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AQDS immobilized solid-phase redox mediators and their role during bioelectricity generation and RR2 decolorization in air-cathode single-chamber microbial fuel cells

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ABSTRACT

The application of immobilized redox mediators (RMs) in microbial fuel cells (MFCs) is an emerging technology for electricity generation with simultaneous azo dye decolorization due to facilitated electrons transfer from bacteria to anodes and azo dyes. The use of immobilized RMs avoids the requirement of their continuous dosing in MFCs, which has been the main limitation for practical applications. Two strategies of anthraquinones-2,6-disulphonic salt (AQDS) immobilization, AQDS immobilized with polyvinyl alcohol particles and AQDS immobilized on anodes by electropolymerization, were evaluated and compared to achieve simultaneous reactive red 2 (RR2) dye reduction and bioelectricity generation. The AQDS immobilized by electropolymerization showed the highest power density (816 ± 2 mW/m^2) and extent of RR2 decolorization (89 ± 0.6%). This power density is one of the highest values yet achieved in the presence of a recalcitrant pollutant, suggesting that immobilization was important for enabling current generation in the presence of RR2.

1. Introduction

Environmental problems generated by the textile industry have received increased attention for several decades because this industrial sector is one of the largest generators of contaminated effluents. It is estimated that 10–15% of dyes used in the dyeing process do not adhere to the fibers and end up in the textile effluent [1]. Azo dyes represent the most important dye class used in the textile industry. They are characterized by the presence of one or more azo chromophores (N = N) and bonds between two or more aromatic rings [2]. Azo dyes are removed by a non-specific reduction mechanism under anaerobic conditions, but the low rate of degradation is the primary issue when adopting anaerobic reactors to remove these compounds from industrial wastewaters [3].

The main limitation for azo dye reduction under anaerobic conditions is the transfer of reducing equivalents generated by the cells during the metabolic process to the azo dye. Redox mediators (RMs) such as quinones (redox active groups that are very abundant in naturally occurring humic substances) can accelerate the transfer of electrons during the reductive biotransformation of pollutants containing electron-accepting groups, such as azo dyes [4]. RMs have increased the reductive conversion rates by one to several orders of magnitude, and in some cases, they have been indispensable for reactions to take place [5]. During the reductive biotransformation of pollutants, the redox group present in humic substances or their quinone model compounds accept electrons derived from the microbial oxidation of organic compounds, such as phenol [5], benzene [6], and vinylchloride [7] for their reduction. The reduced redox group then donates electrons to organic pollutants (e.g. azo dyes, polyhalogenated compounds and nitro-substituted aromatic compounds) to be reoxidized to a state that accepts electrons again. RMs do not necessarily have to be supplied abundantly in anaerobic bioreactors to accelerate the reductive biotransformation of azo dyes, as they are regenerated during electron transfer process. Continuous dosing of the RMs would be expensive, and also result in the continuous discharge of the biologically recalcitrant RMs. Application of non-dissolved RMs and effective methods to immobilize them have recently been explored. RMs have been immobilized by entrapment in calcium alginate, polyvinyl alcohol–H_3BO_3, and agar [8], by covalent binding on ceramsites [9] or polyurethane foam [10], adsorption on metal oxide nanoparticles [11], electrostatic attraction on the surface of anion exchange resins [12], and by electropolymerization on activated carbon felt [13]. In the presence of immobilized RMs such as humic substances or quinones, higher removal rates of azo dyes have been observed in fed batch [12–13] and continuous flow high-rate anaerobic reactors [14]. Immobilized RMs are able to mitigate inhibitory effects caused by compounds contained in textile effluents, by adsorbing a fraction of these compounds and accelerating their reduction, which is

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relevant considering the great complexity and variability in the composition of textile wastewaters [15].

Microbial fuel cells (MFCs) are currently being examined for both renewable energy production and wastewater treatment as they can be used to generate electrical power from biodegradable organic matter using exoelectrogenic microorganisms [16]. MFCs have been examined as a technology for decolorization of azo dyes due to the dual advantages of dye treatment and simultaneous electricity generation [17–20]. Adding RMs into solution using single chamber, air-cathode MFCs accelerated the rate of dye degradation compared with a RM-free MFC, with glucose as the readily biodegradable substrate [17,20–21]. Rates of dye decolorization [22] and power densities [23–25] were also improved by adding AQDS to the anode. A polypyrrole/AQDS modified active carbon felt anode increased the decolorization rate (KE-3B) of dye 3.2-fold in comparison with an unmodified anode [22]. However, the effect of this composite anode on the performance of the MFC was not reported. When AQDS was immobilized in polypyrrole (PPy) films, electrocatalytic activities were improved, as well as stability of MFCs, compared to tests using a spontaneously adsorbed monolayer of AQDS [25]. Using a two-chamber MFC with an AQDS-modified carbon felt anode, the power density using a pure culture of *Shewanella decolorationis* S12 produced a maximum power density of 1303 mW/m^2, which was 13 times larger than the control with an untreated anode [23]. However, the use of a pure culture to treat a wastewater would not be feasible due to the growth of other anaerobic microorganisms, and the ferricyanide catholyte which was used in these previous tests would not be possible for practical applications.

Two groups of bacteria have been identified to be responsible for transfer of the electrons, azo dye-degrading bacteria, and electrochemically active bacteria on the MFC anode. The combined interactions of these microorganisms together enhanced azo dye degradation [20]. However, power densities can be adversely affected by the toxicity of the dyes and their decolorization products, and competition for the growth substrate by both groups of bacteria [19,26]. One drawback of immobilization on the anode is that the RM is not available to azo dye-degrading bacteria present in the solution, which could reduce rates of azo dye decolorization. Alternatively, electricity generation could benefit from enhanced activity of the electricity-generating biofilm due to the availability of the RMs to the anode biofilm [23].

The aim of this investigation was to evaluate whether immobilized AQDS as the solid-phase RM could achieve both simultaneous bioelectricity generation and azo dye reduction in air-cathode MFCs. While previous studies have reported on immobilized dye reduction, the impact on power production has not directly been examined. Reactive red 2 (RR2) was for tests here as this particular azo compound has been reported to be very recalcitrant and also very toxic to anaerobic consortia, resulting in the collapse of treatment in high-rate anaerobic bioreactors [14]. An important difference between RR2 and other commonly examined azo dyes [17–21] in MFCs is that neither RR2 or its decolorization products act as RMs. Thus, while power densities obtained in previous studies would have benefitted from the presence of the mediators in solution. Two strategies of immobilization were evaluated and compared: AQDS immobilized with polyvinyl alcohol (PVA), and immobilization on the anode surface by electropolymerization. To our knowledge, the present study constitutes the first demonstration of immobilized AQDS serving as an effective solid-phase RM with good power densities achieved along with effective and improved rates of a highly recalcitrant pollutant like RR2 in air cathode, single-chamber MFCs.

2. Materials and methods

2.1. Chemicals

Polyvinyl alcohol (PVA, average molecular weight 89,000–98,000) and Reactive Red 2 (RR2, 40% purity) were purchased from Sigma-Aldrich. Anthraquinone-2,6-disulfonic acid disodium salt (AQDS) with a purity of 90% was obtained from Acros Organics. All other chemicals were of reagent grade.

2.2. Immobilization with polyvinyl alcohol (PVA)

A suspension of AQDS (10% w/v) was mixed with PVA (7% w/v) and heated to 80 °C to dissolve the PVA. The AQDS-PVA solution was then cooled at −20 °C for 1 d to form a solid that was cut into squares (~1 to 2 mm^2 each) to produce particles containing the mediator. The squares were mixed in H_3BO_3 (5% w/v) for 1 h to ensure crosslinking of the PVA, and then rinsed at least 3 times using distilled water. The amount of AQDS was calculated from the total mass of AQDS-PVA obtained after cooling, with 2.55 g of PVA-AQDS material containing 0.5 g of AQDS, with these amounts of materials added to the solution of the MFCs.

2.3. Immobilization by electropolymerization

The pyrrole monomer was purified two times by distillation prior to use. The electropolymerization of a PPy/AQDS composite film on a carbon fiber brush electrode [22] was performed in a conventional three-electrode electrochemical cell with two compartments. A carbon brush was used as the working electrode, with a stainless steel mesh (50 × 50 mesh) counter electrode, and an Ag/AgCl reference electrode. The working electrode compartment contained 30 mL of PBS with 0.1 M PPy monomer and 0.024 M AQDS [22]. The counter electrode compartment was filled with 30 mL of 0.1 M H_2SO_4. A constant potential of 0.8 V was applied for the anodic polymerization in a stirred N_2-gas saturated solution [24]. The time required for electropolymerization (30 min) was controlled using a potentiostat (VMP3, Biologic Science Instrument). The electropolymerized electrodes were rinsed three times with distilled water and kept in distilled water saturated with N_2 gas before use.

2.4. MFC design and operation

Single chamber, air-cathode, cubic-shaped MFCs were constructed as previously [27], with an anode chamber 3-cm in diameter and 28 mL volume, and operated in a temperature controlled room (30 ± 1 °C). Each reactor contained one graphite fiber (PANEX33 160 K, ZOLTEK) brush anode (14 mm diameter by 25 mm length) made using two wound titanium wires and an activated carbon air cathode [28]. The MFCs were inoculated with anaerobic sludge (2 g VSS L^{-1}) from the Pennsylvania State University wastewater treatment plant and fed with a synthetic textile wastewater containing RR2 (0.3 mM), glucose as the fuel (1 g L^{-1}), and a 50 mM phosphate buffer solution (pH = 7.1) containing minerals and vitamins [27].

Five types of single-chamber MFCs were simultaneously operated (in duplicate) to evaluate the relative effectiveness of immobilized AQDS as a solid-phase redox mediators. The first four MFCs test reactors contained: 1) AQDS immobilized with PVA particles (AQDS/PVA); 2) AQDS immobilized on the anode by electropolymerization (AQDS-anode); 3) a combination of both approaches, with immobilized AQDS on the PVA particles and with electropolymerization of AQDS on the anode (AQDS/PVA-anode); and 4) no AQDS addition. One additional MFC was operated to evaluate the relative impact of immobilization versus soluble mediators by adding the AQDS directly into the solution (AQDSs).

Before addition of azo dye, all MFCs were operated over several cycles at a fixed external resistance of 1000 Ω. Once stable cycles of voltage were observed, the resistance was decreased to 200 Ω to increase the current. RR2 was then added to all MFCs at the same final concentration (0.3 mM), and the MFCs were again operated for several cycles until repeatable cycles of voltage were produced. AQDS/PVA particles were initially equilibrated with a more concentrated RR2 solution

($5\ g\ L^{-1}$) in order to ensure that particles added to the reactor did not appreciably deplete the soluble concentration of RR2. The amount of RR2 adsorbed on AQDS/PVA after equilibrium was ≤1% of RR2 initially added. The anode solution was refreshed when the voltage decreased below 20 mV. The results shown are based on duplicate reactors, with the results given as averages and standard deviations.

2.5. Toxicity of RR2

To evaluate the possible toxic effects of RR2 and its reduction products, three consecutive experimental conditions were tested using the control lacking AQDS after it had been operated for 6 months. Stage (i) was the recovery stage when RR2 was no longer added. Stage (ii) consisted of addition of exoelectrogenic microorganisms to investigate the impact on recovery of current output. In stage (ii) the medium was refreshed only one time with pre-acclimated bacteria from another MFC that had been running in fed batch mode for over 6 months. Stage (iii) was an investigation of the effect of RR2 (0.3 mM) addition on exoelectrogenic microorganisms. For each stage, the MFCs were operated for five fed-batch cycles. In all stages glucose was used as substrate ($1\ g\ L^{-1}$).

2.6. Analyses

The voltage (V) across an external resistor was monitored at 20 min intervals using a multimeter (Model 2700, Keithley Instruments, USA) and used to calculate the current based on the voltage drop across the resistor. Power density curves were obtained by varying the external resistor from open circuit to 10 Ω. Reactors were first operated under open circuit conditions for at least 2 h prior to each polarization test, and with an interval of 30 min at each resistance. Current (I), power (P) and Coulombic efficiency (CE) were calculated as previously described [29], while the power density was obtained by normalizing to the projected surface area of one side of the cathode ($7\ cm^2$).

CV tests were conducted using a potentiostat (VMP3, Biologic Science Instrument) with the anode as the working electrode, the cathode as the counter electrode, and an Ag/AgCl reference electrode. All the voltammograms were recorded with a scan rate of $20\ mV\ s^{-1}$.

Color removal was monitored by withdrawing samples (1 mL) at a time interval of 2 or 4 h during a single batch cycle, followed by sample centrifugation at $3220 \times g$ for 10 min to remove suspended biomass from the anode solution. Samples were diluted prior to measurement of the absorbance as needed. RR2 concentration was determined photometrically by UV/visible spectrophotometry (Shimadzu UV-1800) at a wavelength of 539 nm.

3. Results and discussion

3.1. Power production in the presence of AQDS, prior to RR2 addition

Electricity generation was not adversely affected by the presence of AQDS (in the absence of RR2) on the anode surface, immobilized on PVA particles in the reactor, or in solution in the MFC solution (Fig. 1). The maximum current over a fed-batch cycle was 1.1 ± 0.01 mA with the AQDS/PVA MFCs, which was similar to that with AQDS in solution (AQDSs, 1.1 ± 0.03 mA), and the control MFC lacking AQDS (1.01 ± 0.03 mA). Similar current densities were also obtained for the two cases with AQDS added by electropolymerization to the anode (1.2 ± 0.04 mA, AQDS/PVA-anode; 1.2 ± 0.02 mA, AQDS/anode) (Table 1), although the duration of the cycles was longer when AQDS was added to the anode (Fig. 1).

CV tests were used to verify the presence of the PPy/AQDS composite film on the carbon brush anodes. The voltammogram of the PPy-modified carbon brush electrode showed a larger current against the unmodified anode (Fig. 2) reflecting an increased surface area of the modified electrode due of incorporation of PPy [23]. The larger current of PPy/AQDS-modified anode than the PPy-modified anode (Fig. 2) was likely due to the redox reactions of AQDS, with an oxidation peak at −0.39 V (vs. Ag/AgCl) and a reduction peak at −0.59 V (vs. Ag/AgCl), in 1 M KCl solution (Fig. 2). The presence of these two peaks confirmed the successful incorporation of AQDS into the PPy matrix.

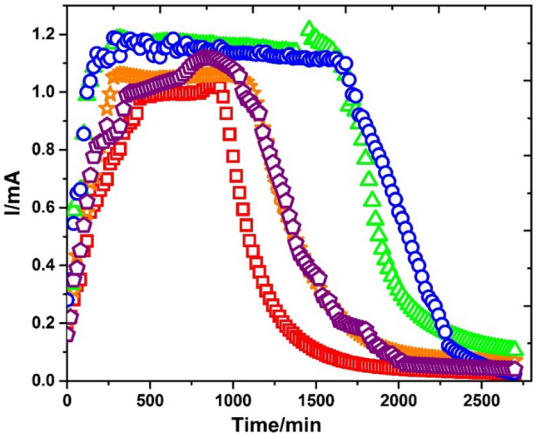


Fig 1. Bioelectricity generation by MFCs with and without immobilized addition of AQDS after 3 months of operation: MFC lacking AQDS (control, □, AQDS on PVA particles (AQDS/PVA, ☆), AQDS on the anode by electropolymerization (AQDS-anode, △), AQDS on the PVA particles and with electropolymerization of AQDS on the anode (AQDS/PVA-anode, ○), AQDS in solution (AQDSs, ⌂).

3.2. Current and power production in presence of RR2

During the first cycles of RR2 addition to the MFCs, the presence of RR2 only slightly reduced the maximum current for AQDS addition to the anode by electropolymerization (1.02 ± 0.05 mA, AQDS/anode), or with AQDS on PVA particles (1.08 ± 0.04 mA, AQDS/PVA-anode) (Fig. 3a). However, addition of RR2 greatly altered the shape of the voltage profiles for these two cases. For example, the time required to reach the maximum current output for the AQDS-anode tests was also much longer (15 h) than that required in the absence of RR2 (5 h). For the other test conditions where AQDS was not on the anode, RR2 substantially reduced the peak current and changed the shape of the voltage profile, with maximum currents of 0.80 ± 0.1 mA (AQDSs), 0.44 ± 0.0 mA (AQDS/PVA), and 0.50 ± 0.1 mA (Control).

The MFCs were operated until they exhibited reproducible cycles of voltage output (~3 months). The maximum current output produced by MFCs with AQDS on the anode were similar to each other, and much higher than those obtained with the other MFCs, with 1.1 ± 0.02 mA (AQDS/anode) and 1.1 ± 0.03 mA (AQDS/PVA-anode) (Fig. 3b and Table 1). These power densities were also similar to those originally obtained before the addition of the RR2. In contrast, the current output reached was much lower for the other cases, with a 58% lower current of 0.46 ± 0.03 mA with AQDS in solution (AQDSs), and 67% lower with AQDS on particles (AQDS/PVA, 0.35 ± 0.01 mA) or in the absence of AQDS (Control, 0.35 ± 0.02) (Fig. 3b). The percent of the substrate converted to electricity was also higher with AQDS on the anode, with a CE = 15 ± 2% for both cases (AQDS/anode, and AQDS/PVA-anode). Lower CEs were obtained in the other cases, ranging from 11 ± 1% (AQDSs) to 5.8 ± 1.1% (AQDS/PVA) (Table 1). The gradual decline of current output in control (without AQDS) and AQDS solution (AQDSs) was presumably due to RR2 toxicity. Previously it was reported that RR2 and its reduction product were extremely toxic to methanogens in the absence of RMs. For example, the use of immobilized humic substances as RMs in an UASB reactor feed with phenol as substrate and RR2, increased the decolorization efficiency of RR2 (~90%), the extent of phenol oxidation (~75%), and improved reactor stability compared to a control UASB reactor operated without immobilized humic substances that collapsed after 120 d of dye introduction [14]. In that

Table 1
Comparative MFC performance during the simultaneous bioelectricity generation and RR2 decolorization under all conditions evaluated.

Treatment	Maximum current output (mA)			Maximum power density (mW/m^2)[a]	CE (%)	K (h^{-1})	Decolorization (%)
	Without RR2	With RR2	Steady-state RR2	Steady-state RR2			
Control	1.1 ± 0.03	0.50 ± 0.1	0.35 ± 0.02	204 ± 2	6.1 ± 1.8	0.034 ± 0.01	37 ± 0.6
AQDS/PVA	1.1 ± 0.1	0.44 ± 0.1	0.35 ± 0.01	221 ± 6	5.8 ± 1.1	0.052 ± 0.03	52 ± 2
ADQS-anode	1.2 ± 0.02	1.02 ± 0.05	1.1 ± 0.02	816 ± 2	15 ± 2	0.14 ± 0.04	89 ± 0.6
AQDS/PVA-anode	1.2 ± 0.04	1.08 ± 0.04	1.1 ± 0.03	773 ± 5	15 ± 2	0.16 ± 0.02	92 ± 1.5
AQDSs	1.1 ± 0.03	0.80 ± 0.1	0.46 ± 0.03	505 ± 2	11 ± 1	0.13 ± 0.03	86 ± 1

[a] Power density normalized by the cathode surface area. The results shown are averages from duplicate measurements. (External resistance of 200 Ω, 1 g L^{-1} glucose, and 0.3 mM RR2).

study, the adsorption of RR2 on an anion exchange resin helped to mitigate the inhibitory effects of RR2 and maintain the redox reactions. In the present study, it is likely that the increase in the electron transfer rate was the principal mechanism to maintain the current output in the AQDS/anode and AQDS/PVA-anode.

Based on power density curves obtained from polarization data, the reactors with AQDS on the anode also had the highest maximum power densities, with 816 ± 2 mW/m^2 for the AQDS-anode and 773 ± 5 mW/m^2 for the AQDS/PVA-anode (Fig. 3c). The next highest power density was produced with the AQDS dissolved in solution with 505 ± 2 mW/m^2. The MFCs with the AQDS on the PVA produced a much lower power maximum power density of 221 ± 6 mW/m^2 (AQDS/PVA), which was similar to that of the control lacking AQDS (204 ± 2 mW/m^2, Control). This demonstrated the presence of AQDS on particles did not ameliorate the adverse impact of RR2 on the exoelectrogenic bacteria producing power. Thus, the AQDS on the electrode surface produced the best improvement in power production of all cases examined here.

The maximum power densities obtained here using AQDS on the anode were higher than those previously reported in most studies using RMs dissolved in solution, demonstrating the importance of having the RM on the anode in presence of recalcitrant pollutants. For example, 67 mW/m^2 was generated using an air cathode MFC with AQDS in solution (0.005 M) and 77 mW/m^2 with humic culture acids (5 g L^{-1}) [30]. In a two-chamber MFC, 52 mW/m^2 was generated with 2 g L^{-1} of humic acid [30]. However, a higher maximum power density of 1303 mW/m^2 was obtained using a pure of *Shewanella decolorationis* S12 in a two-chamber MFC, with a PPy/AQDS-modified carbon felt anode and a ferricyanide catholyte [25]. While this power density was 38% higher than that obtained here, the two results cannot be directly compared due to differences in the substrate, microorganisms and type of cathode. The maximum current densities that can be produced in an MFC, even in the absence of RMs and recalcitrant pollutants, varies widely and depends on the microorganisms, substrate, materials,

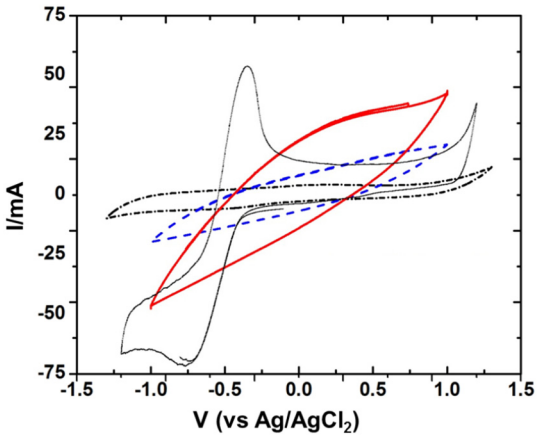


Fig. 2. Cyclic voltammograms in PBS (50 mM, pH 7.1) and 5 mM of AQDS in 1 M KCl: Unmodified anode (▬ · ▬ · ▬), PPy-anode (▬ ▬ ▬), AQDS/PPy-anode (——), AQDS in 3 M KCl (——).

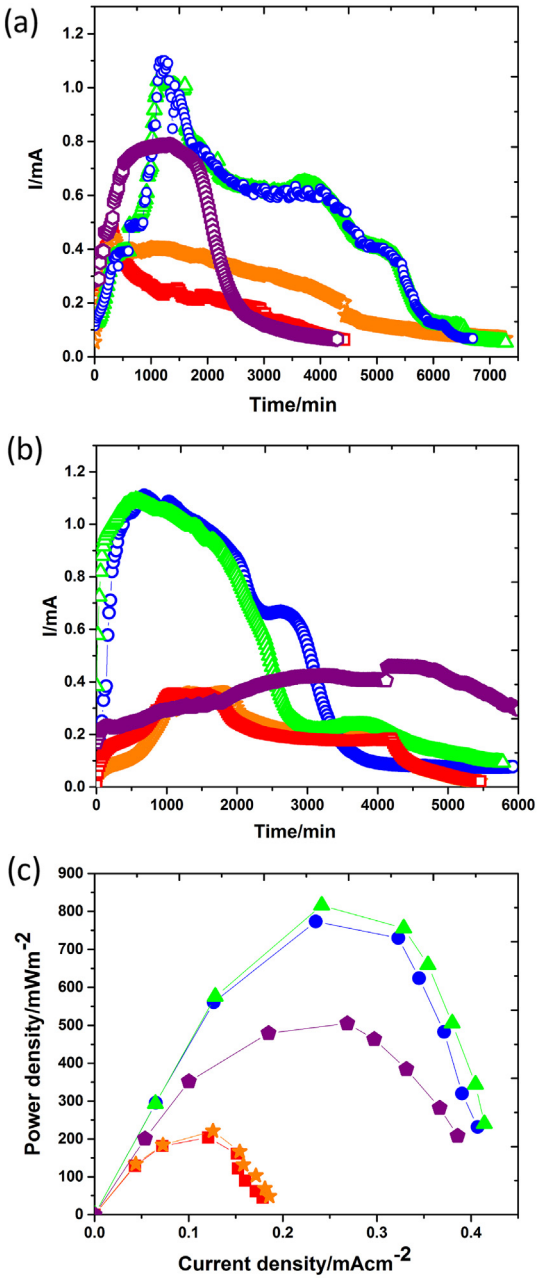


Fig. 3. Bioelectricity generation by MFCs with and without immobilized addition of AQDS. (a) Current production immediately after addition of RR2, and (b) after steady-state (reproducible cycles of current); (c) power density curves in the presence of RR2 after acclimation. MFC lacking AQDS (control, □), AQDS on PVA particles (AQDS/PVA, ★), AQDS on the anode by electropolymerization (AQDS-anode, Δ), AQDS on the PVA particles and with electropolymerization of AQDS on the anode (AQDS/PVA-anode, ○), AQDS in solution (AQDSs, ⌂). (External resistance of 200 Ω, 1 g L^{-1} of glucose, and 0.3 mM RR2).

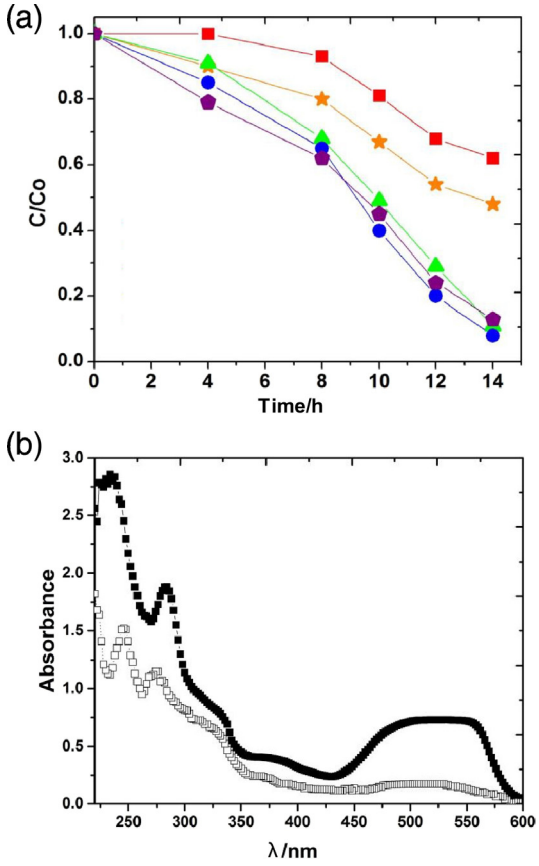


Fig.4. (a) Decolorization of RR2 (0.3 mM) after steady-state conditions were obtained in the presence of RR2: MFC lacking AQDS (control, □), AQDS on PVA particles (AQDS/PVA, ☆), AQDS on the anode by electropolymerization (AQDS-anode, Δ), AQDS on the PVA particles and with electropolymerization of AQDS on the anode (AQDS/PVA-anode, ○), AQDS in solution (AQDSs, ⬠). (b) UV–vis spectra of feed solution from AQDS-anode containing RR2 (0.3 mM) during one reduction cycle: initial solution (■), final solution (□).

configuration and solution chemistry [27,31]. In most studies acetate is used as the fuel. MFCs with glucose or other fermentable substrates typically produce less power than those with acetate [32]. In studies with acetate the anode biofilm has been shown to be predominately colonized by *Geobacter sulfurreducens* [33], which cannot use glucose as a substrate for growth. Thus, power depends on the fermentation of the substrate to volatile fatty acids (primarily acetate) that can be used by *Geobacter* sp. to produce power. In the previous tests with *S. decolorationis* S12 the substrate (lactate) could be directly used to produce current, and ferricyanide was used as the electron acceptor. Both of these conditions which would be expected to improve power production compared to conditions examined here, as the glucose used here would need to be fermented for power generation, and ferricyanide can produce higher power densities than those using oxygen reduction with an air cathode [34]. In another study, 270 mW/m^2 was generated with the reduction of the azo dye Congo Red using a riboflavin modified anode, and 320 mW/m^2 was generated with a humic acid-graphite anode modified by electropolymerization, with glucose as the substrate [35]. Congo red has been widely evaluated as this azo dye and its decolorization products serve as RMs to enhance electron transfer [17–21, 35]. In contrast to results with Congo Red, RR2 did not impact the current generated here as it is not a RM. However, even with these differences in the ability of the azo dye to function as an RM, and the addition of RMs as AQDS, riboflavin and humic acids in that previous study, the power here was still 60% larger than that previously obtained.

The immobilization of AQDS on electrode surface was shown here to improve power in air cathode MFCs, with the further addition of AQDS on the PVA particles having no noticeable impact. The modification of the anode with PPy likely increased the working surface area of the anode, and the presence of AQDS enhanced electron transfer efficiency from the bacteria to the anode, which could have increased the number of active bacteria attached to the anode [23–25].

3.3. RR2 decolorization in presence of AQDS

Decolorization of RR2 was consistent with first-order kinetics (k_{d1}) for all experimental conditions ($r^2 \geq 0.90$ in all cases, Table 1). The catalytic effect of AQDS immobilized on the anode could be confirmed by the increased of decolorization rates (k_{d1}) based on the extent of RR2 removal after 14 h on incubation (Table 1). For instance, the reactors with AQDS on the anode increased ~4.1-fold the k_{d1} value for the AQDS-anode and ~4.7-fold the k_{d1} value for the AQDS/PVA-anode as compared with reactor lacking AQDS. The catalytic effect of AQDS immobilized was also favorably reflected by a greater extent of decolorization of RR2 (Fig. 4a), with 89 ± 0.6% of RR2 decolorization for the adsorbed mediator (AQDS-anode) and 92 ± 1.5% for the immobilized mediator (AQDS/PVA-anode) after 14 h of incubation. These extents of decolorization were similar to that obtained with AQDS in solution (86 ± 1%, AQDSs), although as noted above the power production was greatly reduced when the mediator was added to the solution. There was much less decolorization with the AQDS/PVA (52 ± 2%) or the control (37 ± 0.6), which required >30 h to achieve 100% decolorization.

3.4. Reduction products of RR2 and toxicity

The reduction of RR2 to its main product was evident based on changes in UV–vis spectra of samples taken from the AQDS-anode MFC at the beginning and end of a fed-batch cycle (16 h). The characteristic band of the RR2 azo dye at ~539 nm decreased considerably after treatment (Fig. 4b), indicating cleavage of the azo bonds (—N═N—). Under anaerobic conditions, the complete reduction of the RR2 involves the transfer of four electrons to produce two aromatic amines as final products, and one of these aromatic amines is aniline [14]. The peak detected at 250 nm is associated with the presence of aniline, and thus this peak provides evidence of azo bond cleavage forming this compound (Fig. 4b). Both RR2 and aniline are extremely toxic to methanogens at concentrations even lower than those used here [3,36], and its presence has caused the collapse of high-rate anaerobic bioreactors [14]. Here, however, enhanced RR2 reduction and aniline production occurred due to AQDS immobilization on the anode along with current generation.

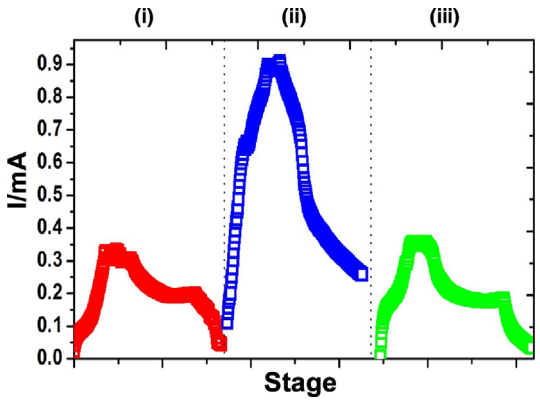
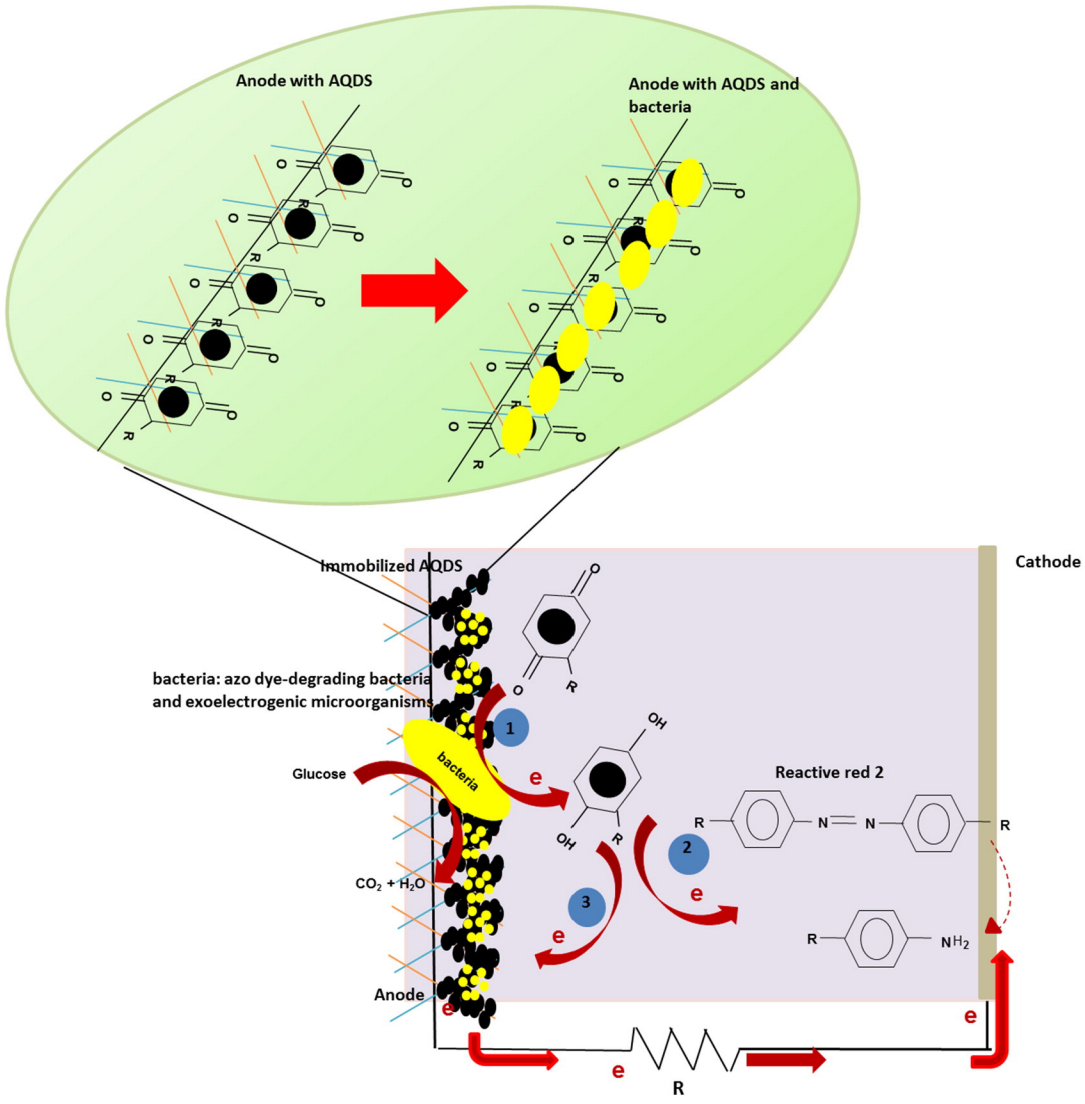


Fig. 5. Exploration of the toxic effects of RR2 and its reduction products to exoelectrogens, using the original control (no AQDS addition) (all tests with 1 g L^{-1} of glucose). Stage (i) shows current during the recovery stage when RR2 was no longer added. Stage (ii) shows current generation following re-inoculation of the reactor with exoelectrogenic microorganisms, demonstrating that recovery of current generation required a fresh inoculum. In Stage (iii), RR2 (0.3 mM) was again added, resulting in a decline of current generation to previous levels.



Scheme 1. Proposed mechanisms for the simultaneous bioelectricity generation and RR2 reduction mediated by immobilized AQDS on the anode surface: (1) AQDS is reduced by the redox component on the cell surface or those released from azo dye-degrading bacteria and exoelectrogenic microorganisms present in the anode; (2) reduced AQDS then transfer the reducing equivalents to the RR2 for its reduction; (3) once RR2 has been reduced, AQDS transfers electrons to the anode to generate electricity. (Black larger ovals are AQDS; small yellow circles are bacteria; not to scale).

During dye decolorization, the voltage in the control (without AQDS) and AQDS solution (AQDSs) could decrease due the competition for electrons by exoelectrogenic microorganisms on the anode or activity repression of exoelectrogenic microorganisms because of the toxicity of RR2 [37–38]. Therefore, three consecutive experimental conditions were tested using the Control lacking AQDS after 6 months of its continuous operation, to determine if a lower current generation was due to toxic effects of RR2 and its reduction products. Fig. 5 shows the changes of current output at all the experimental stages. During the first stage (i), when RR2 was removed the current increased only slightly from 0.35 ± 0.02 mA to 0.4 ± 0.02 mA (Fig. 3b, Table 1). During the second stage (ii), once exoelectrogenic microorganisms were re-inoculated into MFC, the current output recovered (0.9 ± 0.04 mA) to a level similar to that obtained prior to RR2 addition (0.4 ± 0.02 mA). In contrast to this result, during the stage (iii), when RR2 was again added to this reactor, the current output decreased to 0.3 ± 0.03 mA after five fed-batch cycles. It can be deduced from these results that the RR2 or its reduction products were toxic to the exoelectrogenic microorganisms, and that the toxic effects were irreversible due to the need to re-innoculate the MFC. This conclusion relative to the toxicity of the RR2 and its products is consistent with previous reports showing the toxicity of RR2 to other anaerobic consortia such as high-rate anaerobic bioreactors [14]. The use of the AQDS on the anode likely resulted in quinone-reducing microorganisms on the anode that helped to mitigate the inhibitory effects of RR2 by increasing the rate and extent of RR2 reduction [23].

3.5. Mechanisms of RR2 and electricity generation in MFCs

The decolorization of azo dye has previously been attributed to mainly to the suspended biomass while electricity generation is usually associated with the anodic biofilm [20,31]. An inadequate supply of electrons will result in a slow azo reduction and lower current generation. Theoretically 4 mol e^- are necessary to reduce 1 mol of RR2. Therefore, to reduce 0.3 mmol of RR2 (total moles added to the reactor), 1.2 mmol e^- are needed. The amount of AQDS added (1.21 mmol) provided 2.42 mmol e^-, therefore sufficient electron donor was provided. Thus, the lack of an impact of the AQDS on the PVA particles suggests that this was not due to a lack of electron donor in the PVA particles. It is more likely that the AQDS embedded within the PVA material could not be readily accessed by the microorganisms.

Previous investigations suggested that one drawback of immobilization of RMs on the anode surface was that the mediators could not

readily be used by suspended bacteria (azo dye-degrading bacteria), which would result in a lack of improvement in decolorization of azo dye, but enhanced electricity generation due to the availability of RMs to anode-attached bacteria [20]. The results obtained here, however, do not support that view. The RR2 removal was improved with mediators on the anode, suggesting that azo dye-degrading bacteria and exoelectrogenic microorganisms were both able to reduce AQDS immobilized on the anode. There are other reports that support favorable interactions of azo dye-degrading bacteria and exoelectrogenic microorganisms. For example, simultaneous Congo red degradation and bioelectricity generation were obtained in one MFC study [39], where *Azospirillum*, *Methylobacterium*, *Rhodobacter*, *Desulfovibrio*, *Trichococcus*, and *Bacteroides* were more abundant in the presence of Congo red than its absence. These bacteria might use aromatic amines as carbon and energy sources for growth under micro-aerobic conditions, or reduce azo dyes [40]. Some *Desulfovibrio* are also capable of generating electricity [41]. In a more recent study, the addition of suspended RMs into an MFC during the reduction of Congo red changed the composition of the anodic microbial community, and stimulated the growth of species belonging to *Chlorobi*, *Endomicrobia and Firmicutes*, members related to heterotrophic and azo dye degraders [39]. Thus, it appears that azo dyes can be degraded by bacteria on the anode, and that the presence of dye degrading microorganism is favorable for electricity generation by exoelectrogens.

The proposed mechanisms for the simultaneous bioelectricity generation and RR2 reduction mediated by immobilized AQDS on the anode surface is shown in Scheme 1. The AQDS electropolymerized on the anode (AQDS/anode) is reduced by the redox enzyme on the cell surface or those released from AQDS, reducing microorganisms (azo dye-degrading bacteria and exoelectrogenic microorganisms) present in the anode biofilm. The reduced AQDS then transfers the reducing equivalents to the RR2 for its reduction or to the anode to generate electricity. In anaerobic digesters, the microbial reduction of the immobilized RM by microorganisms in the anaerobic sludge is the rate-limiting step during the reductive azo reduction of RR2 [42]. The reduction of the immobilized RM depends on the interaction between the fixed catalysts and quinone-reducing microorganisms. Thus, the development of a biofilm of quinone - reducing microorganisms, specifically azo dye-degrading bacteria over the surface of anode, helped to overcome this limitation and improve dye degradation rates.

4. Conclusions

Immobilized AQDS on the anode served as effective solid-phase redox mediator for simultaneous bioelectricity generation and degradation of RR2 dye in air-cathode, single chamber MFCs. The immobilization of AQDS on the electrode surface increased the rate of RR2 reduction and improved conversion of glucose into current compared to controls. The development of a biofilm of quinone reducing microorganisms on the electrode surface was also essential to improve RR2 degradation rates. Electrode modification using RMs opens new windows for exploring practical applications of degradation of recalcitrant pollutants coupled with bioelectricity production using MFCs.

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