

# Gravity settling of planktonic bacteria to anodes enhances current production of microbial fuel cells



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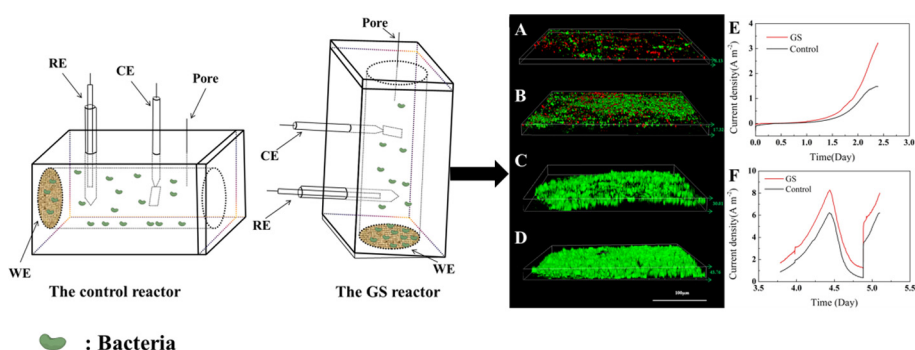
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## HIGHLIGHTS

- Gravity settling (GS) of bacteria to anodes reduces the startup time by 12%.
- GS increases the current density by 29% to  $8.41 \pm 0.13 \text{ A m}^{-2}$ .
- GS decreases the charge transfer resistance and increases anodic biomass.
- GS also increases the electroactivity, especially at early stage biofilm formation.

## GRAPHICAL ABSTRACT



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## ABSTRACT

How to acclimate a highly electroactive biofilm in short time is the bottleneck to improve the power output of microbial fuel cells (MFCs). Here we demonstrated that a simple method, the gravity settling (GS) of planktonic bacteria, is cost effective to improve MFC performance instead of physical and chemical treatment of anodes. The startup time is 12% shorter, and the maximum current density increases by 29% to  $8.41 \pm 0.13 \text{ A m}^{-2}$  than that of the control. Cyclic voltammeteries at different growth stages show that GS has a remarkable improvement (66%) on limiting current at the lag stage than at exponential (32%) and mature stages (24%), which was due to the 73% decrease in charge transfer resistance. Biofilm analysis further reveals that the GS promotes biofilm electroactivity per protein in addition to the accumulation of more biomass by gravitational settling, especially at the very beginning of electroactive biofilm formation. Our findings provide new knowledge on MFC startup, which is also important to enhance power densities of large scale MFCs in the future.

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## 1. Introduction

Microbial fuel cell (MFC) is a promising and environmental friendly technology, which has drawn a wide concern over the past two decades. MFCs, more extended to bioelectrochemical systems,

can directly produce electricity [1], hydrogen gas [2] or hythane [3] through the degradation of biomass. It is considered as a new technology of sustainable energy system. Currently, the highest power produced in an air-cathode MFC reached  $4.7 \text{ W m}^{-2}$  [4], and the further increase of power density requires a substantial improvement on both anode and cathode catalytic activity. As reviewed previously, the cathode performance is mainly limited by the high overpotential of oxygen reduction reaction, where efficient

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catalysts should be exploited [5]. While for the bacterial anode, the acclimation and colonization of highly electroactive biofilm on the surface of electrode is the most critical for its performance [6]. It had been found that the mixed bacterial community usually exhibited a higher electroactivity than that of a single strain [7]. Since the bacterial electron transfer mechanism is still under debate [8–10]. How to efficiently enrich a highly electroactive mixed bacterial community from inoculum (such as wastewater, soil extract or sediment) is of great interest to this field [11,12]. It was found that the types of inoculum influenced the microbial component and its activity [13], and the power density can be increased by 6 times from 0.6 to 4.31 W m<sup>-2</sup> when the anode was repeatedly inoculated by the acclimated microbial consortia [14]. In spite of the bacterial source, the initial attachment of bacteria to anodes is very important to both the power output and startup time. The removal rates of chemical oxygen demand (COD) in wastewater increase with current densities [15], so the power/current densities of MFCs also closely relate with pollutants removal. However, limited information is reported to date.

The electroactive biofilm in MFC is formed from the initial attachment of planktonic bacteria. During this process, the initial attachment of planktonic cells requires a relative stable fluid state. However, most of reported large-scale systems were operated with recirculation or stirring, which may interrupt the formation of electroactive biofilm at the very beginning, no matter whether a planar or 3-D anode is used [16,17]. It is not a problem until the system is scaled up. For example, a 1000 L MFC inoculated with wastewater required 60 days to startup [18], a much longer time compared to the 140 h for a 28 mL reactor [19]. To accelerate the startup and increase current density, electron acceptors such as Fe<sup>3+</sup> had been used to stimulate the growth of exoelectrogens [20]. Anodes can be also modified by conductive coatings to increase specific surface area [21–24], providing more areas available for bacterial attachment. However, these treatments increase the cost of electrodes, making MFCs less competitive than traditional technologies. Therefore, from an engineering perspective, finding a cost-effective approach to reduce startup time and increase current density without any treatments is urgently needed.

In order to attach more planktonic cells to anode without recirculation or stirring, here a simple strategy was tested to enhance MFC current density by gravity settling of planktonic bacteria. The comparison was performed through setting anodes horizontally (to receive precipitant bacteria with a maximum area, marked as gravity settling, GS) versus the vertical setting (marked as the control, see Fig. 1A). Using three electrode bioelectrochemical systems (BESs), the startup time, current density and electrochemical performance were compared. The biomass growth as well as spatial biofilm topography in systems with two different anode settings was also investigated.

## 2. Experimental section

### 2.1. Construction and operation of three electrode reactors

In order to avoid influences from the cathode, three electrode reactors were designed. Similar as single chambered MFCs reported previously, reactors were constructed by a cylindrical chamber (4 cm in long and 3 cm in diameter) with an effective volume of 28 mL and 7 cm<sup>2</sup> of cross section area [25]. The reactor had two holes to fix the reference electrode (RE) and the counter electrode (CE) (Fig. 1A). The working electrode (WE) was a carbon cloth connected to a titanium wire, facing to a platinum plate (1 cm<sup>2</sup>) as the CE. An Ag/AgCl (3.5 M KCl) with a porous vycor frit (diameter of 1 cm) was placed close to WE as the RE [26]. Prior to use as anode,

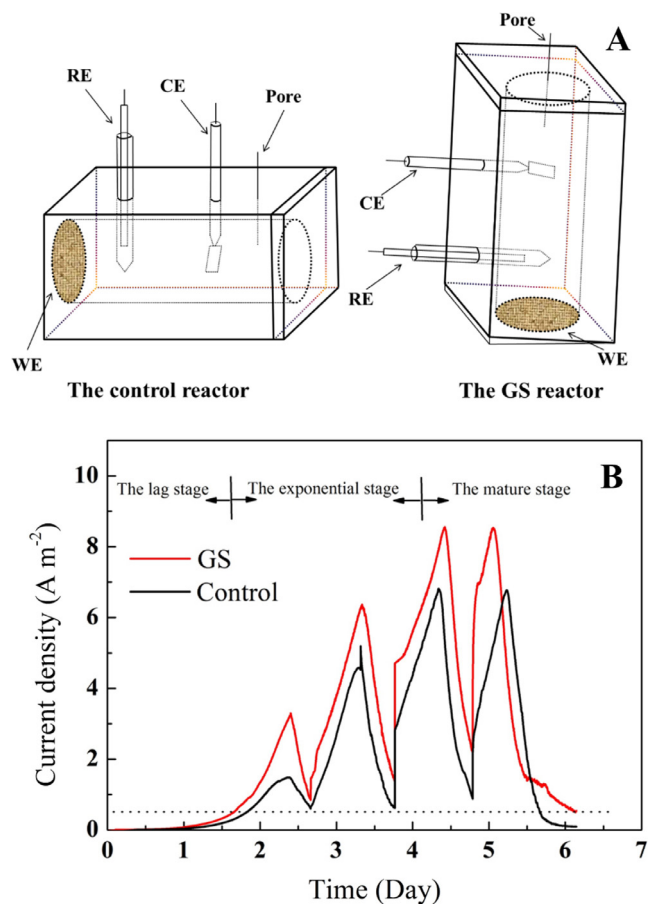


Fig. 1. Sketch of the control and GS reactors (A) and current outputs after inoculation in different reactors (B).

carbon cloth was cleaned by acetone to remove organic contamination and then rinsed by ethyl alcohol and distilled water [27]. With the same configuration, anodes were placed vertically and horizontally to have two different types of reactors (Fig. 1A). In control reactors, anodes were set on one side of reactors as reported previously [28]. While for GS reactors, anodes were fixed at the bottom. Three electrodes were connected to a potentiostat (CHI 1000C, Chenhua Instruments, Shanghai, China). The WE potential was poised at 0 V (vs. Ag/AgCl) to accumulate electroactive biofilm [29].

All reactors were inoculated using the mixed effluents from MFCs fed with acetate in our laboratory. The medium contained 50 mM phosphate buffer solution (PBS, Na<sub>2</sub>HPO<sub>4</sub>, 4.576 g L<sup>-1</sup>; NaH<sub>2</sub>PO<sub>4</sub>, 2.132 g L<sup>-1</sup>; NH<sub>4</sub>Cl, 0.31 g L<sup>-1</sup>; and KCl 0.13 g L<sup>-1</sup>), 12.5 mL L<sup>-1</sup> trace mineral, 5 mL L<sup>-1</sup> vitamin solution and 1 g L<sup>-1</sup> sodium acetate [30]. The medium was flushed for 20 min using nitrogen (80%)/carbon dioxide (20%) gas to remove dissolved oxygen before filling into reactors. All reactors were operated at a constant temperature of 25 ± 1 °C in an incubator. Each type of anode (control and GS) was operated in four repeats. The potential effect of 4 cm water pressure on exoelectrogens in GS is neglected since exoelectrogens were isolated from marine sediment (>10 cm under water) [31].

### 2.2. Electrochemical analyses

The polarization curve was obtained according to linear sweep voltammetry (LSV) using a potentiostat (Autolab PGSTAT 302N, Metrohm, Switzerland) [32]. The WE, RE and CE were the same

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