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## Short Communication

# Effect of copper ions on glucose fermentation pathways in bioelectrochemical system



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#### HIGHLIGHTS

- Changes in fermentation products, electrical output, and methane generation were discussed.
- $\bullet$  The glucose fermentation pathways were strongly influenced by  $\mbox{Cu}^{2+}$  addition.
- The metabolic reaction of glucose fermentation altered from mixed acid type to acetogenesis type.
- The flow of electrons from acetate to anode decreased with increase of Cu<sup>2+</sup> concentration.
- A decrease in electricigens accompanied by an increase in fermenters was observed.

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#### G R A P H I C A L A B S T R A C T



### ABSTRACT

Toxic metal ions were previously found to be effective removed by anodic biofilms under the coexistence of organics in bioelectrochemical system (BES). However, the effect of toxic metal ions on the organics fermentation pathways remains unclear. To investigate the pathway systematically, the glucose fermentation pathways were discussed under different  $Cu^{2+}$  concentrations. After introducing  $Cu^{2+}$ , more acetate and less propionate were observed, implying that the metabolic reaction of glucose fermentation altered from mixed acid type to acetogenesis type. This pattern produced more "food" (acetate or hydrogen) for methanogens, thus, the methane content increased by 19.67%, 39.51%, and 27.71% in the presence of 0.1, 1, and 7 mg L<sup>-1</sup> Cu<sup>2+</sup> compared to the blank, respectively. Increased Cu<sup>2+</sup> concentrations resulted in the decrease of current production, which was associated with the decrease of electricigen (*Geobacter*). Consistent with the change of fermentation type, the fermenters (*Klebsiella* and *norank\_f\_norank\_o\_Bacteroidales*) that related to the production of acetate increased, while the dominant methanogens (*Methaospirillum*) didn't decrease until the Cu<sup>2+</sup> concentration reached 7 mg L<sup>-1</sup>. © 2021 Published by Elsevier Ltd.



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#### 1. Introduction

BES is a rapidly developing technology that combines biological metabolism with electrochemistry to achieve pollutant degradation and valuable products recovery (Bakonyi et al., 2018). In recent years, BES has been widely used in the treatment of wastewater, such as organic pollutants, toxic metals and inorganic salt (Wu et al., 2018). Electroactive biofilm (EAB) was the core of BES, since BES relies on EAB as biocatalysts to promote the conversion of organics into power (Yuan et al., 2020). As the "food" for BES are complex in nature, their biodegradation requires cooperation between different functional groups: fermenters, homoacetogens, electricigens and methanogens, in which key microbial populations have been identified (Lovley, 2008). For example, fermenters such as Rhodopseudomonas (glucose), Plobacter (lactic) and Clostridium (cellulose) and electricigens such as Shewanella, Geobacteraceae, and Pseudomonas in the anode biofilm have been reported previously (Gorby et al., 2006; Lovley et al., 2008; Kiely et al., 2011; Rabaev et al., 2004).

It's well known that most common methanogens and electricigens are limited to use a few substrates (e.g., acetate and hydrogen) and most substrates (e.g., sugar ethanol or cellulose) could not be used by methanogens and electricigens directly (Kiely et al., 2011; Prathap et al., 2010). Thus, good acidification performance of fermenters was required. With glucose as substrates, the initial fermentation product primarily were acetate and hydrogen or more reductive products, such as propionate, butyrate, with small amount of hydrogen (Table 1) (Freguia et al., 2008). Acetate and hydrogen are ideal substrates not only for electricigens but also for methanogen, as the anode chamber is anaerobic which provides the possibility for methanogens to grow (Parameswaran et al., 2009). This resulted in competition between methanogens and electricigens for these by-products. The consumption of these byproducts eliminating the inhibition of glucose fermentation and makes the fermentation proceed or even faster (Freguia et al., 2008). Each step of organic matter degradation by different functional microbes constitutes the whole fermentation pathways.

When BES was utilized in actual wastewater treatment, the environmental factors also could affect the fermentation pathways, thus influencing BES performance. For instance, Temudo et al. (2008) demonstrated that higher pH values (7.0–8.5) were favorable for alcohol generation, while lower pH increased the production of butyrate/acetate. Jiang et al. (2018) found that a more negative oxidation reduction potential (ORP) led to higher production of acetic acid, H<sub>2</sub> and CH<sub>4</sub>, while increasing the ORP from -1.0 V to 0.6 V (versus Ag/AgCl) significantly reduced acetic acid generation by 33% and methanogenesis by 68% in mixed culture electro-fermentation. Besides, Mahmoud et al. (2017) examined the effect of NH<sup>4</sup> on the glucose fermentation pathways in a microbial electrolysis cells, demonstrating that the existence of NH<sup>4</sup> (0.02–0.18 g/L) minimized the production of H<sub>2</sub> and CH<sub>4</sub> but

increased the electricity output.

Notably, toxic metals were widely found in municipal sewage, which bring about the toxicity effect of toxic metals on EAB (Ngah and Hanafiah, 2008), therefore, many efforts were performed on this topic. For instance, low dosage of toxic metals (e.g., 0.1 µg/L  $Cu^{2+}$  or 0.1  $\mu g/L-1$  mg  $L^{-1}$   $Cd^{2+}$ ) were found to significantly improved the power generation in BES as it promoted the electron shuttle mediated extracellular electron transfer (Xu et al., 2016). Higher dose toxic metals inhibited the anode performance which seems to through its toxicity to electricigens. Specifically, a decreased in Geobacter accompanied by an increase in Stenotrophomonas were observed in the anodic biofilms with addition of  $10 \text{ mg } \text{L}^{-1} \text{ Cd}^{2+}$  or  $\text{Cu}^{2+}$  (Zhang et al., 2018a, 2018b). Most of these studies are focused on extracellular electron transport, power output and microbial diversity. However, the insight into how the toxic metals regulate fermentation pathways in the anodic chamber remains unclear.

Therefore, dual chamber BES reactors were constructed to explore this issue. Due to the frequent observation of  $Cu^{2+}$  in municipal sewage (Ngah and Hanafiah, 2008), it was selected as the representative toxic metal to be introduced into the anode chamber. Glucose was used as a model substrate, since it's the hydrolysis product of most organics. Electrochemical, chemical and microbial sequencing tools were combined. Changes in fermentation products, electrical output, and methane generation respects to time as a function of anodic microbes response were discussed. This's very relevant to the application of BES, since fermentation was the basis for the pollutants degradation and electricity output of EAB.

#### 2. Materials and methods

#### 2.1. Reactor setup and inoculation

A two-chamber (41.25 mL) reactor as previously described (Zhang et al., 2018a) was set up, with a square graphite felt (3 cm  $\times$  2 cm  $\times$  0.5 cm) as its anode electrode and a titanium wire as cathode electrode respectively. On top of the anode, there is a cylindrical gas collector (1.3 cm in diameter and 2.5 cm in height) sealed with a butyl bottle stopper. Each reactor was equipped with a saturated calomel electrode (SCE), which was fixed 0.5 cm away from the anode electrode, and the anode potential was controlled at -0.3 V (vs SCE). All reactors were placed in thermostat with a temperature of 30 °C.

We inoculated the BES's anode chamber with 4 mL anaerobic sludge with 5 mM glucose growth medium as the sole carbon source. Every liter of growth medium (initial pH of 7) containing: 5 mM glucose, 50 mM PIPES buffer, 0.31 g of NH<sub>4</sub>Cl, 0.13 g KCl, 10 mL trace elements (Table S1), 5 mL vitamins (Liu et al., 2017). All growth mediums were purged with high purity nitrogen ( $\geq$ 99.9%) for 15 min prior to feeding to achieve an anaerobic condition. We acclimatized the biofilm by continuously feeding the growing

Table 1

All fermentation types of glucose and their reaction equations

Types	Bioreactions	$\Delta G^{\circ} \prime^a (kJ/mol)$				
(1) Mixed acid	glucose $\rightarrow$ acetate <sup>-</sup> + propionate <sup>-</sup> + CO <sub>2</sub> + H <sub>2</sub> + 2H <sup>+</sup>	-287				
(2) Acetogenesis	$glucose + 2H_2O \rightarrow 2acetate^- + 2CO_2 + 4H_2 + 2H^+$	-216				
(3) Butyric	glucose $\rightarrow$ butyrate <sup>-</sup> + 2CO <sub>2</sub> + 2H <sub>2</sub> + H <sup>+</sup>	-264				
(4) Homolactic	glucose $\rightarrow$ 2lactate <sup>-</sup> + 2H <sup>+</sup>	-197				
(5) Alcoholic	glucose $\rightarrow$ 2ethanol + 2CO <sub>2</sub>	-235				

Source of Gibbs free energies of formation: Data refer to pH 7 and 298 K. The Gibbs free energy depends on the anodic potential; here we reported values for a near-optimum anodic potential of +200 mV SHE.

biofilm with growth medium in batch run (24 h for one cycle). When the current stabilized continuously for at least three cycles, we considered that biofilm acclimation had been completed. All BES experiments were performed in duplicate.

#### 2.2. Batch run of BES with different $Cu^{2+}$ concentration

After acclimation, the nutrient solutions containing different  $Cu^{2+}$  contents were added to individual reactors. We selected 0.1 and 1 mg L<sup>-1</sup> of Cu<sup>2+</sup> which were close to the concentration in the actual environment in this study (Ngah and Hanafiah, 2008). To obtain more information about Cu<sup>2+</sup> impact on glucose fermentation pathways, other two dosages of Cu<sup>2+</sup> (0 and 7 mg L<sup>-1</sup>) were also performed. The liquid in both chambers were replaced by fresh nutrient solutions once 24 h. After continuous operation for 7 batches with Cu<sup>2+</sup> addition, we studied the glucose fermentation pathways by timing sampling in the eighth cycle (72 h for one cycle).

#### 2.3. Analytical methods

**Chemical analysis.** The liquid phase volatile fatty acids were quantified by high-performance liquid chromatography (HPLC; model LC-16, Shimadzu, Jiangsu, CHINA) with a C18 packed column (Wondasil, 250 mm\*5- $\mu$ m, Japan) after filtration through a 0.22- $\mu$ m polyether maple membrane filter. We used 10 mM sodium dihydrogen phosphate and pure methanol as mobile phase at a constant flow rate of 0.8 mL/min. We analyzed the H<sub>2</sub> and CH<sub>4</sub> content of 250- $\mu$ L of gas extracted with a gas-tight syringe (Hamilton, 500- $\mu$ L, Switzerland) using a gas chromatograph (GC9800, Kechuang Corporation, Shanghai, CHINA). Nitrogen was the carrier gas. Temperature conditions for detector, injection, and column were 220, 150, and 110 °C, respectively. We determined the residual glucose content in the liquid phase by sulfuric acid-anthrone method. Each measurement was calibrated with a five-point standard curve.

**Electrochemical metrological analysis.** The current was recorded every 10 s with a multichannel potentiostat (CHI1000C, Shanghai CH Instrument Company) using chronoamperometry. Cyclic voltammogram (CV) of the electrode biofilms were performed with workstation (CHI660E Shanghai CH Instrument Company) in a three electrode system. We set the parameters of the electrochemical workstation to scan the voltage between - 0.6 V and 0.5 V at a rate of 5 mV s<sup>-1</sup>.

**Microbial community analysis.** In the interest of studied the effect of Cu<sup>2+</sup> on the microbial community of anodic biofilms. The biofilms in different reactors were scraped off the anode electrodes on the end of experiments. We extracted the microbial DNA using a PowerSoil DNA isolation kit according to the manufacturer protocol and amplified the 16S RNA genes by PCR using the bar-coded primer set 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), which target at the V4 hypervariable region. PCR amplification, sequencing and data analysis refer to previous study (Mahmoud et al., 2017).

#### 2.4. Calculations

The electron balances were calculated by quantifying the equivalent number of electrons per fermentation products and glucose with the following equivalences: 8 e– equiv per mol of acetate, 14 e– equiv per mol propionate, and 24 e– equiv per mol glucose respectively (Mahmoud et al., 2017). The electric quantity of the circuit was obtained by integrating the Current-Time curve, calculated as current =  $\int I dt$ .

#### 3. Results and discussion

# 3.1. Framework for explaining metabolism of glucose by anodic biofilms for BES in a response to $Cu^{2+}$

As illustrated in Fig. 1A, the blank reactor (0 mg  $L^{-1}$ ) showed good reproducible performances within 7 batch cycles, with a maximum current density of  $1.06 + 0.052 \text{ A/m}^2$ , indicating that EAB had been domesticated on the electrode (Yu et al., 2017). However, with addition of  $Cu^{2+}$  (0.1, 1 and 7 mg  $L^{-1}$ ) into the anodic chamber, the current gradually decreased, which might be due to the toxicity of Cu<sup>2+</sup> to EAB (Abourached et al., 2014). Specifically, in the BESs fed with solutions containing 0.1 and 1 mg  $L^{-1}$  Cu<sup>2+</sup>, no apparent changes in the maximum current density were observed within the first three cycles. However, the maximum current density dropped by 20% and 27% at seventh cycles compared with the first cycle, respectively, probably ascribed to the copper accumulation during the foregoing cycles. In the BESs fed with a  $Cu^{2+}$  concentration of 7 mg  $L^{-1}$ , the maximum current density decreased slightly from the second cycles, and the seventh cycles dropped by 52% compared with the first cycles.

Fig. 2 shows the electrons distribution from glucose to the possible electron sinks throughout the experiments with different Cu<sup>2+</sup> concentrations. Among BESs reactor, acetate and propionate production rapidly increased to the peak and associated with completely glucose degradation within 7 h of operation, suggesting that mixed acid type fermentation has occurred in the anodic biofilm (Table 1). Specifically, without  $Cu^{2+}$ , acetate and propionate accounted for 39.53% and 12.51% of the total electrons supplied from glucose after 7 h of operation, respectively. When introducing 0.1 and 1 mg  $L^{-1}$  Cu<sup>2+</sup>, the proportion of acetate increased to 41.53% and 45.53% and the proportion of propionate decreased to 10.51% and 8.60%, respectively. These results implied that the fermentation in the anode chamber may transform from mixed type to acetogenesis type in a response to  $Cu^{2+}$  toxicity. Notably, when adding 7 mg  $L^{-1}$  Cu<sup>2+</sup> into the anodic chamber, a more obvious effect on glucose fermentation was observed. Specifically, acetate production increased to 49.52% of the total glucose's electrons significantly within 7 h, which was 1.25 times higher than the blank. Simultaneously, propionate production decreased to 5.5% of the total electrons, which was much lower than 0.1 and 1 mg  $L^{-1}$ .

The current generation increased continuously to a peak and associated with the gradually consumption of acetate below the detection limit at the 72 h. Specifically, without  $Cu^{2+}$ , current increased by 24.57% and acetate decreased by 39.53% within 72 h. suggesting that electrons (about 62% of electrons from acetate) flow from acetate to anode. However, when introducing 0.1 and 1 mg L<sup>-1</sup>  $Cu^{2+}$ , the proportions were decreased to 52.66% and 48.03%, respectively. Furthermore, when adding 7 mg  $L^{-1}$  Cu<sup>2+</sup> into the anodic chamber, current generation was inhibited obviously, with only 43.05% of the electrons supplied from acetate routed to the anode. These results indicated that the acetoclastic electricigenesis was inhibited by Cu<sup>2+</sup> toxicity. CV results show that the corresponding catalytic current decreased along with the increase of Cu<sup>2+</sup> concentration (Fig. 1B). Notably, the catalytic current dropped obviously when introducing 7 mg  $L^{-1}$  Cu<sup>2+</sup> to the anodic chamber compared to the blank. The lower catalytic current at higher concentration of Cu<sup>2+</sup> demonstrated that the inhibitory effect of Cu<sup>2+</sup> on electricigens.

Due to the low proportion (<1%) of methane in the total electrons supplied by glucose, we show all the methane data in a single graph (Fig. 3). This low proportion of methane probably due to the applied voltage (-0.3 V), since methanogens requires a reductive environment where potential should be less than -0.527 V (vs SHE) for its growth (Zhao et al., 2019). Among BESs reactor, methane



Fig. 1. (A) Current generation profile of BESs versus time. (B) CV curve with substrates addition at different Cu<sup>2+</sup> concentration in corresponding BES anode chamber, with a scanning rate of 5 mV s<sup>-1</sup>.



Fig. 2. Electron distribution in BESs fed with 5-mM glucose at different Cu<sup>2+</sup> concentrations: (A) 0 mg L<sup>-1</sup>; (B) 0.1 mg L<sup>-1</sup>; (C) 1 mg L<sup>-1</sup>; (D) 7 mg L<sup>-1</sup>.

slowly accumulated within 7 h, then increased rapidly and peaked after 24 h. Specifically, without Cu<sup>2+</sup>, methane slowly accumulated within 7 h, then rose rapidly to a plateau (7.38  $\pm$  0.21 g/m<sup>3</sup>) after 24 h. When 0.1 and 1 mg L<sup>-1</sup> Cu<sup>2+</sup> were introduced, the peak values of methane concentrations were stabilized at 8.44  $\pm$  0.36 and 10.16  $\pm$  0.34 g/m<sup>3</sup>, respectively. This patterns probably due to the fact that the above fermentation produced more methanogens' favorite foods (acetate or hydrogen). However, the maximum methane contents obtained in the BESs fed with 7 mg L<sup>-1</sup> was 9.23  $\pm$  0.42 g/m<sup>3</sup>, lower than the 1 mg L<sup>-1</sup> but still higher than the blank. These results implied that the Cu<sup>2+</sup> concentration over to 7 mg L<sup>-1</sup> contributed to inhibited effect on methanogenesis, which

probably due to the toxicity suppression to methanogens. From the above results, it's known that the fermenters are more resistant to  $Cu^{2+}$  toxicity than methanogens and electricigens.

#### 3.2. Distribution of microbial population in BESs

The microbial communities among the BES reactors were compared by high throughput sequencing. The alpha diversity indexes between different samples were shown in Table 2. The shannon index in the blank was lower than that in the reactors with  $Cu^{2+}$  addition, and the ace and chao indexes in the reactors with  $Cu^{2+}$  addition was higher than that in the blank. All the facts



Fig. 3. Methane concentration among BESs fed with 5 mM glucose at different  $\mbox{Cu}^{2+}$  concentrations.

 Table 2

 Alpha diversity index between different samples.

Percent of community abundance on phylums level

0.8

0.6

0.4

0.2

0.0

0

A

Samples	Sobs	Shannon	Simpson	Ace	Chao	Coverage
0	151 ± 4.55	1.91 ± 0.05	0.26 ± 0.007	192.84	182.20	0.999141
0.1	183 ± 5.28	$2.17 \pm 0.05$	$0.21 \pm 0.007$	222.56	211.28	0.999144
1	$191 \pm 5.44$	$2.23 \pm 0.06$	$0.19 \pm 0.005$	225.77	215.60	0.999163
7	$210 \pm 6.03$	$2.46 \pm 0.07$	$0.17 \pm 0.003$	234.36	229.19	0.999412

suggested that the presence of  $Cu^{2+}$ , especially a high level of  $Cu^{2+}$ , raised the microbial richness and diversity significantly. This may be due to the abundance of *Proteobacteria* decreased whereas other microorganisms increased with the decrease of  $Cu^{2+}$  concentrations (Fig. 4A).

The sequences analyses of the V4 region of bacterial 16S rRNA gene in the biofilm samples were shown in Fig. 4. At the phylum level, the majority of microbes were found to be belonged to four phyla: *Bacteroidetes, Proteobacteria, Euryarchaeota, and Firmicutes.* Many microbes in the four phyla were reported to be widely

 $\frac{0.1}{Cu^{2+}} \frac{1}{(mg L^{-1})}$ 

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presented in the BESs, and plenty members of phyla *Proteobacteria* were known to perform anode respiration (Zhang et al., 2011). The lower abundance of *Proteobacteria* with higher  $Cu^{2+}$  concentrations agreed with the facts that  $Cu^{2+}$  suppressed electricity generation. On the contrary, *Firmicutes* and *Bacteroidetes*, which were known to ferment sugar (Ren et al., 2010). Their proportion increased with the increased  $Cu^{2+}$  concentrations, which was consistent with our above finding that the degradation of glucose wasn't inhibited by these dosages of  $Cu^{2+}$ . Notably, the presence of *Euryarchaeota* was corresponding to the detected methane in the gas samples, as most members of them were known to methanogenesis (Xing-pan et al., 2019).

Among the Proteobacteria, Alphaproteobacteria and Deltaproteobacteria were the two most abundant subgroups. The prevalent genus inside Deltaproteobacteria bacteria was Geobacter, which had been found to oxidized acetate and transmitted the electrons onto electrode (Bond, 2002). As shown in Fig. 4B, biofilm samples from blank reactor had 43% of the total genus sequences pertain to *Geobacter*, while samples from 0.1 to 1 mg  $L^{-1}$  Cu<sup>2+</sup> had 40% and 36% of the total sequences, respectively. The relative abundance of Geobacter decreased with the increase of Cu<sup>2+</sup> concentration, which was consistent with the results of maximum current density and current. Notably, the abundance of Geobacter in the biofilm sample from 7 mg  $L^{-1}$  Cu<sup>2+</sup> decreased obviously to 9%, while the electric current declined was not great. This pattern probably due to the presence of unclassified o Rhizobiales, which belongs to Alphaproteobacteria and has been reported as an electricigens (Zhang et al., 2019b). Their relative abundance increased with higher  $Cu^{2+}$  concentration. This may be attributed to the fact that unclassified\_o\_Rhizobiales are more tolerant to copper toxicity than Geobacter, as unclassified\_o\_\_Rhizobiales prefer to be metabolically versatile which allowing them to adapt quickly to new environments (George et al., 2017). The third most abundant class in Proteobacteria was Gammaproteobacteria, among which Klebsiella and Aeromonas were the most abundant genera. Klebsiella has been reported to have the ability to ferment glucose to produced acetic acid (Lay et al., 2012). Its relative abundance increased with the increase of  $Cu^{2+}$  concentration, indicating that the addition of Cu<sup>2+</sup> promoted the accumulation of acetate. The presence of *Aer*omonas implied that the working chamber did not remain strictly anaerobic, and oxygen may be transferred from another chamber through the CEM (Pham et al., 2003). This may be the main reason for the losses of electrons (about 50%) in the system.



Fig. 4. Microbial community sequencing results. (A) Microbial community distribution at the phylum level. Phyla with less than 1% of total sequences are grouped as "others". (B) Microbial community distribution at the genus level. Genera with less than 1% of total sequences are grouped as "others".

Among the *Bacteroidetes*, norank\_f\_norank\_o\_*Bacteroidales* and *Microbacter* were the two most abundance genera. The relative abundance of norank\_f\_norank\_o\_Bacteroidales significantly rose from 4.5% to 12.2% with increasing Cu<sup>2+</sup> concentration, which was known as acetic acid-producing fermenters (Zhang et al., 2014), supporting the above results that Cu<sup>2+</sup> altered glucose fermentation towards to production of more acetic acid (Table 1). The genus *Microbacter*, has been reported to utilize kinds of sugars as carbon sources and produced acetate (Sanchez-Andrea et al., 2014). We observed no obvious decrease in the abundance of Microbacter among the BESs, suggesting that these species were not susceptible to Cu<sup>2+</sup>. The two genera were widely distributed on anaerobic system and played a critical role in the glucose fermentation. These phenomena further confirmed that the degradation efficiency of glucose mentioned above was not affected by the addition of  $Cu^{2+}$ . The main genus affiliated to Euryarchaeota was Methaospirillum, which was known as obligate hydrogentrophic methanogens (Zhang et al., 2019a). When adding 0.1 and 1 mg  $L^{-1}$  Cu<sup>2+</sup>, the abundance of Methaospirillum and the contents of methane increased, demonstrating that methanogenesis was not inhibited by the  $Cu^{2+}$  below 1 mg  $L^{-1}$ . The increased abundance of *Meth*aospirillum may be due to the accumulation of acetic acid. However, further increasing of  $Cu^{2+}$  concentration to 7 mg  $L^{-1}$  resulted in no increase of Methaospirillum abundance, indicating that methanogenesis would be affected by these concentrations of  $Cu^{2+}$ .

Beta diversity (Fig. 5) demonstrated a distinct microbial dynamic variety between reactors with different  $Cu^{2+}$  addition. The data of weighted PCoA reflected that principal components PC1 explained 93.69% of the total bacterial community variations. The high value of the PC1 indicated that samples of different groups can be separated by the first principal component partition. As this is a single factor experiment, increasing Cu<sup>2+</sup> concentration correlated with the PC1 vector. The trend along PC1 was related with the increased relative abundances of the orders Rhizobiales, Bacteroidales, Enterobacteriales, Methanomicrobiales, and Clostridales at elevated Cu<sup>2+</sup> concentration. To the contrary, the relative abundances of Desulfuromonadales decreased in response to the increase of Cu<sup>2+</sup> concentration. Several members of *Rhizobiales*, and *Desul*furomonadales were well-known electricigen. The decrease of these orders was consistent with the results of maximum current density and current. Simultaneously, the increase of Bacteroidales and



*Clostridales*, well-known acetic acid-producing fermenters, supports the point that the introduction of  $Cu^{2+}$  altered glucose fermentation towards the production of more acetate. In particular, the sample blank and 0.1 had the nearest distance, and the greater the concentration difference between the samples, the farther the distance was in the PC1 vector.

#### 4. Conclusion

The glucose fermentation pathways were strongly influenced by Cu<sup>2+</sup> addition. Firstly, the relative abundance of Klebsiella and norank\_f\_norank\_o\_Bacteroidales were increased by Cu<sup>2+</sup> at 0.1 and 1 mg  $L^{-1}$ , therefore the fermentation type transformed from mixed acid type to acetogenesis type. Consequently, the methane concentrations were increased 19.67% and 39.51% compared to the blank in the presence of 0.1 and 1 mg  $L^{-1}$  Cu<sup>2+</sup>. However, further increasing  $Cu^{2+}$  concentrations to 7 mg  $L^{-1}$  didn't contribute to the production of more methane. This pattern was consistent with the change of the relative abundance of Methaospirillum. Notably, the current intensity decreased linearly with the increase of the Cu<sup>2+</sup> concentration, which was associated with the decrease of electricigen (Geobacter). Further investigation revealed that the existence of  $Cu^{2+}$  increased the microbial diversity, and the effect seems to be dosage dependent. The changes of microbial diversity and some functional bacteria were responsible for the change in glucose fermentation pathways. This study provided a better understanding of the effect of copper on the organics fermentation pathways in BES, which is helpful for the development of an efficient BES for toxic metals wastewater treatment.

#### Credit author statement

Yaping Zhang: Project administration, Writing - Review & Editing, Supervision Yangao Xu: Investigation, Formal analysis, Writing - Original Draft Xi Chen: Investigation, Resources Caiyun Chen: Investigation Jian Sun: Conceptualization Xiaoyan Bai: Visualization Yong Yuan: Writing - Review & Editing, Supervision.

#### **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.chemosphere.2021.129627.

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