EXPLORING MICROBIAL FUEL CELL-ASSISTED BIOREMEDIATION OF TEXTILE DYES: ENERGY CONVERSION

Bor-Yann Chen1*, Bin Xu2, Pei-Lin Yueh1, Ke Han1, Lianjie Qin2, Chuang-Chuan Hsueh1, Yufeng Xia3

1 Department of Chemical and Materials Engineering, National I-Lan University, I-Lan 26047, Taiwan
2 School of Environmental and Materials Engineering, Yan-Tai University, YanTai 264005, China
3 School of Chemical Engineering, Hua-Qiao University, Xiamen 361021, China
*Corresponding Author Email: bychen@niu.edu.tw

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ARTICLE DETAILS

ABSTRACT

Prior studies indicated that -OH and/or –NH2 substituent containing auxochrome compounds (e.g., 2-aminophenol and 1-amino-2-naphthol) could act as electron shuttles (ESs) to stimulate wastewater decolorization and bioelectricity generation in microbial fuel cells (MFCs). This study provided first- attempt to disclose how and why thionine-associated textile dyes (i.e., azure A and azure C) could also own such redox-mediating capabilities in MFCs. Due to the presence of iminium part as mediating group, –N(CH3)2 or –N(CH3) H substituent could effectively mediate electron transport compared to –NH2 substituent for bioelectricity generation in MFCs. For dye-bearing wastewater treatment, the presence of electron-mediating textile dyes (e.g., thionine, azure A and azure C) in MFCs is promising to stimulate biogradation of organics and bioelectricity generation. With such ESs as stimulants, using MFC as operation strategy would be cost-effective for wastewater treatment as oxidation of organic pollutants could be automatically accelerated.

KEYWORDS

Electron shuttles, textile dyes, bioelectricity generation, microbial fuel cells.

1. INTRODUCTION

As treatment of wastewater containing myriads of textile dyes through conventional methods still had disadvantages, remediation alternatives of reducing operation cost and promoting process efficiency gradually became more attractive than ever [1]. Moreover, global warming and spiraling energy prices have significantly affected international economy and national security [2]. Therefore, exploring renewable green energy for sustainable development is inevitably of great importance to reduce the dependence on imported fossil fuels. As a matter of fact, industrial wastewater usually contains myriads of organic matter and apparently it can be utilized as a fuel source for energy recycling. In fact, among all renewable green energy, biomass-based energy would be more environmentally-friendly. For example, microbial fuel cells (MFCs) are novel bioelectrical devices that can directly transform chemical energy from oxidation of organics to bioelectricity via a series of electrochemical reactions catalyzed by microbes [3].

For augmentation of bioelectricity-generating capability of MFCs, one of the most intriguing alternatives prevailing recently was exogenous supplementation of electron shuttles (ESs) with low toxicity potency. In fact, ESs (or redox mediators) are organic molecules that can reversibly be oxidized and reduced to serve as electron carriers among multiple redox reactions. Thus, redox mediators could enhance the efficiency of dye decolorization as the primary electron donor to decolorization of azo dye. With ESs, electron flux in MFCs can be augmented to achieve higher bioelectricity-generating capability. In particular, due to chemical structure effect as mentioned elsewhere, the presence of functional groups in the proximity of redox mediators directly affected the performance of bioelectricity generation as well as reductive decolorization [4]. For example, the stronger electron withdrawing group (e.g., -SO3Na) near azo bond(s) could effectively assist decolorization performance (e.g., methyl orange) to be taken place. In addition, -SO3Na showed better capability to pull electron(s) towards azo bond(s) than -COONa, it significantly attenuated electron density near azo bond and therefore facilitated color removal.

However, prior study also pointed out that decolorized intermediates with different functional substituents resulted in significant difference in biotoxicity potency as well electron-shuttling characteristics [5]. For instance, hydroxyl (-OH) and amino (-NH2)- group bearing aromatics (e.g., 2-aminophenol (2AP), benzene-1,2- diaminobenzene (b12d), 1,2-diaminobenzene (12db), were all found to be promising redox mediators due to low toxicity potency to bioelectricity-generating bacteria [6-8]. These all suggested that electron- shuttling capabilities and biotoxicity potency of candidate mediators were strongly due to chemical structures. Regarding this perspective, this study selected non-azo textile dyes with different substituents (e.g., -NH2, -NH(CH3), -N(CH3)2) as models for comparative analysis upon their redox- mediating capabilities.

Moreover, this study extended to explore whether non-azo textile dyes or derived intermediates can also act as redox mediators in MFCs. In fact, some researchers mentioned that both methylene blue and neutral red could act as redox mediators to enhance power-generating capabilities of Escherichia coli and Actinobacillus succinogenes- bearing MFCs [9,10]. Regarding non-azo textile dyes, thionine is a strongly staining metachromatic dye widely used for biological staining and a well-known
electron-shuttling mediator for bioelectricity generation \[11,12\]. Although thionine-related compounds chemically own capabilities to mediate electron transport, whether they are also biochemically-feasible to enhance bioelectricity generation of electrochemically active microbes in MFCs is still remained open to be explored \[13\]. For example, azure A is a phenothiazine dye that is chemically formed by oxidation of methylene blue.

That is wastewater decolorization might form intermediate(s) to accelerate dye bioremediation due to autocatalysis. This work chose thionine, azure A and azure C as candidate mediators of textile dyes to disclose the mysteries for industrial applications. Of course, exogenous supplementation of textile dyes to wastewater during practical treatment will not be allowed. However, if such textile dyes (e.g., thionine, azure A or azure C) originally present in dye-bearing wastewater, using MFC as operation strategy would be more promising for color removal due to mediator stimulation. In addition, as textile dye(s) may play as electron shuttles, fed-batch or continuous modes of MFC operation inevitably are more appropriate than batch mode of operation for cost-effective biodegradation of textile dye(s) via energy conversion.

2. MATERIALS AND METHODOLOGY

2.1 MFC Construction

Membrane-free air cathode single-chamber MFCs using seed strains Proteus haureri ZMd44, Klebsiella pneumoniae Zmd31 and Aeromomas hydrophila NIU01 were constructed in cylindrical tubes made by polymethyl methacrylate (PMMA) (cell sizing ID = 54 mm, L = 95 mm) with the operating volume of ca. 220 mL (refer to prior study-JTICE 41 (2010) 682–688 for schematic configuration). Porous carbon cloth (Ctech™) (without waterproofing or catalyst) with a projected area of ca.22.9 cm² (i.e., π/2.72) on one side were used as anode electrodes. The air cathode sized almost identical to the anode consisted of a polytetrafluoroethylene (PTFE) diffusion layer (Ctech™) on the air-facing side.

2.2 Inhibition Inspection via Respirometry

Toxicity effects of test ESs on cellular respiratory activity associated to microbial cell viability were inspected individually using automated Columbus Micro-Oxymax Respirometer equipped with CO₂ sensors. The measurement relied on the circulation of air through closed-system testing bottles whereas the liquid in bottles remain static (i.e., mobile liquid- flowing gas respirometer) \[14\]. As cumulative amount of CO₂ present in blank bottle was nearly negligible (ca. 1-2 mg) during testing different time courses of CO₂ production were simply due to the absence or presence of existing toxicant(s) in cultures. For respirometric experiments, a loopful of test microbes (e.g., P. haureri ZMd44) seed taken from an isolated colony on an LB streak plate was precultured in 50 mL Bacto LB culture (Miller (Luria–Bertani) (per liter; 10 g Bacto tryptone, 5 g Bacto yeast extract, 10 g sodium chloride) for 12 h (i.e., late exponential growth phase) at 30°C, 125 rpm using a water bath shaker (SHINKWANG, SKW-12). Then, 0.5 mL precultured broth was inoculated into 50 mL bottles containing fresh LB broth and test textile dyes at different concentrations for respirometric experiments at 30°C, 110 rpm. The calibration of respirometer was conducted by using standard CO₂(g) at 0.5% prior to experiments. Note that all MFCs were operated in membrane-less single chamber.

2.3 Cyclic Voltammetric determination

Cyclic voltammetry of candidate mediators (e.g., thionine, malachite green) was performed using an electrochemical workstation (Jiehan 5600, Taiwan) at 10 mV s⁻¹ scan rate. The working, counter, and reference electrodes were a glassy carbon electrode (0.07 cm²), platinum electrode (6.08 cm²), and a Hg/HgCl₂ electrode filled with saturated KCl(aq), respectively. The glassy carbon electrode (GCE, ID = 3 mm; model CH1104, CH instruments Inc., USA) was successively polished with 0.05 μm alumina polish and then rinsed with 0.5 MH₂SO₄ and deionized water prior to use. Experiments were performed in phosphate buffer solutions (PBS; pH = 7.0) at 0.1M and the solutions were purged with nitrogen for 15 min prior to analysis. The scanning rate was 10 mV s⁻¹ over the range from 0.4 to -0.6 V. The redox potentials recorded as Hg/HgCl₂ reference electrode were corrected by 0.241 V (i.e., E₀ of Hg/HgCl₂) to the standard hydrogen electrode (SHE).

2.4 Electrochemical Measurement

For comparative analysis upon prior studies, electrochemical impedance spectroscopy (EIS) (HIOKI 3522-50, Japan) measurement was conducted on steady-state open circuit potential distributed with an amplitude of 10 mV at the frequency range of 104–5×10⁻3 Hz \[15\]. Collected data were analyzed using the software for Nyquist plot (Zview 2.6b, Jiehan Tech.). Regarding stable power generation measurement, cell voltage was automatically measured (set 1 data point per minute) using a data acquisition system (DAS 5020; Jehan Technology Corp.) via external resistance Rout=1KΩ to compare with prior results \[15\]. The power densities (P) and current densities (I) were determined using linear sweep voltammetry (LSV) measurement and corresponding voltages were recorded by using a multimeter.

3. RESULTS AND DISCUSSION

According to Equation (1), the rankings of biotoxicity potency of thionine-associated chemicals (in mg L⁻¹) were 0.2 × LB (blank) < azure A (40) < azure C (40) < thionine (40) < azure A (120) < azure C (120) < thionine (120) (Figure 1). Apparently, respirometric profiles suggested that the presence of such textile dyes expressed biotoxicity potency to microbial cells in MFCs. In addition, mediator present in relatively less toxic doses could stimulate higher bioelectricity-generating capabilities in MFCs. That is, the promising concentration of redox mediator to enhance power-generation of MFCs should be below toxic threshold to maximize biomass-based energy production.

3.1 Dose-Response Assessment

Figure 1: Cumulative CO₂ production for bacteria ZMd31, ZMd44, NIU01 during the course of toxicity tests with thionine, azure A and azure C (accumulated at 30 h) calculated CO₂ [T data after \[16\]. Since bioelectricity-generating capability of MFC depends not only upon electrochemical potential, but also biotoxicity potency of existing chemicals, dose-response evaluation of three chemicals to microbes in MFCs needed to be first inspected \[14\]. To clarify the toxicity potency to different bacteria, 40 and 120 mg L⁻¹ thionine, azure A and azure C were supplemented to bacterial cultures for bio- respirometric assessment. According to time-series profiles of CO₂ respirometric data. The relationship of CO₂ accumulation can be presented as

\[
m_{\text{CO}_2}(t) = m_{\text{CO}_2}(T) \cdot e^{(-\alpha t)}
\]

where m_{\text{CO}_2} was cumulative amount of CO₂ at time t, m_{\text{CO}_2} [T was saturated cumulative amount (at t→∞) of CO₂, and α was decay rate constant for CO₂ production \[17\].

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**Power Generation Analysis**

![Cyclic voltammograms for redox processes of thionine (A), azure C (B) and azure A (C) in 0.1 M phosphate-buffered solution (PBS) at pH 7.0 (scan rate = 10 mV s⁻¹; data modified from [16]).](image)

**Figure 2**

To determine whether model dyes (i.e., thionine, azure C and azure A) own capabilities to enhance bioelectricity generation in MFCs, cyclic voltammograms (CVs) of these compounds were implemented for feasibility study in 0.1 M phosphate-buffered solution (PBS) at neutral pH (Figure 2). Cyclic voltammetry profiles clearly showed that three chemicals all could perform such redox-mediating characteristics as electron shuttles (ESs).

Aware that ESs can mediate electron transfer in quasi-reversibility for augmentation of electron flux in fuel cells. According to cyclic voltammetry, the forward scan could produce specific current peaks for augmentation of electron flux in fuel cells. As shown in Figure 2, under identical scan conditions, azure A could produce the highest peak current, then azure C and thionine (i.e., azure A > azure C > thionine).

![Comparison of power density of ZMd31-seeded, ZMd44-seeded and NIU01-seeded MFC supplemented with 40 mg L⁻¹ thionine, azure C and azure A (Blank MFC without mediator supplementation) (original data after [16]).](image)

**Figure 3**

To disclose whether thionine-related textile dyes could act as exogenous ESs, comparative analysis upon power density-curves based on polarization data of MFCs supplemented with test dyes were conducted (Figure 3). As a matter of fact, when 40 mg L⁻¹ thionine, azure A and azure C were supplemented to Klebsiella pneumoniae ZMd31-seeded MFC, the power-generating capability increased from 35.68±1.47 to 59.18±0.87 mW m⁻² (ca. 165.9% increase), from 35.68±1.47 to 68.27±3.53 mW m⁻² (ca. 191.3% increase) and from 35.68±1.47 to 148.55±3.81 mW m⁻² (ca. 416.3% increase), respectively. Similarly, regarding P. hauseri ZMd44-seeded MFC, the power-generating potential increased from 70.70±0.79 to 83.39±0.28 mW m⁻² (ca. 117.9% increase) at 40 mg L⁻¹ thionine supplemented. Moreover, for 40 mg L⁻¹ azure C and azure A added, the power-generating efficiency increased from 70.70±0.79 to 101.11±4.74 mW m⁻² (ca. 143.1% increase) and 123.33±0.54 mW m⁻² (ca. 174.4% increase), respectively. In addition, for ZMn31-seeded MFC, the power-generating efficiency increased from 28.04±0.23 to 36.06±3.04 mW m⁻² (ca. 128.6% increase) at 40 mg L⁻¹ thionine supplemented.

Moreover, for 40 mg L⁻¹ azure C and azure A supplemented, the power-generating efficiency increased from 28.04±0.23 to 66.11±2.58 mW m⁻² (235.9% increase) and 111.79±1.65 mW m⁻² (398.7% increase), respectively. To confirm data reproducibility, experiments at least in duplicate (i.e., replicate (I) and (II) in Figure 3) were implemented to confirm such stimulating characteristics of three test chemicals. In summary, apparently these thionine-based textile dyes could effectively stimulate electrochemically functioning anodic biofilm in MFC to perform excellent efficiencies of bioelectricity generation. Evidently, compared to thionine, azure C and azure A seemed to be more promising to be an electron-shuttling mediator to enhance power generation as revealed in Figure 3.

As aforementioned, redox mediator can enhance electron transfer, Park and Zeikus also demonstrated that electricity generation in a glucose-fed MFC could be enhanced by about 10-fold than mediator-free MFC when neutral red (a redox mediator) was added [10, 18]. According to our results, the electrochemical capabilities of azure A was better than azure C and thionine. This could be explained by the characteristics of their redox potentials and chemical structures.

As revealed in Figure 2, the redox potential $E'_0$ can be calculated as:

$$E'_0 = \frac{1}{2}(E_{pa} + E_{pc}) \quad \text{(vs. SCE)}$$

where $E_{pa}$ and $E_{pc}$ were peak potentials at reduction and oxidation, respectively. The $E'_0$ of thionine, azure C and azure A were -0.125V, -0.170V and -0.195V, respectively. Theoretically, the $E'_0$ of mediator should lie between primary electron donor and final electron acceptor, thus electron can be transferred by mediator. The redox potential of NADH is ~0.32V (vs. NHE), which sets to the limits of redox mediator’s application. That is, the redox potential of mediator should be higher than ~0.32V to extract electrons from NADH [3]. In order to maximize the power output of the MFC, the redox potential of the mediator should be as low as possible compared to redox potential of NADH. The more negative potential of azure A suggested that it was easier to be oxidized (refer to Campbell and Farrell’s “Biochemistry” Chapter 20, 5th Ed.). These all supported that azure A could easily shuttle electrons to the anode for effective power generation as shown herein.

### 3.2 Electrochemical Impedance Evaluation

To uncover that thionine-based textile dyes could play as electron-shuttling mediators in MFCs, electrochemical impedance spectroscopy (EIS) data measurements were also carried out to compare the resistance characteristics before and after supplementation of test dyes to MFCs (Figure 4). For comparison, Nyquist-plot analyses were implemented for ZMd31-seeded, ZMd44-seeded, and NIU01-seeded MFCs with supplementation of dyes at 40 mg L⁻¹. Apparently, total internal resistance $R_i$ (i.e., Total $R$=Relec+$R$in+$R$diff) and $R$in+$R$diff (i.e., kinetic and diffusion resistance; [2]) decreased with appropriate supplementation of thionine, azure C and azure A (Figure 4). That was in consistent with prior findings, revealing that supplemented appropriate
concentrations of electron shuttles (ESs) could gradually decreased Total Rin and Rkin + Rdif [7]. Moreover, higher electron transfer efficiencies of azure A and azure C were very likely due to the increased flux of electron transfer mediated by thionine-associated dyes from microbes to electrode and proteomically expressed microbes in biofilms on the anode (Figure 4). These pointed out that these three dye could all play crucial roles as ESs to skyrocket efficiencies of bioelectricity-generation in MFC as anticipated [17, 20].

Figure 4: Comparison of various resistances from Nyquist plots of electrochemical impedance spectra by ZMd31-seeded, ZMd44-seeded, and NIU01-seeded MFCs bearing supplemented concentration at zero (Blank), thionine, azure C and azure A (40 mg L-1) (Relec, Rkin+Rdiff and Total Rin denoted Electrolyte Resistance, Kinetic and Diffusion Resistance and total internal resistance, respectively) (original data after [16]).

3.3 Mechanisms of Electron-Shuttling mediators

As aforementioned, apparently thionine, azure C and azure A all expressed electron-shuttling capabilities and could be applied to MFCs for enhancement of bioelectricity generation. As matter of fact, redox mediators can be reversibly inter-converted between their reduced and oxidized form(s) for mediating electron-transfer in electricity generation. Thus, the current is controlled by both charge transfer and mass transport [21]. Theoretical description of Butler–Volmer equation and Cottrell equation could be used to indicate an n electron process as:

\[ |E_{pc} - E_{pa}| = \frac{57 \text{ mV}}{n} \]  

where \(E_{pc}\) and \(E_{pa}\) were peak potentials at reduction and oxidation, respectively. As the ratio of the peak currents passed at reduction (\(i_{pc}\)) and oxidation (\(i_{pa}\)) is near unity, these test dyes were thus electron-shuttling mediators due to the presence of reversible couples as anticipated (refer to http://en.wikipedia.org/wiki/Cyclic_voltammetry). As shown in Table 1, azure A (\(n \approx 1.90\)) owned higher capability to transfer electrons than azure C (\(n \approx 0.95\)) and thionine (\(n \approx 1.16\)), suggesting the most promising electron-shuttling potential of azure A. Note that such an electron-transferring capability is also strongly dependent upon operation conditions of test medium (e.g., different results for strongly acidic at 0.1 M H2SO4 and neutral pH PBS condition herein) [22,23]. However, the mysteries of higher capability of electron transfer for azure A were still remained open for the follow-up inspection.

Figure 5: Cyclic voltammograms for (a) thionine and (b) azure C at different scan rate; (c) Linear relationship of peak currents vs. (scan rate)1/2 (data after [16]).

Table 1: List of redox peak potential and parameters for electron transfer in cyclic voltammograms (data modified after [16]).

| Test ES | \(E_{pa}\)(mV) | \(E_{pc}\)(mV) | \(|E_{pc} - E_{pa}|\)(mV) | Number of electrons transferred \(n\) |
|---------|----------------|----------------|--------------------------|-----------------------------------|
| thionine | -149.2          | -99.9          | 49.3                     | 1.16                              |
| azure C  | -199.9          | -139.9         | 60.0                     | 0.95                              |
| azure A  | -209.9          | -179.9         | 30.0                     | 1.90                              |

a. Absolute potential of the oxidation peak
b. Absolute potential of the reduction peak
c. \(n = 57 \text{ mV} / |E_{pc}-E_{pa}|\) for an n electron process

For thionine and azure C, electron-transfer capabilities were nearly identical (i.e., \(n \approx 1\)). That is, mass diffusion would be more significant. However, molecular weights of both chemicals are nearly identical (i.e., thionine [263.75] and azure C [277.77]), pure molecular diffusion would be negligible. Therefore, to compare the effect of overall diffusion, cyclic voltammetry could be used. As literature [22, 23] mentioned, Randles–Sevcik equation could be used to describe the effect of scan rate on the peak current \(i_p\) in cyclic voltammetry:

\[ i_p = 2.69 \times 10^{5} n^{3/2} A D^{1/2} C^{1/2} v^{1/2} \]  

where \(i_p\) (amps), \(A\) (cm2), \(D\) (cm2/s), \(C_0\) (mole/cm3), \(v\) (V/s) and \(n\) denoted the peak current, electrode area, diffusion coefficient, concentration, scan rate and number of electrons transferred, respectively.
At nearly identical values of C0, A and n, the ratio of peak current ip and square root of scan rate v1/2 (i.e., ip/v1/2) is proportional to the diffusion coefficient D.

As indicated in Figure 5(a)-(c), the ratio of both diffusion coefficients could be determined via linear plots of ip vs. v1/2 with actual values of n and C0 (i.e., Dazure C + Dazure A=19.29). Evidently, redox mediation could cause major structural changes in the analyte for diffusion. Here, when –H was replaced by –CH3, hydrogen bonding of redox intermediate(s) and water molecules could be significantly attenuated and thus the diffusion of azure A was more kinetically favorable.

Moreover, as indicated in Figure 6, for different mediators supplemented to NIU01-seeded MFC, the response time to achieve 300 mA m−2 for blank, thionine, azure C and azure A were ca 353, 210, 91 and 47 s, respectively. This finding also confirmed the results as aforementioned in Figure 3, 4 regarding the ranking of these chemicals.

According to the proposed electron-shuttling mechanism, thionine, azure A and azure C could act as electron shuttles due to reversibility reduced and oxidized states as shown in forms (1) and (2) in Figure 7. Using different bacteria-bearing MFCs, the ranking of electron-shuttling capabilities of thionine, azure C and azure A were azure A > azure C > thionine (Table 2). The finding could be explained by the reasons as follows: When electron shuttles were oxidized, the positive charge formed on sulfur as indicated in form (3) (Figure 7) would be delocalized on the carbons (i.e., forms (4) and (5)) and nitrogen (i.e., form (6)) via resonance to reversibly interconvert to more stable intermediates as indicated in forms (4)-(6) (Figure 7). In particular, the oxidized intermediate with the positive charge on nitrogen of the iminium part (C=N+R1R2) (i.e., form (6) in Figure 7) would be significantly stabilized as more methyl group(s) were attached to nitrogen through inductive effect and hyperconjugation effect [24]. That is, when nitrogen had more positive charges, nitrogen would intensely attract electron density from methyl group toward itself by inductive effect to stabilize such an unstable electron-deficient condition. Moreover, the oxidized intermediate having positive charge nitrogen (i.e., with a vacant p orbital) attached with more highly substituted environment would also be more stable by hyperconjugation which is stabilized among a vacant p-orbital and CH s-orbital bonds on neighboring carbons (i.e., those methyl groups attaching to nitrogen).

When the oxidized intermediates of electron shuttles are more stable, Arrhenius activation energy to trigger the formation of such oxidized intermediates for electron-mediating reaction would be lower, and thus redox reaction of electron shuttles would apparently be faster. That is, the electron-transfer rate of supplementing more highly substituted nitrogen (i.e., azure A) to MFCs would be faster due to lower activation energy barrier for energy extraction (Table 2).

**Figure 6:** Time courses of current densities for NIU01-seeded MFC supplemented with different dye mediators (data after [16]).

**3.4 Comparison of Substituent-Affected Mediating Capabilities**

According to the proposed electron-shuttling mechanism, thionine, azure A and azure C could act as electron shuttles due to reversibility reduced and oxidized states as shown in forms (1) and (2) in Figure 7. Using different bacteria-bearing MFCs, the ranking of electron-shuttling capabilities of thionine, azure C and azure A were azure A > azure C > thionine (Table 2). The finding could be explained by the reasons as follows: When electron shuttles were oxidized, the positive charge formed on sulfur as indicated in form (3) (Figure 7) would be delocalized on the carbons (i.e., forms (4) and (5)) and nitrogen (i.e., form (6)) via resonance to reversibly interconvert to more stable intermediates as indicated in forms (4)-(6) (Figure 7). In particular, the oxidized intermediate with the positive charge on nitrogen of the iminium part (C=N+R1R2) (i.e., form (6) in Figure 7) would be significantly stabilized as more methyl group(s) were attached to nitrogen through inductive effect and hyperconjugation effect [24]. That is, when nitrogen had more positive charges, nitrogen would intensely attract electron density from methyl group toward itself by inductive effect to stabilize such an unstable electron-deficient condition. Moreover, the oxidized intermediate having positive charge nitrogen (i.e., with a vacant p orbital) attached with more highly substituted environment would also be more stable by hyperconjugation which is stabilized among a vacant p-orbital and CH s-orbital bonds on neighboring carbons (i.e., those methyl groups attaching to nitrogen).

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**Figure 7:** Scheme of reversible electron shuttling of thionine, azure C and azure A through resonance effects of positive charge transfer among various oxidized forms [16].

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<tr>
<th>Iminium part*</th>
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<td>R1=CH3, R2=CH3</td>
<td>High</td>
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*Iminium part:

4. CONCLUSION

This study revealed that model thionine-based textile dyes (e.g., thionine, azure C and azure A) could act as electron-shuttling mediator(s) to enhance capabilities of reductive decolorization and bioelectricity generation. Apparently, –N(CH3)2 or –N(CH3)3 H substituent could perform better shuttling capabilities than –NH2 substituent to mediate electron transport in MFC. In addition, similar chemicals with –N(CH3)2 or –N(CH3)3 H substituent are very likely to have promising electron-mediating capabilities of azure A and azure C. Follow-up studies on efficiencies of various ISs for electron transfer will be implemented for system optimization.

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REFERENCES


