Short Communication

Enhanced azo dye removal through anode biofilm acclimation to toxicity in single-chamber biocatalyzed electrolysis system

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**Highlights**

- Azo dye toxicity could shock the bioanode electrochemical activity.
- Anode biofilm was acclimated to azo dye toxicity by gradient increase of azo dye concentration.
- Enhanced azo dye removal in single-chamber biocatalyzed electrolysis system after bioanode acclimation.
- Removing IEM reduced the Rin 3 times than in the presence of IEM by EIS.

**Abstract**

Azo dye is widely used in printing and dyeing process as one of refractory wastewaters for its high chroma, stable chemical property and toxicity for aquatic organism. Biocatalyzed electrolysis system (BES) is a new developed technology to degrade organic waste in bioanode and recover recalcitrant contaminants in cathode with effective decoloration. The ion exchange membrane (IEM) separate anode and cathode for biofilm formation protection. Azo removal efficiency was up to 60.8%, but decreased to 20.5% when IEM was removed. However, expensive ion exchange membrane (IEM) not suitable for further practical application, bioelectrochemical activity of bioanode is sensitive to the toxicity of azo dye. A gradient increase of azo dye concentration was used to acclimate anode biofilm to pollutant toxicity. The azo removal efficiency can be enhanced to 73.3% in 10 h reaction period after acclimation. The highest removal efficiency reached 83.7% and removal rates were increased to 8.37 from 3.04 g/h/L of dual-chamber. That indicated the feasibility for azo dye removal by single-chamber BES. The IEM cancellation not only decreased the internal resistance, but increased the current density and azo dye removal.

**1. Introduction**

A large amount of dyeing wastewater is produced in dyeing enterprises annually (Parikh et al., 2005). High toxicity and chroma make it difficult to remove by traditional methods of wastewater treatment. Among all kinds of dye pollutants, the azo dyes are 80% of total amount of organic dyes. Its chromophore is azo bonds, that could decrease water transmittance and then result in destruction of ecosystem when azo dye enters the rivers, seas, etc. (You et al., 2010). Effective electron reduction methods target at azo bonds and transfer to the amidogen so that high chroma can be removed (Sun et al., 2009).

Biocatalyzed electrolysis system (BES) is newly developed device with bio-electrons transport and reductions at bio-electrode. The electrons are produced by exoelectrogens from organic (glucose, acetate, etc.) degradation and transported to anode, through external circuit to cathode (Heilmann et al., 2006). At the cathode, oxygen and proton is an electron acceptor generating electrical energy (Logan et al., 2006) and hydrogen energy in initial research (Liu et al., 2008; Wang et al., 2011). In recent years, the scope of electron acceptor has expanded, such as carbon dioxide (Nevin et al., 2011), nitrate (Guoqiang et al., 2012), perchlorate (Butler et al., 2010), nitrobenzene (Mu, 2009) or other pollutants (Tao et al., 2012; Shen et al., 2012; Zhan et al., 2012). Whether in energy generation or pollutants removal, the BES is becoming more recognized on environmental problems and issues.

The stability of bioanode electrochemical activity is the basis of BES. In previous studies, the toxicity of pollutants do not influence the anode biofilm in dual-chamber BES, because the ion exchange membrane (IEM) prevents anode biofilm from toxicity of azo dyes in cathode chamber. However, IEM is a costly component of BES, it could cause pH gradient and the increase of internal resistance, that is not suitable for use in engineering application. Although membrane removal is cost-effective for single chamber BES, the toxicity of pollutants could influence the anode biofilm, which...
results in the increase of anode potential and the decrease of electric current density.

The aim of this paper is to investigate the azo dye Amido black 10B how to influence the anode biofilm electrochemical activity, and then influence electron transport and azo dye removal efficiency in the the whole system after the IEM removal. A method was found out to make anode biofilm to acclimatize the azo dye toxicity by gradient increasing of azo dye amido black 10B the aim in this study is to investigate the membrane-free feasibility for azo dye removal, comparing performance of both with-membrane and membrane-free in BES.

2. Methods

2.1. BES structure

The laboratory scale bioreactor was constructed by plexiglas, its outside shape was rectangle (13 cm×8 cm×8 cm), both anode and cathode are carbon brush (ID 4.5 cm × L 4.0 cm), after installation of cathode and anode both chamber volumes are 70 ml in this laboratory scale reactor (Fig. 1). A saturated calomel electrode (SCE) reference electrode was placed in the anode chamber. The DC power supply between the anode and cathode is 0.5 V. The external resistor is 10 Ω.

2.2. Artificial wastewater

The composition of artificial wastewater was as follows (g/L): Na₂HPO₄·12H₂O, 11.55; NaH₂PO₄·2H₂O, 2.77; NH₄Cl, 0.41; KCl, 0.14. In dual-chamber BES, acetate was added to the artificial wastewater as electrons donor at the anode chamber, the concentration is 10 mM; amido black 10B was added to the artificial wastewater as objective pollutant at the cathode chamber (Mu et al., 2009), the concentration is 100 mg/L. In single chamber BES, both acetate and amido black 10B were added to the artificial wastewater solution simultaneously, the concentration is same to the dual-chamber solution. All the reaction solution need to pump nitrogen for 15 min to clear the oxygen.

2.3. Inoculation and acclimation

The bioanode was incubated in a MFC with acetate as carbon resource in laboratory previously (Wang et al., 2011). The inoculation active sludge was collected from a local municipal wastewater treatment plant in Harbin, China.

First, the bioreactors were set up using IEM to separate anode and cathode for prove that the azo dye can be reduced at cathode, five dual-chamber BESs was used. Artificial dye wastewater without acetate was injected to cathode chamber. Acetate was as electron donor at anode chamber. The azo dye removal efficiency was tested in 2 months.

IEM is a component of dual-chamber BES, not suitable for practical application. That is why single-chamber BES was attempted to use for azo dye removal. So first the IEM was removed from all five dual-chamber BESs. Three bioanodes were acclimated for the anode biofilm, the anode potential and the potential recovery time were monitored. Two bioreactors were as control without any operation.

The steps of anode biofilm acclimation are as follows: (1) Removing the IEM of dual-chamber BES; (2) replace the artificial wastewater with amido black 10B concentration gradient of 10, 30, 50, 70, 100 mg/L. (3) According to the initial anode potential and the normal potential recovery time, to judge the effect of anti-toxic acclimation.

2.4. Blank control experiments

Three acclimated bioanodes and two un-acclimated bioanode all were installed in a new single-chamber BES with a new cathode of carbon brush, in order to retain the identity of cathode because abiciocathode might change into biocathode, the anode biofilm microorganisms could arrive to the surface of cathode in single-chamer BES during the anode biofilm acclimation.

2.5. Analysis of reactor performance

The current was monitored every 15 min by measuring the voltage of resistor using voltage collection instrument. The potential was obtained from two electrode system of reference and anode or cathode electrode. Acetate concentration was measured by gas chromatography equipped with a polar capillary column (HP-INNOWAX 19095N30 m×0.53 mm×1 μm, Agilent Co., Ltd., USA) at oven temperature of 250 °C with a flame ionization detector at 300 °C. The amido black 10B concentration was measured by spectrophotometry, the maximum absorption wavelength is 618 nm. The EIS (Electrochemical impedance spectroscopy) method was used to characterize the performance of BESs (He et al., 2009), frequency range were taken from 100 kHz to 10 mHz using a 10 mV sine wave. The data obtained from the EIS was simulated by ZsimpWin3.10 soft.

3. Results and discussion

3.1. Anode biofilm formation in absence of azo dye

Before anode biofilm acclimation, the dual-chamber BES was tested for azo dye removal, amido black 10B was injected to the cathodic chamber. The anode potential was −0.51 V(vs SCE),

![Fig. 1. (A) Schematic diagram of dual-chamber BES. (B) Schematic diagram of single-chamber BES.](image-url)
average current was 0.253 mA. The azo dye removal efficiency was 60.8% in 10 h, that indicated the amido black 10B was reduced noticeably at cathode. When removed IEM from BES, artificial wastewater was injected the BES without azo dye. The anode potential was −0.51 V, is same to before IEM removed, that indicated the IEM did not influence the anode biofilm in absence of azo dye.

3.2. Anode biofilm acclimation

After removed IEM, the azo dye concentration of 10 mg, 30 mg, did not influence the anode potential and the time that recover to the normal potential status when anode solution replaced, but increased to azo dye of 50 mg/L, the initial anode potential was disturbed badly, the initial potential increased to −0.28 V (Fig 2). In the previous experiments, the anode potential was about −0.5 V (vs. SCE) as a stable status for a BES. Additionally, 3.5 h of the anode potential recovery time that also indicated the azo dye of 50 mg/L could shock the anode biofilm electrochemical activity (normal time is shorter than 1.25 h), so 50 mg/L azo dye acclimation was repeated three times, and so on, until the azo dye concentration increased to the final concentration of 100 mg/L. Whether the initial potential or normal potential recovery time, they were close to the status that normal solution replaced with azo dye of 0 mg, then, the anode biofilm acclimation was achieved.

When biofilm acclimation end, the changes of performance was investigated in BES by batch experiment. As seen from Fig. 3a, the anode potential was decreased to about −0.52 V, this change was very little after anode acclimation; but, the current was several times larger than that in the dual-chamber BES, the 10 h average current is from 0.253 mA to 0.466 mA.

The azo dye removal efficiency in 10 h reaction time was 83.7% and 60.8% in the single-chamber and dual-chamber BES, respectively (Fig 3b). On the other hand, the azo dye initial mass in the single-chamber BES is two times more than in the dual-chamber BES while their concentration was same (100 mg/L). In 10 h reaction time, average azo dye removal rate was from 3.04 mg/h LTV (TV is the total BES volume) to 8.37 mg/L in from dual-chamber to single chamber BES. The azo dye removal efficiency and rate were both increased.

As seen from power consumption of azo dye removal per gram, the electrical energy consumption in the single-chamber BES is lower than the dual-chamber BES (see Table 1); In the single-chamber BES, based on the electric quantity at the electrocircuit and azo dye removal mass, the electron recovery efficiency for azo dye removal at cathode was 152.7%, which is far above 100% theoretically. These indicated another reaction witch accelerated the azo dye removal in the single-chamber BES, but it did not use the electrons from the electrocircuit. The traditional anaerobic role as the other cause is suggested. In dual-chamber BES, the cathodic condition is inorganic, no electron donor, no microbe, the anaerobic fermentation cannot happen. In short, both cathodic chamber and anodic chamber become one chamber, the microbe and substrate can touch the azo dye directly, so the BES is like a anaerobic reactor. In addition, a great quantity of microbe was observed in the residual solution of single-chamber BES.

From these aspects, the traditional anaerobic role may be involved in the single-chamber BES. The electrons from the acetate did not only arrive to the anodic surface, but to the azo dye molecule, bringing the diversity to the BES.
3.3. Enhanced azo dye removal after toxicity acclimation

The acclimated and un-acclimated anode biofilms for azo dye removal results is shown in Fig. 4a. When 100 mg/L azo dye was injected into the new single-chamber BES, the anode potential increased quickly in the control BES with un-acclimated bioanode, until above 0.15 V, it demonstrates that the anode biofilm was shocked by azo dye toxicity, not suitable to accept the electron from the acetate oxidation in anode.

The current was less far than that in the acclimated BES that indicated bioanode could not play its function as well. The removal efficiency of azo dye in BES with un-acclimated bioanode was 73.3% in 10 h, the dye solution became colorless closely. For the control, the removal efficiency decreased to 20.5% (Fig. 4b). From the anode potential, current density and azo removal efficiency, all the tests indicated that the acclimated anode biofilm is applicable in single-chamber BES for azo dye removal, its effect is better than of un-acclimated anode biofilm.

The EIS (Electrochemical impedance spectroscopy) experiment was also used to test the internal resistance \( R_{\text{in}} \) of BESs. Fig. 5 showed the Nyquist plots of three different BESs and the data obtained from EIS was simulated with equivalent circuit \( (R(QR)(QR)) \). The \( R_{\text{in}} \) of control BES with un-acclimated anode was 15 KΩ, but it is just 923 Ω when the anode was acclimated. On the other hand, it should be also realized that the presence of IEM would make the \( R_{\text{in}} \) three times to the absence of IEM (3123 Ω). From the simulating results, removing the IEM can decrease the \( R_{\text{in}} \) distinctly.

Recent studies have proved that it is effective to use the cathodic reduction for recalcitrant contaminants in BES (Mu et al., 2009), though BES is in its infancy. Compared with biological and chemical technologies, BES is more efficient and targeted than anaerobic process with lower demand of electrical energy to azo dye removal. BES is now developed quickly and demonstrated the principle and technology for specific azo dye treatment process. The great potential for engineering application is to plant BES in any developed treatment systems to strength removal of a kind of specific contaminants, that will save investment and avoid unnecessary waste.

4. Conclusions

Fundamentally, the objective of dual-chamber to single chamber is for the practical application further, which lead to remove the expensive IEM for the BES cost reducing. Anode biofilm acclimation enhanced anti-toxic and electrochemical activity of anodic communities, in contrast, the un-acclimated anode biofilm was failed for azo dye removal when exposed directly in single-chamber BES. From dual-chamber to single chamber, the azo dye removal efficiency increased from 60.8% to 83.7% in 10 h reaction period. This study indicated the way for anode biofilm acclimation from two-chamber to single chamber reduced the costs and improved the azo dye removal efficiency.

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