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**Complete removal of heavy metals with simultaneous efficient treatment of etching terminal wastewater using scaled-up microbial electrolysis cells**

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## **Abstract**

The treatment of actual low and high strengths etching terminal wastewater (ETW) from plating and electronic industry meeting national discharge standards is demonstrated in laboratory scale (1 L) and in scaled-up (40 L) microbial electrolysis cells (MECs). Both cylindrical single-chamber MECs achieved complete removal of heavy metals and efficient treatment of organics using either low strength ETW at an hydraulic retention time (HRT) of 5 d, or high strength wastewater at HRTs of 7 d (1 L) or 9 d (40 L). The removal rate of organics and heavy metals increased by 36-fold and scaled almost with the reactor volume ratio of 40. Electrode potentials in the scaled-up MECs (40 L) were resilient to the wastewater strength. Bacterial communities on both anodes and cathodes of the 1 L and the 40 L reactors experienced a selective shock and a significant community change after switching from low to high strengths wastewater, although reactor performance was effectively maintained. This study demonstrates complete removal of multiple heavy metals with simultaneous efficient wastewater treatment in MECs of different scales meeting China national discharge standards and provides a plausible approach for simultaneous removal of value-added products (e.g., heavy metals) and efficient treatment of practical etching terminal wastewater.

**Keywords:** etching terminal wastewater; microbial electrolysis cell; influent strength; hydraulic retention time; bacterial community

## 1. Introduction

The rapid increase in market demand for electronic and electrical equipment generates globally approximately one billion cubic meters of etching wastewater [1-2]. The etching and cleaning processes of printing circuit boards equipment produces (after passing through solvent extraction, chemical precipitation, chemical reduction, and/or even membrane technology) etching terminal wastewater (ETW) which usually contains low concentrations of dissolved recalcitrant organics (e.g., triethanolamine oleic soap, 3-nitrobenzenesulfonate, dodecylbenzenesulfonate, tetramethyl ammonium hydroxide) and heavy metals (e.g., Cu(II), Ni(II), Zn(II), Cr(VI)) [3-5]. Such wastewater has often been processed in municipal treatment plants, after payment of a fee based on the volume discharged [1-2]. However, increasingly stricter national legislation has been limiting the amount of ETW that can be discharged to municipal treatment plants. Moreover, the sustainability consideration demand the etching industry to implement innovative water treatment processes to allow complete removal of heavy metals with simultaneous efficient treatment of its organic load.

Microbial electrolysis cells (MECs), on the other hand, are devices that uses a small amount of electrical energy supply or voltage to allow the oxidation of organic/inorganic species by exoelectrogenic microorganisms at the anode, and the reduction of oxidized substrates either electrochemically or electrotrophically at the cathode [6-7]. MECs have recently gained tremendous attention for its ability to extract energy from the organic matters contained in actual wastewater, including

winery, urban and urine (Table S1). These demonstration studies have been performed in small reactor volumes ranging from 0.04 – 910 L (Table S1). The treatment of industrial wastewaters containing recalcitrant organics, such as coal gasification and landfill leachate, has also been explored using single-chamber or dual-chamber MEC reactor configurations, but always in small reactor volumes (e.g., 0.3 – 1.4 L). Often the chemical oxygen demand (COD) removals in such systems (e.g., 2 – 60%) has been low due to high toxicity and high initial pollutant load [8]. Thus, scaling up of MECs utilizing a high pollutant influent has proven to be difficult.

On the other hand, pairing scaled-up MECs with the treatment of effluents with a relatively low recalcitrant organics content and thus less toxicity, such as in ETW, might represent a new promising method to achieve efficient and complete treatment of the organics matter in this wastewater. Simultaneously, heavy metals in such wastewater can be removed at the cathodes, thus MECs can also provide an emerging and sustainable technology for the reduction and removal of many heavy metals from aqueous solutions [7,9]. While early studies have mainly focused on the conceptual removal of single heavy metals in biotic or abiotic cathodes of dual-chamber MECs, most of recent studies have explored the removal of heavy metals from simulated wastewaters, using single or stacked small reactors with working volumes in the range 0.012 – 1.0 L (Table S1). These studies have provided insightful information on the potential of MECs for the treatment of simulated simple wastewater. Nevertheless, the

removal of recalcitrant organics in complex real wastewaters has scarcely been reported.

To advance the MECs technology to the next level, it is essential to investigate the removal of multiple heavy metals from actual industrial wastewaters with simultaneous treatment of recalcitrant organics in large volume MEC reactors. Scaled-up single-chamber MECs have been explored for treating simulated wastewater containing W(VI), Mo(VI) and acetate [10] in a 40 L MEC. However, it is arduous to find in-depth studies that have considered the treatment of actual industrial wastewaters containing multiple heavy metals and recalcitrant organics at these reactor scales. Besides the application using actual industrial wastewater, the comparison of the behavior of MECs at different reactor scales should provide valuable information for scaling-up MECs units, as already shown for the treatment of domestic wastewater [11].

In this study, the removal of multiple heavy metals (Cu(II), Ni(II), Zn(II) and Cr(VI)) with simultaneous efficient treatment of both low and high strengths recalcitrant organics in ETW was investigated in small (1 L) and large-scale (40 L) cylindrical single-chamber MECs operated in one-pass continuous flow under different hydraulic retention times (HRTs). The system performance was analyzed using multiple characterization methods including cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), morphological observation by a scanning electron microscope (SEM), X-ray photo-electron spectroscopy (XPS). The

variation of bacterial communities developed on the cathodes and the anodes in both the 1 L or 40 L MECs, under either low or high strengths ETW, were analyzed using 16S rRNA gene sequencing. One objective of this study was to demonstrate complete removal of heavy metals and achieve efficient ETW treatment in MECs meeting national discharge standards. This study overall brings a step forward the practical application of MECs for effective treatment of actual ETW, contributing to sustainable development of the electronic and semiconducting industry.

## **2. Materials and methods**

### *2.1 Reactor construction*

Duplicate cylindrical single-chamber small-scale (1 L; 8 cm inner diameter; 20 cm high) and large-scale (40 L; 34 cm inner diameter; 45 cm high) MEC reactors were made of polyvinyl chloride (PVC) and constructed with concentric electrodes, as previously described [10] (Fig. S1 in Supporting Information (SI)). The reactors working liquid volumes were 38 L and 0.8 L, respectively. The inner cathodes with dimensions (1 L: 4.5 cm diameter and 283 cm<sup>2</sup> total area; 40 L: 30.5 cm diameter and 4310 cm<sup>2</sup> total area) and the outer anodes (1 L: 7.5 cm diameter and 471 cm<sup>2</sup> total area; 40 L: 33.5 cm diameter and 4734 cm<sup>2</sup> total area) of both reactors (1.0 cm distance between the edges) were made of graphite felt (0.5 cm thick, Sanye, Beijing, China) supported on stainless steel mesh (type 304 SS, McMaster-Carr, OH). The meshes of anodes and cathodes were connected by an external circuit at an applied

voltage of 0.8 V (Leici, Shanghai). A small resistor of 10  $\Omega$  was connected in series with power supply to allow calculation of the current. All potentials were collected using a data acquisition (PISO-813, Hongge Co., Taiwan). The anode and the cathode potentials, as well as, the circuital currents were shown either as averages over operational periods or as a function of the operational time. A Ag/AgCl reference electrode (197 mV vs. standard hydrogen electrode, SHE) was inserted in each reactor around the upper part of the reactor and close to either the cathode or the anode to obtain separate measurements of the anode and cathode potentials, with all potentials reported here vs. SHE. Reactors were wrapped with aluminum foil to exclude light to avoid the growth of algae.

## *2.2. Inoculation and operation*

The inoculum originated from existing MECs, operated for one year at a substrate to microorganisms ratio of  $0.87 \pm 0.13$  g COD/(g SS·d), and with an equivalent volume of a nutrient solution containing trace elements [10]. The reactors were inoculated feeding with progressive ETW/nutrient ratios, including nutrient only, 1 : 1, 3 : 1, 7 : 1, 18 : 1, and ETW only with each lasting at least 3 – 5 d. Before feeding, the solutions were deoxygenated by sparging nitrogen for 30 min. The composition of the low and high strength ETW wastewater, collected from the company TOTO Ltd. (Dalian, China) producing faucets, is reported in [Table 1](#).



The reactors were firstly acclimated using low strength ETW. The acclimation process was completed when the electrode potential reached a steady-state, typically after two additional consecutive fed-batch cycles operation with each lasting 3 – 5 days. The reactors were then shifted to a continuous flow operational mode, where low strength ETW was pumped (BT100-2J, Lange, China) into the 1 L or 40 L MECs at an HRT of 5 d for 30 d. After the acclimation period, the feed stream to the reactors was switched to receive high strength ETW maintaining the same HRT of 5 d, for further 30 d operation. Following this and based on the insufficient treatment of high ETW in the 1 L MECs at the HRT of 5 d, the HRT in both the 1 L and 40 L MECs were purposefully prolonged to 7 d to achieve a higher treatment level. The treatment of high strength ETW under this condition met the China national discharge standards in the 1 L reactor, however, insufficient treatment in the scale-up 40 L MECs required, a longer HRT of 9 d. Thus, the performance of 1 L (7 d) and 40 L (9 d) MECs were compared since both met the constraint of the China national discharge standards. All experiments were carried out at room temperature of  $25 \pm 3$  °C.

The cathodes did not reach heavy metal saturation, under the experimental conditions used, which was estimated to occur only after processing 56 L (low strength ETW) and 17 L (high strength ETW) in the 1 L reactors, and 861 L (low strength ETW) and 270 L (high strength ETW) in the 40 L reactor, based on adsorption saturation assessment similar to adsorption of Cr(VI) on graphite felt [12].

Two controls were operated: operation of the 1 L or 40 L reactors under open circuit conditions (OCCs), to examine the removals of recalcitrant organics and multiple heavy metals in the absence of circuital current, and without inoculation (abiotic anode and cathode) to examine the effect imparted by the electrochemically active bacteria in these systems.

### *2.3. Analytical methods and calculations*

H<sub>2</sub> (m<sup>3</sup>/m<sup>3</sup>/d) was measured daily using a gas chromatograph (GC7900, Tianmei, Shanghai), and normalized to the reactor working volume and operational time (d) [13]. The reactor influent and effluent were sampled and filtered through a 0.22- $\mu$ m pore diameter syringe filter. Heavy metals of Cu(II), Ni(II), Zn(II) and Cr(VI) were measured by inductive coupled plasma atomic emission spectrometer (ICP-AES, PerkinElmer Optima 2000 DV, USA). COD was measured using the manganese method due to its reliable accuracy at low organics levels [14] and organics removal was expressed as either percent COD removal (%) or COD removal rate (mg/L/d). The concentrations of NH<sub>4</sub><sup>+</sup>-N in the actual ETW were determined according to the Nessler reagent colorimetry method whereas the concentrations of NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, sulphur compounds, and Cl<sup>-</sup> were measured using an ion chromatograph (ICS-600, Thermo Scientific). The total nitrogen was measured using a DR/600 colorimeter (HACH Co. Ltd., USA) according to the manufacturer's instructions. Solution conductivity and pH were measured via a conductivity probe and meter (DDS-307,

Leici, Shanghai), and a pH probe and meter (PHS-3C, Leici, Shanghai), respectively.

The anodic coulombic efficiencies ( $CE_{an}$ , %) was defined by the ratio between the measured electric current and the theoretical electric current calculated from the measured COD removal. The cathodic  $CE$  ( $CE_{ca}$ , %) was defined as the ratio of the circuit charges utilized for hydrogen production and reduction of heavy metals (e.g., Cu(II), Ni(II), and Zn(II)) and the total circuit charges [15,16]. Energy consumption and energy efficiencies (%) were calculated according to Eqs. S1–2 [16].

The organic compounds in the ETW were qualitatively confirmed by a high performance liquid chromatograph with an APCI (–) ion trap mass spectrometer (Agilent HPLC – MS 6410). Electro-spray ionization (ESI) was operated in a positive mode, with the scan mass range set from 70 to 1000.

Acute toxicity of the low and high strengths wastewater and of the reactors influent/effluent was assessed using a Microtox Toxicity Analyzer (DXY-3, Nanjing Institute of Soil Science, Chinese Academy of Science) and the toxicological response was based on the bioluminescence inhibition rate of *Photobacterium phosphoreum* T3 bacterium [17] purchased from the Nanjing Institute of Soil Science, Chinese Academy of Science.

Morphologies of the electrodes were examined using a scanning electronic microscopy (SEM) (QUANTA450, FEI company, USA). X-ray photoelectron spectroscopy (XPS; Kratos AXIS Ultra DLD) was used to determine the valences of the material deposited over the electrodes.

Samples collected from the middle of the anodes and cathodes were examined using SEM (QUANTA450, FEI company, USA), whereas CV and EIS analysis were conducted using a potentiostat (VSP, BioLogic). The electrodes bacterial community composition was examined using high-throughput 16S rRNA gene sequencing technology [10] (see details in SI). The taxonomies for 16S rRNA genes obtained in this study were deposited in the NCBI SRA (<http://www.ncbi.nlm.nih.gov/sra>) with the BioProject ID of PRJNA809576. PICRUSt bioinformatics software enabling the function prediction of uncultivated microbes and applicable in detecting the 16S rRNA marker gene sequences of bacterial genomes, was used to ascertain the direct evidence of microbial communities' functional capabilities [18]. PICRUSt analysis based on KEGG database was conducted to help deep understanding the microbial functions in these systems. KEGG was performed by BLASTP according to KEGG (<http://www.genome.jp/kegg/>).

One-way ANOVA in SPSS 19.0 was used to analyze the statistical differences among the data, and all of the data indicated significance levels of  $p < 0.05$ .

### **3. Results and discussion**

#### *3.1 Organic compounds in etching terminal wastewater*

The organic matter in the ETW was assessed by HPLC-ESI-MS. The mass/charge ratios of 415 and 437 at a retention time of 16.76 min were attributed to triethanolamine oleic soap (Fig. S2A and B), which has been previously reported in

metal-working fluids as biodegradable and as sole source of carbon or nitrogen for bacterial growth [3,19]. Similarly, the mass/charge ratios of 415 and 473 at 14.70 min belonged to 2-naphthalenesulfonic acid formaldehyde polymer sodium salt (Fig. S2C and D), which was biodegraded by conventional microorganisms including but not limited to *Arthrobacter* sp. 2AC, *Comamonas* sp. 4BC and *Cunninghamella polymorpha* [20]. While it was impractical to determine the entire organic composition of the actual ETW, the presence of these two biodegradable organic compounds supports the acclimation of specific electrochemically active bacteria for the degradation of the ETW organic matter.

### 3.2 Reactor startup

During the MECs start-up process, following inoculation with the different ratios of low strength wastewater and nutrient, the cathode potentials of both 1 L (Fig. 1A and Fig. S3A) and 40 L (Fig. 1B and Fig. S3B) reactors shifted to less negative values as the composition gradually approached that of the real wastewater, whereas the anode potentials generally stabilized at the initial stages of the start-up process and increased thereafter. Stable cathode potentials (1 L:  $\sim -0.620$  V; 40 L:  $\sim -0.404$ ), anode potentials (1 L:  $\sim -0.090$  V; 40 L:  $\sim 0.280$  V) and circuitial currents (1 L:  $\sim 2.7$  mA; 40 L:  $\sim 1.9$  mA) were achieved using low strength wastewater (Fig. 1 and Fig. S3). The adverse influence of the increased size of reactor on system performance was

similar to the loss of circuital current as reactor size was increased using domestic wastewater or acetate as fuels in other studies [11].

### 3.3 Low strength etching terminal wastewater

After the start-up process was completed, using low strength wastewater, the performance of both 1L and 40 L reactors was similar, achieving almost complete heavy metals removal (Fig. 2A), and efficient COD removals (1 L:  $74.0 \pm 6.6\%$ , rate:  $25.8 \pm 2.0$  mg/L/d; effluent COD:  $43.5 \pm 7.5$  mg/L; 40 L:  $70.1 \pm 1.1\%$ , rate:  $24.4 \pm 0.4$  mg/L/d; effluent COD:  $48.4 \pm 1.7$  mg/L;  $p = 0.62$ ). The amount of hydrogen production was significantly higher in the 1 L reactor ( $0.0094 \pm 0.0003$  m<sup>3</sup>/m<sup>3</sup>/d versus  $0.00027 \pm 0.0001$  m<sup>3</sup>/m<sup>3</sup>/d) (Fig. 2B), mainly ascribed to the appreciable higher circuital current in the 1 L than the 40 L ( $2.70 \pm 0.10$  mA vs.  $1.88 \pm 0.17$  mA,  $p = 0.03$ ) (Fig. 2D) and thus bacterial community compositions as subsequently discussed, consistent with other reports on adverse impact of scaling-up reactor using acetate or domestic wastewater as fuels [11]. Similar electrode potentials of both reactors ( $p$ : 0.50 (cathode) and 0.41 (anode)) implied their non-influence during this scale-up. In addition, the total chromium in the effluent of all experiments was always below the detection limit. The concentrations of SO<sub>4</sub><sup>2-</sup> (1 L:  $0.96 \pm 0.03$  mg/L; 40 L:  $1.50 \pm 0.01$  mg/L) and S<sup>2-</sup> (1 L:  $0.01 \pm 0.01$  mg/L; 40 L:  $0.09 \pm 0.02$  mg/L) in the effluents were always appreciably lower than those in the influents, whereas NO<sub>3</sub><sup>-</sup>-N in the effluents was totally removed under all experimental conditions, while negligible changes of aqueous NH<sub>4</sub><sup>+</sup>-N (1 L:  $0.12 \pm 0.02$  mg/L; 40 L:  $0.10 \pm 0.01$

mg/L) was observed. Mapping the pathways of these N species such as the occurrence of dissimilatory nitrate reduction to ammonium in this complex system might need to be further investigated using N isotope tracing methods coupled with kinetics data. The effluents of both reactors met the China national wastewater discharge standards (GB25467-2010). Electrode potential is closely related with the thermodynamic overall free energy of the electron transfer reactions, whereas circuit current usually suggests varying degrees of dynamic mass transfer inhibition [21]. Thus, the similar removals of heavy metals and organic matter in both 1 L and 40 L MECs, consistent with the insignificant changes in electrode potential, confirm the critical role of electrode potential rather than circuit current in both reactors on the treatment of this strength wastewater. Similarly, electrode potential was found to be more influential in a 0.2 L MECs dual-chamber cathode used for the selective recovery of Pb and Zn from simulated wastewater [22].

The increase in effluent pH (Fig. 2C) might be ascribed to the transport of other ionic species, which promoted the consumption of protons from water autohydrolysis and thus increased the liquid media alkalization in both the 1 L and 40 L MECs (Fig. 2B), similar to other MECs for other contaminants removal [23,24].

Under OCCs, significantly lower contaminants removals were achieved along with complete Cr(VI) removal (Fig. 2A) and a lower COD removal (Fig. 2B). Similarly, in the abiotic controls, heavy metals removals of  $11.7 \pm 1.5\%$  (Zn) –  $15.9 \pm 2.0\%$  (Cu) in 1 L MECs and  $12.4 \pm 1.3\%$  (Cu) –  $16.4 \pm 2.1\%$  (Ni) in 40 L reactors

were also observed whereas organics were negligibly removed in these reactors. Thus, higher system performance was only obtained in the biotic MECs under a closed circuit system.

The assessment of the acute toxicity of the low strength ETW using the bioluminescence test reported inhibition rates of  $51.9 \pm 3.8\%$  (Fig. S4) and confirmed the general recognition of the apparently toxicity of this wastewater [17]. After treatment in the 1 L or 40 L MECs the luminescence inhibition rates of the effluents were significantly suppressed ( $15.7 \pm 3.1\%$ ) suggesting efficient detoxification of ETW, while under OCCs the luminescence inhibition rates in both reactors decreased to the range  $28.4 \pm 1.0 - 30.2 \pm 1.6\%$ .

### *3.4 High strength etching terminal wastewater*

The removal of the heavy metals was effectively totally achieved in the 1 L reactor using the same HRT of 5 d and high strength wastewater while COD decreased to  $78 \pm 1\%$  ( $121 \pm 2$  mg/L/d) (Fig. S5). However, the effluent (COD 165 mg/L, Cu(II) 0.51 mg/L, Zn(II) 0.51 mg/L, Ni(II) 0.64 mg/L) exceeded the permissible China national wastewater discharge residuals standards, despite higher circuit currents ( $3.8 \pm 0.4$  mA) and similar electrode potentials ( $p$ : 0.412 (cathode) and 0.914 (anode)) (Fig. S5) to those recorded with the low strength wastewater (Fig. 2D).



Increasing the HRTs to 7 d (1 L) and to 9 d (40 L) achieved total removal of heavy metals (Fig. 3A) and appreciable higher organics removal (1 L:  $88.9 \pm 0.8\%$ , rate:  $98.2 \pm 0.9$  mg/L/d; 40 L:  $88.7 \pm 1.0\%$ , rate:  $76.1 \pm 0.9$  mg/L/d) (Fig. 3B) from this high strength wastewater and significantly higher than under OCCs.  $\text{NO}_3^-$ -N in the effluent was below the detection limit with the other parameters below national wastewater discharge standards ( $\text{NH}_4^+$ -N:  $0.32 \pm 0.01$  mg/L (1 L),  $0.26 \pm 0.01$  mg/L (40 L);  $\text{SO}_4^{2-}$ :  $0.98 \pm 0.00$  mg/L (1 L),  $1.08 \pm 0.01$  mg/L (40 L); sulfide:  $0.33 \pm 0.01$  mg/L (1 L),  $0.50 \pm 0.02$  mg/L (40 L)). In addition, lower similar effluent conductivities ( $0.68 - 0.69$  mS/cm) with higher pHs (1 L:  $7.15 \pm 0.02$ , 40 L:  $7.22 \pm 0.05$ ;  $p = 0.323$ ) (Fig. 3C) were reasonably observed. The higher effluent pHs in comparison to the values reported with the low strength wastewater (Fig. 2C) might also be attributed to the transport of more other ionic species under this high strength ETW and thus led to more media alkalization [23,24].

Evident different circuital currents (1 L:  $3.20 \pm 0.05$  mA, 40 L:  $2.75 \pm 0.01$  mA,  $p = 0.02$ ) (Fig. 3D) might explain the variable hydrogen production (Fig. 3B) whereas similar cathode ( $p = 0.78$ ) and anode ( $p = 0.10$ ) potentials were also observed (Fig. 3D). For each reactor, the high and low strengths wastewater led to insignificant changes in electrode potentials ( $p$ :  $0.71 - 0.84$  (1 L) and  $0.23 - 0.73$  (40 L)) and evident changes in circuital current ( $p$ :  $0.049$  (1 L) and  $0.019$  (40 L)) (Fig. 2D; Fig. 3D). These results in concert demonstrate the cathode and anode potentials could be maintained after scaling-up, using both low or high strengths wastewater and

sufficient HRT. Previous studies have suggested that smaller rather than large size MECs perform more efficiently for the treatment of domestic wastewater and that the electrode potentials normally varies with the increase in MEC size [11]. This study demonstrates that this is not happening for the treatment of both low and high strengths ETW in these scaled-up reactors.

The acute toxicity was reduced by 60% in both reactors from  $74.1 \pm 2.2\%$  at the reactors influent to  $29.0 \pm 2.3\%$  (1 L) and  $30.2 \pm 1.6\%$  (40 L) (Fig. S4) at the effluents, slightly lower than the 69% observed with the low strength wastewater. These results demonstrate that effective treatment of high strength ETW can be achieved in the scaled-up MEC using sufficiently longer HRTs, while maintaining steady electrode potentials, providing sufficient interaction of the water contaminants with the exoelectrogens and the electrotrophs, although the population of these may vary during scaling up, as subsequently interpreted.

Based on contaminants removal rate (1 L: 0.08 g COD/d, Cu(II): 3.86 mg/d, Ni(II): 2.43 mg/d, Zn(II): 1.66 mg/d, Cr(VI): 0.24 mg/d; 40 L: 2.87 g COD/d, Cu(II): 143 mg/d, Ni(II): 89.9 mg/d, Zn(II): 61.2 mg/d, Cr(VI): 8.9 mg/d), the 40 L reactors at HRT of 9 d achieved 36-fold improvement compared to the 1 L MECs at 7 d, which is quite close to the scale-up volume ratio of 40. This scaled-up and trade-off was thus worthwhile and provided insightful information for scaling up MEC for the treatment of actual high strength ETW.

### 3.5 CV and EIS analysis

The reduction onset potentials of Cu(II), Ni(II) and Zn(II) on both electrodes of the 1 L (Fig. 4A; Table S2) and the 40 L (Fig. 4B; Table S2) MECs were in most cases negligibly different under low strength wastewater influents, and shifted to more positive values using the high strength wastewater, consistent with the kinetics results (Figs. 2 – 3). This suggests that less energy was required for the reduction of heavy metals under high strength wastewater, which was attributed to the higher rate of mass transfer of organics as “fuels” to the electrodes resulting from the higher concentration gradient between the bulk and the electrode surface. This behavior has also been observed in other bioelectrochemical systems for the reduction of single Cr(VI), Au(III) or Co(II) [7,9,25,26].

The more positive reduction onset potential observed with the biotic rather than the abiotic electrodes indicates a decrease in the overall free energy of the electron transfer reaction, mainly due to bacterial interactions with the electrode surface [21]. An exceptional reduction onset potential of Ni(II) (cathode:  $-0.250$  vs.  $-0.160$ ; anode:  $-0.240$  vs.  $-0.200$ ) in the 40 L MECs was shifted to more negative values under the high strength influent conditions (Fig. 4B; Table S2), likely ascribed to the general higher intrinsic toxicity of Ni(II) on the exoelectrogens/electrotrophs in comparison to the toxicity of Zn(II) and Cu(II). Other studies have shown that 500 mg/L of Zn(II) [27] or 120 mg/L of Cu(II) [28] have been individually and bio-catalytically removed in bioelectrochemical systems as in this study, however the removal of Ni(II) is more

efficiently performed under abiotic conditions due to its intrinsic toxicity towards exoelectrogens [29]. In contrast to Ni(II) and Zn(II), the reduction peak potentials of Cu(II) and reduction peak currents always increased in response to a higher strength influent (Fig. 4A and B; Table S2), consistent with the kinetic results (Fig. 3).

With regard to organics oxidation, high strength wastewater invariably enhanced oxidation peak currents in both reactors (Fig. 4A and B; Table S2). Oxidation onset potentials of organics on both electrodes of the 1 L reactors shifted to less positive values in response to high strength wastewater, similarly as in the cathodes but conversely to the anodes of the 40 L MECs. Oxidation peak potentials in most cases decreased in response to high strength wastewater with an exceptional slight increase on the anodes of 40 L MECs. These results may reflect the progressive occurrence of multiple and complex organics oxidation reactions on both electrodes of the 1 L and 40 L MECs.

While the diffusional resistance  $R_{\text{dif}}$  always dominated the internal resistance, the solution and electrode resistance  $R_s$  of the cathodes was higher than those of the anodes or the abiotic controls in the 1 L reactors (Fig. 4C; Table S2). All resistances diminished when the reactors were fed with high strength wastewater influent and increased in the 40 L reactors (Fig. 4D; Table S2). The decreased internal resistance in all its components ( $R_s$ ,  $R_{\text{dif}}$  and  $R_{\text{ct}}$  (charge transfer resistance)) with high strength wastewater, in both 1 L and 40 L reactors, is consistent with the higher circuital currents (Fig. 3D) and the more positive reduction onset potentials (Fig. 4A – B;

Table S2) observed. This implies mass transfer limitations with low strength wastewater and the importance of providing a sufficient rate of mass transfer of “substrates” to the cathode/anode surfaces in order to achieve a high system performance. This can be achieved by increasing shear around the electrodes, although, this must be balanced against bacteria adhesion to the electrodes. The higher circuit currents (Figs. 2D and 3D) observed in the 1 L reactor compared to the 40 L reactor at both low and high strength ETW, resulted from the lower internal resistance in all its components recorded in the 1 L reactor (Fig. 4C and D; Table S2).

### *3.6 Electrodes morphology evolution and analysis of metal precipitates*

SEM observations of the electrodes after operation reported the formation of a higher fraction of irregular-shaped aggregates on the cathodes (Fig. S6A, C, E and G) in comparison to the anodes (Fig. S6B, D, F and H) resulting from different chemical and biochemical reactions occurring on the cathodes and on the anodes [27]. These were more accentuated when the system was fed with high strength (Fig. S6C, D, G and H) rather than low strength (Fig. S6A, B, E and F) wastewater and is consistent with achieving a higher net metals removal rates with high strength wastewater (Figs. 2 and 3).

The predominant components of the precipitates on the cathodes and the anodes were examined by XPS. Reduced products of each heavy metal were dominantly observed on the cathodes of the 1 L reactors (Cu(0): 75%; Ni(0): 74%; Zn(0): 52%)

(Fig. 5A, E, I and M; Table S3), and these were higher than the equivalent observed in the 40 L reactors (Cu(0): 60%; Ni(0): 60%; Zn(0): 47%) (Fig. 5C, G, K and O; Table S3). These results reflect a more cathodic reductive capability in the smaller reactors (1 L rather than the 40 L), consistent with the circuital currents (Fig. 3D). All anodes, however, were invariably loaded with Cu(II), Ni(II) and Zn(II) (Fig. 5B, D, F, H, J and L; Table S3) with an exceptional amount of the reductive product of Cr(III) (51 – 58%) (Fig. 5N and P; Table S3). This might be explained by the similar electrostatic behaviours of Cu(II), Ni(II) and Zn(II), converse to the more preferable diffusion of the negatively charged  $\text{Cr}_2\text{O}_7^{2-}$  to the oxidative anodes, and thus the preferential formation of the reductive products of Cr(III) under the same electric field-driven conditions [26]. These reductive products of heavy metals on the cathodes were similar to those previously observed in anaerobic cathodes of dual-chamber MECs utilized for the recovering of single-component heavy metals (e.g., Cd(II), Co(II), Cu(II), Cr(VI)) from simulated aqueous catholyte [25,28,30].

### 3.7 Alteration of microbial community diversity

From eight generated libraries, a total of 264531 non-chimeric and quality-filtered reads were obtained and these sequences were assigned into 3024 OTUs with a distance limit of 0.03 (Table S4). The rarefaction curves reached a plateau (Fig. S7), indicating sufficient sequencing coverage, shown as 99.6 – 100% of the bacterial communities using the Good's Coverage estimators (Table S4).

The cathodes exhibited a higher microbial community diversity than the anodes since the Shannon indices increased in both the 1 L reactors at low strength influents (4.22 vs. 3.84) and the 40 L reactors at high strength influents (4.73 vs. 3.94) (Table S4). This result reflects variable strategic compromises of either less toxic low strength influents in the 1 L reactors or high rate of substrate mass transfer in the high strength influent in the 40 L reactors for diversifying the bacterial communities, consistent with the kinetic results (Figs. 2 – 3).

Both reactor size and influent strength induced remarkable differences in the bacterial communities at phylum (Fig. S8A) or genus (Fig. 6A) levels based on Weighted Fast UniFrac principle coordinates analysis. The variable degrees of the different grouped quadrant of the bioanodes and the biocathodes of each reactors with the same strength influents at either phylum (Fig. S8A) or genus (Fig. 6A) levels, reflected the anodic and the cathodic influences on the bacterial community compositions. A dendrogram constructed on the basis of community phylogenetic lineages further demonstrated differences and evolving diversities in these microbial communities at phylum level (Fig. S8B) or class level (Fig. 6B).

The bacterial community was totally composed of 15 phyla, with the more dominant *Proteobacteria* on the cathodes (64.9%) than the anodes (59.3%) of the 1 L reactors fed with high strength ETW, both of which were higher than 44.0 – 45.0% with low strength wastewater (Fig. S9A). This implies an increased amount of *Proteobacteria* in the 1 L MECs originally acclimated to low strength wastewater.

Conversely, a decrease of the predominant *Proteobacteria* was observed on both the cathodes (45.4% vs. 70.6%) and the anodes (40.2% vs. 58.1%) of the 40 L reactors after feeding with high strength wastewater. These results collectively demonstrated a different evolution of the *Proteobacteria* in the 1 L and 40 L reactors in response to the high strength wastewater. Converse to the *Proteobacteria*, *Firmicutes* was predominant in the 1 L reactor at low rather than high strengths wastewater (anode: 31.5% vs. 13.3%; cathode: 28.0% vs. 3.4%), implying lower bacterial survival when the reactors were operated with the high strength wastewater. In the 40 L reactor, however, the switch to the high strength wastewater strongly stimulated the relative abundance of *Firmicutes* (anode: 32.7% vs. 2.0%; cathode: 15.8% vs. 2.5%). A similar stimulus was also observed for cathodic *Candidatus saccharibacteria* (1 L, 12.9% vs. 0.2%) or *Planctomycetes* (40 L, 9.1% vs. 0.3%), and anodic *Planctomycetes* (1 L, 7.4% vs. 0.3%) or *Bacteroidetes* (40 L, 16.7% vs. 3.1%) when the reactors were switched to high strength wastewater.

Four classes (*Alpha-*, *Beta-*, *Gamma-*, and *Delta-*) within *Proteobacteria* were invariably observed from all bacterial communities (Fig. S9B). High strength wastewater led to a relative increase in cathodic *Betaproteobacteria* (41.9% vs. 22.4%), *Gammaproteobacteria* (7.2% vs. 1.9%) and *Alphaproteobacteria* (10.7% vs. 7.3%), and anodic *Alphaproteobacteria* (36.7% vs. 10.2%) and *Deltaproteobacteria* (11.3% vs. 3.3%) in the 1 L reactor. However, in the 40 L reactor, only *Deltaproteobacteria* increased on the anodes (8.6% vs. 1.9%) with negligible changes



on the cathodes (approximate 16.7%) after reactors switched to high strength wastewater.

For 40 L reactors switched from low to high strengths wastewater, dominant genera of *Rhodopseudomonas* (cathode: 4.9%; anode: 2.8%), *Dechloromonas* (cathode: 1.4%; anode: 2.3%), *Aquabacterium* (cathode: 1.9%; anode: 1.6%) and unclassified *Parachlamydiaceae* (cathode: 0.3%; anode: 0.5%) were shifted to *Cloacibacterium* (cathode: 1.7%; anode: 12.8%), *Methylocystis* (cathode: 1.1%; anode: 4.7%), *Brevundimonas* (cathode: 1.8%; anode: 0.5%), *Streptophyta* (cathode: 1.0%; anode: 0.3%), *Sulfuriferula* (cathode: 0.1%; anode: 1.1%), unclassified *Veillonellaceae* (cathode: 1.7%; anode: 2.2%) and unclassified *Betaproteobacteria* (cathode: 2.4%; anode: 0.1%) (Fig. 7). This trend was different from the 1 L reactors with predominant *Dechloromonas* (16.3%), *Ferribacterium* (10.1%), *Tolumonas* (3.5%), *Sphaerotilus* (3.1%), *Reyranella* (1.1%), *Piscinibacter* (1.3%), unclassified *Enterobacteriaceae* (2.2%) and unclassified *Betaproteobacteria* (1.7%) on the cathodes, and *Methylocystis* (5.1%), *Methylobacterium* (4.6%), *Aquicella* (1.8%), unclassified *Deltaproteobacteria* (9.4%), unclassified *Planctomycetaceae* (5.3%), unclassified *Veillonellaceae* (2.3%) and unclassified *Parachlamydiaceae* (1.1%) on the anodes after it was switched to the high strength wastewater. The reduction and even disappearance of some genera along with an increase or emergence of other resistant genera on both electrodes of the 1 L and the 40 L reactors after switching to high strength wastewater, indicates a selective shock and an evolving development on

the microbial community. Both anode and cathode of the 40 L shared more number of same genera (24) than those of 1 L (11) at high strength wastewater, converse to those at low strength wastewater (40 L: 19; 1 L: 22), indicating the likely occurrence of unique bidirectional electron transfers in these genera with heavy dependence on reactor volume and wastewater strength.

Bacteria most similar to *Geobacter* have been documented as core genera in many microbial communities degrading either recalcitrant organics or removing heavy metal [25,30,31]. The abundance of these genera decreased more rapidly when high strength rather than low strength wastewater was fed to the MECs (1 L; cathode: 0.0 vs. 4.5%; anode: 0.2% vs. 7.1%. 40 L; cathode: 0.4% vs. 15.5%; anode: 0.5% vs. 0.8%) (Fig. 7). The ecological roles of these bacteria in these reactors cannot be ascertained based on their presence and it is still difficult to directly associate changes and activities of these specific genera even using rDNA based techniques [6,26]. However, the increase or emergence of some resistant genera along with the decrease and even disappearance of the others demonstrates that the shock of switching from low to high strengths ETW altered the composition of the microbial communities on the electrodes.

Gene function prediction of these bacterial communities was analyzed with PICRUSt software based on KEGG database [18]. Functions related to size of MECs at equal low influent strength of ETW, including environmental information processing (membrane transport and signal transduction), genetic information

processing (translation, replication and repair), cellular processes (cell motility and cellular signaling), and metabolism (energy metabolism such as nitrogen metabolism and sulfur metabolism, and metabolism of cofactors and vitamins) in both electrodes of the 1 L MECs were all predictably increased than those in 40 L MECs (Fig. 8A). These along with the general increased hydrogenase (Fig. 8B) might in concert explain the higher hydrogen production rate in the 1 L MECs compared to the 40 L MECs (Fig. 2B), consistent with the observations in other bioprocesses [32,33]. With high strength ETW, however, the functions were mainly related to metabolism and cellular processes (Fig. 8A), where xenobiotics biodegradation and metabolism, lipid metabolism, carbohydrate metabolism, and metabolism of terpenoids and polyketides of the 1 L MECs anodes were more enriched than the cathodes, all of which were invariably higher than those in the 40 L MECs. This result corroborates with higher COD removal rate in 1 L than that in 40 L MECs ( $98.2 \pm 0.9$  mg/L/d vs.  $76.1 \pm 0.9$  mg/L/d) with variable HRTs (1 L: 7 d; 40 L: 9 d) (Fig. 3B).

In addition, antioxidant enzymes of catalase, superoxide dismutase, superoxide dismutase, glutathione peroxidase and glutathione S-transferases were varying degrees induced in both 1 L and 40 L reactors (Fig. 8C), well consistent with the stressful circumstance of multiple heavy metals and recalcitrant organics in these low and high strengths ETW. These antioxidant enzymes reportedly played a cogent role in the detoxification of heavy metals through multiple mechanisms of intracellular accumulation and precipitation, active metal uptake, and valence transformation in

many microorganisms [34]. Collectively, these results clearly indicated that both the shift of the microbial community composition and metabolic changes of these functional bacteria on the cathodes and the anodes of the 1 L and 40 L reactors in response to the low and high strengths ETW, might have contributed to the efficient system performance.

It is difficult to exactly assign roles to each genus in these systems since microorganisms have complex symbiotic and even parasitic interactions. Thus dominant species cannot be directly related with their large contribution and minority species might largely function in bacterial community [6,35,36]. For such complex ETW, exoelectrogens might release electrons from the organics oxidation with multiple reaction steps, whereas electrotrophs reduced oxidative heavy metals. The mutually beneficial cooperation among exoelectrogens, electrotrophs and parasitic bacteria for utilizing breakdown products also cannot be excluded [35-37]. Further advances in molecular tools for analysis of the in situ physiological status of cells as well as isotope tracing methods, should make it possible to better map the routes for electron flux, the degradation of recalcitrant organics, the removal of heavy metals, and even the N and S metabolisms during the treatment of ETW through these electromicrobiomes.

With such low-strength wastewater, the  $CE_{an}$  in the 1 L reactor was appreciably higher than in the 40 L reactor, while the  $CE_{ca}$  followed a significant opposite trend (Table 2). The  $CE_{ca}$  in the 40 L reactor reaching up to 927% demonstrated that the

reduction of the multiple heavy metal, nitrate and sulfate species in the ETW not only was resulted from the flow of electrons from the cathode but also was augmented by the anaerobic microbial community. Such occurrence has also been reported for the degradation of other recalcitrant organics such as nitrobenzene, 4-bromophenol, pyridine or even synthetic coal gasification wastewater containing phenol, methylphenols and xylenols, in single-chamber MECs or up-flow biocatalyzed electrolysis reactor [8,23,38,39]. With high-strength wastewater the  $CE_{an}$  decreased compared to the results obtained with low ETW in both the 1 L and 40 L (Table 2), implying lower exoelectrogenic electron extraction from reductive substrates, consistent with results of treatment of many other recalcitrant organics in MECs [8,23,38,39]. The lower energy consumption and higher energy efficiency in the scaled-up 40 L reactor compared to the 1 L reactor and at the high than low ETW demonstrated a more efficient process in the former. Such results also exceeded those recorder in anaerobic systems (eg., 2 kWh/kg COD) for aerators in activated sludge [40] as well as other electrochemical systems for removal of a range of refractory organics [39], implying a significant advantage of the MECs in terms of process efficiency and operational cost over other systems.

The present study has demonstrated that cylindrical 1 L and 40 L single-chamber MECs can achieved complete removal of multiple heavy metals (Cu(II), Ni(II), Zn(II) and Cr(VI)) and significant organics removal of 70 – 74% from both low and high strengths ETW. The effluent from these reactors contained low concentrations of

heavy metals (Cu(II): 0.03 – 0.29 mg/L; Zn(II): 0.01 – 0.19 mg/L; Ni(II): 0.06 – 0.10 mg/L; Cr(VI): 0.0 mg/L), COD (44 – 48 mg/L), and other contaminants (NH<sub>4</sub><sup>+</sup>-N: 0.10 – 0.32 mg/L; SO<sub>4</sub><sup>2-</sup>: 0.96 – 1.50 mg/L; sulfide: 0.01 – 0.50 mg/L) and met the national wastewater discharge standard of China.

Superior system performance in CCCs than those in OCCs and abiotic controls confirmed the importance of closing the external circuit to achieve efficient system performance, whereas similar performance in 1 L (HRT: 7 d) and 40 L (HRT: 9 d) MECs with high strength wastewater reflected the worthwhile compromise of using longer HRT and larger reactors for efficient ETW treatment. The appreciable 36-fold improved contaminants (organics and heavy metals) removal rate in the 40 L reactors with HRT of 9 d rather than the 1 L MECs at 7 d, confirmed that the MECs performance almost scales-up with the reactors volume ratio. This provides insightful information for further large-scale MECs applications for the treatment of actual ETW. The stability of the cathode and anode potentials as the reactor was scaled-up with both low or high strengths wastewater, can be taken as an indicator for efficient contaminants removal. Such occurrence sharply contrast with other studies reporting that smaller rather than larger size MECs are more efficient for the treatment of domestic wastewater treatment and that the electrode potentials normally varies with the increase in MEC size [11].

Many previous studies have used small-size single/dual-chamber MECs for the removal of either simulated heavy metals [9,22,41] or for the treatment of (simulated)

industrial wastewaters containing recalcitrant organics, such as from coal gasification, landfill leachate, or ethanolamine [8]. Compared to these reports, the present study for the first time suggests the feasible option of a larger-scale single-chamber MECs for complete removal of multiple heavy metals with simultaneous efficient mineralization of organics in actual ETW. This study not only provides an effective and sustainable approach for the treatment of this complex actual industrial produced wastewater, but also brings MECs closer to commercialization as a technology for actual industrial terminal wastewater treatment with concomitant value-added products recovery.

The one-pass, flow-through HRTs of 5 or 9 d used in this study compared favourably with the HRTs of up-flow anaerobic blanket reactors (5.2 – 240 h) used for the treatment of industrial wastewaters [8,42] although the HRTs somewhat exceed those of conventional wastewater treatment plants. The further practical implementation of the proposed process in industry depends on demonstrating the long-term stability of these reactors under variable environmental conditions, and requires a comprehensive analysis of the process economics relative to conventional anaerobic or simpler electrochemical processes.

The use of the specific electrode surface area parameter has been advocated for scaling up of modular and commercial microbial fuel cells using chemical catalyst air-cathodes and in most cases acetate as a fuel, where oxygen reduction and cathode materials limit performance. The electrode specific surface area for cylindrical geometry scales with  $2r^{-1}$ , where  $r$  is the electrode radius [43]. The lower specific

surface area of the 40 L reactor compared to the 1 L reactor (cathode: 13 m<sup>2</sup>/m<sup>3</sup> vs. 89 m<sup>2</sup>/m<sup>3</sup>; anode: 12 m<sup>2</sup>/m<sup>3</sup> vs. 53 m<sup>2</sup>/m<sup>3</sup>) cannot support the relative performance of both reactors for ETW treatment. Thus scaling up of MECs using the sole concept based on geometrical similarity on the radius of the electrode should be cautioned particularly in face of the complexity of treating actual industrial ETW. Much exploration in this direction is still in need.

Both anodes and cathodes of the 1 L and the 40 L reactors experienced a selective shock and an evolving development of the microbial community with metabolic regulations of the functional bacteria metabolisms after switching from low to high strengths wastewater. The variable numbers of same genera on both electrodes of either 1 L or 40 L MECs with presumable occurrence of bidirectional electron transfers may provide alternative origins for unearthing new indigenous bacteria with capability of bidirectional electron transfers, which have recently been observed only with pure cultures of *Shewanella loihica* or *Anabaena siamensis* used for nitrate removal or hydrogen evolution [44].

#### **4. Conclusions**

This study for the first time has demonstrated the effective treatment of real etching terminal wastewater in MEC reactors of different scales operated under one-pass continuous flow. The effluents from these reactors using either low or high strengths influents met the national wastewater discharge standards of China. The



stability of the treatment process was confirmed by the stability of the cathode and anode potentials as the process conditions were varied. The MECs performance almost scales-up with the reactors volume ratio up to 40 L. The strength of the wastewater largely influenced the compositions of the bacterial communities and contributed to system performance under the different operating conditions. This study demonstrates complete removal of multiple heavy metals with simultaneous efficient wastewater treatment in MECs of different scales, and provides a plausible approach for simultaneous recovery of value-added products (e.g., heavy metals) and efficient treatment of practical etching terminal wastewater.

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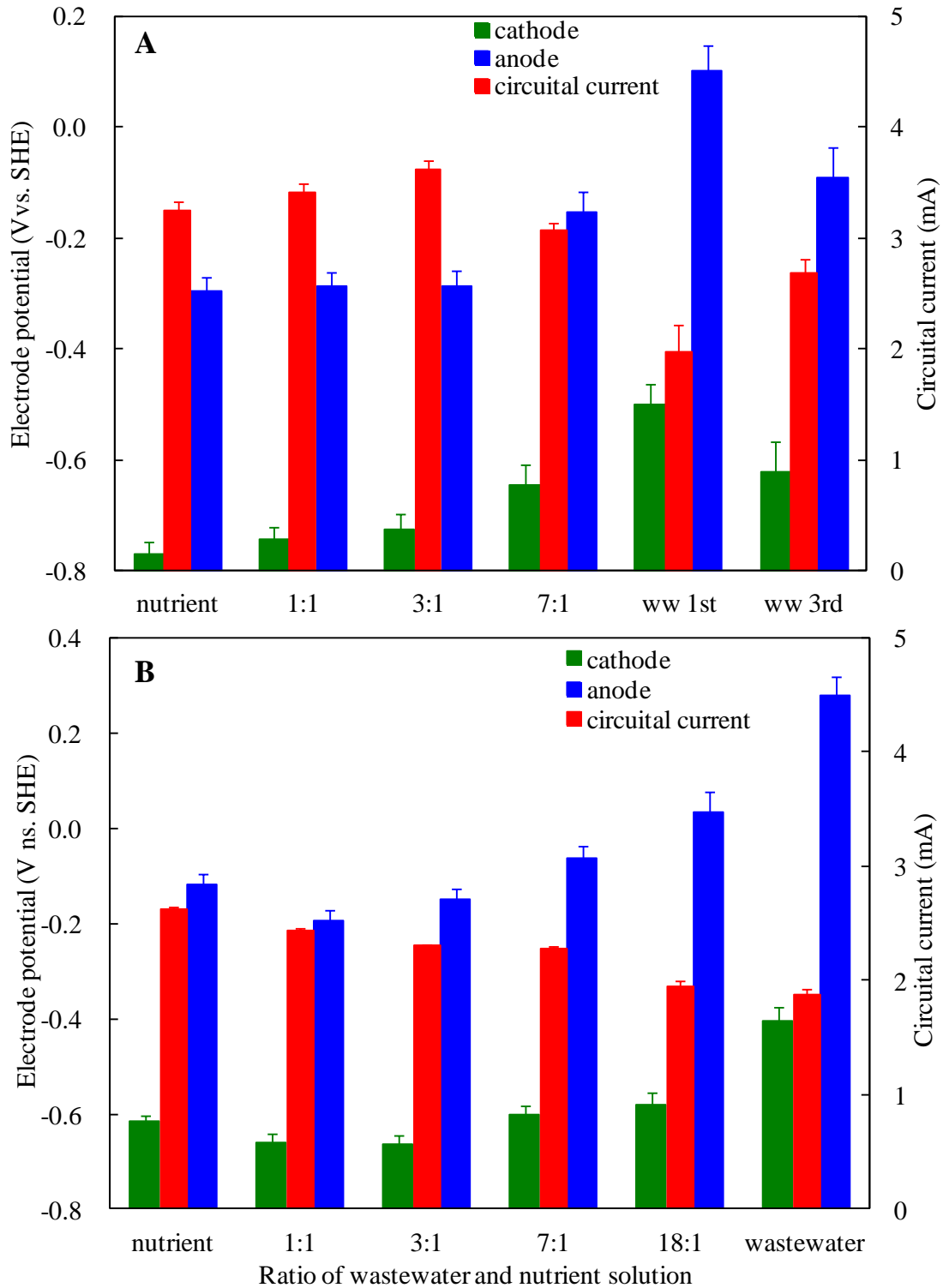
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**Table 1** Composition of actual etching terminal wastewater

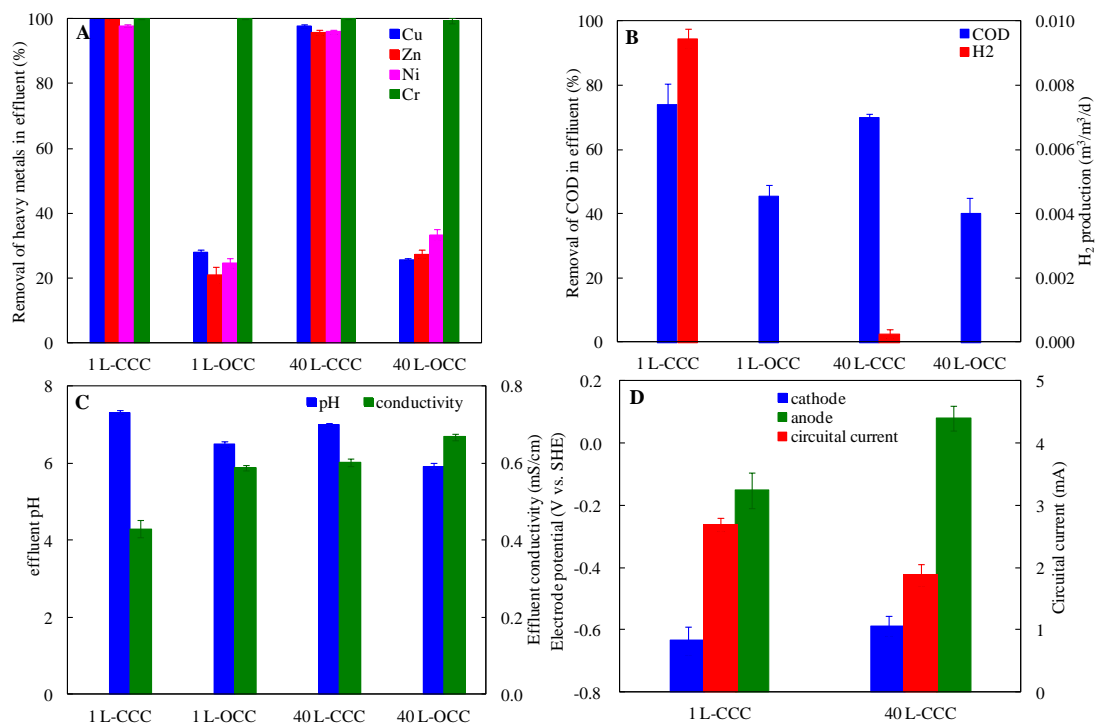
Loading	Parameter	Value	Loading	Parameter	Value
Low strength wastewater	Organics (SCOD)	174 ± 10 mg/L	High strength wastewater	Organics (SCOD)	773 ± 7 mg/L
	Cu(II)	13.9 ± 0.6 mg/L		Cu(II)	33.8 ± 1.1 mg/L
	Zn(II)	4.4 ± 0.3 mg/L		Zn(II)	14.5 ± 0.9 mg/L
	Ni(II)	2.6 ± 0.3 mg/L		Ni(II)	21.3 ± 0.6 mg/L
	Cr(VI)	1.4 ± 0.3 mg/L		Cr(VI)	2.1 ± 0.4 mg/L
	NH <sub>4</sub> <sup>+</sup> -N	0.08 ± 0.00 mg/L		NH <sub>4</sub> <sup>+</sup> -N	0.10 ± 0.00 mg/L
	NO <sub>3</sub> <sup>-</sup> -N	0.68 ± 0.04 mg/L		NO <sub>3</sub> <sup>-</sup> -N	2.63 ± 0.18 mg/L
	sulfide	1.04 ± 0.09 mg/L		sulfide	3.21 ± 0.6 mg/L
	SO <sub>4</sub> <sup>2-</sup>	3.06 ± 0.02 mg/L		SO <sub>4</sub> <sup>2-</sup>	5.68 ± 0.01 mg/L
	total chlorine	0.79 ± 0.01 mg/L		total chlorine	2.25 ± 0.01 mg/L
	pH	2.98 ± 0.03		pH	3.10 ± 0.02
	conductivity	0.71 ± 0.03 mS/cm		conductivity	1.11 ± 0.01 mS/cm

**Table 2** Anodic and cathodic  $CE$ s, energy consumption and energy efficiency of the 1 L and 40 L MECs fed with low or high strengths ETW.

Reactor	Parameter	Low loading ETW	High loading ETW
1 L	Circuit current (mA)	$2.70 \pm 0.10$	$3.20 \pm 0.05$
	$CE_{an}$ (%)	$93.9 \pm 2.2$	$30.4 \pm 1.5$
	$CE_{ca}$ (%)	$40.9 \pm 1.3$	$65.2 \pm 1.0$
	Energy consumption (kWh/kg COD)	$1.52 \pm 0.06$	$0.48 \pm 0.03$
	Energy efficiency (%)	$71.2 \pm 2.3$	$145 \pm 6$
40 L	Circuit current (mA)	$1.88 \pm 0.17$	$2.75 \pm 0.01$
	$CE_{an}$ (%)	$69.7 \pm 3.2$	$1.2 \pm 0.1$
	$CE_{ca}$ (%)	$927 \pm 13$	$445 \pm 17$
	Energy consumption (kWh/kg COD)	$0.033 \pm 0.008$	$0.014 \pm 0.003$
	Energy efficiency (%)	$98.7 \pm 2.4$	$127 \pm 5$

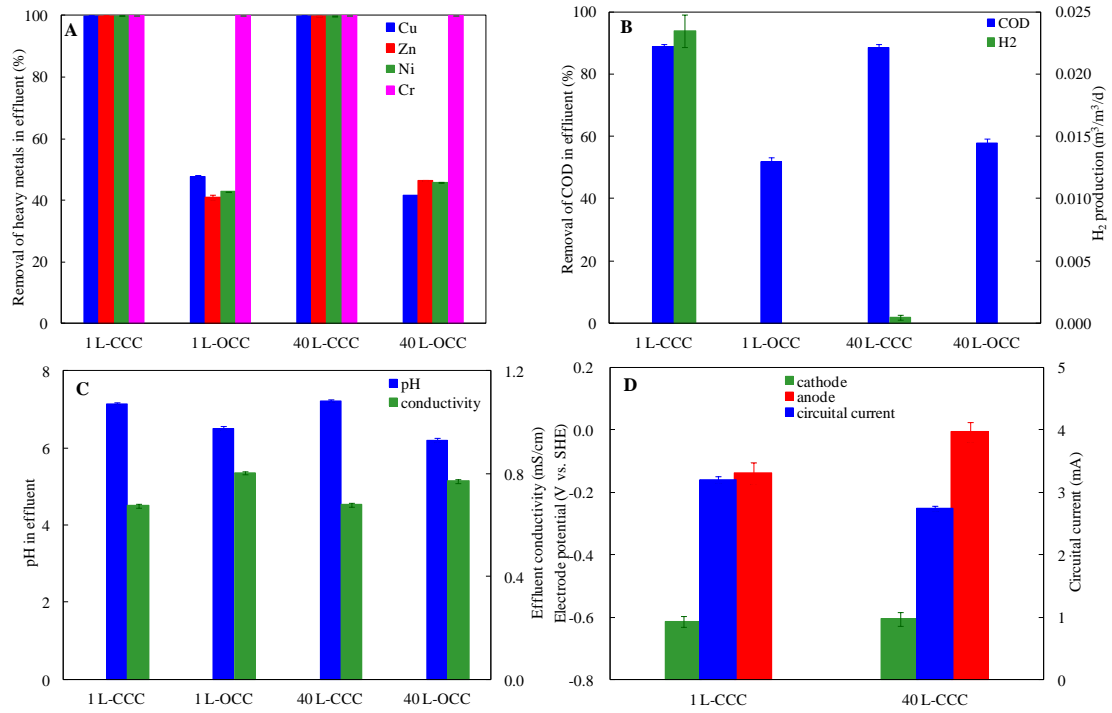


**Figure 1** Electrode potentials and circuit currents of MECs with 1 L (A) or 40 L (B) fed with different ratios of low strength of etching terminal wastewater and nutrient solution during startup period.

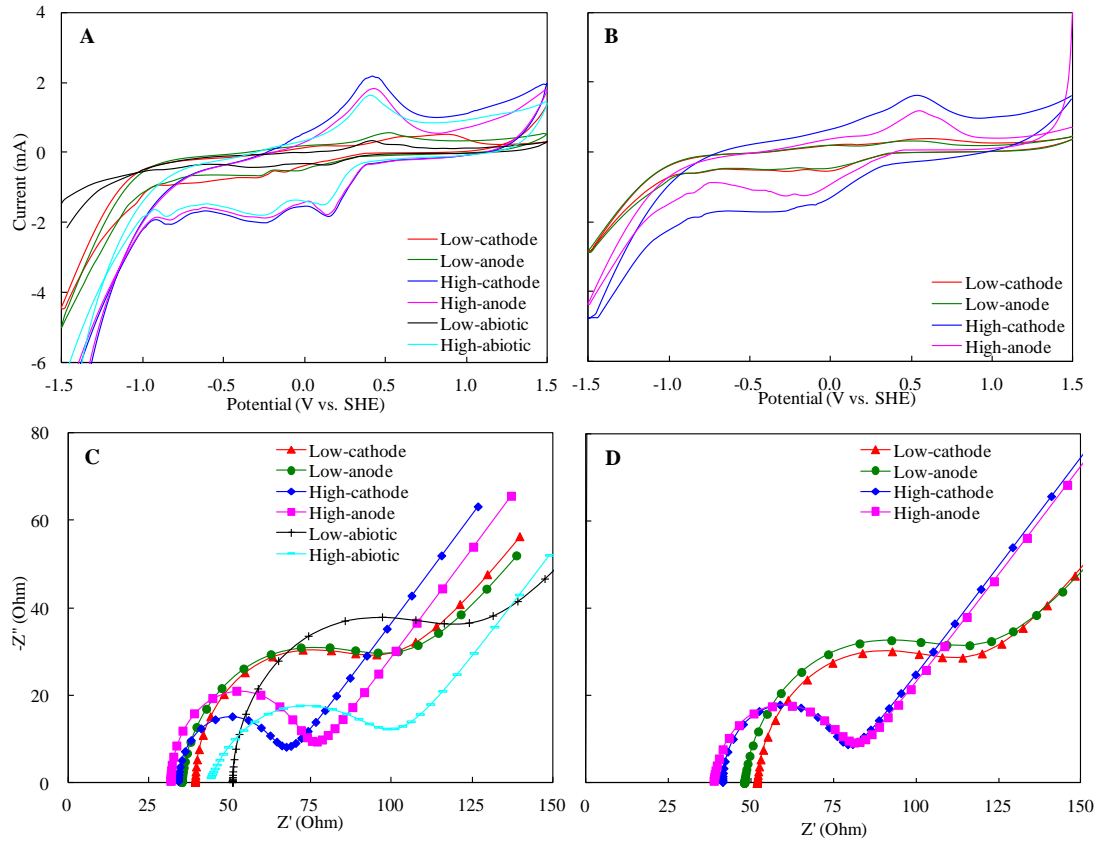


**Figure 2** Comparison of heavy metal removal (A), COD removal and hydrogen production (B), effluent pH and conductivity (C), and electrode potential and circuit current (D) of MECs at 1 L or 40 L fed with low strength of etching terminal wastewater (HRT: 5 d).

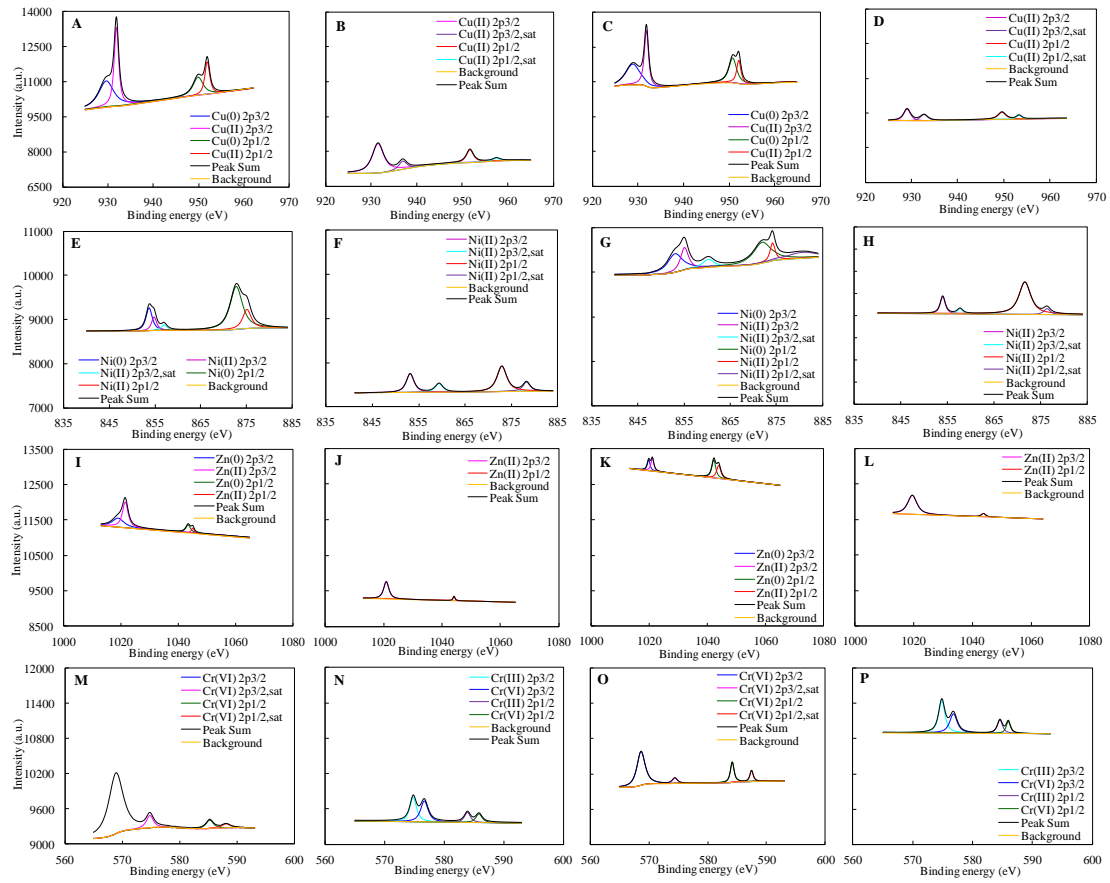




**Figure 3** Comparison of removal of heavy metals (A) and COD (B) in the effluent, hydrogen production (B), effluent pH and solution conductivity (C), and electrode potential and circuit current (D) of MEC reactors at 1 L (HRT: 7 d) or 40 L (HRT: 9 d) with high strength influent of etching terminal wastewater.

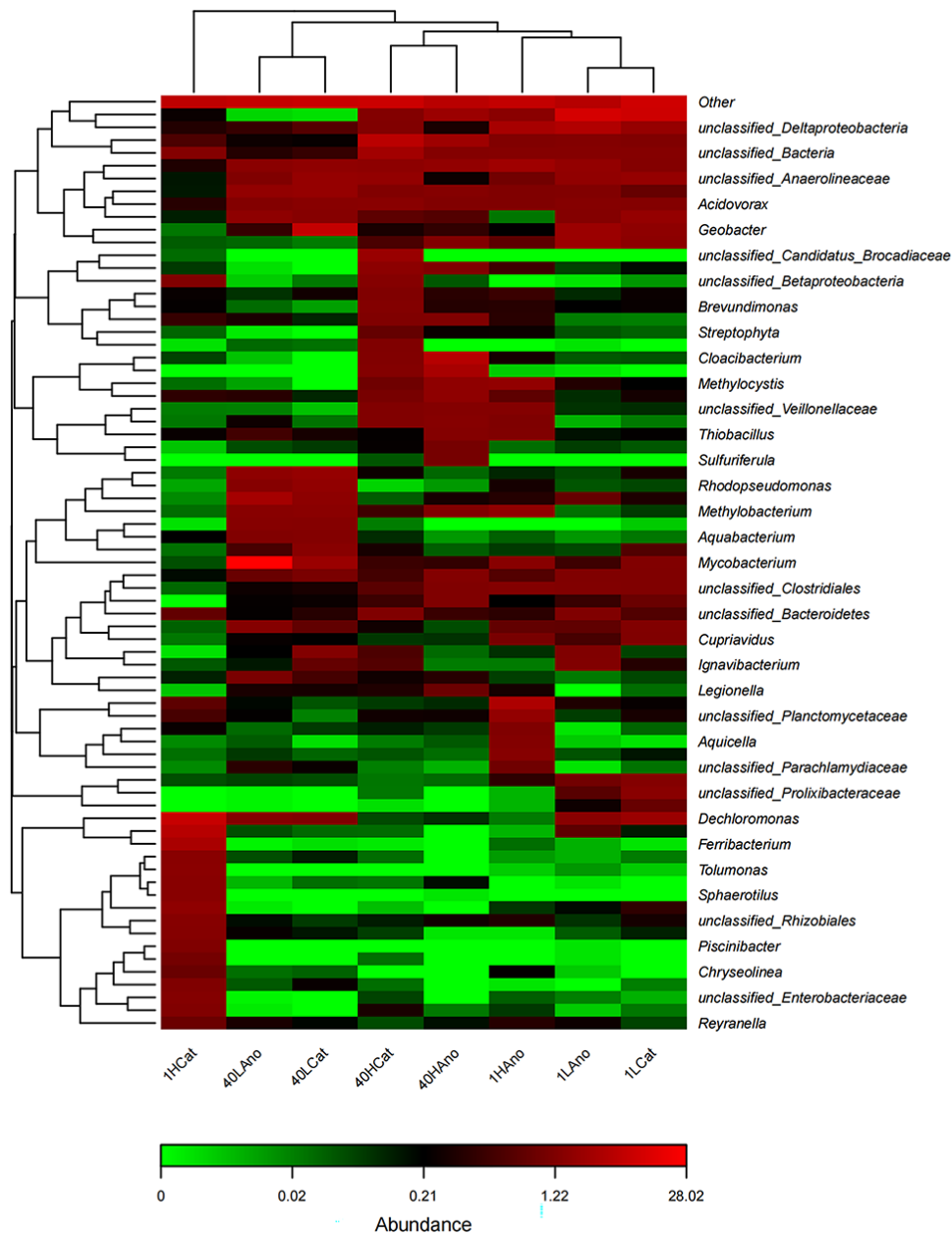


**Figure 4** Comparison of CV test (A and B) and Nyquist plots of EIS spectra (C and D) of the bioanodes and the biocathodes of the 1 L (A and C) or 40 L (B and D) MECs fed with low or high strengths etching terminal wastewater.

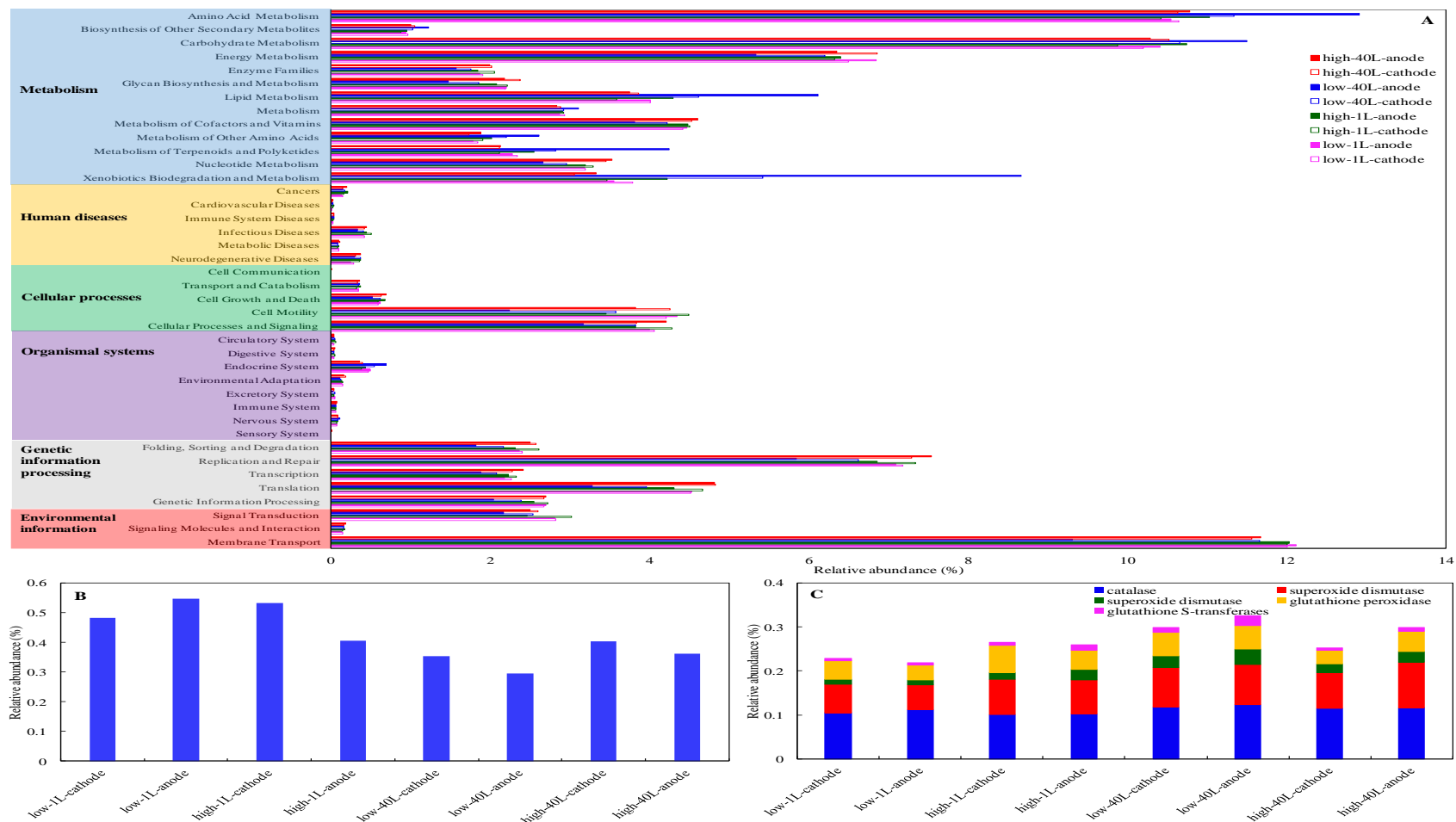


**Figure 5** XPS spectra of Cu (A – D), Ni (E – H), Zn (I – L) and Cr (M – P) precipitates on the cathodes (A, C, E, G, I, K, M and O) and the anodes (B, D, F, H, J, L, N and P) of MECs with 1 L (A, B, E, F, I, J, M and N) or 40 L (C, D, G, H, K, L, O and P) at the end of 30 d operation and fed with high strength etching terminal wastewater.





**Figure 7** Comparison of relative abundance of bacterial reads retrieved from the cathodes and the anodes of 1 L or 40 L MECs fed with low or high strengths etching terminal wastewater (thus abbreviated as 1LCat, 1LAno, 1HCat, 1HAno, 40LCat, 40LAno, 40HCat and 40HAno, respectively) and classified at the genus level. Genera that represent less than 1.0% of the total bacterial community composition were classified as “others”.



**Figure 8** Relative abundances of metabolic pathways on KEGG categories (levels 2) (A), and relative abundances of hydrogenases (B) and antioxidant enzymes of catalase, superoxide dismutase, superoxide dismutase, glutathione peroxidase and glutathione S-transferases (C) for samples of the cathodes and the anodes of 1 L or 40 L MECs fed with low or high strengths etching terminal wastewater.



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