Short Communication

Efficient azo dye decolorization in a continuous stirred tank reactor (CSTR) with built-in bioelectrochemical system

Min-Hua Cui^a, Dan Cui^b, Lei Gao^a, Hao-Yi Cheng^b, Ai-Jie Wang^{a,b,*}
^a State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, PR China

^b Key Laboratory of Environmental Biotechnology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, PR China

HIGHLIGHTS

- A newly designed CSTR-BES was developed for efficient azo dye removal.
- BES module took an important role for increasing decolorization rate.
- The effects of anaerobic sludge and acetate concentration were investigated.

ARTICLE INFO

Article history:

Received 12 June 2016

Received in revised form 28 July 2016

Accepted 29 July 2016

Available online 30 July 2016

Keywords:

Continuous stirred tank reactor (CSTR)

Bioelectrochemical system (BES)

Azo dye

Sludge concentration

Sodium acetate concentration

ABSTRACT

A continuous stirred tank reactor with built-in bioelectrochemical system (CSTR-BES) was developed for azo dye Alizarin Yellow R (AYR) containing wastewater treatment. The decolorization efficiency (DE) of the CSTR-BES was $97.04 \pm 0.06\%$ for 7 h with sludge concentration of 3000 mg/L and initial AYR concentration of 100 mg/L, which was superior to that of the sole CSTR mode (open circuit: $54.87 \pm 4.34\%$) and the sole BES mode (without sludge addition: $91.37 \pm 0.44\%$). The effects of sludge concentration and sodium acetate (NaAc) concentration on azo dye decolorization were investigated. The highest DE of CSTR-BES for 4 h was $87.66 \pm 2.93\%$ with sludge concentration of 12,000 mg/L, NaAc concentration of 2000 mg/L and initial AYR concentration of 100 mg/L. The results in this study indicated that CSTR-BES could be a practical strategy for upgrading conventional anaerobic facilities against refractory wastewater treatment.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Textile industry is one of the most important industries in national economy in China. However, textile factories discharge a large amount of dyestuffs containing wastewater (Cui et al., 2016b) with high chromaticity, which triggers severe environmental pollutions, such as biological toxicity, “three-induced” (carcinogenicity, teratogenicity, mutagenicity), unpleasant appearance, etc. Azo dye is the most widely used synthetic dyestuffs, about 10% of which are unemployed and discharged in aqueous effluent (Sandhya and Swaminathan, 2006). On account of aerobic stability of azo dye, anaerobic treatment processes are usually employed for decolorization to cater the subsequent mineralization (Shaul et al., 1991). In the practical scene, the continuous stirred tank reactors (CSTRs) are pervasively applied

in the textile wastewater remediation (Jeihanipour et al., 2013). However, CSTR is still suffering from disadvantages like low efficiency and time-consuming for azo dye removal (Chen et al., 2007).

Bioelectrochemical systems (BESs) have attracted more attentions in the recent years for enhancing transformation of various pollutants, such as, nitro-compounds (Shen et al., 2014), halogenated pollutants (Kong et al., 2015), sulfate (Coma et al., 2013), as well as azo dyes (Cui et al., 2016a). BESs could reduce the over potential by catalysis of electro-active microorganisms and improve reaction kinetics greatly. While, BESs still have many challenges towards to scaling-up and application. The effective way for BES application is coupling it to conventional anaerobic units rather than entirely replacing them. Few attempts about the coupling systems have been performed. Cui et al. reported that coupling BES into anaerobic baffled reactor (ABR) improved by 8.2% of azo dye decolorization (Cui et al., 2014). A coupled microbial electrolysis cell-upflow anaerobic sludge blanket (MEC-UASB) was built to removal organic matters efficiently (Zhang et al., 2013). Remarkably, these cases focused on the upflow (plug flow)

* Corresponding author at: State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (SKLUWRE, HIT), P.O. Box 2614, 202 Haihe Road, Harbin 150090, PR China.

E-mail address: waj0578@hit.edu.cn (A.-J. Wang).

reactors and neglected the completely mixed reactor configurations (CSTR etc.) that were widely used in application. Recently, a CSTR was integrated with microbial electrochemical system to treat high strength artificial wastewater and simultaneously recover electricity and methane energy (Wang et al., 2015). It is essential to evaluate the performance of CSTR-BES, which could provide an optional strategy for upgrading current CSTR-like facilities to efficiently treat refractory wastewaters.

In this study, a CSTR with built-in BES (CSTR-BES) was developed for azo dye containing wastewater treatment. The influence of initial azo dye on the performance of BES was investigated. To identify the specific function of BES in the CSTR-BES system, different modes were operated to compare the performance of CSTR-BES with and without sludge inoculation, and at open and closed circuit, respectively. Besides, the effect of anaerobic active sludge concentration and acetate concentration were also evaluated for the CSTR-BES.

2. Materials and methods

2.1. Reactor configuration

Six identical cylindrical glass bioelectrochemical systems (BESs) with each working volume of 750 mL were manufactured for this study (SI, Fig. S1). Graphite fiber brushes (L 5 cm $\times$ Φ 5 cm, TOHO TENAX, Co., Ltd., Japan) were used for both anodes and cathodes. All graphite fiber brushes were pretreated according to the literature (Feng et al., 2010). Titanium wire (1 mm in diameter, Baoji LiXing Titanium Group Co., Ltd., China) used as current collector was stretched out of the reactors and connected with circuit. During the experiment, each reactor was operated with external resistance of 10 Ω and applied voltage of 0.5 V using a DC power supply (FDPS-180, Fudan Tianxin Scientific and Educational Instruments Co., Ltd, Shanghai, China). A saturated calomel electrode (SCE) (+247 mV vs. standard hydrogen electrode) was used as reference electrode. Anodes, cathodes and reference electrodes were connected to a data acquisition system (Keithley 2700, Keithley Co. Ltd., U.S.). Electrode potentials and currents were recorded at every 10 min.

2.2. Inoculation and operation

The anodes were inoculated using the mixed inoculation consisting of effluent from a long-term operated BES and 10 mL activated sludge from Taiping municipal wastewater treatment plant (Harbin, China). Sodium acetate (NaAc, 500 mg/L) and AYR (25 mg/L) were added as electron donor and acceptor, respectively. Each reactor was equipped with a magnetic rotor and placed on a six-position magnetic stirrer (84-1, Shanghai Meiyongpu Instruments Co., Ltd., China) for homogeneous mixture. The medium was replaced every two days in the first two weeks for biofilm formation which can continuously generate electrons.

The composition of medium was NaAc (500–2000 mg/L), AYR (60–200 mg/L), phosphate buffer solution (50 mmol/L), KCl (0.13 g/L), NH_4Cl (0.31 g/L), distilled water, trace element solution (10 mL/L) and vitamin solution (10 mL/L) (Wolin et al., 1963). O_2 was wiped off from the medium using N_2 (purity of 99.99%) for 30 min. Before replacing medium of reactors, magnetic stirrer was stopped for 30 min to deposit sludge. Half of the supernatant (approximately 375 mL) was drawn out and replaced by fresh medium. A N_2 gas bag was connected to the headspace to balance pressure and maintain anaerobic atmosphere. Anaerobic active sludge was collected from a long term operated ABR-BES reactor and adjusted to desired concentration (Cui et al., 2014).

This study was operated in three stages: at stage I, the effect of initial AYR concentration (30, 50, 75 and 100 mg/L) on decolorization performance in sequencing batch mode, membrane-less BES was evaluated as control. At stage II, 3000 mg/L (mixed liquor suspended solids) anaerobic sludge was introduced into BES to build a CSTR-BES. The superiority of CSTR-BES for azo dye removal was identified. NaAc consumption during AYR decolorization was evaluated at both closed and open circuit. At stage III, with initial AYR concentration kept at 100 mg/L, the effect of NaAc concentration on AYR decolorization efficiency in CSTR-BES was probed at sludge concentration of 3000, 6000, 9000 and 12,000 mg/L. Each operational condition was run in three parallels and at least three repetitions. All reactors were operated at ambient temperature ($23 \pm 2^\circ\text{C}$).

2.3. Chemicals

Alizarin Yellow R (AYR) with an azo bond and a nitro group was used as the model azo dye (Commercial purity grade, Shanghai Sangon Biotech Co., Ltd., China). p-phenylenediamine (PPD) as the main by-product of AYR bioreduction (Cui et al., 2012) was purchased from Aladdin Industrial Corporation (analytical reagent).

2.4. Analytical methods

Samples taken from the sampling ports were centrifuged at 3000 rpm for 10 min firstly to deposit sludge and the sludge pellets were returned to the corresponding reactor. The liquid supernatant was immediately filtered through 0.45 μm filters (Tianjin Jinteng Experiment Equipment Co., Ltd., China). AYR concentration was quantified with a UV-vis spectrophotometer (UV-1800, Shanghai Meipuda instrument Co., Ltd., China) at wavelength of 374 nm (Cui et al., 2012). PPD was measured with a high performance liquid chromatography (HPLC, e2695, Waters Co., U.S.) equipped with an UV-vis detector (model 2489, Waters Co., U.S.) and a C18 column (5 μm ; 4.6 mm $\times$ 150 mm, Symmetry, Waters Co., Ltd., U.S.). Acetate (Ac^-) was analyzed using a gas chromatograph (GC, 6890 N, Agilent, Inc., U.S.) equipped with a flame ionization detector (FID) and Stabilwax-DA column (30 m $\times$ 0.32 mm $\times$ 0.5 mm) at oven and injector temperatures of 60 $^\circ\text{C}$ and 250 $^\circ\text{C}$, respectively. He carrier gas and N_2 makeup gas were used for the GC-FID.

2.5. Calculation

AYR decolorization efficiency (DE,%) and decolorization rate (DR, $\text{g/m}^3\cdot\text{d}$) were calculated based on the difference between the initial AYR concentration and measured effluent AYR concentration, as shown in Eqs. (1) and (2):

$$\text{DE} = \frac{C_0 - C_t}{C_0} \times 100\% \quad (1)$$

$$\text{DR} = \frac{(C_0 - C_t) \times 24}{t} \quad (2)$$

where C_0 and C_t are the initial AYR concentrations and measured effluent AYR concentration, separately, mg/L; t is the treating time, h.

3. Results and discussion

3.1. Decolorization in CSTR-BES

At stage I, BES without sludge introduction was operated in batch mode and continuously stirring to improve mixing and mass transfer. The effect of initial AYR concentrations (30, 50, 75,

100 mg/L) on the decolorization of BES was investigated. As shown in Fig. 1, AYR could be efficiently removed and its decolorization efficiency (DE) increased linearly during the first 4 h, but after then, the increase rates of DE slowed down and reached above 90% at 7 h without obvious difference at the variation of initial AYR concentration. Higher initial AYR concentration led to lower DE in a short period, e.g. DE at 4 h declined from 90.96 ± 0.63 to $46.71 \pm 4.53\%$ when initial AYR concentration increased from 30 to 100 mg/L. AYR anaerobic degradation pathway in BES has been previously reported (Cui et al., 2012). PPD and 5-aminosalicylic acid (5-ASA), two products of AYR decolorization, were produced via azo bond cleavage. As shown in SI Fig. S2, PPD concentration in BES after 7 h gradually increased from 11.68 ± 0.20 to 34.75 ± 3.48 mg/L with the rising of initial AYR concentration. Correspondingly, PPD recovery efficiency fluctuated between 93.88 ± 2.74 and $105.66 \pm 1.82\%$ indicating that the removal AYR was completed reduced via azo bond cleavage instead of absorption or other pathway.

The current magnitude reflected the rate of electrochemical reactions. As shown in SI Fig. S3, similar current variation trends were obtained regardless of initial AYR concentrations that increased from 30 to 100 mg/L. Current was reached to peak value quickly after replacing fresh medium and then slightly decreased to plateau with the consumption of electron donor (acetate). Peak current value reached 5.37 mA at initial AYR concentration of 30 mg/L, which sharply dropped to trough as low as 1.4 mA five hours later. The peak current increased to 6.99 mA with the rising of initial AYR concentration to 100 mg/L. Higher initial AYR concentration led to later emergence of peak current. Peak current appeared at 10 and 20 min for 30 and 50 mg/L of initial AYR concentration, respectively, which turned up at 1 h for 75 and 100 mg/L. This delay was probably due to the temporary inhibition of high concentration AYR to electrode-respiratory bacteria. With the degradation of AYR, less toxic products were produced and the inhibition was released.

At stage II, 3000 mg/L (mixed liquor suspended solids) anaerobic active sludge was introduced into BES to build CSTR-BES. Four

different operational modes were tested for comparison: CSTR-BES at closed circuit (CSTR-BES-Closed), CSTR-BES at open circuit (CSTR-BES-Open), sole BES without sludge introduction at closed and open circuit (named as BES-Closed and BES-Open in short, respectively). The influent AYR concentration was kept at 100 mg/L. As shown in Fig. 2, the sequence of AYR removal efficiency was CSTR-BES-Closed > BES-Closed > CSTR-BES-Open > BES-Open. The DE at 7 h of CSTR-BES-Closed was $97.04 \pm 0.06\%$ with 6% higher than that of BES-Closed ($91.37 \pm 0.44\%$). Dramatic improvement was emerged at 4 h, DE was $63.71 \pm 0.38\%$ in CSTR-BES-Closed, which was 17% higher than that in BES-Closed ($46.71 \pm 2.19\%$). The superior performance of CSTR-BES-Closed than BES-Closed was attributed to the addition of active sludge and thus some part of AYR was reduced via anaerobic biological reaction. The DEs of CSTR-BES-Open and BES-Open at 7 h were much lower than those of closed assays, which were only 54.87 ± 4.34 and $40.74 \pm 0.68\%$, respectively, indicating that the external power supply and the utilization of cathode as persistent electron donor for azo dye did accelerate the AYR reduction even in the CSTR-BES. In addition, the significant promotion of CSTR-BES at closed mode than open mode implied that BES took a leading role in removal AYR in a short time, which could overcome the disadvantage of slow rate of traditional anaerobic process.

Comparing the consumption of NaAc (co-substrate) among the four operational modes showed that sludge addition and closed circuit mode both required more NaAc (SI, Fig. S4). It was indubitable that more microorganisms in additive active sludge would consume more electron donor. In CSTR-BES-Closed and BES-Closed, as NaAc was the sole electron donor for anodic oxidation and further drove the reaction on the cathode, 11.5 and 4.6% more NaAc were consumed, but 42.2 and 50.6% higher AYR removal efficiencies were obtained, respectively, compared to those in open circuit at 7 h. The co-substrate utilization rates of CSTR-BES-Closed and BES-Closed were 0.72 g NaAc/g AYR and 0.59 g NaAc/g AYR, which were 1.13 and 1.28 g NaAc/g AYR in open circuit, indicating that

Fig. 1. AYR concentration (A) and decolorization efficiency (B) variation in BES.

Fig. 2. AYR concentration (A) and decolorization efficiency (B) at different operational modes.

Table 1

Effect of sludge and co-substrate concentration on AYR removal efficiency of CSTR-BES in 4 h.

DE _{4h} (%)		NaAc concentration (mg/L)			
		500	1000	1500	2000
Sludge concentration (mg/L)	0	46.71 ± 0.38	49.86 ± 2.65	53.15 ± 1.41	58.95 ± 2.36
	3000	63.71 ± 2.19	68.13 ± 1.45	70.37 ± 1.34	75.44 ± 1.34
	6000	65.88 ± 1.34	70.50 ± 3.43	73.52 ± 3.51	81.21 ± 1.89
	9000	64.08 ± 3.21	75.56 ± 1.94	79.26 ± 0.84	85.80 ± 4.41
	12,000	69.00 ± 2.88	76.64 ± 4.37	84.96 ± 1.64	87.66 ± 2.93

lower electron equivalent was needed in the system with built-in BES. Thus, the operational cost would be significantly reduced that further emphasized the importance of BES.

3.2. Effect of sludge and NaAc concentration on decolorization in CSTR-BES

At stage III, concentrations of anaerobic active sludge and NaAc were evaluated to improve the availability of CSTR-BES. DE at 4 h (DE_{4h}) was selected for evaluating the performance of CSTR-BES at tested conditions as shown in Table 1. In the sole BES without sludge addition, higher NaAc led to higher DE_{4h}, which increased from 46.71 ± 0.38 to 58.95 ± 2.36% with NaAc concentration increasing from 500 to 2000 mg/L. However, only 12% improvement of DE was obtained with 4 times increase of NaAc concentration (from 500 to 2000 mg/L). This indicated that electron donor (NaAc) for the anode was not the main limitation for azo dye removal in the sole BES. In theory, 35.71 mg/L NaAc was needed for the complete reduction of 100 mg/L AYR. Excess supply of NaAc was not an efficient way to improve the performance of BES. This also proved the characteristic of low electron donor requirement of BES (Mu et al., 2009).

In the CSTR-BES with 3000 mg/L active sludge, DE was improved by 17% compared to that in the sole BES at 500 mg/L of NaAc. Anaerobic biological reduction of azo dye with NaAc as electron donor further improved DE in the CSTR-BES. Although DE was positive correlated to the concentration of anaerobic activity sludge, the CSTR-BES was insensitive to further increase anaerobic sludge concentration at NaAc concentration of 500 mg/L. DE_{4h} merely promoted by 5.29% with the anaerobic sludge concentration increased from 3000 to 12,000 mg/L. The indistinctive decolorization improvement seems triggered by the insufficient co-substrate (NaAc) concentration. When the anaerobic sludge concentration increased to 12,000 mg/L, the CSTR-BES reactor was much more sensitive to the concentration of NaAc, DE_{4h} was improved by 18.66% and up to the highest of 87.66 ± 2.93% along with NaAc concentration increased from 500 to 2000 mg/L, which implied that more biomass in the reactor required more co-substrate for AYR reduction. Increasing either anaerobic sludge or NaAc concentrations could improve decolorization performance of CSTR-BES, higher NaAc concentration pandered to concentrated sludge.

4. Conclusions

A continuous stirred tank reactor with built-in bioelectrochemical system (CSTR-BES) was proved as an efficient and promising process for azo dye wastewater treatment. Decolorization efficiency (DE) of CSTR-BES was superior to sole CSTR or sole BES. Higher concentration of sludge or NaAc was benefit for enhancing AYR decolorization. The highest DE of CSTR-BES at 4 h was 87.66 ± 2.93%, at sludge concentration of 12,000 mg/L and NaAc concentration of 2000 mg/L. These results indicated that coupling

BES into CSTR could effectively improve reactor performance and become a practical strategy for upgrading conventional anaerobic processes.

Acknowledgements

This research was supported by the National Nature Science Foundation for Distinguished Young Scholars (Grant No. 51225802), the National Nature Science Foundation of China (Grant No. 51508551), the China Postdoctoral Science Foundation (Grant Nos. 2015M580140, 2016T90142), the Ministry of Environmental Protection of the People's Republic of China (Major Science and Technology Program for Water Pollution Control and Treatment) (No. 2014ZX07204-005), the "Hundred Talents Program" of the Chinese Academy of Sciences (Grant No. 29BR2013001) and the Jiangsu Key Laboratory of Chemical Pollution Control and Resources Reuse in NJUST (Grant No. 30920140122008).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2016.07.135>.

References

- Chen, B.Y., Wang, M.Y., Lu, W.B., Chang, J.S., 2007. Use of active consortia of constructed ternary bacterial cultures via mixture design for azo-dye decolorization enhancement. *J. Hazard. Mater.* 145 (3), 404–409.
- Coma, M., Puig, S., Pous, N., Balaguer, M.D., Colprim, J., 2013. Biocatalysed sulphate removal in a BES cathode. *Bioresour. Technol.* 130, 218–223.
- Cui, D., Guo, Y.Q., Cheng, H.Y., Liang, B., Kong, F.Y., Lee, H.S., Wang, A.J., 2012. Azo dye removal in a membrane-free up-flow biocatalyzed electrolysis reactor coupled with an aerobic bio-contact oxidation reactor. *J. Hazard. Mater.* 239–240, 257–264.
- Cui, D., Guo, Y.Q., Lee, H.S., Wu, W.M., Liang, B., Wang, A.J., Cheng, H.Y., 2014. Enhanced decolorization of azo dye in a small pilot-scale anaerobic baffled reactor coupled with biocatalyzed electrolysis system (ABR-BES): a design suitable for scaling-up. *Bioresour. Technol.* 163C, 254–261.
- Cui, M.-H., Cui, D., Lee, H.-S., Liang, B., Wang, A.-J., Cheng, H.-Y., 2016a. Effect of electrode position on azo dye removal in an up-flow hybrid anaerobic digestion reactor with built-in bioelectrochemical system. *Sci. Rep.* 6, 25223.
- Cui, M.-H., Cui, D., Liang, B., Sangeetha, T., Wang, A.-J., Cheng, H.-Y., 2016b. Decolorization enhancement by optimizing azo dye loading rate in an anaerobic reactor. *RSC Adv.* 6 (55), 49995–50001.
- Feng, Y., Yang, Q., Wang, X., Logan, B.E., 2010. Treatment of carbon fiber brush anodes for improving power generation in air-cathode microbial fuel cells. *J. Power Sources* 195 (7), 1841–1844.
- Jeihanipour, A., Aslanzadeh, S., Rajendran, K., Balasubramanian, G., Taherzadeh, M.J., 2013. High-rate biogas production from waste textiles using a two-stage process. *Renewable Energy* 52, 128–135.
- Kong, D., Liang, B., Yun, H., Cheng, H., Ma, J., Cui, M., Wang, A., Ren, N., 2015. Cathodic degradation of antibiotics: characterization and pathway analysis. *Water Res.* 72, 281–292.
- Mu, Y., Rabaey, K., Rozendal, R.A., Yuan, Z.G., Keller, J., 2009. Decolorization of azo dyes in bioelectrochemical systems. *Environ. Sci. Technol.* 43 (13), 5137–5143.
- Sandhya, S., Swaminathan, K., 2006. Kinetic analysis of treatment of textile wastewater in hybrid column upflow anaerobic fixed bed reactor. *Chem. Eng. J.* 122 (1–2), 87–92.
- Shaul, G.M., Holdsworth, T.J., Dempsey, C.R., Dostal, K.A., 1991. Fate of water soluble azo dyes in the activated sludge process. *Chemosphere* 22 (1–2), 107–119.

- Shen, J., Xu, X., Jiang, X., Hua, C., Zhang, L., Sun, X., Li, J., Mu, Y., Wang, L., 2014. Coupling of a bioelectrochemical system for p-nitrophenol removal in an upflow anaerobic sludge blanket reactor. *Water Res.* 67C, 11–18.
- Wang, H., Qu, Y., Li, D., Zhou, X., Feng, Y., 2015. Evaluation of an integrated continuous stirred microbial electrochemical reactor: wastewater treatment, energy recovery and microbial community. *Bioresour. Technol.* 195, 89–95.
- Wolin, E.A., Wolin, M.J., Wolfe, R.S., 1963. Formation of methane by bacterial extracts. *J. Biol. Chem.* 238, 2882–2886.
- Zhang, J., Zhang, Y., Quan, X., Chen, S., 2013. Effects of ferric iron on the anaerobic treatment and microbial biodiversity in a coupled microbial electrolysis cell (MEC)–anaerobic reactor. *Water Res.* 47 (15), 5719–5728.