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# Quaternary Ammonium Compound in Anolyte without Functionalization Accelerates the Startup of Bioelectrochemical Systems using Real Wastewater

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ABSTRACT

Quaternary ammonium has been demonstrated to be an efficient functional group for anodic material modification in bioelectrochemical systems (BESs). However, not all anode materials can be easily functionalized with quaternary ammonium. Here QAC of 0.05 M, 0.01 M and 0.001 M is directly added in anolyte instead of complex material functionalization. The startup time of 0.01 M (91 ± 0.5 h) and 0.001 M (101 ± 1 h) using real wastewater were 29% and 21% shorter than 128 ± 1.5 h of the no QAC control. Coulombic efficiency increased by 95% from 37 ± 1% (control) to 72 ± 2% (0.01 M), while the startup was obviously inhibited up to 0.05 M due to its biotoxicity. Cyclic voltammetry reveals that 0.01 M had a 23% higher peak current density than the control with a broader redox window observed. High-throughput sequencing confirmed that QAC imposed a selective stress on anodic microbial community. This provides a simple method to accelerate BES startup for anodes that not be easily modified.



## 1. Introduction

Bioelectrochemical systems (BESs), a promising and environmentally friendly technology compared to conventional waste treatment methods, uses microorganisms as catalysts to generate electricity (microbial fuel cells, MFCs) or produce valuable chemicals (microbial electrolysis cells, MECs). The characterization of degrading a broad range of organic substrates makes BESs draw growing attention in world-wide application of wastewater treatment [1]. However, a common limitation of long startup time and low current generation efficiency remains a major issue in restricting practical industrialization of BESs, especially when using real wastewaters. For example, a 1000 L microbial electrolysis cell needed to be inoculated with domestic wastewater and required 60 days for startup [2]. In the past decade, bacteria capable of direct electron transfer have been thoroughly studied and some representative strains possessing strong electrochemical activity are isolated from anodic biofilms. Diverse range of bacteria are confirmed as exoelectrogens, including five classes of *Proteobacteria* as well as *Firmicutes*,*Bacteroidetes* and *Acidobacteria* [3–7]. Though the mechanisms in pure culture level are increasingly revealed, considerably less is known about mixed biofilms. From an engineering perspective, the process of exoelectrogens acclimation from mixed inoculum such as real wastewaters is virtually of great value since it closely related to the electrogenic performance and startup time.

To date, approaches of anode surface modification have been widely used in BES, which have solid improvement on BES performance, such as conductive PANI (polyaniline, a polymer) coating [8], heat treatment [9], surface functionalization [10] and so on. It was discovered that glassy carbon anodes with grafting $—N^+(CH_3)_3$, $—OH^-$ and $—SO_3^-$ functional groups by electrochemical reduction of aryl diazonium effectively shortened startup time by 38%, 32% and 30% with a time of 23 d, 25.4 d and 25.9 d respectively, compared to 37.2 d with $—CH_3$ modified [11]. Conductive polymers *i.e.* poly (pyrrole-alky ammonium) modified anodes by electropolymerization also reduced the startup time by approximately 50 h than the control with 200 h [12]. Carbon cloth (CC) anodes were modified by electrochemical oxidation with nitric/sulfuric acid (CC-NS), ammonium nitrate (CC-AN) and ammonium sulfate (CC-AS), all of which presented acceleration of startup period and demonstrated higher current compared to the unmodified CC [13].

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Among these modified methods, quaternary ammonium compound (QAC) is one of the most commonly used and effective functional group for a fast startup because of its excellent cationic surfactant and anion exchange capacity. It can be also used at cathode to accelerate $OH^-$ transfer towards the bulk electrolyte in BESs [14]. However, it is also widely known as a disinfectant for its sterilization capability in many industrial fields, with bactericidal concentration usually below 1000 ppm (*i.e.* 0.005 M) [15]. QAC's specific molecular structure causes membrane lysis and protoplast distortion of bacterial cell, and thereby damages cell integrity [16]. There has reported that QAC modification on the catalyst layer of air-cathode in a MFC can effectively inhibit the growth of cathodic biofilm [17]. However, not all the bacteria are target spots of QAC because of its selectively antimicrobial property. It has been proved that Gram-negative bacteria and biofilm bacteria are generally more resistant to QAC compared with Gram-positive and single-living bacteria in suspension [18]. Therefore, the use of QAC in a BES possibly has a selective effect on planktonic bacteria and the formation of exoelectrogenic biofilm.

Despite the engineering feasibility of methods of surface modification, there would be some anode materials (such as metals) that not capable to be modified. Different from other works, here we added different dosage of QAC directly into the anolyte and monitored the electrochemical performance during biofilm formation using real wastewater as the inoculum, dispensing with the complicated modification operations. To the best of our knowledge, this study validated the selective impact of QAC on the bacterial community that directly enriched from wastewater for the first time, rather than that merely enhanced biofilm attachment and electron transfer efficiency by the change of physicochemical properties of anode surface. The final population of bacterial communities were also compared.

## 2. Experimental

### 2.1. BESs construction and operation

The single chamber BESs were constructed from a cylindrical glass container (AiDa, Tianjin, China) with an effective volume of 100 mL, 5 cm in diameter and 5 cm in height. The chamber was tightly sealed by a circular lid made of polytetrafluoroethylene (PTFE) with only three holes left where electrodes inserted. As shown in Fig. S1, the graphite rod (1 cm diameter, 10 cm length) with exposed area of 13.35 $cm^2$ was used as the anode (working electrode), placed facing to a platinum plate (1 cm × 1 cm) as counter electrode. An Ag/AgCl (3.5 M KCl) reference electrode was inserted in the center. We sheltered Ag/AgCl electrode with a porous vycor frit as the salt bridge to prevent deviation of referential potential as previously described [19]. Prior to use, new graphite-rod electrodes were pretreated by sand paper to polish and sonicated in 1 M HCl and 1 M NaOH for 10 min respectively to clean the oil or metal ion contamination, then rinsed by distilled water several times. The electrodes were connected to a multichannel potentiostat (CHI 1000C, CH Instrument, Shanghai, China), which applied a constant potential (0 V *vs.* Ag/AgCl) at each anode to cultivate biofilm and record currents. All BES cells were run at an average temperature of 32 ± 2 °C with two similar cells worked in duplicate.

### 2.2. Inoculum and startup

Real municipal wastewater collected from a domestic wastewater treatment plant (Xianyang Road Wastewater Treatment Plant, Tianjin, China) at primary settling tank was the mixed bacterial inoculum. Before inoculation, wastewater with chemical oxygen demand (COD) of 600 mg $L^{-1}$ was firstly sealed-stored in the fridge at 4 °C overnight to deplete the residual oxygen in the wastewater, creating a fully anaerobic conditions for exoelectrogens. Growth medium mixture (anolyte) of 100 mL contained 50 mL of wastewater as inoculum, 50 mM of phosphate buffer solution (PBS, $Na_2HPO_4$, 4.09 g $L^{-1}$; $NaH_2PO_4{\cdot}H_2O$, 2.93 g $L^{-1}$; $NH_4Cl$, 0.31 g $L^{-1}$; KCl, 0.13 g $L^{-1}$), 0.5 g $L^{-1}$ of sodium acetate as the additional electron donor and a certain concentration of QAC (0.05 M, 0.01 M, 0.001 M). The QAC used here was $C_6H_{15}Cl_2NO$ solution (69%, molecular weight of 188 g $mol^{-1}$, Dongying J&M Chemical Co., Ltd., Shandong, China). Cells without QAC were operated as controls. The values of solution conductivity of 0.05 M, 0.01 M, 0.001 M and control were listed in Table S2. The BES filled with growth medium mixture was bubbled continuously by nitrogen/carbon dioxide gas (4:1) for 10 min to flush out dissolved oxygen. Every 2 days, when nutrients were exhausted, anolyte of all cells were refreshed to form a complete cycle. This was repeated for 3 cycles until current density reached 0.5 A $m^{-2}$, thereafter anolyte medium was changed to ordinary formulation that consisted of 50 mM PBS, 1 g $L^{-1}$ sodium acetate, vitamins (5.0 mL $L^{-1}$) and trace minerals (12.5 mL $L^{-1}$) [14] without wastewater and QAC.

### 2.3. Electrochemical and chemical analysis

Cyclic voltammetry (CV) measurements were conducted by the potentiostat with a three electrode system as mentioned before. The biofilm developed on graphite rod surface was used as the working electrode, platinum plate as the counter electrode and Ag/AgCl electrode protected with salt bridge as reference electrode. All potentials here were referenced to Ag/AgCl (3.5 M KCl, 0.2 V *vs.* SHE, according to the manufacture). CVs were performed within a potential window ranging from −0.6 V to 0 V at a scan rate of 1 mV $s^{-1}$ since Liu et al. found that c-type cytochromes (c-Cyts) did not present redox peak responses any more above 0.2 V *vs.* SHE in a *Geobacter* dominated BES [20]. Fig. S4 proved this conclusion that the potential window of all redox peaks was below 0 V when CVs were scanned from −0.8 to 0.3 V. CVs in this study were therefore scanned from −0.6 to 0 V. The current was normalized to the exposure area (13.35 $cm^2$) of the anode surface. CV curves with the turnover current (2 h after medium refreshment) and starving current were both carried out. For the CV at cycle end, the current decreased to baseline levels, accompanied with low metabolic rates of bacteria and irreversible redox reaction of electroactive components. Here we called it "cycle end CVs" in this starvation condition but not entire nonturnover condition.

The total Coulombs were available by integrating the current ($I$, A) over time ($t_b$, s), Coulombic efficiency (CE) thus can be calculated for a fed-batch system by the following equation:

$$C_E = \frac{8\int_0^{t_b} I\mathrm{d}t}{FV\Delta\mathrm{COD}} \quad (1)$$

where $C_E$ is the Coulombic efficiency, 8 is a constant for COD calculation, which is based on the fraction of the molecular weight of $O_2$ (32 g $mol^{-1}$) and the number of reduced electrons (4) per mole of $O_2$. $F$ represents Faraday's constant (9.65 × $10^3$ C $mol^{-1}$) and $V$ is the volume of anode chamber (mL) [21].

### 2.4. Community analysis of the anodic biofilm

Biofilms adhered on the graphite anodes were sampled at the end of cycle 5 by scraping the surface repeatedly using a sterile blade for high-throughput MiSeq Illumina sequencing of the 16S rDNA. In order to keep a sufficient DNA concentration, the samples collected from parallel BESs were combined. Bacterial communities of all samples including the wastewater inoculum were then