



## Enhanced azo dye decolorization and microbial community analysis in a stacked bioelectrochemical system



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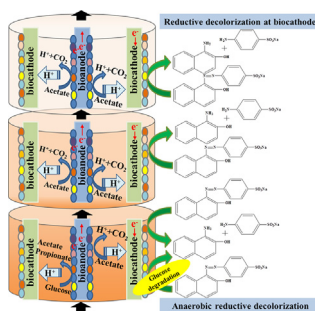
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### HIGHLIGHTS

- Stacked BES with sleeve-type modules was developed to assess scale-up performance.
- It achieved high azo dye decolorization extent at high loading rates.
- The performance improved as the number of stackable modules increased.
- Sequential removal of pollutants was achieved in each module.
- Enriched microbial community contributed to electron transfer and decolorization.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

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### ABSTRACT

In this study, a novel, single-chamber bioelectrochemical system (BES) with stacked modules was developed to improve azo dye decolorization. The decolorization extent of BES with three modules ( $80.3 \pm 3.1\%$ ) was about 15% and 33% higher than that with two modules ( $65.6 \pm 4.5\%$ ) and one module ( $47.1 \pm 3.9\%$ ) at an influent Acid Orange 7 (AO7) loading rate of  $250 \text{ g m}^{-3} \text{ d}^{-1}$ , demonstrating the feasibility of enhanced decolorization in scale-up BES through increasing the number of stacked modules. Moreover, the contribution of each module to dye decolorization, chemical oxygen demand (COD) removal and volatile fatty acids (VFAs) changes, showed the sequential utilization of pollutants in the stacked BES. Furthermore, analysis of the microbial communities showed that *Pseudomonas*, *Geobacter*, *Comamonas*, *Meniscus*, *Bellilinea*, *Achromobacter*, and *Paludibacter* were significantly enriched and resulted in the specific microbial community structures of the biocathode, meanwhile *Geobacter* and *Acinetobacter* were enriched in the microbial community of the bioanode, which mainly contributed to the electron transfer and/or azo dye decolorization. The results of this study demonstrate the potential of stacked BES for the accelerated deployment of large-scale BES applications for the treatment of refractory waste streams such as azo dye-bearing wastewater.

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## 1. Introduction

Azo dyes account for > 70% of the global industrial dyestuff demand (~9 million tons) and have been widely used in a number of industries, such as textiles dyeing, leather, plastics, cosmetics, food, and paper printing [1,2], causing a serious environmental problem and public health concern. Advanced oxidation processes (AOPs) have been proposed as the most effective processes to successfully remove azo dyes from wastewaters [3,4]. In comparison to advanced oxidation methods, biological treatment is considered as an alternative approach with low-input, cost-effective and environmentally-safe [5]. In recent years, bioelectrochemical systems (BES) as a promising technology has attracted significant attention for their use in wastewater treatment, to remove organic pollutants or transform them into degradable products by taking advantage of both biological and electrochemical processes [6–11]. In order to enhance the processing capacity of wastewater treatment in BES reactors, researchers have attempted many methods, such as reducing electrode overpotentials by employing electrode materials with catalysts [12,13], decreasing electrode spacing with novel reactor designs [14,15], decreasing mass transfer resistance with modified membranes [16,17], increasing solution conductivity with optimal operational conditions [18,19], and promoting specific mechanisms through microbial community development [13,20].

However, further efforts are needed for practical applications of the BES technology, especially the scale-up of BES reactors. One approach for the scale-up is to increase the physical dimension of individual BES reactors. However, scaling-up via increasing the reactor size carries the inherent challenge of increased internal resistances, which is volume-dependent [21,22]. This is an inherent difficulty of BES reactor scale up. Another approach is to connect a number of small-size units. Recent studies indicated that scaled-up microbial fuel cells (MFCs) exhibited improved power density by stacking a series of miniaturized MFCs in comparison with increasing the size of an individual MFC [23,24], demonstrating a more successful BES scale up with stackable modules than by increasing the size of an individual BES. Thus, further consideration should be given to the development of stacked BES with a certain number of bioelectrochemical modules as a way of increasing working volume able to accommodate realistic flow rates.

In this study, stacked BES reactors were developed with a number of stacked bioelectrochemical modules to investigate the extent of azo dye wastewater treatment in comparison with a BES reactor scaled up via increasing reactor size. This study aimed to evaluate the effect of module numbers on the stacked BES performance, to investigate the contribution of each bioelectrochemical module to the overall pollutant removal, and to analyze the effect of each module on the microbial communities in stacked BES.

## 2. Material and methods

### 2.1. Configuration of the stacked BES

In order to investigate the effect of stacked bioelectrochemical modules for BES scale-up on the azo dye wastewater treatment, three different single-chamber BES reactors with the same working volume (2 L, ID 8 cm × H 60 cm) were set up using one, two or three bioelectrochemical modules (BES-1#, Fig. 1A; BES-2#, Fig. 1B; and BES-3#, Fig. 1C, respectively). The bioelectrochemical modules were connected in parallel. As previously described, the sleeve-type bioelectrochemical module was made with carbon brush with inner anode and outer cathode displayed as surrounding electrode deployment [14]. Each module was separated by a flange and a silicone ring to ensure tightness. The influent entered the bioelectrochemical modules at the bottom and exited at the top as shown in Fig. 1. Sampling ports near the outlet were placed at each module of the stacked BES reactors (Fig. 1). The anode, cathode and the reference electrode were connected to a data acquisition system (Keithley 2700, Keithley, Co., USA) with an

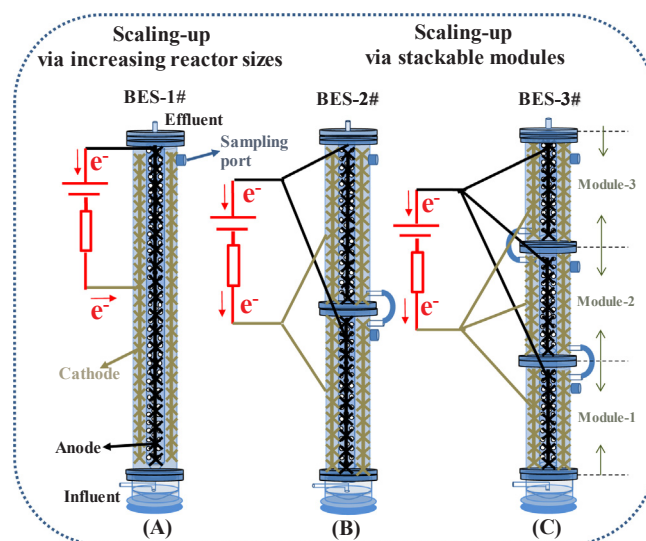


Fig. 1. Schematic diagram of stacked bioelectrochemical system with different module numbers.

external resistance of  $20\ \Omega$ , to record electrode potential and current every 30 min.

### 2.2. Operation of the stacked BES

Activated sludge obtained from the Taiping wastewater treatment plant (Harbin, China) was used as the inoculum of the BES reactors. Acid orange 7 (AO7), characterized by one azo bond ( $-N=N-$ ), was used as an azo dye model. It is the most common dye that has been widely used in industries and has been extensively researched in various studies with different treatment methods. The stacked BES reactors were fed with an influent containing AO7 ( $50\text{--}250\ \text{mg L}^{-1}$ ), glucose ( $1\ \text{g L}^{-1}$ ), phosphate buffer solution ( $50\ \text{mM}$ ), KCl ( $0.13\ \text{g L}^{-1}$ ),  $\text{NH}_4\text{Cl}$  ( $0.31\ \text{g L}^{-1}$ ), trace element solution ( $1\ \text{mL L}^{-1}$ ), and a vitamin solution ( $1\ \text{mL L}^{-1}$ ) [25]. Experiments were performed with applied voltage of  $0.5\ \text{V}$  and temperature of  $25 \pm 4\ ^\circ\text{C}$ . Before stacking, the six bioelectrochemical modules were inoculated and operated independently, fed with AO7 ( $50\ \text{mg L}^{-1}$ ) to achieve biofilm formation at the anode and cathode. After a steady-state was observed for a period of at least 10 d, the modules were stacked as shown in Fig. 1 by constructing BES-1#, BES-2#, and BES-3# with one, two, and three modules, respectively. The flow rate was controlled with a peristaltic pump (BT100-1L/YZ1515, Longer Pump Co., China).

Firstly, the performance with different loading rates in the different stacked BES reactors was investigated in terms of decolorization extent and electrochemical characteristics. With a hydraulic retention time (HRT) of 24 h, the influent AO7 concentration was gradually increased from 50 to 100, 150, 200 and  $250\ \text{mg L}^{-1}$ , resulting in a loading rate of 50, 100, 150, 200 and  $250\ \text{g m}^{-3}\ \text{d}^{-1}$ , respectively. Secondly, the performance of each module (Module-1, Module-2, and Module-3) in the stacked BES-3# was investigated to assess the contribution of each individual module in terms of decolorization extent, chemical oxygen demand (COD) removal and volatile fatty acids (VFAs) formation. With the same influent AO7 concentration of  $200\ \text{mg L}^{-1}$ , the HRT was gradually decreased from 24 to 12, 8, 6, and 5 h, and the corresponding influent AO7 loading rates increased from 200 to 400, 600, 800, and  $1000\ \text{g m}^{-3}\ \text{d}^{-1}$ . Each operational condition was maintained for at least two weeks. Finally, the biofilm microbial communities of bioanode and biocathode were analyzed to determine the microorganism distribution of each module in the stacked BES after stable operation with  $1000\ \text{g m}^{-3}\ \text{d}^{-1}$  AO7.

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