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Enhanced nutrient removal from mixed black water by a microbial ultra-low weak electrical stimulated anaerobic-two stage anoxic/aerobic process

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ABSTRACT

Efficient nutrient removal from mixed black water is under challenge in the context of global pollution control and circular economy. The anaerobic-anorexic-aerobic process is a widely used biological nutrient removal technology for wastewater treatment, yet its denitrification capacity is limited by the low biodegradable organics to nitrogen (C/N) ratio due to prior degradation of mixed black water. We have previously proved that weak electrical of 0.2 V could stimulate microbial denitrification of black water using an external electrical supply to ensure voltage stability. To reduce the dependence on additional electrical and to ensure a stable voltage output, we built a microbial fuel cell (MFC) and a microbial electrolytic cell (MEC) embedded in the anaerobic-two stage anoxic/aerobic (A-(A/O)²) system in this study, where the MFC degrades organic matter and generates electricity first. Then, the electroactive bacteria in the MEC catalyze denitrification at low carbon levels, which is affected by microbial ultra-low weak electrical stimulation (MUIWES) generated by MFC. Results showed that the removal rates of total nitrogen (TN) and total phosphorus (TP) were up to 91.3% and 98.3% respectively at a stimulation voltage of 0.1 V. Fluorescence spectroscopy revealed the formation of aromatic proteins and an increase in tightly bound-extracellular polymeric substances (TB-EPS), suggesting the involvement of these compounds in electron transfer. Community analysis disclosed the activation of autotrophic denitrifying bacteria and the inhibition of most heterotrophic denitrifying bacteria.

1. Introduction

Mixed black water refers to effluents from septic tanks which contains feces, urine and flushing water [36]. Treatment of mixed black water is challenging because it is difficult to separate feces and urine from the flushing water while excessive loads of flushing water increases the cost of transportation and treatment of the wastewater [16]. Moreover, human feces contain high concentrations of organic pollutants and pathogenic microorganisms, which causes water pollution and disease

transmission [15]. Simultaneous control of organic pollutants and pathogenic microorganisms in the mixed black water is generally complicated and expensive [40]. According to statistics, the emission of black water from toilets in China amounts to 1.44×10^7 tons daily. On average, 1 ton of black water from toilets can pollute 220 tons of clean water with valuable substances such as nitrogen and phosphorus [23]. Therefore, advanced technologies treating mixed black water while recovering resources from it are necessary in the context of the circular economy [20].

Abbreviations: A-(A/O)², Anaerobic-two-stage anoxic/aerobic; BES, Biological electrochemical system; MFC, Microbial fuel cell; MEC, Microbial electrolytic cell; DC, Direct current; MUIWES, Microbial ultra-low weak electrical stimulation; EG, Experimental group; CK, Control check; A/O, Anoxic/oxic; COD, Chemical oxygen demand; NH₄⁺-N, Ammonia nitrogen; TN, Total nitrogen; PO₄³⁻-P, Phosphate; TP, Total phosphorus; NO₃⁻-N, Nitrate; NO₂⁻-N, Nitrite; EPS, Extracellular polymeric substance; LB, Loosely bound; TB, Tightly bound; PCR, Polymerase Chain Reaction; OTUs, Operational taxonomic units.

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The modified anaerobic-anoxic-aerobic processes (A²/O) are commonly used to treat mixed blackwater [4,26,28,58]. For example, the process is inverted to avoid the adverse effect of return nitrate on anaerobic phosphorus release and the two-stage anoxic-aerobic process is extended with longer hydraulic retention time to enhance the treatment of blackwater. But denitrification efficiency is usually limited due to the prior degradation of carbon microbial nutrients in the septic tank, the unstable microbial diversity and the activity of phosphorus-releasing bacteria competing for carbon sources [38,48]. Moreover, when the hydraulic load is insufficient, phosphorus removal will be reduced because aeration reduces the PHB content in phosphate-accumulating organisms (PAOs) cells, resulting in a decrease in phosphorus uptake, which prevents PAOs absorbing extracellular phosphate to synthesize polyphosphate effectively [3]. The efficiency can be improved by the embedding of a biological electrochemical system (BES), in which an electrochemical reaction is driven by active microorganisms located at the surface of electrodes [2,7,14,29,53]. For instance, electricity-producing microorganisms such as *Geobacter*, *Shewanella*, *Proteobacteria*, and *Bacteroidetes* are attached to the anode of MFC and produce extracellular polymers (EPS) as electron acceptors to maintain their survival [43]. The generated EPS could then form biofilms to degrade substrate in sewage and generate electrons that can be further used for the reduction processes [21]. For example, electrons reach the cathode via the external circuit to reduce NO₂⁻ or NO₃⁻ electron acceptors, thus reducing the competition for carbon sources between phosphorus-releasing bacteria and denitrifying bacteria (including autotrophic denitrifying bacteria and heterotrophic denitrifying bacteria) and improving the efficiency of nutrient removal [39,59].

Previous studies coupling microbial fuel cells (MFCs) with conventional wastewater treatment processes such as wetland and upflow anaerobic sludge tanks have shown that the combined processes achieve significant sludge reduction due to the small fraction of COD (chemical oxygen demand) used for microbial growth compared to conventional processes, but are limited by their low power supply efficiency and pollutants removal efficiency [37,63]. Many scholars are currently treating black water and generating microbial electricity simultaneously with MFCs, but the N removal is not significant [2,7,14]. Therefore, we hypothesized that a stepwise system combining both MFC and microbial electrolytic cell (MEC) with anoxic-oxic compartments would enhance sludge reduction and denitrification and avoid the competition between autotrophic and heterotrophic denitrifying bacteria. As a first trial to stimulate microbial denitrification in mixed black water treatment, we used a direct current (DC) supply to provide 0.2 V of power to the MEC unit to supplement the unstable power production and low output voltage of the MFC unit [32]. Later potassium persulfate is also dosed as cathodic solution to improve the electrochemical activity of the MFC and to ensure the stable supply of ideal power to the MEC [24]. However, these modifications directly or indirectly increase processing costs. Ideally, the applied current intensity should not be too strong to avoid inhibiting microorganisms and denitrification [64].

Actually, most investigations have studied electrical stimulation at voltages of 0.1–1.5 V [22,33,49], whereas little is known about the effects of microbial ultra-low weak electrical stimulation (MUIWES) (0–0.1 V). Therefore, to reduce the dependence on additional electrical providing and to ensure a stable voltage output, this study built a microbial fuel cell (MFC) and a microbial electrolytic cell (MEC) embedded in the anaerobic-two stage anoxic/aerobic (A-(A/O)²) system, where the MFC degrades organic matter and generates electricity first. Then, the electroactive bacteria is enhanced by MUIWES generated by MFC and then catalyzes denitrification at low C/N levels in the MEC unit. To the best of our knowledge, this is the first study to explore the role of MUIWES (0–0.1 V) in the coupled A-(A/O)²-MFC-MEC process.

In this study a novel A-(A/O)²-MFC-MEC system to remove N (nitrogen), P (phosphorus) and COD from mixed black water using MFC voltages at 0, 0.03, 0.05, 0.07 and 0.1 V was studied. Extracellular polymers (EPS) and changes in the abundance and structure of microbial

populations were also examined. When the A-(A/O)²-MFC-MEC process is applied to actual projects in the future, it can largely reduce the dosage of external organics and the excess electricity generated can be used to enhance performances of biological treatment units to achieve the economic and social benefits by saving energy consumption and reducing carbon emissions.

2. Materials and methods

2.1. Experimental setup

The diagram of the experimental device and process flow chart is demonstrated in Fig. 1. Anaerobic tank 1, Anoxic tank 1, Aerobic tank 1, Anoxic tank 2, and Aerobic tank 2 in the A-(A/O)² process are denoted as Anaerobic 1, Anoxic 1, Oxidic 1, Anoxic 2, and Oxidic 2, respectively. Both the experimental group (EG) and the control check (CK) use reactors with nearly identical conditions, except that the CK is not equipped with MFC and MEC. The EG is composed of an anaerobic zone, two anoxic zones, two oxic zones and a sedimentation tank. The Anaerobic 1 is coupled with an MFC, and the Anoxic 2 is coupled with an MEC to achieve better black water treatment efficiency. The reactors are made of plexiglass with the thickness of 5 mm. The length, width, and height of each chamber are 22 cm, 15 cm, and 10 cm, respectively, and the total volume is 12 L. The first anoxic tank is separated by a porous plate in the middle, and the left compartment (Anaerobic 1) builds the MFC, which serves as power supply for the MEC. The right compartment (Anoxic 1) receives the internal return flow from Oxidic 1. Anaerobic 1 is equipped with a 5 × 8 cm wire mesh, a 5 × 6 × 10 cm carbon brush, and an external 5000 Ω resistor is used in the MEC with an electrode distance of 4 cm. The electrode is fixed on the top partition of the reactor by a titanium wire, and the two ends of the resistance wire are connected to two ends of the electrode by a titanium wire mesh. Anaerobic 1 and Anoxic 1 have outlets in the middle and upper parts, with sampling points and mixed liquid suspended solid (MLSS) return points on the top. The cathode and anode electrodes of the MEC are made of 5 × 8 cm steel wire mesh and 5 × 8 cm carbon felt, respectively. The electrode distance is 4 cm. The electrode is fixed on the separator on the upper side of the reactor by titanium wire.

The MFC electrode voltage IT8800 series DC electronic load control was used to collect voltage data every 30 s. The biomass sludge was magnetically stirred in the anoxic tank and anaerobic tank at a speed of 300 rpm. Oxygenation disks were placed at the bottom of the oxic tanks.

2.2. Wastewater quality and sludge inoculation

Real mixed black water samples were taken from the septic tank of a teaching building in University of Shanghai for Science and Technology (old campus at East Guoshun Road). Quality of the actual mixed black water is determined each week since year 2015. Artificial wastewater was used to simulate the mixed black water and used as the influent for the laboratory scale A-(A/O)²-MFC-MEC reactors. The approximate composition of artificial wastewater is in mg/L: COD 1050–1150, ammonia nitrogen (NH₄⁺-N) 50–60, total nitrogen (TN) 50–60, and phosphate (PO₄³⁻-P) 10–12. Detailed composition of the artificial nutrient solution, and composition of trace elements in the stock solution are shown in Supplementary Tables S1 and S2. Nutrient solution and a weak acid buffer reagent were included in the artificial water to keep the pH of the artificial water distribution between 7.0 and 8.0.

The sludge inoculated was taken from the Eastern District Sewage Treatment Plant of Yangpu District, Shanghai, China. The sludge was cultivated and domesticated into aerobic sludge and anaerobic sludge under corresponding conditions, then inoculated in the aeration tank and anaerobic tank, respectively. Sludge concentration in the system was 3100 ± 100 mg/L.

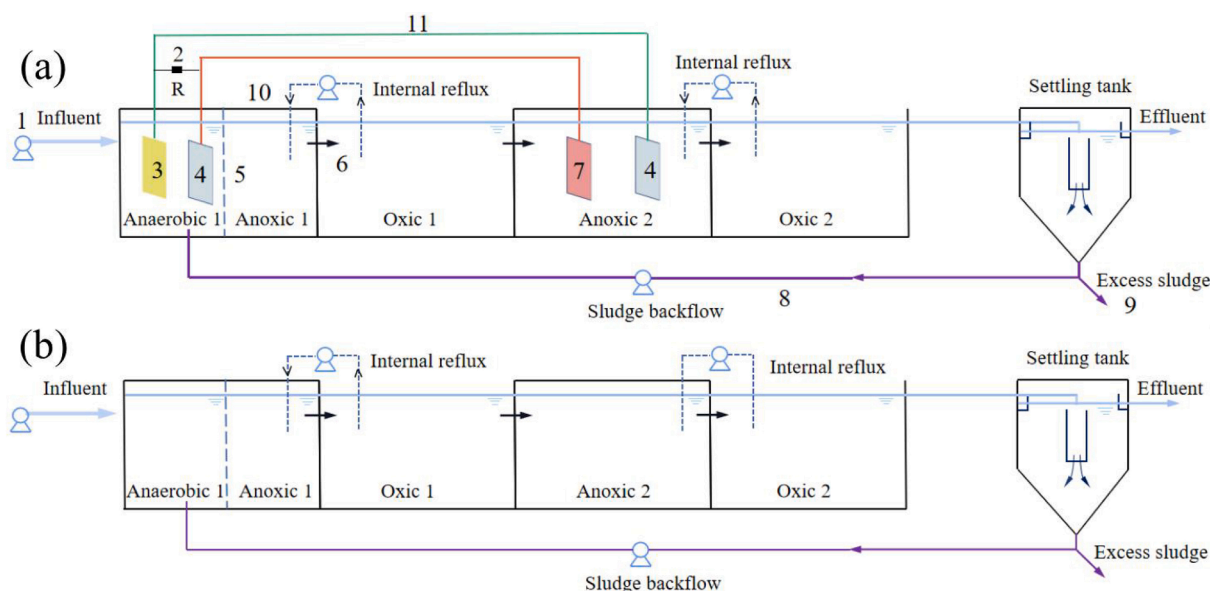


Fig. 1. a) Anaerobic-two-stage anoxic/aerobic (A-(A/O)²) reactors equipped with a microbial fuel cell (MFC) in the Anaerobic 1 compartment, and a microbial electrolysis cell (MEC) in the Anoxic 2 compartment, and (b) Control check (CK) without MFC and MEC. The electricity generated in the MFC powers the MEC to complete the removal of N and P. 1) Peristaltic pump, 2) resistance wire, 3) carbon brush electrode, 4) wire mesh electrode, 5) porous partition, 6) sewage flow direction, 7) carbon felt electrode, 8) external sludge return flow, 9) excessive sludge flow, 10) internal sludge return flow, 11) microbial weak electricity flow from the MFC.

2.3. Pre-treatments of the electrodes and system parameter optimization

The 5 cm × 8 cm carbon felt electrode from Jingzhou Haote New Material Co., Ltd was washed with deionized water, soaked 1 h in 1 M NaOH, washed with deionized water, dried in an oven at 50°C for 12 h, soaked in 1 M HCl for 1 h, and rinsed with deionized water before drying. The 5 × 6 × 10 cm carbon brush electrode from Jingzhou Haote New Material Co., Ltd, was firstly washed with deionized water and soaked for 24 h in acetone, after that it was burnt at 400°C~600°C in a muffle furnace for 30 min and rinsed in deionized water and finally dried at 50 °C. The operation parameters of the A-(A/O)²-MFC-MEC system were sequentially optimized, including the optimization of single and double chamber MFCs, the internal reflux ratio (100%, 200%, 300%), and the hydraulic retention time (12 h, 24 h, 36 h).

2.4. Analytical methods

2.4.1. Determination of conventional water quality indicators

TN, NH₄⁺-N, nitrate (NO₃⁻-N), nitrite (NO₂⁻-N), total phosphorus (TP), COD and pH were measured according to the standard methods [1]. Pollutants removal efficiencies were calculated as follows:

$$R = (C_0 - C_1)/C_0 \times 100\% \quad (1)$$

where C₀ is the influent pollutant concentration (mg/L), C₁ denotes the effluent pollutant concentration (mg/L), and R depicts the removal efficiency.

2.4.2. Extraction and determination of extracellular polymer substances

EPS of the sludge is extracted by the thermal extraction method [5]. Typically, 40 mL of post-experimental sludge is centrifuged for 5 min at 4000 rpm, and the supernatant is discarded. The precipitate is washed twice with 0.01 M phosphate buffered solution at pH 7.4 then centrifuged. 15 mL of 0.05% NaCl and 40 mL of 70 °C-preheated 0.05% NaCl solution are then added to the precipitate to obtain a suspension at about 50°C. After centrifugation under a reciprocating shaker for 5 min at 4000 rpm, the organic matter in the supernatant is separated and considered as a solution of loosely bound (LB)-EPS. 0.05% NaCl is added to the sludge centrifuge tube to make up the volume to 40 mL. The tube

is water-bathed at 60 °C for 30 min, then centrifuged at 4000 r/min for 15 min, before collected and considered as a solution of tightly bound (TB)-EPS. LB and TB solutions are passed through a 0.45 μm filter membrane, then the filtrate containing purified LB and TB are analyzed by a three-dimensional fluorescence spectrometer (Hitachi, F-700, Japan). Parameter settings of the spectrometer are shown in Supplementary Table S3.

2.4.3. Microbial community analysis

5–10 g of sludge samples from the carbon felt electrode in the MEC (anoxic tank) were taken for static precipitation and put into a sterile centrifuge tube of 10 mL for microbial community analysis when the process performance is stable. The genomic DNA of the samples were triply extracted using cetyltrimethylammonium bromide, and then mixed to establish the amplicon libraries of bacteria. An appropriate amount of sample DNA is added to the centrifuge tube, and the sample is then diluted to 1 ng/μL with sterile water. The diluted genomic DNA is used as a template with specific primers 515F (5' -GTG CCA GCM GCC GCG G-3') and 806R (5' -GGACTA CHV GGG TWT CTA AT-3') in the Barcode, Phusion® High-Fidelity Polymerase Chain Reaction (PCR) Master Mix with GC Buffer of New England Biolabs. High-efficiency and high-fidelity enzymes are used to ensure the efficiency and accuracy of amplification based on the sequencing area. The Uparse software is used to cluster all clean reads of samples. By default, the sequences are clustered into operational taxonomic units (OTUs) with 97% consistency. At the same time, according to the algorithm principle, the sequence with the highest OTUs frequency was selected as the representative sequence of OTUs. The Alpha diversity reflect the richness and diversity of the microbial community within the sample. Illumina sequencing data was submitted to the NCBI, under the study accession number PRJNA783741, and can be viewed by the following URL: <https://www.ncbi.nlm.nih.gov/biosample/23444611>.

3. Results and discussion

3.1. Pollutants removal from mixed black water

We studied the removal of COD, N, and P in a new process

comprising A-(A/O)² reactors equipped with a MFC providing electricity to a MEC (Fig. 1). To accelerate the electron transfer efficiency and improve the carbon source utilization efficiency, this study firstly optimized the parameters and results which indicated that the best configuration and parameters for further experiments were single-chamber MFC, 200% for internal return ratio, 25% for external return ratio, and 24 h for hydraulic retention time respectively (Supplementary Table 4 and supplementary Fig. S1-Fig. S5). The stability of the MFC performance was also confirmed by supplementary Fig. S6.

We then studied the removal of COD, TP, TN, and NH₄⁺-N at MEC voltages of 0, 0.03, 0.05, 0.07, and 0.1 V by changing the external resistance value (Fig. 2). Results demonstrated that at EG-0 V the COD removal rate was only 2.23% higher than CK (Fig. 2A). The reason for this finding is that although the microorganisms enriched in MFC used organic matter to generate electricity and accelerate the removal of organic matter, the final effluent COD removal rate of CK was 95.87%, which was close to maintain the normal survival level of microorganisms, so the embedding of the MFC had no significant effect on the enhancement of the final effluent COD [52]. Similarly, at EG-0 V, the COD removal efficiency was already very high up to 98.1%, thus increasing the stimulation voltage values did not significantly improve the removal efficiency.

TP and NH₄⁺-N removal showed similar trends. With already high removal rates of 98.5% for P and 99.6% for NH₄⁺-N at EG-0 V, P removal increased by 22.2% and NH₄⁺-N removal by 7.8% versus the CK. This is consistent with the 48.0–67.0% removal rate of P observed using MFC embedded in an A/O reactor [55]. The remaining removable COD, NH₄⁺, and TP reach the concentration for normal survival of microorganisms, so the effect of regulating voltage on the effluent concentration is not significant.

The removal of TN increased sharply from the CK (80.0%) to EG-0 V (88.7%), then increased progressively with MUIWES MEC: 89.0% at 0.03 V, 90.2% at 0.05 V, 90.9% at 0.07 V, and 91.3% at 0.1 V (Fig. 2C). This represents an improvement of 15.2% versus a previous study

showing 76% of TN removal [32]. Overall, it is found that the effluent quality of the mixed black water after the MUIWES treatment was better than standard discharging limits[45].

Xie et al. [55] studied the MFC-Anaerobic-Anoxic-Aerobic reactor and found that the microbial community structure changed along the direction of water flow. Therefore, studying the changes of pollutants and microorganisms in the A-(A/O)²-MFC-MEC system along the water flow direction and influencing factors under the stimulation of weak voltage is of great significance to understand the processing mechanism of the coupled system.

Concentrations of COD, TP, NH₄⁺-N and TN at each step of the treatment chain are shown in Fig. 3 (All MUIWES data are shown in supplementary Fig. S7). Results showed that COD removal was predominant during Anaerobic 1 and Anoxic 1 steps. This is explained by the MFC that induces the growth of electricity-producing bacteria and decomposes organic matter into small molecules, which further feeds denitrifying bacteria in the Anoxic 1 [19]. The TP concentration decreased continuously along the flow direction. Interestingly, the TP concentration of EG both decreased significantly in Anaerobic 1 and Anoxic 2. In Anaerobic 1, it is mainly due to dilution by MLSS return flow. In addition, the intensely decomposition of organic matter by MFC led to an increase in pH, which was suitable for the conversion of P into small insoluble phosphate [57]. The decrease of TP in Anoxia 2 is caused by the dilution of internal reflux and the phosphorus removal by anoxic denitrification using NO₃⁻ as electron acceptor. Furthermore, it was shown that there was a synthesis of poly-β-hydroxybutyrate and release of phosphorus at higher COD concentrations, leading to inhibition of “phosphorus uptake”[27]. Since the COD of CK was higher than that of EG, TP removal by EG was greater than CK in Anoxia 2.

Profiles of NH₄⁺-N and TN show similar trends. The decrease of NH₄⁺-N in Anaerobic 1 was mainly due to the dilution by the MLSS return flow, and the decrease in Anoxic 1 was mainly due to the dilution by the internal return flow. Surprisingly, NO₃⁻-N was absent and NO₂⁻-N concentration was very low in Aerobic 1. This is tentatively explained by

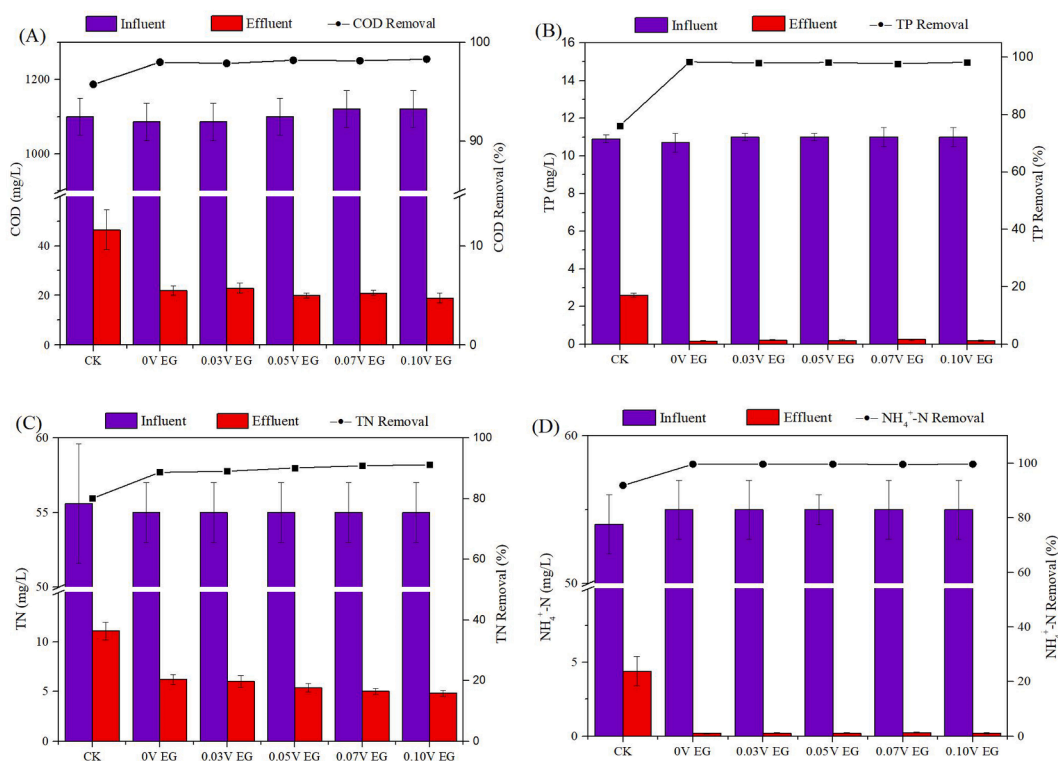


Fig. 2. Total removal in mg/L and percent of the (A) chemical oxygen demand (COD), (B) total phosphorus (TP), (C) total nitrogen (TN), and (D) ammonia nitrogen (NH₄⁺-N) in A-(A/O)² system equipped with a MFC providing microbial ultra-low weak electrical stimulation (MUIWES) to a MEC. CK: control without MFC and MEC. Voltage refers to voltage applied to the MEC.

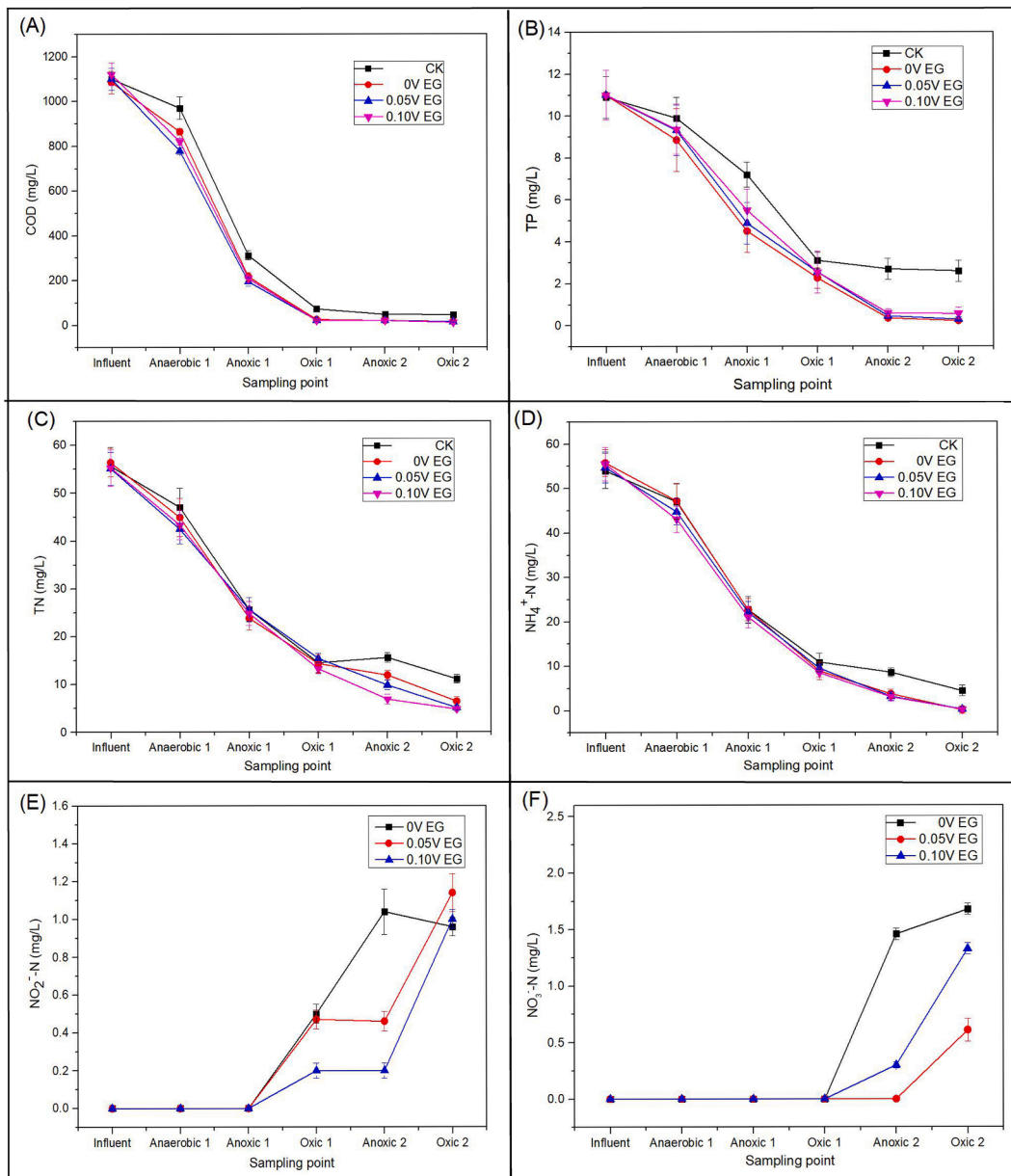


Fig. 3. (A) COD, (B) TP, (C) TN, (D) NH₄⁺-N, (E) nitrite (NO₂⁻-N) and (F) nitrate (NO₃⁻-N) in the successive treatment steps of the A-(A/O)² system equipped with a MFC providing MUIWES to a MEC. CK: control without MFC and MEC. Voltage refers to voltage applied to the MEC.

aerobic denitrification supported by COD reduction.

3.2. Role of MUIWES in TN removal and EPS formation

As indicated in Fig. 3, TN removal was mainly effective in the Anoxic 2 reactor equipped with MEC. Here, TN, NO₂⁻-N, and NO₃⁻-N concentrations kept decreasing with increasing voltage values. The TN removal rate increased from 78.8% at 0 V to 87.6% at 0.1 V. Therefore, this study assumes that multiple microorganisms compete and cooperate under MUIWES, inducing multiple nitrogen removal mechanisms such as anodic ammonia oxidation, heterotrophic denitrification, autotrophic denitrification, and/or anaerobic ammonia oxidation, resulting in effective treatment of low C/N ratio wastewater [24]. In addition, MUIWES may increase the electron transfer process which enhances the denitrification capacity [32]. Since Pili or pili like compounds containing microorganism is activated and its electrical conductivity is increased in the presence of weak electrical stimulation, the stimulation of weak electricity increases the amount of aromatic amino acids in EPS.

Then the efficiency of electron transfer can be improved and the performance of denitrification would be ultimately enhanced. Overall, findings in this study show that MUIWES induce a significant effect on TN removal.

EPS had unique structures, and some have biological activity. On the one hand, EPS can protect microbial cell bodies. On the other hand, it can also be used as a carbon source for cell metabolism [41,47]. Changes in EPS in the denitrifying sludge of the MEC were analyzed by three-dimensional fluorescence (Fig. 4). Results showed the fluorescence intensity and area of the three-dimensional fluorescence spectrum LB fluorescent region (0.00 V > 0.05 V > 0.10 V) and the corresponding TB (0.00 V < 0.05 V < 0.10 V). As MUIWES increased, the LB/TB decreased. These results were consistent with the phenomenon that the sludge LB/TB reduced from 0.66 to 0.62 after microcurrent stimulation reported by [60]. LB-EPS decreased and TB-EPS increased under weak electrical stimulation at 0.2 V, but the total amount of LB-EPS and TB-EPS proteins changed very little [10]. The LB-EPS located in the outer layer of granules extends from the TB-EPS with a highly porous and dispersible

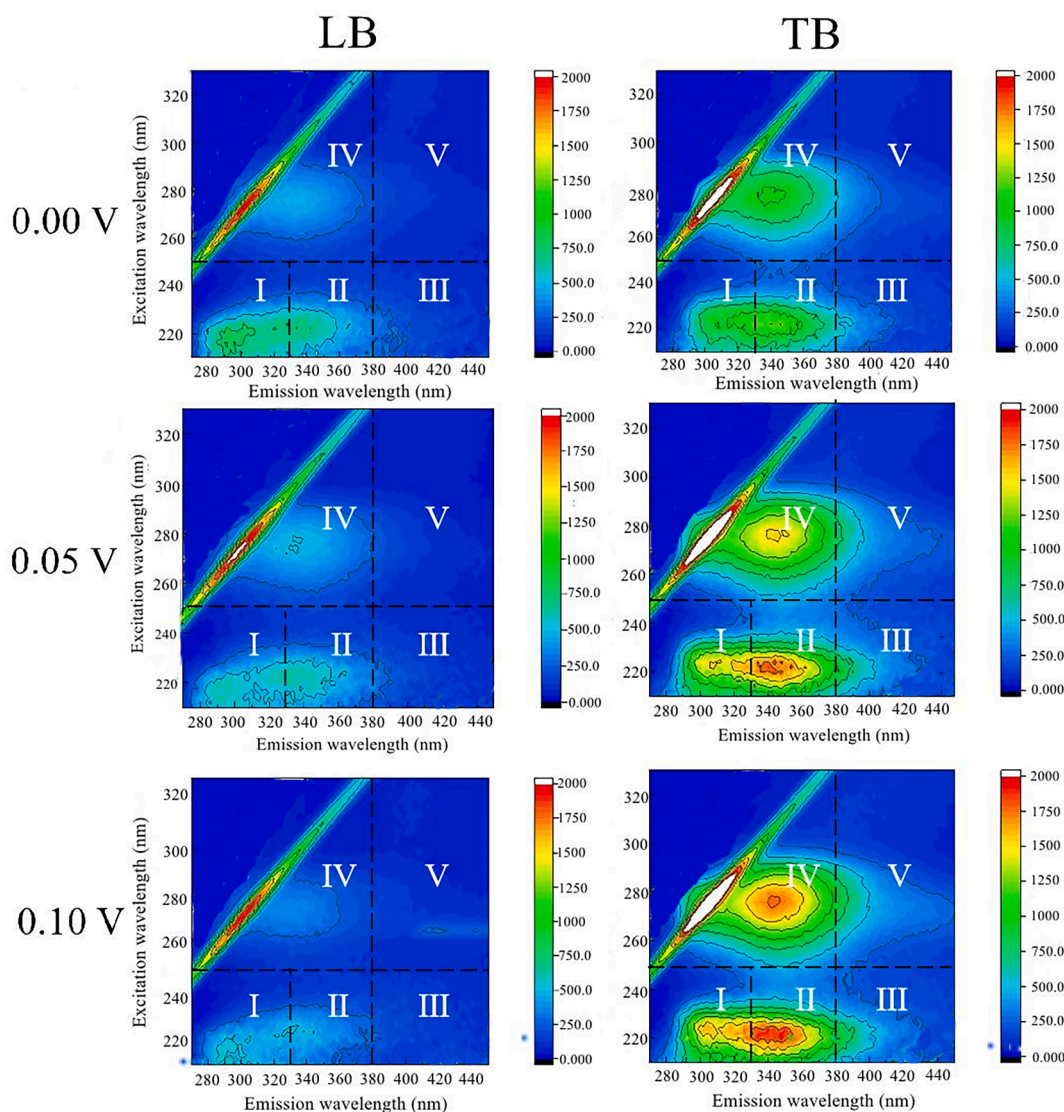


Fig. 4. Loosely bound-extracellular polymeric substance (LB-EPS) and tightly bound-extracellular polymeric substance (TB-EPS) in the denitrifying sludge of the MEC unit, analysed by fluorescence, versus MUIWES.

structure while the TB-EPS located in the core region of the granules are highly compact and rigid [60]. Therefore, this study speculate that the metabolic activity of microorganisms increases at 0.1 V MUIWES could result in the possible conversion of LB-EPS that is too far from microbial cells for metal-like electron transfer, to TB-EPS that is more tightly bound to cells[56]. This ultimately enhances the electron transfer between microbial cells.

According to the division of fluorescence spectra, regions I and II are representing mainly aromatic protein substances, and their content increases with increasing voltages (Fig. 4). This is important because EPS are involved in electron energy transfer[25]. Since the microbial cells are encapsulated in EPS, and are thus not in direct contact with the external environment, EPS contains many microbial secretions such as polysaccharides, proteins, and humic substances that act as electron mediators [13]. These substances have semi-conductive properties, and in particular, play a very important role in electron transfer [34,46]. The main components of EPS proteins are extracellular enzymes, most of which are electrically active or acting as electron shuttles [12]. Therefore, the increase of proteins observed in this study may lead to more extracellular enzymes involved in extracellular electron transfer. On the other hand, EPS proteins play an important role in cell adhesion and aggregation and contribute to the maintenance of sludge structure

stability [35].

Region IV represents microbial byproducts and protein-like substances, which increases with MUIWES (Fig. 4). Protein-like substances include a variety of cellular chromophores that mediate electron transfer between fermenting bacteria and methanogens [44]. Overall, results in this study suggested that applying MUIWES MEC induced the conversion of LB-EPS to TB-EPS, and in turn, enhanced electron transfer and improved denitrification. Microbial community growth was closely related to sewage treatment, sludge performance, and EPS contents [42,54].

3.3. Probing the mechanism of MUIWES denitrification from a biological perspective

Further structural diversity and similarity analysis of the MEC nitrifying sludge microbial community structural is required to verify the MUIWES MEC denitrification mechanism. Table 1 indicates the statistics of the Alpha diversity indices. Results show that most indices show similar trends along the successive treatment steps of wastewater, with a minimum diversity in Anaerobic 1. The Ace and Chao 1 indexes indicated that the diversity of bacterial communities gradually increased in the order of Anaerobic 1, Anoxic 1, Oxidic 1, Anoxic 2 and Oxidic 2. The high

Table 1
Statistics of the Alpha diversity indexes for each sample.

Sample_name	Observed specie	Shannon index	Ace	Chao 1	Coverage	Simpson index
A 1	530	5.582	555	554	0.998	0.940
A 2	472	5.142	519	521	0.998	0.929
Anaerobic	400	3.792	447	426	0.998	0.784
Anoxic 1	539	5.662	574	569	0.998	0.941
Oxic 1	632	7.066	660	661	0.998	0.983
Anoxic 2	639	6.588	710	921	0.997	0.971
Oxic 2	558	5.833	601	592	0.998	0.960

^a A 1 and A 2 represent CK-Anoxic 1 and CK-Anoxic 2, respectively.

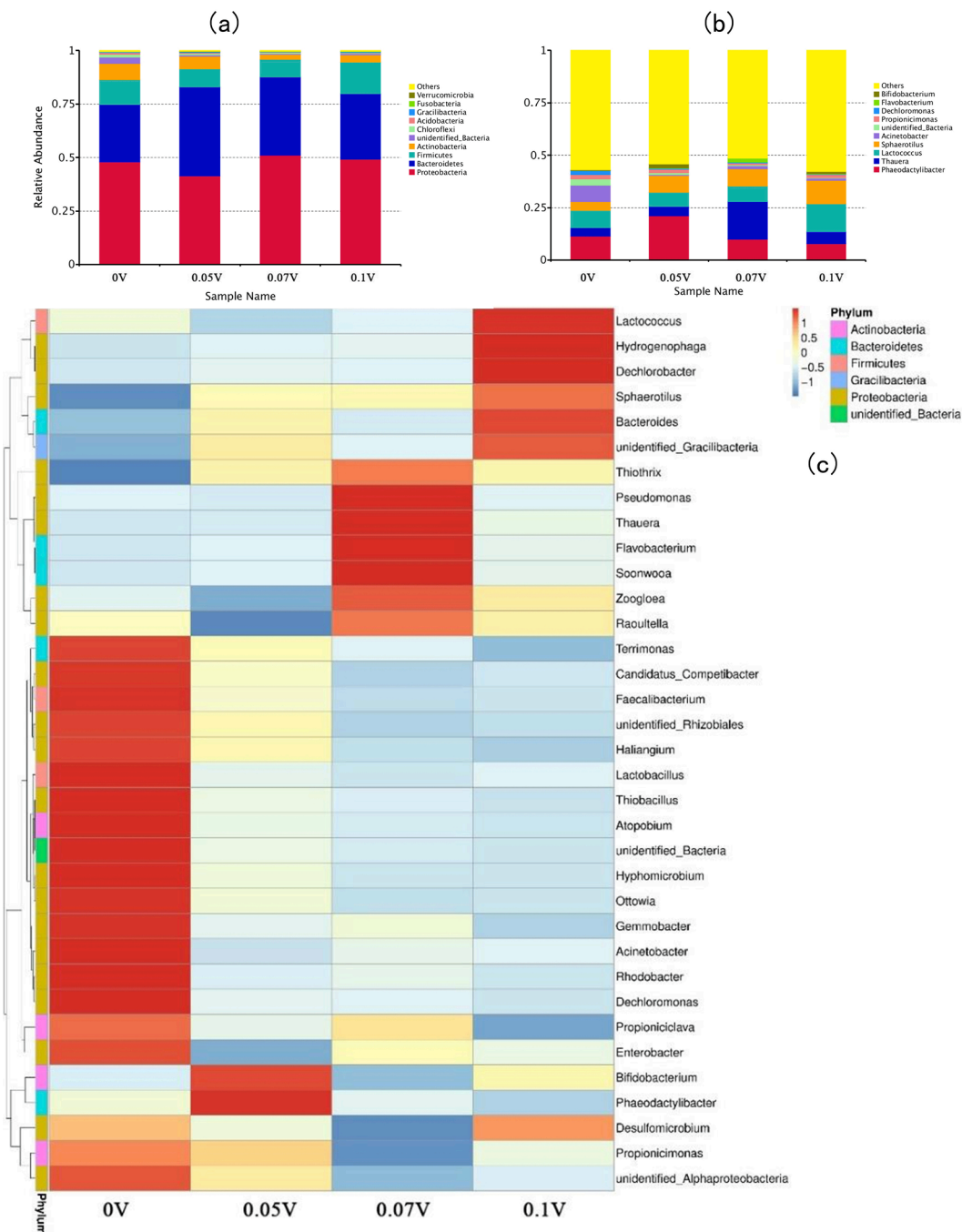


Fig. 5. Relative abundance of phylum-level and genus-level microbial species in the MEC unit at different voltages and phylum level Heatmap. (a): phylum level, (b): genus level, (c): phylum level Heatmap. (ultra-low weak electrical generated from MFC).

diversity observed in Anoxic 2 implied that MUIWES increased the microbial diversity. Therefore, it is necessary to focus on the changes of MEC microbial populations under different MUIWES.

As shown in Fig. 5 (a), the relative abundance of species at the phylum level of the MEC microorganisms at different MUIWES was analyzed and it showed that the dominant species in each sample were ranked in the order of abundance as *proteobacteria*, *bacteriophages*, *saprophytes*, *actinomycetes*, and *staphylococci*. There were differences in the structure of microbial populations at four voltages, with *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* being the dominant species. Electro-producing and hydrogen-producing bacteria were distributed in the four phyla, which facilitated the degradation of organic matter by microorganisms in the MEC unit. *Firmicutes* could release hydrolase, transform organic matter to produce acid, and transfer electrons to the electrode, thus the increase of MUIWES was beneficial to the enrichment of *Firmicutes* [6]. *Propionimonas* was closely related to the removal of phosphorus and has excellent phosphorus absorption characteristics [11]. From the genus level, *Phaeodactylibacter*, *Thaurea*, *Lactococcus*, *Sphaerotilus*, *Acinetobacter*, and *Propionimonas* were the dominant strains in the process (Fig. 5 (b)). Two types of denitrifying bacteria were included, namely the autotrophic denitrifying bacterium (e.g. *Thaurea*) and the heterotrophic denitrifying bacterium (e.g. *Sphaerotilus*, *Acinetobacter*). These were identified by combining the representative autotrophic/heterotrophic microorganisms from previous studies in the field of nitrogen removal and phosphorus removal [32,51,61]. With the increase of MUIWES, the abundance of the autotrophic denitrifying bacterium *Thaurea* increased (4.1% for CK versus 17.8% for EG-0.07 V) and the abundance of the heterotrophic denitrifying bacterium *Acinetobacter* kept decreasing (7.4% for CK versus 0.7% for EG-0.1 V), which is similar to the results of our previous studies where electrical stimulation inhibited the heterotrophic denitrifying bacteria [32]. Interestingly, the heterotrophic denitrifying bacteria *Sphaerotilus* were increasing (4.46% for CK versus 11.15% for EG-0.1 V). This is because the reflux from Oxic-2 to Anoxic-2 brought in a small amount of dissolved oxygen to form a local aerobic zone, and *Lactococcus* synthesized lactic acid as a carbon source through glycolysis to facilitate the survival of *Sphaerotilus* (aerobic denitrification bacteria) [17]. Combined with Fig. 5(c), it was found that the abundance of heterotrophic denitrifying bacteria such as *Ottowia*, *Gemmobacter*, and *Dechloromonas* all decreased with increasing MUIWES [32,51,61], which was consistent with the above analysis. This is because e^- reaching the cathode are used by autotrophic denitrifying bacteria such as electrophiles to reduce NO_2^- and NO_3^- to N_2 [9]; Zhen et al., 2009). With the increase of MUIWES e^- , the abundance of autotrophic denitrifying bacteria is promoted, thus competing with heterotrophic denitrifying bacteria for NO_2^- and NO_3^- electron acceptors [8]. In addition, the electricity producing bacteria degraded part of the COD, which reduced the carbon source required for denitrification by heterotrophic denitrifying bacteria. The abundance of hydrogenotrophic denitrifying bacteria *Hydrogenophage* increased with increasing MUIWES, and it was reported that *Hydrogenophage* could reduce NO_3^- to N_2 using H_2 and H^+ [9]. When the stimulation voltage was lower than 0.07 V, *Thaurea* and *Hydrogenophage* abundance increased with increasing MUIWES. However, when it was increasing from EG-0.07 V to EG-0.1 V, the relative abundance of the autotrophic denitrifying bacteria *Thaurea* decreased and the relative abundance of *hydrogenophage* increased, indicating that the two autotrophic denitrifying bacteria competed fiercely for e^- and the contribution of *hydrogenophage* to denitrification increased. As shown in supplementary Fig. S8, *Anaerolineae* were present in the MEC unit under MUIWES. Previous studies have reported a reciprocal relationship between *Anaerolineae* and anaerobic ammonium oxidation bacteria, possibly surviving through the breakdown of soluble microbial products and EPS produced by anammox bacteria [30]. Therefore it is likely that anaerobic ammonium oxidation is present in this system.

Results showed that under MUIWES, lactic acid produced by glycolysis of *Lactobacillus* as a carbon source increased the abundance of

Sphaerotilus. However, all other heterotrophic denitrifying bacteria were inhibited, with increased abundance for *Thaurea* and the *hydrogenophilic* autotrophic denitrifying bacteria. Conversion of LB-EPS to TB-EPS enhanced electron transfer and improved the denitrification efficiency. The overall decrease of heterotrophic microorganisms facilitated the advanced treatment of low C/N wastewater.

3.4. Denitrification pathway under function of MUIWES

The denitrification pathway occurring in the MEC unit is shown in Fig. 6. It is assumed that anaerobic ammonia oxidation is not included in the calculations because it represents only a small percentage of the microbial population. As shown in Fig. 3, without MUIWES treatment, COD and TN were reduced by 8 mg/L and 3.14 mg/L, respectively; while elevated NO_2^- and NO_3^- effluents indicated lower denitrification activity and calculated removal of COD/TN > 2.5. When 0.1 V of MUIWES was applied, COD and TN were reduced by 12.0 mg/L and 5.2 mg/L, respectively. The low accumulation of NO_3^- and NO_2^- indicated that the activity of autotrophic and/or heterotrophic denitrification was high, and the calculated COD/TN removal was < 2.5. The change in pollutant removal from 0 V to 0.1 V MUIWES was elucidated from the electron donor/electron acceptor perspective. For the electron donor, the reduced 4 mg/L COD produced 0.5 mmol e^- and the reduced 0.28 mg/L NH_4^+ produced 0.12 mmol e^- via the anodic ammonia oxidation pathway [31]. For electron demand, the 1.26 mg/L NO_3^- reduction produced 0.45 mmol e^- and the 0.54 mg/L NO_2^- reduction produced 0.12 mmol e^- , for a total of 0.57 mmol e^- less than the number of electrons supplied [18]. This finding indicated that the presence of anaerobic ammonia oxidation to remove some of the NH_4^+ [50]. Therefore, the excess 0.05 mmol e^- electron donor was attributed to the reaction of anaerobic ammonia oxidation. Calculating anaerobic ammonia oxidation by the electron donor and electron acceptor equation, an additional contribution of 0.21 mg/L NH_4^+ removal from the anaerobic ammonia oxidation at 0.1 V MUIWES was obtained in this study. The reaction generates about 0.05 mg/L of NO_3^- and 0.28 mg/L of NO_2^- . The low contribution of nitrogen removal by anaerobic ammonia-oxidizing bacteria may be achieved by the arrival of electrons via the external circuit to the cathode, which will be reduced to N_2 with NO_3^- and NO_2^- as electron acceptors by the electroautotrophic denitrifying bacteria or the hydrogen autotrophic denitrifying bacteria. This results in a very low concentration of NO_2^- as the anaerobic ammonia oxidation reactant. In addition, MEC anodic ammonia oxidation competes with anaerobic ammonia oxidation for the substrate NH_4^+ . 2.86 mg/L of COD was required to remove 1 mg/L of NO_3^- [62]. 4 mg/L of COD reduction was achieved at 0.1 V MUIWES compared to 0 V for heterotrophic denitrification, so the 4.92 mg/L of TN reduction included 3.52 mg/L of autotrophic denitrification. Thus, 71.5% of the increased TN removal was calculated as autotrophic denitrification at 0.1 V MUIWES.

Based on the discussion above, a complicated MUIWES denitrification pathway involving seven types of microorganisms is presented as follows (Fig. 6). In the anode area, the following mechanisms are involved: (1) Fermentation bacteria decompose the remaining glucose into lactic acid under anaerobic conditions. (2) Electricigens decompose lactic acid and small organic matter to produce e^- and H^+ . At the same time, MUIWES increases the secretion of aromatic proteins and LB-EPS, which enhances the electron transfer effect between microbial cells. (3) Anodic ammonia oxidation oxidizes NH_4^+ to NO_2^- and e^- . (4) Anammox bacteria use NH_4^+ as an electron donor and NO_2^- as an electron acceptor to produce N_2 and a small amount of NO_3^- . At the cathode area, the following mechanisms may be participated: (1) the H^+ gains e^- and forms H_2 . (2) Hydroautotrophic denitrification bacteria use the cathode-generated H_2 and anode-generated H^+ to achieve autotrophic denitrification. (3) Electroautotrophic denitrification bacteria obtains e^- from cathode to achieve denitrification. (4) Heterotrophic denitrification bacteria uses organic matters to achieve traditional denitrification. Various nitrogen species formed in the micro-carbon source wastewater

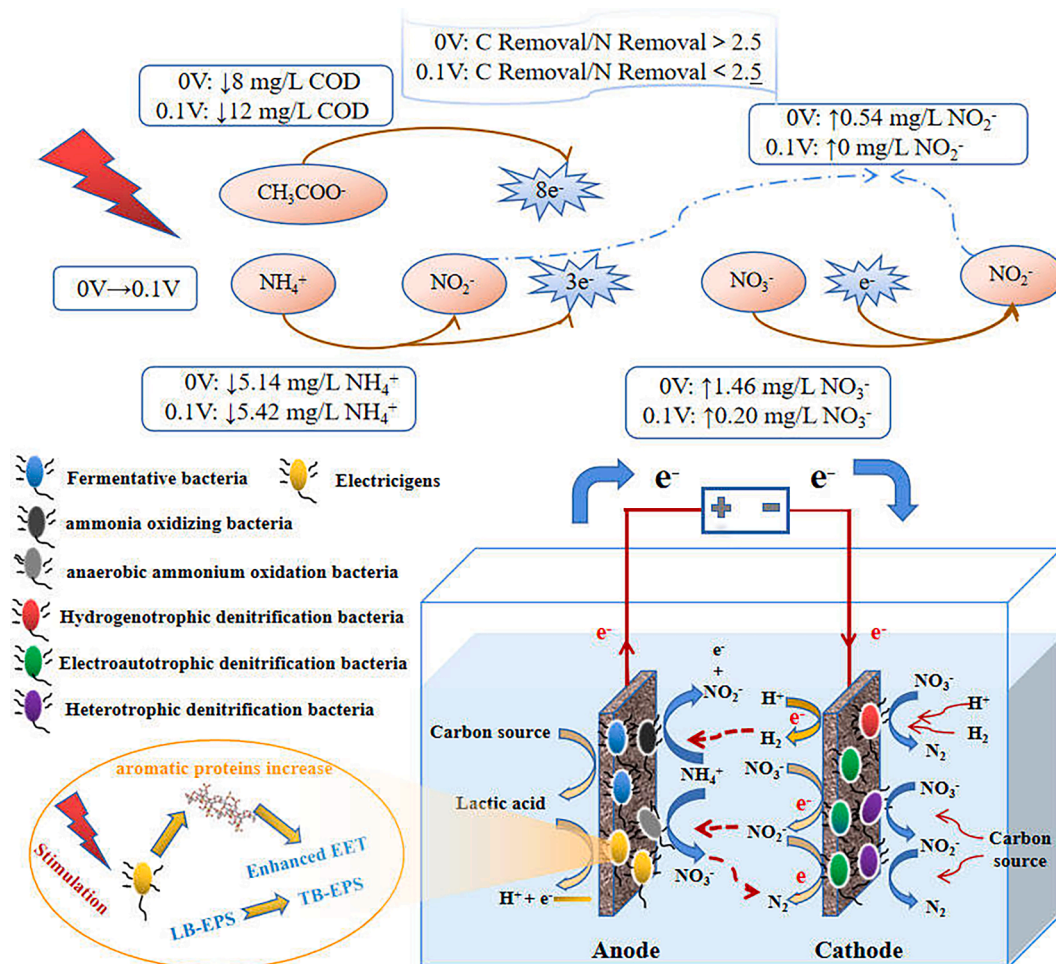


Fig. 6. Demonstration of denitrification pathway under function of MUIWES.

often coexist, which makes it reasonable for several types of denitrification paths to coexist in the MEC unit.

4. Conclusion

In this study, an A-(A/O)²-MFC-MEC system was constructed achieving high TN and TP removal rates of 91.3% and 98.3% under 0.1 V MUIWES. Electroautotrophic denitrification, hydrogenotrophic denitrification, and heterotrophic denitrification were the main pathways with the least accumulation of NO_2^- -N and NH_4^+ -N under 0.1 V MUIWES. With enhanced MUIWES, aromatic proteins increased and LB-EPS converted to TB-EPS, enhancing electron transfer and leading to increased abundance of autotrophic denitrifying bacteria and decreased abundance of heterotrophic denitrifying bacteria. Thus, the challenge of poor nitrification and denitrification performance in the A-(A/O)² process treating low C/N ratio wastewater was solved. If the A-(A/O)²-MFC-MEC system is practically applied to low C/N ratio wastewater treatment projects such as mixed blackwater in the future, MUIWES can be used to partly take place of carbon source feeding and DC power input, and the excess power generated can be used for energy-consuming equipment such as aeration to achieve the economic and social benefits of saving energy and reducing carbon emissions.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cej.2022.134615>.

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1 Supplementary Data

2

3 **Enhanced nutrient removal from mixed black water by a microbial ultra-low weak**
4 **electrical stimulated anaerobic-two stage anoxic/aerobic process**

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12

13 **1. Supplementary descriptions to the main text**

14 **1.1 Anode materials**

15 Studies have shown that some carbon-based materials such as carbon felt, carbon brushes
16 are often used as the anode material for MFC due to good electrical conductivity, flexibility,
17 corrosion resistance, as well as a strong affinity for easier attachment of MFC-producing
18 bacteria growth. However, due to the difference in specific surface area and the gap between
19 carbon felt and carbon brush, the electron transfer efficiency and the mass transfer efficiency
20 of the substrate are different, which eventually affects the effect of electricity production.
21 Therefore, in this experiment, carbon felt and carbon brush anode materials were selected for
22 control.

23 Carbon felt (5 cm×8 cm) and carbon brush (5 cm×8 cm) were pre-treated and placed into
24 the anaerobic tank with a hydraulic retention time of 24 h, an internal reflux ratio of 200%, and
25 an external reflux ratio of 25%. After one week of stable operation, the effect of different anodes
26 on the carbon removal and denitrogenation of the first-stage A/O coupled MFC was
27 investigated. The influent COD concentration was 1050±50 mg/L, and the effluent COD of
28 carbon brush MFC and carbon felt MFC were 80±10 mg/L and 70±10 mg/L, respectively. The
29 effluent NH₄⁺-N and TN removal rates were 71.21%, 68.64%, and 66.24%, 64.86% for carbon
30 brush MFC and carbon felt MFC, respectively. From the results, it can be seen that the
31 microorganisms all formed a stable biofilm system, and the denitrification effect of carbon
32 brush MFC was slightly better than that of carbon felt MFC because carbon brush has a larger
33 specific surface area than carbon felt, which has a higher electron transfer efficiency and is
34 more favorable for the growth of nitrifying denitrifying bacteria. The result is consistent with
35 the studies of Guo et al., 2020 and Xiang et al., 2015. The influent TP concentration was
36 10.50±0.50 mg/L and the carbon brush MFC stable effluent concentration was 0.78 mg/L with

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1 a removal rate of 92.94%, while the carbon felt MFC stable effluent concentration was 1.32
2 mg/L with a removal rate of 85.94%. The phosphorus removal was affected by the double
3 chamber structure which increased the transfer resistance of ions and protons in the solution.

4 The voltage of carbon brush MFC and carbon felt MFC changes with time. The voltage of
5 carbon brush MFC gradually increases and stabilizes at 200 mV, while the voltage of carbon
6 felt MFC gradually decreases and tends to below 100 mV, so the performance of carbon brush
7 MFC is better than that of carbon felt MFC. The carbon felt gap is small, and the
8 microorganisms on the surface of the electrode material gradually increase with the growth of
9 time, especially the growth of heterotrophic microorganisms which are not related to the
10 electricity production, resulting in the blockage of the gap between the granular materials,
11 which will impede the electron transfer between the electrodes and the mass transfer process of
12 the substrate from the main body of the solution to the electrode surface, and increase the
13 internal resistance of the MFC, thus leading to the degradation of the electricity production
14 performance of the MFC. The gap of carbon brushes is large and the increase of microbial film
15 thickness is not enough to obstruct the electron transfer and matrix mass transfer process within
16 two months of operation, so the power density of the carbon brush MFC system is high.

17 18 **1.2 Single/double chamber MFC**

19 The A-(A/O)²-MFC-MEC system was set up with a hydraulic retention time of 24h, an
20 external reflux ratio of 25%, and an internal reflux ratio of 200%. After one week of steady
21 operation, the effect of single and double chamber MFC on the first stage A/O nutrient removal
22 was investigated.

23 The influent concentration was 510±30 mg/L, and the effluent COD of single-chamber
24 MFC and double chamber MFC was 33 mg/L and 45 mg/L, respectively, indicating that the
25 differences in COD removal rates between single/double chamber MFC were not significant.
26 This is because most of the organic matter is used as a carbon source by the microorganisms
27 when denitrification and phosphorus removal are performed (Lu et al., 2012), and this COD
28 concentration is already the organic matter level that maintains the normal activity of the
29 microorganisms inside.

30 The influent concentration was 30.5±2 mg/L, and the effluent NH₄⁺ of single-chamber
31 MFC and double chamber MFC was 4.5 mg/L and 8.25 mg/L, respectively, after the stable
32 operation, with removal rates of 85.25% and 72.95%. The single-chamber removal rate of TN
33 is 12.11% higher than the double chamber removal rate. Because the single-chamber structure
34 is more conducive to e- transfer, nitrification and denitrification are promoted by enhanced
35 electron and proton transfer (Wan et al., 2010). Double-chamber MFC has higher mass transfer
36 resistance limiting the free electron and proton transfer, so the denitrification effect is lower
37 than that of single-chamber MFC (Min et al., 2005).

38 The influent concentration was 5.2±0.2 mg/L, and the effluent TP of single-chamber MFC
39 and double chamber MFC was 0.72 mg/L and 0.61 mg/L, respectively, after the stable operation,
40 with removal rates of 86.15% and 88.27%, indicating that the differences in TP removal rates
41 between single/double chamber MFC were not significant. This is due to insufficient carbon
42 sources in the reactor resulting in the same amount of anaerobic phosphorus release by the
43 phosphate accumulating organisms (PAOs) (Yang et al., 2006).

44

1.3 Internal reflux ratio

The A-(A/O)²-MFC-MEC system has a hydraulic retention time of 24 h, an external reflux ratio of 25%, and a single chamber MFC providing 0.07V MUIWES to a MEC. After one week of stable operation, the effect of internal reflux ratios (100%, 200%, 300%) on nutrient removal from the A-(A/O)²-MFC-MEC system was investigated. The average effluent concentrations of COD with internal reflux ratio of 100%, 200% and 300% were 29 mg/L, 20 mg/L and 12 mg/L, respectively, and the average removal rates were 97.36%, 98.18% and 98.91%, respectively. With the increase of the internal reflux ratio, the internal material circulation and electron transfer are accelerated and the COD removal is enhanced.

The average effluent concentrations of NH₄⁺-N with internal reflux ratio of 100%, 200% and 300% were 0.39 mg/L, 0.22 mg/L and 0.11 mg/L, respectively, and the average removal rates were 99.3%, 99.6% and 99.8%, respectively. It can be seen that the best removal of NH₄⁺-N is achieved with an internal reflux ratio of 300%.

The average effluent concentrations of TN with internal reflux ratio of 100%, 200% and 300% were 8.34 mg/L, 6.5 mg/L and 3.91 mg/L, respectively, and the average removal rates were 84.84%, 88.18% and 92.89%, respectively. Therefore, the increase of the internal reflux ratio reduces the TN concentration in the effluent. The internal reflux is to supply nitrate nitrogen to the anoxic section to be reduced to N₂ as an electron acceptor for denitrification. However, an excessive internal reflux ratio can introduce excessive O₂, which can disrupt the anoxic environment and affect the denitrification effect of the process (Hocaoglu et al., 2010).

The average effluent concentrations of TN with internal reflux ratio of 100%, 200% and 300% were 0.43 mg/L, 0.24mg/L and 0.18 mg/L, respectively, and the average removal rates were 95.9%, 97.71% and 98.29%, respectively. The effluent concentration has reached 0.50 mg/L or less, and the effect of phosphorus removal by internal reflux ratio is relatively small.

1.4 Hydraulic retention time

The removal effects of COD, TP, NH₄⁺-N and TN were enhanced continuously when the hydraulic retention time of 12h was gradually increased to 24h and 36h, but the differences between 24h and 36h were not significant.

The average effluent concentrations of COD with hydraulic retention time of 12h, 24h and 36h were 29 mg/L, 20 mg/L and 12 mg/L, respectively, and the average removal rates were 96.82%, 98.18% and 99.09%, respectively. With the increase of hydraulic retention time, the internal material circulation and electron transfer were accelerated, and the consumption of organic matter was faster (Niu et al., 2018).

The average effluent concentrations of TP with hydraulic retention time of 12h, 24h and 36h were 0.73 mg/L, 0.23 mg/L and 0.25 mg/L, respectively, and the average removal rates were 93.02%, 97.82% and 97.59%, respectively. The hydraulic retention time of 24h and 36h were more effective, but the difference in removal efficiency was not significant. This is due to the absorption of phosphorus by phosphorus-polymerizing bacteria in the aerobic zone, which increases the hydraulic retention time in the aerobic zone and thus improves the removal rate of phosphorus, but the phosphorus concentration in this system is not high and the efficiency of phosphorus removal has reached saturation at too high a hydraulic retention time (Wang et al., 2014).

1 The average removal rates of $\text{NH}_4^+\text{-N}$ with hydraulic retention time of 12h, 24h and 36h
2 were 0.73 mg/L, 0.23 mg/L and 0.25 mg/L, respectively. The effect of different hydraulic
3 retention times on the removal of $\text{NH}_4^+\text{-N}$ was not significant, and the concentration of $\text{NH}_4^+\text{-N}$
4 had reached a very low state.

5 The average effluent concentrations of TN with hydraulic retention time of 12h, 24h and
6 36h were 8.81 mg/L, 6.5 mg/L and 5.01 mg/L, respectively, and the average removal rates were
7 83.98%, 88.18% and 90.89%, respectively. Increasing the hydraulic retention time can
8 strengthen the utilization of degradable organic carbon source and internal carbon source in the
9 wastewater by denitrifying bacteria, which can improve the overall denitrification efficiency of
10 the combined process.

11 The experimental study of anaerobic-two-stage anoxic/aerobic (A-(A/O)²) systems for
12 treating mixed black water by hydraulic retention time (12 h, 24 h, 36 h) showed that the best
13 treatment effect was achieved with a hydraulic retention time of 36 h, but when the hydraulic
14 retention was too large, the treatment capacity would be reduced and the operating cost would
15 be increased. Therefore, both operating cost and treatment effect should be considered. In order
16 to obtain the most energy-saving and high-efficiency effect, 24 h hydraulic retention time was
17 selected as the best.

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1 **Supplementary Table 1** Composition of the artificial nutrient solution

Chemicals	Molecular formula	Content
Glucose	$C_6H_{12}O_6$	0.462 g/L
Sodium acetate trihydrate	$CH_3COONa \cdot 3H_2O$	1.068 g/L
Ammonium Chloride	NH_4Cl	0.192 g/L
Potassium Dihydrogen Phosphate	KH_2PO_4	0.044 g/L
Magnesium sulfate, heptahydrate	$MgSO_4 \cdot 7H_2O$	0.005 g/L
Sodium bicarbonate	$NaHCO_3$	0.050 g/L
Calcium chloride, anhydrous	$CaCl_2$	0.005 g/L
Trace element stock solution	The ingredients are listed in Supplementary Table 2	1mL/L

2
3 **Supplementary Table 2** Composition of the trace element stock solution

Chemicals	Molecular formula	Content
Ferric chloride	$FeCl_3$	0.90 mg/L
Zinc Sulfate	$ZnSO_4 \cdot 3H_2O$	0.12 mg/L
Sodium molybdate	$NaMoSO_4 \cdot 2H_2O$	0.06 mg/L
Manganese Chloride	$MnCl_2 \cdot 4H_2O$	0.06 mg/L
Potassium Iodide	KI	0.18 mg/L
Copper sulfate	$CuSO_4 \cdot 5H_2O$	0.03 mg/L
Calcium chloride	$CaCl_2 \cdot 6H_2O$	0.15 mg/L
Boric acid	H_3BO_3	0.15 mg/L

4
5 **Supplementary Table 3** Parameter settings of the 3D fluorescence spectrometer

Parameters	Set values
Excitation wavelength range	200.0~420.0 nm
Emission wavelength range	250.0~550.0 nm
Excited broadband	2.0 nm
Launch broadband	2.0 nm
Scan rate	1200 nm/min
Excitation unit slit width	2.5 nm
Slot width of transmitting unit	2.5 nm
Photocell voltage	700V

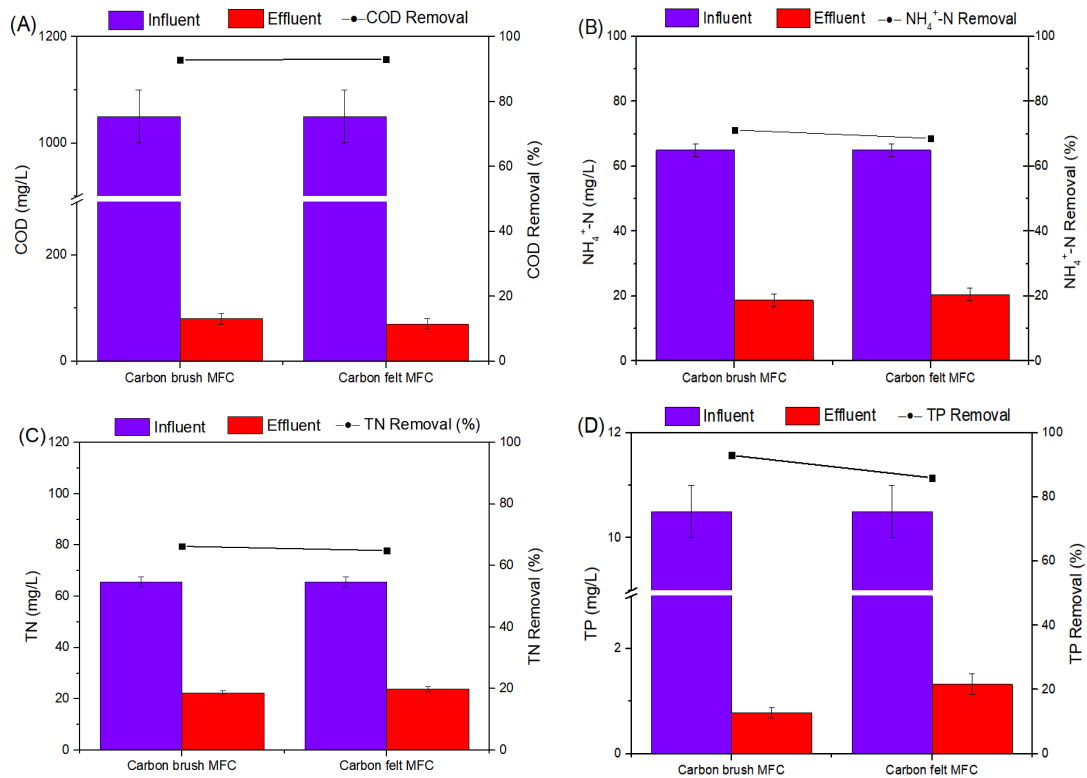
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Supplementary Table 4 A-(A/O)²-MFC-MEC Parameter Optimization

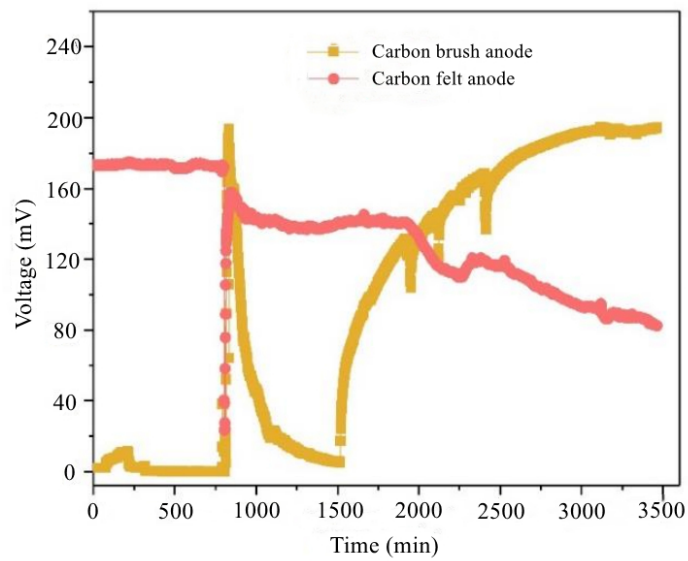
No	Single / double chamber MFC	internal reflux ratio (%)	hydraulic retention time (h)	External reflux ratio (%)	Stimulation voltage (V)	COD removal rate (%)	TN removal rate (%)	TP removal rate (%)
1	Single chamber	200	24	25	0	93.55	71.78	86.11
2	double chamber	200	24	25	0	91.95	60.65	88.24
3	Single chamber	100	24	25	0.07	97.31	84.48	97.31
4	Single chamber	200	24	25	0.07	98.17	88.18	98.17
5	Single chamber	300	24	25	0.07	98.97	92.89	98.97
6	Single chamber	200	12	25	0.07	96.82	83.98	93.02
7	Single chamber	200	24	25	0.07	98.18	88.18	97.82
8	Single chamber	200	36	25	0.07	99.09	90.89	97.59

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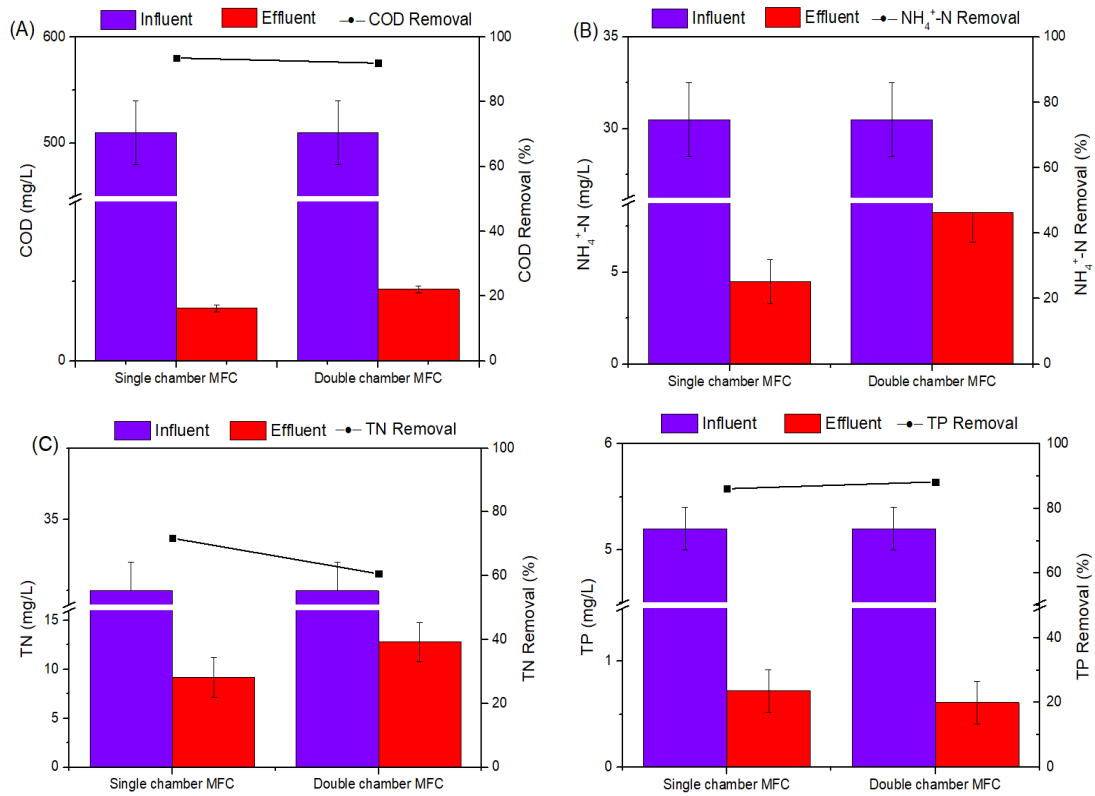
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Supplementary Figure S1 Total removal in mg/L and percentage of the COD (A), NH₄⁺-N (B), TN (C) and TP (D) in the A-(A/O)² system equipped with different anode MFCs



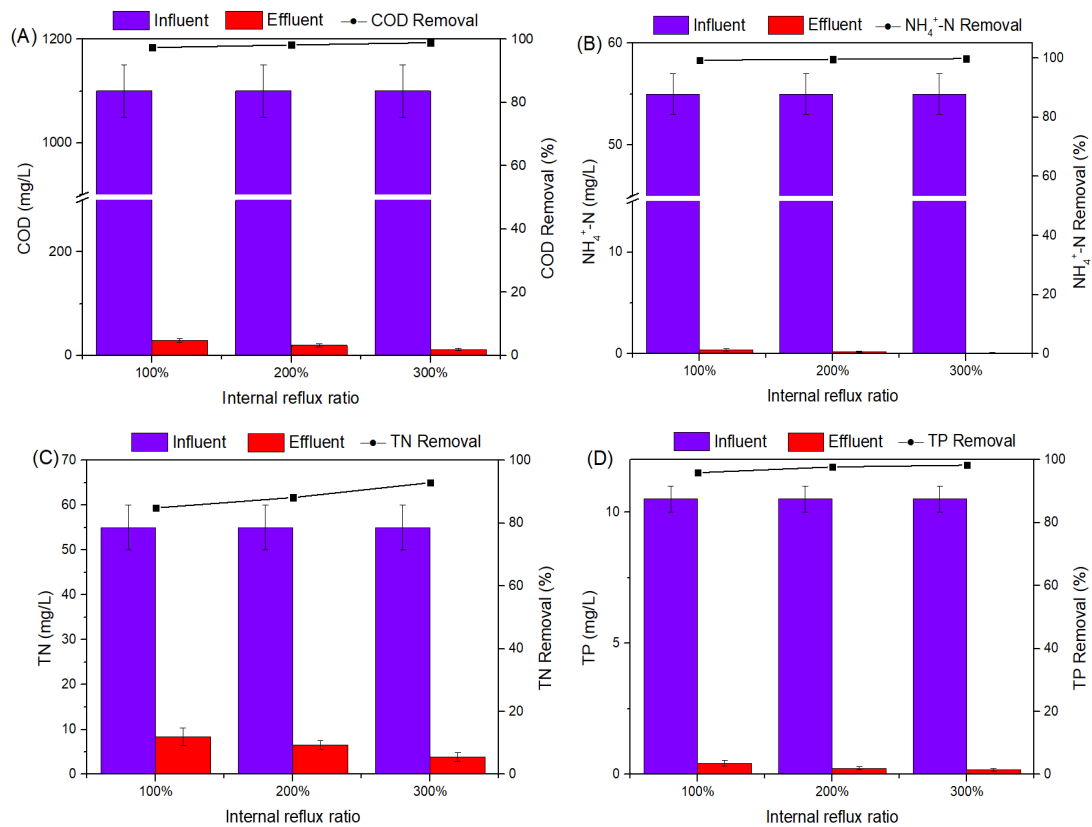
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Supplementary Figure S2 Variation of electricity production with time for different anodes of MFC



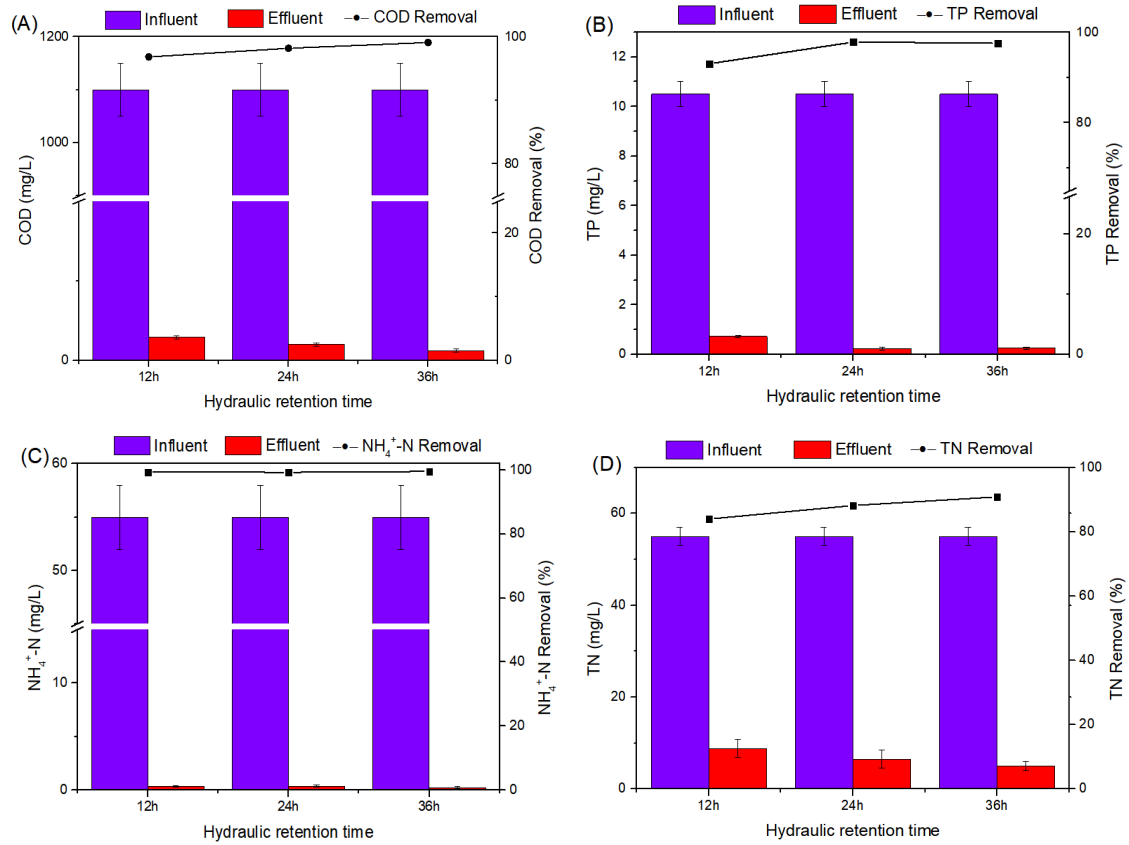
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Supplementary Figure S3 Total removal in mg/L and percentage of the COD (A), NH₄⁺-N (B), TN (C) and TP (D) in the A-(A/O)² system equipped with different anode MFCs

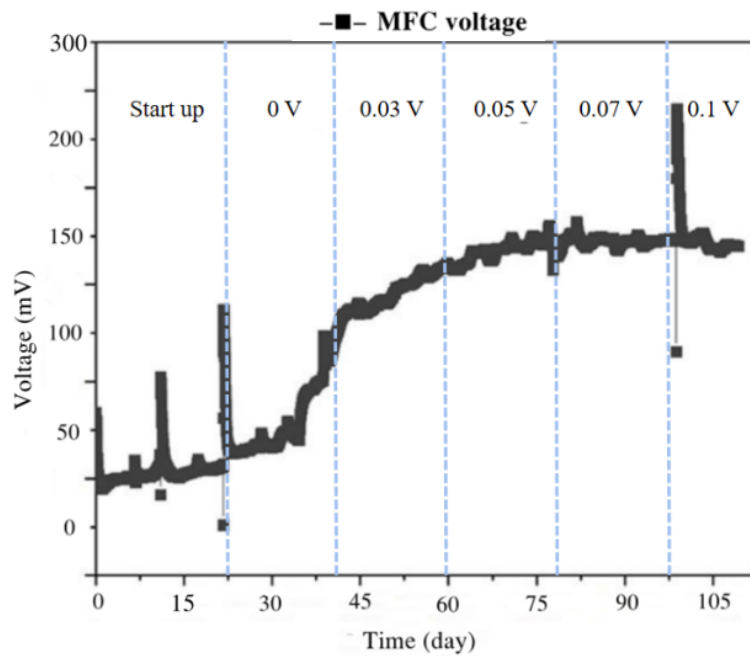


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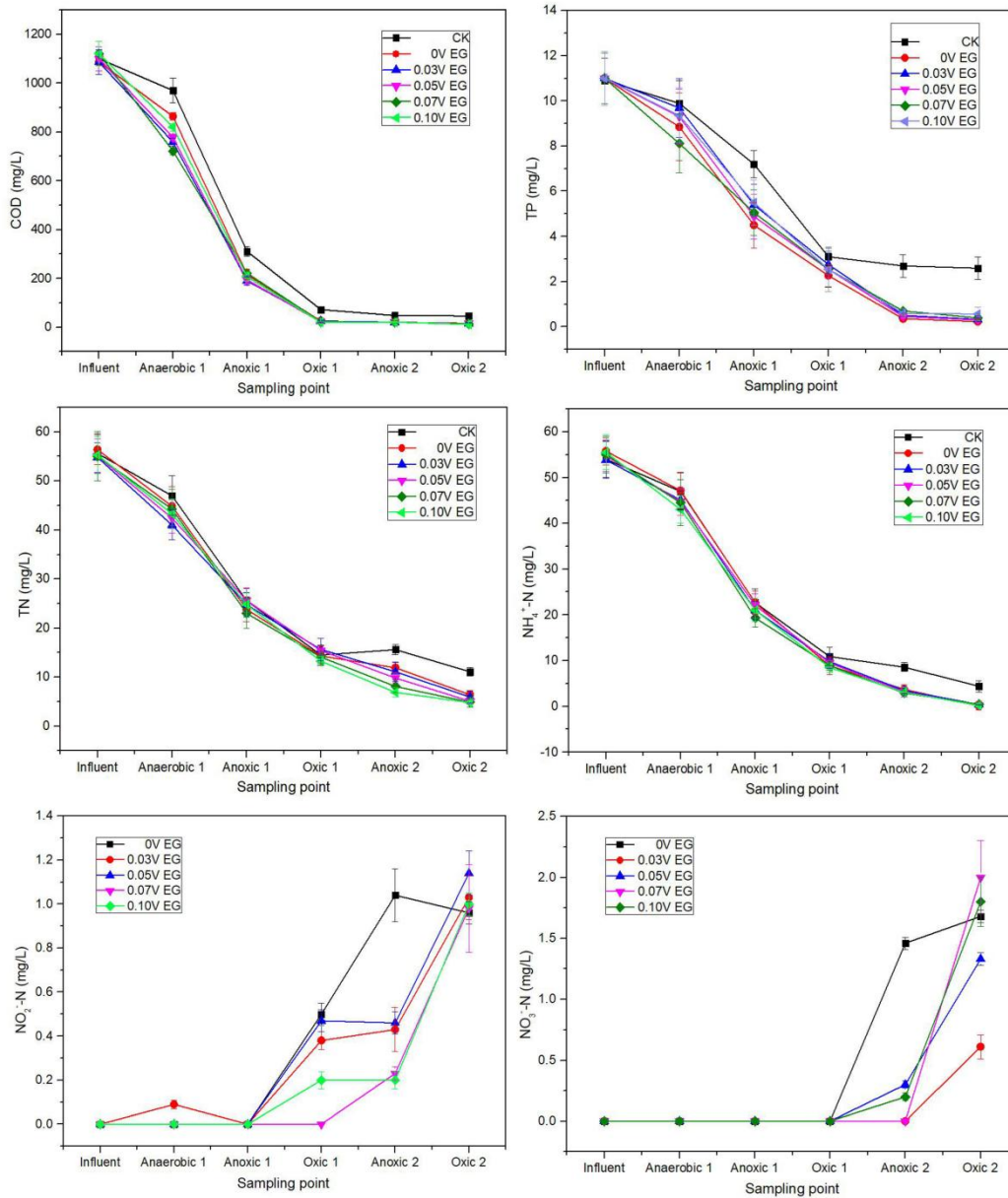
Supplementary Figure S4 Total removal in mg/L and percentage of the COD (A), NH₄⁺-N (B), TN (C), and TP (D) in the A-(A/O)² system equipped with a MFC providing MUIWES to a MEC. Voltage refers to voltage applied to the MEC.



1
 2 **Supplementary Figure S5** Total removal in mg/L and percentage of the COD (A), TP (B),
 3 $\text{NH}_4^+\text{-N}$ (C) and TN (D) in the A-(A/O)² system equipped with a MFC providing MUIWES to
 4 a MEC. Voltage refers to voltage applied to the MEC.

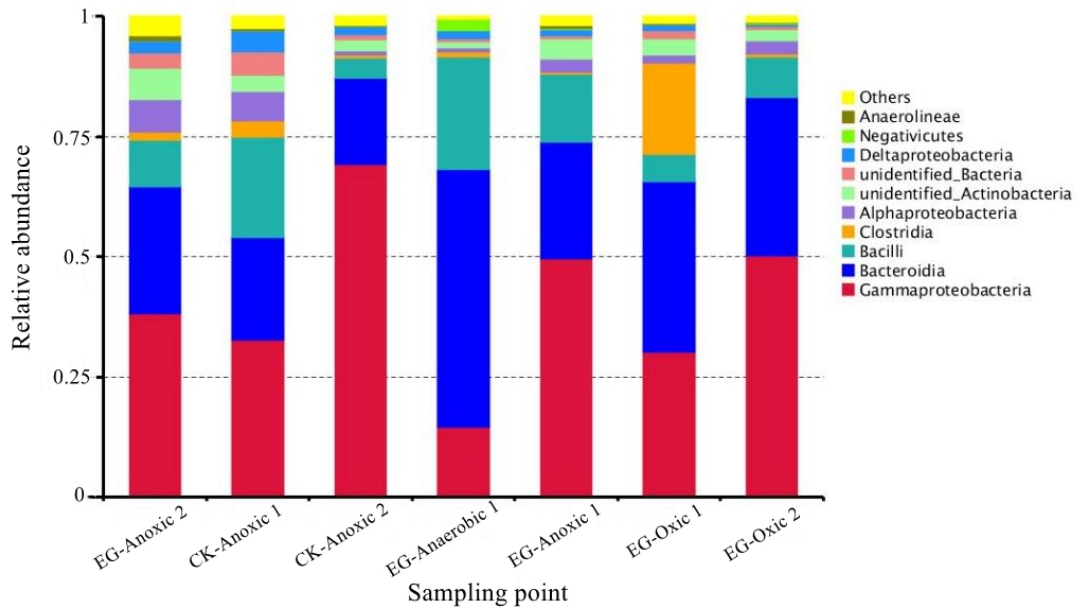


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 6 **Supplementary Figure S6** MFC power production varies with MUIWES
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Supplementary Figure S7 The concentrations of COD, TP, TN, NH₄⁺-N, NO₂⁻-N and NO₃⁻-N in a typical operation point at different MUIWES



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Supplementary Figure S8 Relative abundance of two craft species at the class level