 0.15
TP	4 ± 0.20	2.94 ± 0.01	0.39 ± 0.09	2.91 ± 0.02	0.15 ± 0.08	3.02 ± 0.01	0.32 ± 0.02

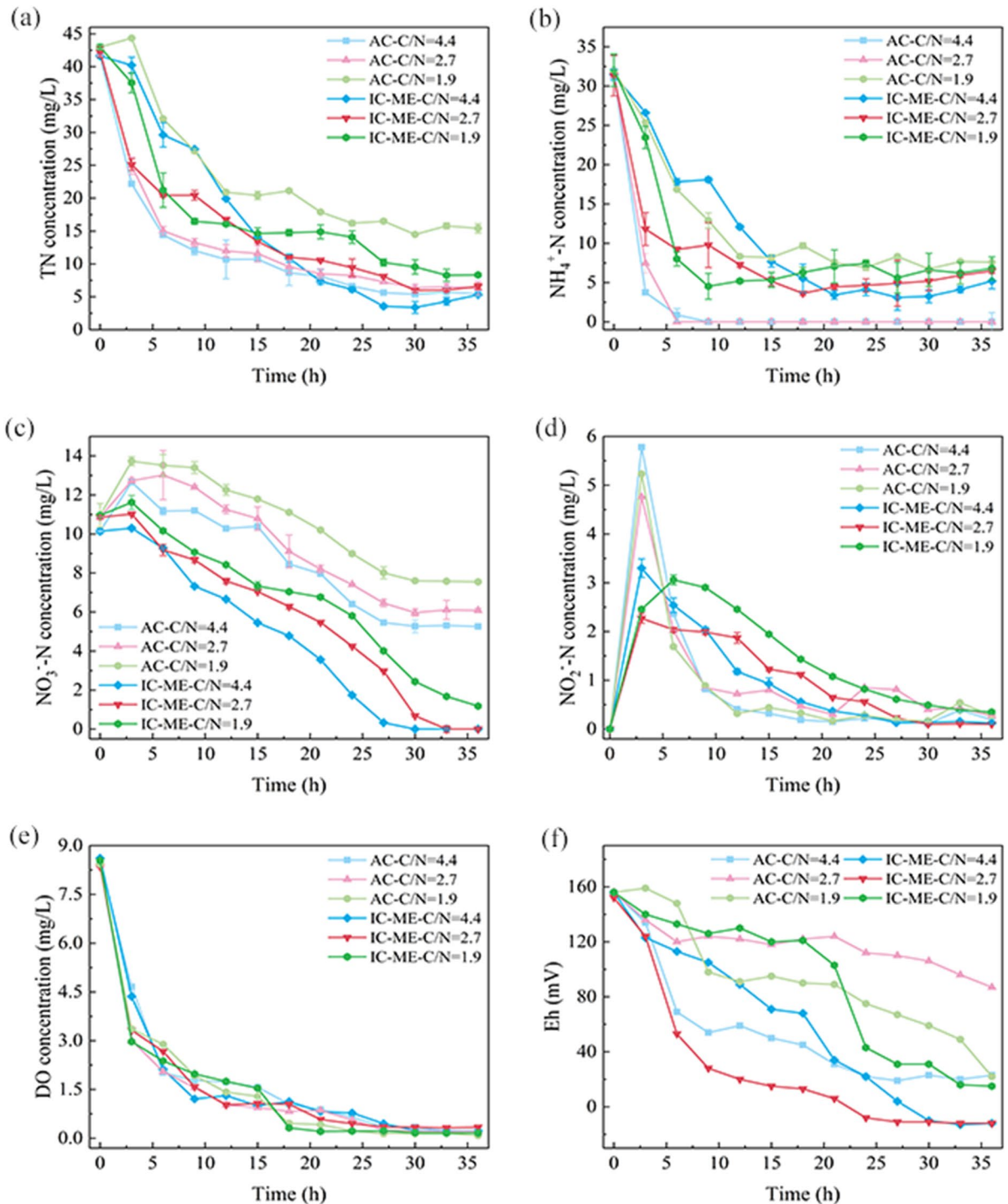


Fig. 3 Concentration changes of each index in the IC-ME system within 36 h. **a** TN concentration; **b** Ammonia nitrogen concentration; **c** Nitrate concentration; **d** Nitrite concentration; **e** DO; **f** ORP

The effluent concentration of $\text{NH}_4^+\text{-N}$ in the AC system is low, and the concentration drops to 0 mg/L when C/N ratios are 4.4 and 2.7 (Fig. 3b), which may be caused by the adsorption and nitrification of $\text{NH}_4^+\text{-N}$ by AC (Bja et al., 2020; Le et al., 2021). The effluent concentration of $\text{NH}_4^+\text{-N}$ in the IC-ME system tends to decrease rapidly at first and then rise slowly, mainly due to Fe/Fe^{2+} -mediated nitrate reduction to $\text{NH}_4^+\text{-N}$ (Si et al., 2020). The concentration of $\text{NH}_4^+\text{-N}$ rapidly decreases over 0–6 h as the reaction time increases. When the HRT was extended from 6 to 36 h, the $\text{NH}_4^+\text{-N}$ removal rate did not increase significantly, with C/N ratios of 1.9 and 2.7. This may be because the system lacks a sufficient source of carbon and the dissolved oxygen content is declining, inhibiting nitrification. The removal rates of $\text{NH}_4^+\text{-N}$ were 78.75%, 79.36%, and 83.52% for C/N ratios of 1.9, 2.7, and 4.4, respectively, and the final effluent concentrations were 6.81 ± 1.67 , 6.47 ± 0.28 , and 5.19 ± 0.98 mg/L, respectively. They all met the first-level B standard (8 mg/L) of “Discharge Standard of Pollutants from Urban Sewage Treatment Plants” (GB18918-2002) and the first-level discharge standard (8 mg/L) of Guangxi, “Emission Standards for Water Pollutants from Rural Domestic Sewage Treatment Facilities” (DB45/2413–2021).

A trend of increasing and then decreasing $\text{NO}_3^-\text{-N}$ effluent concentration can be seen in both AC and IC-ME systems (Fig. 3c), which is due to the conversion of $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$ by nitrification in the early stage. In both systems, the IC-ME system showed significantly better nitrate removal (89.25%, 100%, and 100% at C/N ratios of 1.9, 2.7, and 4.4, respectively) than the AC system (31.09%, 44.01%, and 48.13%). At an HRT of 33 h, the $\text{NO}_3^-\text{-N}$ effluent concentrations of influence C/N ratios of 2.7 and 4.4 were reduced to 0 mg/L (100% removal rate). Under an influent C/N ratio of 1.9, the heterotrophic denitrification was hindered by the lack of carbon source to provide electrons, and the $\text{NO}_3^-\text{-N}$ removal effect was reduced. With the increase in the C/N ratio, the removal efficiency of $\text{NO}_3^-\text{-N}$ increases significantly. When the C/N ratio increases from 1.9 to 2.7, the removal efficiency of $\text{NO}_3^-\text{-N}$ increases from 89.25 to 100%. The main reason is that the IC-ME reaction promotes the transfer of electrons, supplying inorganic electron donors (Fe^{2+} and $\text{H}_2/[\text{H}]$) to the whole micro-electrolytic system, which in turn drives chemical and biological autotrophic denitrification to occur together (Sda et al., 2019). Iron filings can cause the stimulation of aerobic denitrification (Hong et al., 2018) and enhance the adsorption of

$\text{NO}_3^-\text{-N}$ by activated carbon (Le et al., 2021). In conclusion, at a low C/N ratios, the addition of iron filings can achieve the efficient removal of nitrate. Figure 3d shows the variation of $\text{NO}_2^-\text{-N}$ concentration during 36 h in HRT. $\text{NO}_2^-\text{-N}$ accumulated rapidly for each C/N ratio in both systems from 0 to 3 h, with the most $\text{NO}_2^-\text{-N}$ accumulated at a C/N ratio of 4.4 (IC-ME: 3.3 ± 0.19 mg/L and AC: 5.78 ± 0.03 mg/L). The $\text{NO}_2^-\text{-N}$ effluent concentrations gradually decreased for different C/N ratios from 9 to 36 h, and the final concentrations were all lower than 0.4 mg/L.

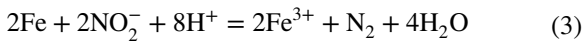
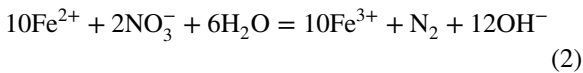
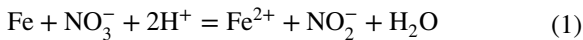
DO plays an important role in $\text{NH}_4^+\text{-N}$ accumulation and $\text{NO}_2^-\text{-N}$ reduction. As shown in Fig. 3e, within an HRT of 9 h, there was no significant difference in DO concentration between the AC system and IC-ME system, and the DO concentration decreased from 8.5 mg/L to below 2 mg/L, which was caused by the rapid consumption of carbon sources and nitrification. Fe and dissolved Fe^{2+} in the IC-ME matrix also consume more available DO, which further suppresses the activity of the nitrifying bacteria and reduces the nitrification performance. When the DO is lower than 2 mg/L, nitrification is difficult to proceed (Grady, 2011). From 9 to 36 h, DO continued to decrease, and the lowest concentration was 0.16 mg/L, thus forming a state of hypoxia and inhibiting nitrification. Additionally, in line with the findings of Deng et al. (Sda et al., 2019), high DO concentrations prevented denitrification caused by Fe–C micro-electrolysis. With the decrease of DO concentration, denitrification was gradually enhanced, and $\text{NO}_3^-\text{-N}$ removal rate increased (Fig. 3c). ORP can indirectly reflect the content of oxides and the activity of microorganisms in the iron-carbon micro-electrolysis device. As shown in Fig. 3f, ORP decreased continuously with the prolongation of time because the oxidation of $\text{NH}_4^+\text{-N}$ and Fe^{2+} consumed DO. In the case of anoxia, the water environment has a strong reduction ability, so the IC-ME system has a better nitrate removal effect.

3.2.2 Overall Performance Analysis of IC-ME for Nitrogen Removal

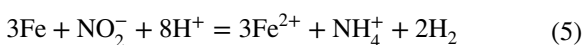
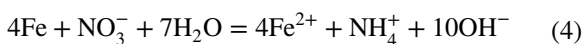
In the iron-carbon micro-electrolysis reaction, iron filings and activated carbon particles constitute many tiny galvanic cells, and Fe^{2+} and $[\text{H}]$ can be generated in the reaction process. The electrode reaction product $[\text{H}]$ has high activity and can degrade part of the

pollutant. At the same time, a large amount of Fe^{2+} and OH^- can be produced by Fe-carbon micro-electrolysis, and products such as $\text{Fe}(\text{OH})_3$ and $\text{Fe}(\text{OH})_2$ with strong adsorption performance can be generated under the condition of sufficient oxygen.

As shown in Fig. 3b, within 0–9 h, the effluent concentration of $\text{NH}_4^+\text{-N}$ decreases rapidly, while the effluent concentration of $\text{NO}_3^-\text{-N}$ increases correspondingly, indicating that IC-ME can quickly realize nitrification. In this process, the effluent concentration of $\text{NO}_2^-\text{-N}$ keeps rising because the oxidation capacity of $\text{NO}_3^-\text{-N}$ is greater than that of $\text{NO}_2^-\text{-N}$, which makes it easier to obtain electrons for reduction. The reduction rate of $\text{NO}_3^-\text{-N}$ is higher than that of $\text{NO}_2^-\text{-N}$, resulting in the accumulation of $\text{NO}_2^-\text{-N}$ (Ys et al., 2019). The reaction process of $\text{NO}_3^-\text{-N}$ reduction is shown in Eqs. (1) and (2), and the $\text{NO}_2^-\text{-N}$ reduction process to N_2 is shown by Eq. (Siantar et al., 1996):



Within 9–36 h, due to the decrease of DO concentration, nitrification was weakened, and the concentration of $\text{NH}_4^+\text{-N}$ increased continuously, becoming the main source of TN in the effluent water. According to the study of Cheng et al. (Cheng et al., 2007a), Fe can reduce part of $\text{NO}_3^-\text{-N}$ to $\text{NH}_4^+\text{-N}$. The study of Shen et al. (Ys et al., 2019) also showed that part of $\text{NO}_2^-\text{-N}$ could also be reduced to $\text{NH}_4^+\text{-N}$, resulting in the accumulation of $\text{NH}_4^+\text{-N}$ in the effluent water. This result is consistent with the experimental results in Sect. 3.2.1 above. The reaction process in which $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ are reduced to $\text{NH}_4^+\text{-N}$ is shown in Eqs. (4) and (5).



The process of low C/N ratio wastewater denitrification by IC-ME is influenced by both physical and biological denitrification. As shown in

Fig. 3c–d, the concentrations of $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ decreased continuously from 9 to 36 h. In addition to REDOX conversion to N_2 and $\text{NH}_4^+\text{-N}$ with Fe, heterotrophic and autotrophic denitrifying bacteria may also be involved in denitrification. The study (Jia et al., 2021) showed that the abundance of denitrifying microorganisms on the surface of iron-carbon fillers was higher than that of other fillers. An appropriate amount of iron can promote the growth and reproduction of Anammox bacteria (Chen et al., 2014a, 2014b; Zhang et al., 2018), promote the formation of extracellular polymers, and improve the denitrification performance of the system. Autotrophic denitrifying bacteria can use Fe^{2+} and [H] produced by micro-electrolysis as electron donors and $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ as electron acceptors to reduce N_2 (Eqs. (6) and (7)) (Xing et al., 2016).



Therefore, the transformation of nitrogen pollutants in the micro-electrolysis process is as follows: $\text{NH}_4^+\text{-N}$ is transformed into $\text{NO}_3^-\text{-N}$ by nitrifying bacteria and nitrosation, and $\text{NO}_3^-\text{-N}$ is transformed into $\text{NO}_2^-\text{-N}$ and N_2 by REDOX and microbial denitrification, in which N_2 is the main product. Part of $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ can be reduced from Fe to $\text{NH}_4^+\text{-N}$.

3.2.3 COD Removal

As shown in Fig. 4, the COD effluent concentration of the AC and IC-ME systems shows a rapid decrease and remains stable in the range 0–36 h. Both AC and IC-ME have excellent treatment effects on COD. At 6 h of HRT, most of the COD of each C/N ratio influent has been removed. It can be observed that the C/N ratio has little effect on the COD removal. The AC system with only activated carbon also has a strong removal effect on COD, mainly because the intermolecular hydrogen bond and direct electrostatic attraction of biochar are conducive to COD removal (Zhou et al., 2019). The heterogeneous surface and porous structure of biochar can provide a more favorable habitat for the enrichment of microorganisms and enhance the degradation of organic matter

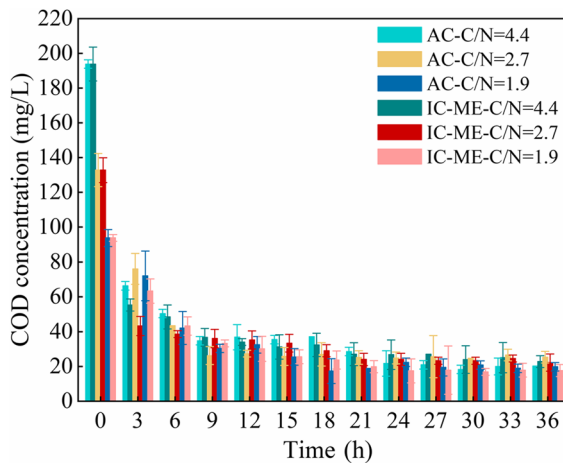


Fig. 4 Effect of AC and IC-ME on COD removal from different low C/N ratio effluents

(Wu et al., 2018). When the HRT is 36 h, the effluent COD concentration of different C/N ratios sewage is below 25.3 mg/L, and the effluent quality meets the first-level A standard ($\text{COD} \leq 50 \text{ mg/L}$) of “Discharge Standard of Pollutants from Urban Sewage Treatment Plants” (GB18918-2002) and the first-level discharge standard ($\text{COD} \leq 60 \text{ mg/L}$) of Guangxi’s “Water Pollutant Discharge Standard for Rural Domestic Sewage Treatment Facilities” (DB45/2413–2021). When C/N ratios are 1.9, 2.7, and 4.4, the COD removal rates in the IC-ME system are 81.25%, 83.31%, and 88.25%, respectively. The COD removal rate in the IC-ME system increases with the increase in the C/N ratio, which is consistent with the research results of Jia et al. (Jia et al., 2021). In this study, AC and IC-ME systems have comparable COD treatment performance, both showing effective and stable removal effects.

3.3 Study on Phosphorus Removal

Figure 5 shows the phosphorus removal performance of the AC system and the IC-ME system at different C/N ratios (4.4, 2.7, and 1.9). The average phosphorus removal efficiency of the IC-ME system (92.93%) was much higher than that of the AC system (26.56%), indicating that the phosphorus removal capacity of the IC-ME substrate was better than that of the AC substrate without the addition of iron filings. This is consistent with the research results of Shen et al. (Ys et al., 2019).

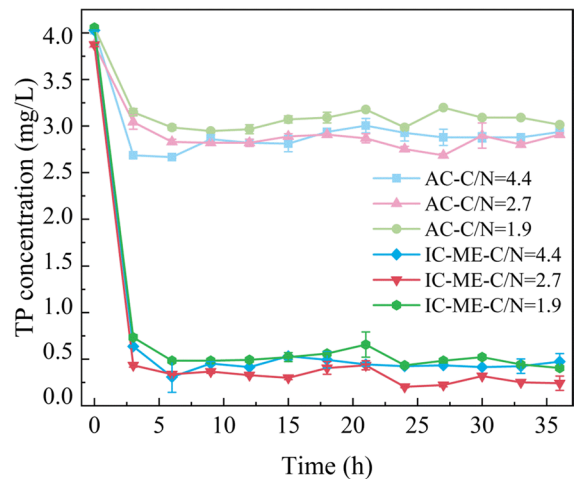


Fig. 5 Effect of AC and IC-ME on TP removal from different low C/N ratios effluents

Both the AC and the IC-ME systems demonstrated a significant reduction in TP effluent concentration during the first 3 h at C/N ratios of 4.4, 2.7, and 1.9, as shown in Fig. 5. This is due to the dissolution of Fe^{2+} and Fe^{3+} in the IC-ME system forming a large amount of iron hydroxide and ferrous hydroxide colloids, which have a very large specific surface area and can adsorb and flocculate organic phosphorus, PO_4^{3-} and FePO_4 , thus achieving the adsorption effect on phosphorus (Cheng et al., 2007b; Coby et al., 2011). In addition, the abundance of Fe^{2+} and Fe^{3+} leads to iron phosphate precipitation (Ys et al., 2019). The reduction of TP concentration in the AC system was mainly due to the adsorption of activated carbon, but the adsorption capacity of activated carbon was limited and ultimately did not achieve perfect phosphorus removal (Si et al., 2021). The TP removal rates of the AC system and IC-ME system were 27.76% (C/N=4.4), 25.28% (C/N=2.7), 26.34% (C/N=1.9), 90.42% (C/N=4.4), 96.16% (C/N=2.7), and 92.2% (C/N=1.9) under different C/N ratios conditions, respectively. This indicates that the TP removal effect of the IC-ME system is always higher than that of the AC system, regardless of the C/N ratio condition. In addition, the removal effects of TP under different C/N ratio influence conditions are comparable and not very different. This means that the influence of the

influence C/N on the TP removal is negligible, and the IC-ME system works well for TP removal. After 36 h, the TP effluent all reached the first-level A standard (≤ 0.5 mg/L) of “Discharge Standard of Pollutants from Urban Sewage Treatment Plants” (GB18918-2002) and the primary discharge standard (≤ 1.5 mg/L) of Guangxi’s “Water Pollutants Discharge Standard for Rural Domestic Sewage Treatment Facilities” (DB45/2413–2021).

3.4 Analysis of Microbial Community Composition

3.4.1 Diversity Index

High-throughput 16S rRNA gene sequencing was used to investigate microbial community diversity in both AC and IC-ME systems. The differences in microbial abundance and diversity between the two systems are shown in Table 2. The structure of the microbial community in the system was altered by the added iron filings, as evidenced by the change in the α -diversity index. The higher the Chao and Ace indices, the greater the microbial richness. The results show that the Chao and Ace indices in the IC-ME system are higher than those in the AC system. The abundance of microorganisms in

the system increases significantly after the addition of iron filings. Shannon and Simpson’s index reflects microbial diversity; The larger the Shannon, the smaller the Simpson and the higher the microbial diversity. Microbial diversity was significantly higher in the IC-ME system with iron-carbon packing than in the AC system with activated carbon packing. This is consistent with the findings of Zhao et al. (Zhao et al., 2019), where the IC-ME system containing iron promotes Chao, Ace, and Shannon indices. Therefore, the bacterial community abundance and diversity are higher in the IC-ME system than in the AC system.

3.4.2 Microbial Community Composition at the Phylum Level

The microbial community composition at the phylum and genus levels is shown in Fig. 6. *Actinobacteria* and *Proteobacteria* are the two most dominant phyla at the phylum level. The order of *Actinobacteria* abundance in the AC and IC-ME systems was IC-ME (48.96%) > AC (40.17%), while the order of *Proteobacteria* abundance was AC (19.36%) > IC-ME (18.01%). From Fig. 6a, it can be seen that the most enriched clades in the IC-ME system due to the addition of iron filings were *Actinobacteria* (48.96%), *Proteobacteria* (18.01%), *Bacteroidetes* (4.66%), *Chloroflexi* (6.21%), and *Firmicutes* (8.62%). Among them, *Proteobacteria* contains many denitrifying bacteria associated with nitrogen and carbon cycling, which facilitate effective COD and nitrogen removal at different influent C/N ratios, and they are commonly found in natural and artificial wetlands

Table 2 Bacterial abundance and diversity

Sample	Ace	Chao	Shannon	Simpson
AC	350.643	345.349	3.3787	0.0745
ICME	548.009	555.303	4.2597	0.0391

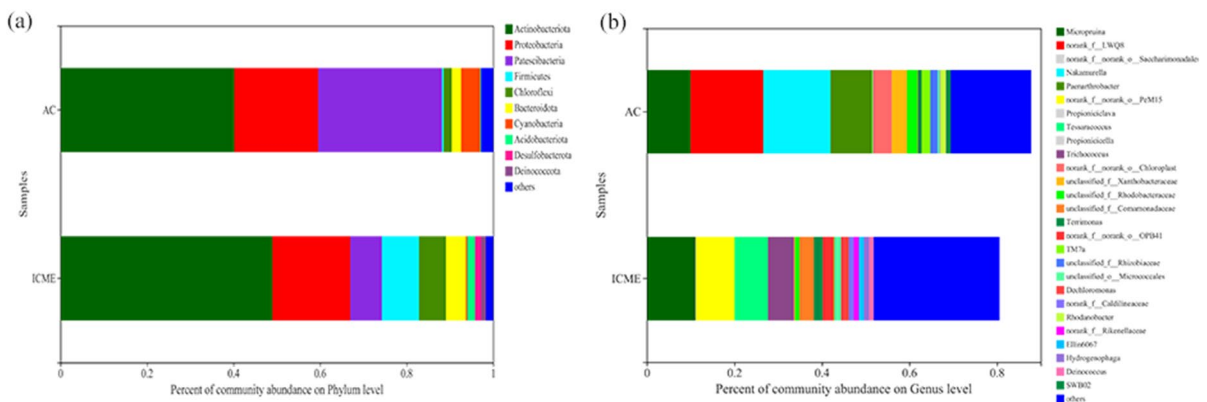


Fig. 6 Community structure of microorganisms in the AC and IC-ME systems. **a** phylum level; **b** genus level

(Ansola et al., 2013; Fga et al., 2020; Yla et al., 2020). The difference in the abundance of *Proteobacteria* between the AC and IC-ME systems is insignificant, suggesting that adding iron filings to the AC does not increase the relative abundance of *Proteobacteria*, but rather decreases it. In the results of a study by Jia et al. (Jia et al., 2021), it was shown that adding biochar increased the relative abundance of *Proteobacteria* and *Actinobacteria*. Nonetheless, the addition of iron filings favored the growth of *Bacteroidetes*, *Chloroflexi*, and *Firmicutes*. *Bacteroidetes* is a common chemical heterotrophic denitrifying bacterium that is highly efficient in degrading organic matter (Navarro-Noya et al., 2013). *Chloroflexi* is a common parthenogenic anaerobic microorganism in anaerobic reactors and is beneficial for establishing an anaerobic environment (Zhang et al., 2012). Under anaerobic conditions, *Chloroflexi* not only absorbs and transforms organic matter, but some complex substrates are also degraded (Miura et al., 2007). Correspondingly, the increased abundance of *Chloroflexi* further enhances the removal of nitrogen and COD by IC-ME. In addition, *Firmicutes* were more abundant in the IC-ME system than in the AC system, presumably because many species belonging to *Firmicutes* are more resistant to environmental stresses (including iron ions) and are readily enriched by electrolytic manipulation (Liu et al., 2020) and because *Firmicutes* are likewise good at removing organic matter (Mkaa et al., 2020; Wei et al., 2018).

3.4.3 Microbial Community Composition at the Generic Level

As shown in Fig. 6b, at the genus level, the IC-ME system has *Micropruina* (11.04%), *norank_f_norank_o_Saccharimonadales* (6.54%), *norank_f_norank_o_PeM15* (8.83%), *Propioniciclava* (8.51%), *Tessaracoccus* (7.64%), *Propionicicella* (4.35%), *Trichococcus* (5.93%), *unclassified_f_Coanomonadaceae* (3.3%), *Terrimonas* (2%), *norank_f_norank_o_OPB41* (2.57%), *Dechloromonas* (1.69%), *Hydrogenophaga* (1.14%), and *Deinococcus* (1.06%), which were significantly higher than the AC system. *Dechloromonas*, a parthenogenic heterotrophic denitrifying bacterium that can use organic carbon sources to enhance nitrate removal (Du et al., 2016), plays an important role in the natural nitrogen cycle and is a powerful Fe(II) oxidizer under mixed nutrient conditions (Chakraborty & Picardal, 2013). The abundance

of *Dechloromonas* in the IC-ME reactor with added iron filings is 1.69% higher than in the AC reactor at low C/N ratio, indicating that the addition of iron filings has a significant effect on the abundance of heterotrophic denitrifying bacteria. Some species in *Dechloromonas* are phosphorus-polymerizing organisms that may contribute to the effective removal of phosphorus from IC-ME systems (Ma et al., 2020).

Hydrogenophaga accounts for 1.14% of the IC-ME system, while it is below the detection limit in the AC system, suggesting that it may play an important role in the IC-ME system. The difference in relative abundances between the IC-ME and AC systems suggests that the presence of *Hydrogenophaga* contributes to the removal of nitrate from the effluent. It has been shown that *hydroophaga* can derive energy from hydrogen oxidation without an organic carbon source (Willems et al., 1989) and undergo hydrogen autotrophic denitrification (Jiang et al., 2018; Qian et al., 2017; Xing et al., 2016). *Hydroophaga* can also accelerate iron corrosion by consuming hydrogen (Feng et al., 2014). In the IC-ME system under study, *Hydroophaga* may degrade nitrates through hydrogen autotrophic denitrification, and the hydrogen produced by iron corrosion can provide an electron donor to *Hydroophaga*, which is the reason for the high abundance of *Hydroophaga* in the IC-ME systems. *Hydroophaga* is widespread in the natural environment (Xiao et al., 2012), abundantly enriched in activated sludge, and *Hydroophaga* can also be detected in drinking water, tap water, and tailings water (Feng et al., 2014; Liu et al., 2018; Xing et al., 2016). As can be seen, the IC-ME system has broad applicability.

3.5 Practical Applications

As of now, four projects in Xing'an County, Guilin City, and Bobai County, Yulin City, for the treatment of domestic sewage and surface source pollution in township and rural areas have promoted and used the findings of the IC-ME process. As an example, a monitoring station in Bobai County, Yulin City, Guangxi, was constructed on July 30, 2020, and started operation on August 1, 2020. It has a treatment scale of 40 m³/day and an area of 160 m². According to data collected from a monitoring station in Bobai County, Yulin City, Guangxi, on the 11 October, 2 December, 18 December, and 30 December 2021, respectively, the average removal rates of TN, NH₄⁺-N, TP, and COD by the IC-ME process were 68.77%, 86.05%, 64.45%,

Table 3 Effectiveness of rural wastewater treatment at a monitoring station in Bobai County, Guangxi

Date	TN (mg/L)			NH ₄ ⁺ -N (mg/L)			TP (mg/L)			COD (mg/L)		
	Influent	Effluent	Removal	Influent	Effluent	Removal	Influent	Effluent	Removal	Influent	Effluent	Removal
11-Oct	56.5	9.3	83.54%	33.74	3.27	90.31%	1.09	0.25	77.06%	125	11.69	90.65%
02-Dec	94.01	18	80.85%	71.95	14.48	79.87%	19.13	5.31	72.24%	117.98	56.02	52.52%
18-Dec	85.39	39	54.33%	81.47	16.98	79.16%	22.25	9.42	57.66%	200.6	31.2	84.45%
30-Dec	87.99	38.4	56.36%	79.35	4.07	94.87%	24.69	12.14	50.83%	237.2	30.6	87.10%

and 78.68% (Table 3). the first-level discharge standard of Guangxi's Emission Standards for Water Pollutants from Rural Domestic Wastewater Treatment Facilities (DB45/2413–2021) was not met by the TN and TP levels in the effluent from a monitoring station in Bobai County, Yulin City, when compared to laboratory effluent. This was mainly caused by the large dispersion of the actual influent water, unpredictable water quality, and the fact that a monitoring station in Bobai County, Yulin City, Guangxi, discharged agricultural wastewater in December, resulting in high influent TN and TP. Farming wastewater is deep-colored, includes large amounts of suspended particles, has high levels of organic matter, and has high levels of bacteria. The majority of solid residues in wastewater are organic compounds, which will make it more difficult to further treat the wastewater, increasing the treatment burden and reducing the treatment impact if solid–liquid separation is not effective. Thus, the IC-ME approach provides great technical support to meet the long-term and ecological goals of treating residential wastewater in townships and rural areas.

4 Conclusion

The IC-ME system constructed with iron filings and activated carbon efficiently removes nitrogen and phosphorus from simulated rural domestic wastewater with a low C/N ratio. At C/N ratios of 1.9–4.4, the average removal rates of NH₄⁺-N, NO₃⁻-N, TN, and TP are 80.54%, 96.42%, 83.10%, and 92.93%, respectively. All the indicators reached the first-level B standard of “Discharge Standard of Pollutants from Urban Sewage Treatment Plants” (GB18918-2002) and the first-level discharge standard of Guangxi's “Water Pollutants Discharge Standard for Rural Domestic Sewage Treatment Facilities” (DB45/2413–2021). The IC-ME system supplies electrons for denitrification, aids in its progression,

and transforms NO₃⁻-N into NO₂⁻-N and N₂, with N₂ serving as the primary product. At low C/N ratios, the IC-ME method may be effective in removing nitrogen from residential sewage in rural areas. Fe²⁺ and Fe³⁺ formed by iron corrosion combine with OH⁻ in the water to form Fe(OH)₃/Fe(OH)₂ colloids which can adsorb and flocculate organic phosphorus, while some Fe²⁺ and Fe³⁺ react with PO₄³⁻ to form phosphate precipitation, thus achieving efficient phosphorus removal. Significant optimization of bacterial community structure was also observed in the IC-ME filler, with *Bacteroidetes*, *Chloroflexi*, and *Firmicutes* significantly more abundant in the IC-ME system than in the AC system, and the IC-ME process has great potential for the removal of nitrogen and phosphorus from rural domestic wastewater with low C/N ratios.

Author Contribution Xiaofang Liang: investigation, methodology, writing—original draft. Dongbo Wang: conceptualization, writing—original draft. Mujin Li: investigation, resources. Dunqiang Liu: investigation. Juncheng Han: writing—review and editing. Qianlan Wei: investigation. Youbin Huang: writing—review and editing. Hu Huang: writing—review and editing. Qingge Feng: resources, supervision.

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Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of Interest The authors declare no competing interests.

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