Biological catalyzed denitrification by a functional electropolymerization biocarrier modified by redox mediator

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ABSTRACT

Electropolymerization biocarriers were prepared by the electropolymerization of polypyrrole (PPy) on an active carbon felt (ACF) electrode using doping anions anthraquinone-2-sulfonate (AQS) or Na₂SO₄. The functional electropolymerization biocarrier (ACF/PPy/AQS) with AQS was used as an immobilized redox mediator for the denitrification process. The characteristics of the electropolymerization biocarriers were analyzed by scanning electron microscope, elemental analyses, Fourier transform infrared (FTIR) spectroscopy and Raman spectroscopy. The results suggested that the denitrification efficiency increased nearly 1.5-fold with ACF/PPy/AQS (0.04 mmol L⁻¹ AQS) compared to the control. A linear correlation was found for the *k* value and the AQS concentration (*C*_{AQS}), which was $k = 624.71C_{AQS} + 83.87$ ($R^2 = 0.9893$). The ORP value stabilized around -200 mV for the denitrification process with ACF/PPy/AQS, which was -25 mV lower than that with ACF/PPy/Na₂SO₄. Repeated-batch operations indicated that the denitrification efficiency with ACF/PPy/AQS maintained over 90% of the original value and exhibited better catalytic activity and durability.

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1. Introduction

Discharged nitrogen-containing compounds can create serious environmental problems such as the eutrophication of rivers and lakes, deterioration of water quality and potential hazards to human or animal health (Wang et al., 2009; Yang et al., 2011). With increasing nitrate pollution and strict limits set by legislation, higher nitrogen removal efficiency has become an interesting topic in wastewater treatment. In recent years, many new biological technologies, such as anaerobic ammonium oxidation (ANAM-MOX) (Jetten et al., 2001; Suneethi and Joseph, 2011) and oxygen-limited autotrophic nitrification-denitrification (OLAND) (Pathak et al., 2007) have been developed based on the new discovery and advancement of microorganism principles. Currently operational cost and low denitrification rate are still limiting factors for the application of these technologies and must be considered in their selection for nitrogen removal processes. Consequently, it is very important to find a novel and economical biological process for nitrogen removal.

During the last two decades, some studies have been conducted to explore the catalytic effects of different redox mediators on the bio-transformation processes of perchlorate, arsenate, nitrate and azo dyes (Van der Zee and Cervantes, 2009; Hong and Gu, 2009).

Redox mediators can reversibly be oxidized and reduced, and have the capacity to serve as electron carriers in multiple redox reactions, and accelerate reactions by lowering the activation energy of the total reaction. The simultaneous conversion of sulfide and nitrate in a denitrifying culture was reported to be enhanced with the addition of some soluble quinoid redox mediators (Aranda-Tamaura et al., 2005), in which continuous dosing implies continuous consumption of soluble redox mediator as well as continuous discharge of biologically recalcitrant compounds. A novel non-dissolved redox mediator catalyzing biological denitrification (RMBDN) was also explored, and the denitrification rate was enhanced about 1.5 fold with immobilized anthraquinone (Guo et al., 2010). However, the non-dissolved redox mediator was not ideal due to low mechanical strength (Guo et al., 2007, 2010). Therefore, a search for an effective method to immobilize a dissolved redox mediator (such as anthraquinone-2-sulfonate (AQS)) in the bioreactor was needed.

The aim of this study was to explore a new denitrification technology with functional electropolymerization biocarriers, which were prepared by the electropolymerization of polypyrrole (PPy) monomer on an active carbon felt (ACF) electrode with doping anions anthraquinone-2-sulfonate (AQS, redox mediator) or Na₂SO₄. The accelerating mechanism of the redox mediator was explained and analyzed for ORP and electron transport. In addition, the biological catalytic activity and durability were also tested by repeated-batch operations.

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Fig. 1. FTIR spectrum of the different electropolymerization biocarriers.

2. Methods

2.1. Chemicals

Pyrrole (Py) monomer (Aldrich, 99%) was distilled twice before use. All solutions were prepared using deionized water. Active carbon felt (ACF) (BET surface of $800 \text{ m}^2 \text{ g}^{-1}$, 4 mm thick) was purchased from Anshan Senxin Activated Carbon Fiber Plant, China. All other chemical reagents were analytical grade and were purchased from Xiandai Ltd. (Shijiazhuang, China).

2.2. ACF/PPy/AQS preparation

The functional electropolymerization biocarriers of ACF/PPy/AQS were prepared as follows: In the working electropolymerization compartment, 150 ml of aqueous solution was placed containing 0.1 M Py monomer and 0.024 M AQS or 0.024 M Na_2SO_4 . A sheet of ACF represented the working electrode with a platinum plate ($2.5 \text{ cm} \times 2 \text{ cm}$) counter electrode. Then, the functional electropolymerization biocarriers were prepared under cyclic voltammetry mode (0.55–0.75 V, 0.005 V/s) controlled by a Voltalab Powersuite/LK98B II (Tianjin Lanlike Electronics Technology Ltd., China) for 3 h. ACF/PPy/AQS was then cleaned twice using distilled water and kept in distilled water saturated with high purity N_2 gas before use. The AQS concentration was calculated by the sum of added sheets of ACF/PPy/AQS and the redox mediator amount of each sheet. The AQS amount of each sheet of ACF/PPy/AQS was about 0.0025 mmol, which was calculated as the change in the added AQS concentration in the electropolymerization solution system.

2.3. Organism and medium

Paracoccus versutus strain GW1 (1416 bp, GU111570) was a heterotrophic denitrification bacterium from “The Hebei Key Laboratory of Environmental Biotechnology”, and was cultivated in a

metal medium (Guo et al., 2010) which consisted of: 1.25 g KHCO_3 , 0.025 g KH_2PO_4 , 0.3 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.00625 g FeSO_4 , and 0.00625 g EDTA per liter and 1 ml L^{-1} of trace elements solution. The trace element solution contained: 15 g EDTA, 0.43 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.24 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.99 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.25 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.22 g $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 0.19 g $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 0.21 g $\text{NaSeO}_4 \cdot 10\text{H}_2\text{O}$, 0.014 g H_3BO_3 , and 0.05 g $\text{NaWO}_4 \cdot 2\text{H}_2\text{O}$ per liter. NO_3^- was used in the form of NaNO_3 .

Strain GW1 was grown for two days at pH 7.0–7.5 and 35 °C (Data is shown in Supporting information (SI) Fig. S1 and Fig. S2). Carbon sources and C/N ratio were sodium acetate and 6, respectively (Fig. S3). The biomass concentration was determined from the optical density (OD) at 660 nm, and the concentration of strain GW1 was adjusted to 5.7 g dry cell weight L^{-1} in all experiments.

2.4. Batch denitrification experiments

An adsorption experiment was conducted to estimate the adsorption capacity for nitrate. ACF/PPy/AQS without bacteria was added into 400 mg $\text{NO}_3^- \text{N L}^{-1}$ synthetic wastewater. The residual $\text{NO}_3^- \text{N}$ concentration in the synthetic wastewater was detected as a function of time until equilibrium.

Specific amounts of ACF/PPy/AQS were placed in a 250 ml growth medium containing designated nitrate concentrations. Denitrification with an identical amount of ACF/PPy/ Na_2SO_4 was also performed as a control experiment. To evaluate the denitrification efficiency, batch denitrification experiments were conducted under different AQS concentrations (0–0.04 mmol L^{-1}) and different initial $\text{NO}_3^- \text{N}$ concentrations (200–700 mg L^{-1}). At the same time, pH and ORP changes were measured to analyze the AQS role during the denitrification process.

2.5. Repeated-batch operations

Repeated-batch operations were conducted to study the reusability of ACF/PPy/AQS in industrial applications. ACF/PPy/AQS

Fig. 2. The effect of different AQS concentration (ACF/PPy/AQS) on the denitrification efficiency (initial nitrate concentration: $400 \text{ mg NO}_3\text{-N L}^{-1}$; 35°C ; pH: 7.0; 0.01 mmol L^{-1} AQS; carbon source: sodium acetate; C/N: 6; $5.7 \text{ g dry cell weight L}^{-1}$).

Table 1

Comparison of documented denitrification rates ($\text{g NO}_3\text{-N g}^{-1} \text{ VSS d}^{-1}$) using different denitrification processes.

Carbon source	Maximum denitrification rate ($\text{g NO}_3\text{-N g}^{-1} \text{ VSS d}^{-1}$)	Type of wastewater	Input $\text{NO}_3\text{-N}$ (mg L^{-1})	References
Sodium acetate	81.29 ^a	Synthetic wastewater	3388	Dhamole et al. (2007)
Acetic acid	1.16 ^a	Stainless steel wastewater	2500	Fernández-Nava et al. (2010)
Sodium acetate	7.76 ^a	Explosive factory	3600	Shen et al. (2009)
Acetic acid	1.2 ^a	Artificial groundwater	2700–8200	Glass and Silverstein (1999)
Acetic acid	45.6 ^a	Synthetic wastewater	>500	Rodríguez et al. (2007)
Methanol	0.486	Synthetic wastewater	500	Bilanovic et al. (1999)
Sodium acetate	108	Synthetic wastewater	200–700	In this study

^a Converted from $\text{g NO}_3\text{-N g}^{-1} \text{ VSS h}^{-1}$ to $\text{g NO}_3\text{-N g}^{-1} \text{ VSS d}^{-1}$.

Fig. 3. The effect of different initial nitrate concentration on the denitrification efficiency with ACF/PPy/AQS (0.01 mmol L⁻¹ AQS; 35 °C; pH: 7.0; carbon source: sodium acetate; C/N: 6; 5.7 g dry cell weight L⁻¹).

was placed into a medium containing 350 mg L⁻¹ NO₃⁻ (pH 7.5) and was then statically incubated at 35 °C for denitrification. After complete denitrification, ACF/PPy/AQS was collected, rinsed twice with sterile deionized water and transferred into a fresh medium containing 350 mg NO₃⁻ N L⁻¹ for the next denitrification experiment. The same procedures were repeated four times.

2.6. Analytical methods

For the batch denitrification experiment, samples were withdrawn at predetermined times and centrifuged at 8000×g for 15 min to remove the insoluble particles. The nitrate concentration in the supernatant was measured colorimetrically using UV spectrophotometry (U-3010, UV/VIS spectrophotometer, HITACHI, Co., Japan). The pH and ORP value change during the denitrification process was measured with a digital pH Meter (Delta-320, China) and an ORP composite electrode (Leici-501, China), respectively.

Elemental analysis was carried out by an Elementar Vario EL III (Germany) to acquire information about the elemental composition and the doping ratio of electropolymerization biocarrier. Infrared spectroscopy was also performed using KBr-pellets with a Fourier transform infrared spectrometer (FTIR, Nicolet5700, Thermo Electron Corporation, USA) to gain the chemical structure of the electropolymerization biocarrier. Raman spectra were recorded with a visible-light Raman spectrophotometer assembled by Dalian Institute of Chemical Physics, Chinese Academy of Sciences. An S-4800-I scanning electron microscope (SEM, Hitachi, Japan,) was used to observe the morphology of the electropolymerization biocarrier.

All experiments were performed in triplicate.

3. Results and discussion

3.1. Characterization of ACF/PPy/AQS

The morphology and microstructure of ACF, ACF/PPy/Na₂SO₄, fresh ACF/PPy/AQS and used ACF/PPy/AQS (triplicate use) were observed with SEM and the results were shown in [Supporting information \(SI\) Fig. S4](#). The surface of both the ACF and ACF/PPy/Na₂SO₄ were smooth, while the surface of fresh ACF/PPy/AQS and used ACF/PPy/AQS had a uniform distribution of particles from electropolymerization with AQS.

The PPy molecule has nitrogen and AQS or Na₂SO₄ have sulfur, so the ratio of nitrogen and sulfur can represent the composition of ACF/PPy/Na₂SO₄ and ACF/PPy/AQS. Results from elemental analysis

indicated the presence of sulfur in each sample of ACF/PPy/AQS and ACF/PPy/Na₂SO₄. The mole ratios of AQS and PPy or Na₂SO₄ and PPy in the electropolymerized matrix were about 5:1.

The FTIR spectra of ACF, ACF/PPy/Na₂SO₄ and ACF/PPy/AQS in the region from 4000 cm⁻¹ to 400 cm⁻¹ are shown in [Fig. 1](#). AQS was verified by the characteristic absorption band of highly conjugated C=O groups, such as quinones and/or carbonyl groups near hydroxyl groups observed at 1678 cm⁻¹ in the spectrum of ACF/PPy/AQS (Li et al., 2008). The bands at 1580 cm⁻¹ and 1457 cm⁻¹ could be assigned to the C–N stretching vibration in the Py ring (Omastová et al., 2004). The band at 1040 cm⁻¹ was due to the symmetric stretching vibration of S=O and has been observed in the spectrum for ACF/PPy/AQS (Tian and Zerbi, 1990). The bands located at 875 cm⁻¹ and 710 cm⁻¹ also corresponded to the stretching vibration of S=O, which was the characteristic absorption band of a sulfonic group. The weak bands observed at 1123 cm⁻¹ and 635 cm⁻¹ in the spectrum of ACF/PPy/AQS corresponded to the C–S bond (Li et al., 2008).

Raman spectral shifts and relative intensity changes in the 900–1200 cm⁻¹ range showed a less drastic difference when comparing ACF and ACF/PPy/Na₂SO₄ ([Fig. S5](#)). But for ACF/PPy/AQS, the band at 1250 cm⁻¹ was due to the symmetric stretching vibration of C=O and the bands at 1065 cm⁻¹, 1092 cm⁻¹, 973 cm⁻¹ and 937 cm⁻¹ corresponded to the symmetric stretching vibration of S=O for the sulfonic group of AQS (Sadowska et al., 2009). The band at 1330–1600 cm⁻¹ was due to graphite (Sadowska et al., 2009).

The results of above analysis showed that this electropolymerization was an effective immobilization method for the dissolved redox mediator AQS.

3.2. The effect of ACF/PPy/AQS on the denitrification efficiency

The adsorption results for ACF/PPy/Na₂SO₄ and ACF/PPy/AQS without microorganisms served as the blank control for the effect of ACF/PPy/AQS on the denitrification experiments. The adsorption of ACF/PPy/Na₂SO₄ and ACF/PPy/AQS all caused little change (0.2%) in the nitrate concentration (400 mg NO₃⁻ N L⁻¹) over 48 h. Therefore, the adsorption of nitrate by ACF/PPy/AQS could be neglected in the experiment in this study.

[Fig. 2](#) showed the denitrification efficiency of ACF/PPy/AQS with different redox mediator concentrations and an initial nitrate concentration of 400 mg NO₃⁻ N L⁻¹. After 14 h of the denitrification reaction, the nitrate concentrations were 137 mg NO₃⁻ N L⁻¹, 102 mg NO₃⁻ N L⁻¹, 72 mg NO₃⁻ N L⁻¹ and 37 mg NO₃⁻ N L⁻¹ at the AQS concentration of 0 mmol L⁻¹, 0.01 mmol L⁻¹, 0.02 mmol L⁻¹ and 0.04 mmol L⁻¹, respectively. The denitrification efficiency was enhanced from 65.75% to 90.75% by ACF/PPy/AQS with 0.04 mmol L⁻¹ AQS ([Fig. 2\(a\)](#)). At the same time, the nitrite concentration first accumulated up to the highest concentration and was then converted into nitrous oxide, nitric oxide and nitrogen gas during the denitrification process. The denitrification steps were sequential and the product of one enzyme is a preceding step for the next one. Fine-tuned regulation of the concentration and activity of the denitrification enzymes was, therefore, required in order to keep the free concentrations of NO₂⁻ and NO below cytotoxic levels. The highest accumulated nitrite concentrations were 86 mg L⁻¹, 107 mg L⁻¹, 132 mg L⁻¹ and 147 mg L⁻¹ at the AQS concentration of 0 mmol L⁻¹, 0.01 mmol L⁻¹, 0.02 mmol L⁻¹ and 0.04 mmol L⁻¹, respectively ([Fig. 2\(b\)](#)). The reduction rate of nitrate to nitrite increased with AQS concentration, and the nitrite reduction rate was also enhanced by AQS. NO₂⁻-reductase was induced in response to the high nitrite concentration after denitrification. The activity of NO₂⁻-reductase gave rise to an initial increase in the NO₂⁻-concentration.

Fig. 4. The pH profile (A, solid symbols) and ORP profile (B, empty symbols) during the denitrification process with ACF/PPy/AQS compared to ACF/PPy/Na₂SO₄ (initial nitrate concentration: 400 mg NO₃-N L⁻¹; 35 °C; initial pH: 7.0; 0.01 mmol L⁻¹AQS; carbon source: sodium acetate; C/N: 6; 5.7 g dry cell weight L⁻¹).

The catalyzed denitrification process with 400 mg NO₃⁻-N L⁻¹ and ACF/PPy/AQS followed a modified pseudo-zero order model with $k = dC_{\text{Nitrate}}/dt$, where the reaction constant k was computed by multiple regression analysis. The reaction constants k (mg NO₃⁻-N g⁻¹ dry cell weight d⁻¹) were 83.04, 90.24, 97.92 and 108 at corresponding AQS concentrations of 0 mmol L⁻¹, 0.01 mmol L⁻¹, 0.02 mmol L⁻¹ and 0.04 mmol L⁻¹, respectively. A linear correlation was found for the k value and the AQS concentration (C_{AQS}), which was $k = 624.71C_{\text{AQS}} + 83.87$ ($R^2 = 0.9893$). The accelerating effect of the redox mediator on the denitrification was similar to the decolorization rate of azo dye (Guo et al., 2007). The maximum removal rate was 108 mg NO₃⁻-N g⁻¹ dry cell weight d⁻¹, which is an attractive rate when compared with other denitrification processes (Table 1).

An initial nitrate concentration in the range of 200–700 mg L⁻¹ was used to study the effect of nitrate concentration on the denitrification rate with the redox mediator. Fig. 3 shows that the denitrification rate was not significantly affected by the initial

concentration in the tested range and followed a pseudo-zero order model, with an average denitrification rate of 78.96 ± 18.96 mg NO₃⁻-N g⁻¹ dry cell weight d⁻¹.

3.3. The effect of ACF/PPy/AQS on the pH and ORP change during the denitrification process

Fig. 4(a) shows that pH increased with nitrate removal for both denitrification processes with ACF/PPy/AQS and ACF/PPy/Na₂SO₄, but the pH value with ACF/PPy/AQS was lower than that with ACF/PPy/Na₂SO₄. This phenomenon might result from the different sources of the needed hydrogen proton for the denitrification process by the redox mediator and the biological denitrification process. For the biological denitrification process, the proton was supplied with the help of coenzymes, like NADPH/NADP⁺, NADH/NAD⁺ and ubiquinol/ubiquinone (Q/QH₂), while for the denitrification process with ACF/PPy/AQS, the proton might come from the reduced redox mediator (Liu et al., 2011).

Fig. 5. Denitrification with repeated batch cycles (initial nitrate concentration: 360 mg $\text{NO}_3\text{-N L}^{-1}$; 35 °C; pH: 7.0; 0.01 mmol L^{-1} AQS; carbon source: sodium acetate; C/N: 6; 5.7 g dry cell weight L^{-1}).

The ORP was an important factor for the effective denitrification process, in which electrons are transferred from a reducing agent to an oxidizing agent until the reaction has reached equilibrium. In this experiment, the high denitrification efficiency with ACF/PPy/AQS increased nearly 1.5-fold in comparison to the control of ACF/PPy/ Na_2SO_4 . The ORP values stabilized around -200 mV for the denitrification process with ACF/PPy/AQS, which was similar to other results (Guo et al., 2007). The ORP stabilized values with ACF/PPy/AQS was -25 mV lower than the control (Fig. 4(b)). When nitrate was consumed completely, the ORP dropped to about -580 mV. The different ORP between ACF/PPy/AQS and ACF/PPy/ Na_2SO_4 might mean that different primarily metabolic or bioreactions took place, namely the denitrification reaction and other bioreactions. The effect of the redox mediator on the ORP during denitrification is similar to the results reported for azo dye decolorization and denitrification processes with different a redox mediator (Guo et al., 2007, 2010).

Theoretically, according to the mechanism of traditional denitrification processes, the transformation of nitrate to nitrogen gas is an oxidation-reduction reaction, in which the transfer of electrons matches with proton flow with the help of coenzymes, like NADPH/ NADP^+ , NADH/ NAD^+ and ubiquinol/ubiquinone (Q/ QH_2). The ORP values of NADPH/ NADP^+ , NADH/ NAD^+ and Q/ QH_2 are -324 mV, -320 mV, and 45 mV, respectively (Rau et al., 2002), and the ORP value of AQS is -218 mV (Kudlich et al., 1997), so the role of AQS may be similar to Q/ QH_2 . The catalyzing mechanism of the redox mediator in denitrification needs to be studied in detail using cell extracts and purified enzymes, which would allow a better understanding of the nature of the process.

3.4. Repeated-batch operations

After four cycles, the denitrification efficiency decreased less than 2% and the functional bio-carrier exhibited good reusability (Fig. 5). This also suggested that the redox mediator on the functional electropolymerization-biocarrier was recycled during the transfer of electrons from an electron donor to the terminal electron acceptor.

4. Conclusion

This study explored a new denitrification process with functional electropolymerization biocarriers of ACF/PPy/AQS. The deni-

trification efficiency with ACF/PPy/AQS (0.04 mmol L^{-1} AQS) increased nearly 1.5-fold compared to the control. A linear correlation of the k value and the AQS concentration was $k = 624.71C_{\text{AQS}} + 83.87$ ($R^2 = 0.9893$) for an AQS concentration of $0\text{--}0.04 \text{ mmol L}^{-1}$. The ORP value with ACF/PPy/AQS (0.04 mmol L^{-1} AQS) was -25 mV lower than that with ACF/PPy/ Na_2SO_4 ; AQS might play a similar role to that of cofactor ubiquinol/ubiquinone (Q/ QH_2) during the denitrification process. Repeated-batch operations exhibited good catalytic activity and durability after four repeated batch experiments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.biortech.2011.12.071](https://doi.org/10.1016/j.biortech.2011.12.071).

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