A direct approach for enhancing the performance of a microbial electrolysis cell (MEC) combined anaerobic reactor by dosing ferric iron: Enrichment and isolation of Fe(III) reducing bacteria

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HIGHLIGHTS

- A new approach was developed to enrich exoelectrogens via dosing Fe(III) in MEC-anaerobic reactor.
- A novel Aeromonas hydrophila was isolated from the anodic biofilm of MEC-anaerobic reactor.
- The isolated strain was capable of Fe(III) reduction, decolorization and electro-activity.

Abstract

Enrichment of microbial functional consortium is critical to strengthen the performance of bio-electrochemical devices for treating industrial wastewaters. This study described a newly enrichment approach for electrochemically active iron reducing bacteria (IRB) through dosing Fe(III) into a MEC combined anaerobic reactor (R1) for dye wastewater treatment. After 51 days operation, reactor R1 presented the highest performance for the degradation of organic matter and dye as compared to the reference reactors without Fe(III). Subsequently, five isolates were obtained from the anodic biofilm of R1, in which a novel IRB related to Aeromonas hydrophila was selected as a model strain due to its highest Fe(III) reducing ability. Cyclic voltammetry and microbial fuel cell (MFC) technology showed that the model strain has the electrochemical activity and electricity generation capability. After inoculating this model strain, the decolorization of three dyes also reached over 90% at an initial concentration of 100 mg/L. This study might provide a novel method to enrich electrochemically active IRB in the bio-electrochemical reactor for treating industrial wastewater.

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1. Introduction

A Microbial electrolysis cell (MEC) as a modified microbial fuel cell (MFC) is a promising technology for the degradation of organic pollutants and hydrogen production [1-3]. In MECs, electro-active bacteria on the anodic biofilm transfer electrons to anode by oxidizing the organic substrate, and electrons combine with protons to form hydrogen at the cathode [4].

The performance of MEC/MFC is directly related to the enrichment of exoelectrogens that were usually found in the anode biofilm [5,6]. Most of exoelectrogens such as Geobacter and Shewanella belong to iron reducing bacteria (IRB) which are capable of reducing Fe(III) via iron respiration approach [7,8]. The electron transfer mechanisms of Fe(III) reduction by IRB are similar to that between exoelectrogens and electrode surface in terms of direct electron transfer via outer membrane cytochrome [9,10] and indirect electron transfer via electron shuttling mediators [11,12]. Therefore, it is believed that some microorganisms with iron respiration ability might have potential electrochemical activity.

Different inoculation conditions make the enriched microbes diverse in MEC. The controlling conditions in terms of appropriate electron donor (i.e. culture-medium), electron acceptor (such as Fe(III)) or anodic material are usually used to enrich and isolate specific exoelectrogens. It was reported that the outer membrane c-type cytochromes of IRB had a high binding affinity to Fe(III) oxide which could be utilized as electron shuttle to reduce distant terminal acceptors [13,14]. Based on the characteristics of Fe(III) oxides, Fe2O3-modified electrode was used as an anode of MFC so as to improve the electricity production significantly as compared to the bare anode [15]. However, the preparation of Fe2O3-modified electrode has technical and economical limitations in
practice. Through incubating anodic biofilm of MEC in an Fe(III) – acetate medium, the enriched exoelectrogens was dosed into a MFC by which 15% of powder densities were improved [16]. It indicated that Fe(III) oxides was efficient for enriching exoelectrogens and further improving the performance of MFC/MEC. Therefore, we speculated that a direct selective strategy via adding Fe(III) oxides in a bio-electrochemical reactor might have a greater potential for enriching Fe(III)-reducing exoelectrogens.

In this study, Fe(OH)₃ power was dosed into a MEC-anaerobic reactor with the aim to enrich Fe(III)-reducing exoelectrogens selectively and directly. To date, there are few reports focus on the method to enrich Fe(III)-reducing exoelectrogens via dosing Fe(III) in a bio-electrochemical reactor. So it is expected to be a novel and useful approach to enrich more electroactive microbes during wastewater treatment. In addition, a novel electrochemically active IRB belong to genus Aeromonas hydrophila was reported and isolated from the anodic biofilm of the MEC-anaerobic reactor. Effects of different factors on the Fe(III) reduction and decolorization and current production ability of this A. hydrophila sp. were investigated.

2. Materials and methods

2.1. The MEC-anaerobic reactor and operation

A pair of carbon felt electrodes (70 mm width × 70 mm length) was inserted into an acrylic plastic up-flow anaerobic blanket reactor (UASB) (280 mm length × 100 mm width × 100 mm height) accompanied with the addition of Fe(OH)₃ powder (30 g, analytical reagent) (hereafter referred to as R1). The working volume of the reactor was 2 L. 30 g Solid Fe(OH)₃ powder is dosed into the 2 L anaerobic reactor in one time. Carbon felt electrodes consist mainly of carbon fiber (carbon content ≥ 95%). The electrodes were supplied by a regulated DC power source, connected in series with a 20 Ω. The control reactor R2 was the same as R1 but without Fe(OH)₃ dosing. Another control reactor R3 was same as R2 but without electrodes.

After being seeded, these three reactors were operated in parallel under a continuous mode at 35 ± 1 °C with a hydraulic retention time (HRT) of 24 h. Synthetic wastewater containing azo dye Reactive brilliant red X-3B (X-3B in abbreviation) was used in this study. Sucrose, NH₄Cl and KH₂PO₄ were added into the azo dye wastewater as the carbon, nitrogen, and phosphorus sources, respectively, to give a COD:N:P ratio of 200:5:1. The trace elements were added according to the following composition: 1 ml/L of a trace element solution containing Zn at 0.37 mmol/L, Mn at 2.5 mmol/L, Cu at 0.14 mmol/L, Co at 8.4 mmol/L, Ni at 0.25 mmol/L, H₂BO₃ at 0.8 mmol/L and EDTA at 3.4 mmol/L. The pH of the influent wastewater was adjusted to 7.5 using NaHCO₃ solution. Seed sludge was obtained from an UASB reactor in our laboratory. The seed sludge had a mixed liquid suspended solids (MLSS) concentration of 15.2 g/L with MLVSS (mixed liquid volatile suspended substances)/MLSS ratio of 0.69.

During the operation, the influent COD and dye concentration were maintained at a fixed value of 2000 and 400 mg/L respectively. At the beginning of the experiment, 30 g Fe(OH)₃ powder was dosed into the reactor R1 once a time. A voltage of 0.6 V was applied to R1 and R2 during 51 days’ operation. The working voltage is significantly lower than the theoretical value of electrolysis of water (1.23 V).

2.2. Isolation and characterization of IRB

The composition of Fe(III) reducing medium used for isolating IRB was as follows: per 1 L solution containing NaAc 24.4 mmol, Na₂HPO₄ 42.3 mmol, KH₂PO₄ 22.0 mmol, NaCl 8.6 mmol, NH₄Cl 18.7 mmol, yeast extract 2.0 g, Wolfe’s vitamin solution 10.0 ml and Wolfe’s mineral solution 10 ml. Fe(III) resource was provided in the form of ferric citrate at 2 mmol of Fe(III) per liter in isolation and 8 mmol in Fe(III) reduction experiments. The final pH of Fe(III) reducing medium was adjusted to 6.9. 50% Luria–Bertani (LB) medium with the pH of 7.2 was used for isolate cultivation under anaerobic condition, which contained (g/L): 5 tryptone, 2.5 yeast extract, 2.5 NaCl. Both mediums were aerated in anaerobic atmosphere of N₂ (100%) for 30 min to remove dissolved oxygen. Before inoculation, both medium were autoclaved at 121 °C for 20 min. The strain was cultivated at 30 °C in both medium.

After 51 days’ operation, anodic biofilm sample was collected from the anode of R1. After 2 months of enrichment and screening, a strain named as XB showed the highest Fe(III) reducing ability among the five isolated strains which was chosen and tested in this study. The genomic DNA of strain XB was extracted using an extraction kit (Biotek Corporation, Beijing, China) according to the manufacturer’s instructions. The 16S rRNA genes were amplified by PCR using a pair of bacterial primers 341F and 901R. The sequence of PCR product was done by TaKaRa Biotechnology Co., Ltd. (Dalian, China) and the sequences were screened against the GenBank database using the BLASTn program to identify the most similar sequences. Then they were aligned by CLASTALX 1.83 and construct phylogenetic trees using the neighbor-joining method by MEGA 3.1.

The growth curve of XB was recorded (at 600 nm absorbance) after inoculating it in 100 ml serum bottles containing 50% LB medium at 30 °C in the dark. The cells of XB were stored frozen before usage. Fe(III) reduction experiments of XB were performed in a 10 ml glass bottle with a screw-top. After activated in the 50% LB medium, the cells (6% w/w) of XB were inoculated into Fe(III) reducing medium (with OD₆₀₀ value of 0.4) at 30 °C in the dark and then Fe(II) concentration in the medium was determined. Effects of different pH (5–10), temperature (20–40 °C) and NaCl concentration (0–5%) on Fe(III) reduction were investigated. Three different azo dyes i.e. Methyl Red (C₁₅H₁₄N₂O₃S), Acid orange 7 (C₁₆H₁₄N₂NaO₄S) and Reactive brilliant red X-3B (C₂₆H₂₆C₇N₆Na₂O₃S₂) at a concentration of 100 mg/L were used to investigate the performance of decolorization by XB. The chemical structure of these three dyes was listed in the Table S1.

2.3. Effects of respiratory chain inhibitors on Fe(III) reduction

Rotenone and capsaicin was used as respiratory chain inhibitors according to the description of Woznica et al. [26]. Rotenone and capsaicin were dissolved in 96% ethanol. Effects of respiratory chain inhibitors on Fe(III) reduction were conducted in 10 ml glass bottles containing Fe(III) reducing medium and the above inhibitors, and its control experiments were done in the same medium but without inhibitors. The inhibition of Fe(III) reduction by inhibitors was expressed as the ratio of Fe(II) concentration in the presence of inhibitors to that of control experiments.

2.4. Cyclic voltammetry (CV) and MFC

To investigate the electrochemical activity of strain XB, CV and MFC technology were used. The detailed steps of CV were conducted according to description by Park and Kim [17]. The operation of MFC was described briefly as follows. The MFC consisted of two identical chambers (50 mm × 40 mm × 15 mm) was separated by a cation exchange membrane. Each chambers had a working volume of 250 ml. Both anode and cathode were made of carbon brush (80 mm × 15 mm, surface area 0.76 m²), which were connected in series with a 1000 Ω. The microbial growth medium (pH 7.0) in the anodic chamber contained 0.31 g/L NH₄Cl, 0.13 g/L
KCl, 2.45 g/L NaH₂PO₄, 4.58 g/L Na₂HPO₄, 0.5–1 g/L NaAC, 10 ml/L vitamin solution and 10 ml/L mineral solution. Vitamin solution and mineral solution were provided according to the description by Lovley et al. [11]. The cathode chamber of MFC was operated with potassium ferricyanide (50 mM K₃Fe(CN)₆ in phosphate buffer (50 mM K₂HPO₄); pH 7.5). The operation of MFC was conducted under sterile environment. Prior to inoculation by strain XB, the above medium were aerated by pure N₂ gas and then sterilized by autoclaving at 121°C for 20 min. To operate the MFC, the microorganism growth medium containing NaAC was added to the anode chamber, and the catholyte containing potassium ferricyanide was added to the cathode chamber. The MFC were operated at a fixed temperature of 30°C.

2.5. Analysis

COD, VSS and TSS were determined according to the standard methods [18]. The concentration of Fe(II) ions was measured using an UV spectrophotometer (China) at an absorbance of 510 nm. Dye concentration was determined by measuring the absorbance of the supernatant at 539 nm (Reactive brilliant red X-3B), 484 nm (Acid orange 7) and 430 nm (Methyl Red). The decolorization rate was calculated using the following equation: Decolorization (%) = (A₀-A₁)/A₀ × 100 (1) where A₀ and A₁ represented the initial and final absorbance of the dyes, respectively. The pH was measured using a pH analyzer (Sartorius PB-20, Germany). The morphology of the sludge was observed by a transmission electron microscopy (TEM; JEM-2000EX, Japan). Real-time potential change was recorded using a digital voltmeter that was linked with a computer. All of the experiments were conducted in triplicates.

Denaturing gradient gel electrophoresis (DGGE) analysis was conducted according to the following procedures. Anodic biofilm was taken from the anode of MFC. The genomic DNA of the sample was extracted using an extraction kit (Biatek Corporation, Beijing, China) according to the manufacturer’s instructions. The general primers for bacteria were GM341F-GC, which contained a 40-bp GC clamp (forward primer) and DS907R (reverse primer) [19]. The detailed steps of DGGE analysis were conducted according to our previous report [20]. Dominant DGGE bands were excised and re-amplified by PCR using the primers described above without the GC clamp. The PCR products were then sequenced by TaKaRa Biotechnology Co., Ltd. (Dalian, China) and the sequences were screened against the GenBank database using the BLASTn program to identify the most similar sequences.

3. Results and discussion

3.1. Effects of Fe(III) on the performance of a MEC-anerobic reactor

The addition of Fe(III) was favorable for the enrichment of IRB that could reduce Fe(III) by oxidizing organic matters through anaerobic respiratory metabolism [21]. Most of IRB e.g. Geobacter [17] and Shewanella [22] were able to convey electrons to anode via nano-wire and outer-membrane cytochrome, playing a key role in the performance of MFCs/MECs [23]. In this study, adding iron hydroxides in a MEC-anerobic reactor (reactor R1) was expected to drive a microbial Fe(III) reduction. Thus, the effects of the Fe(III) on the performance of R1 as well as the enrichment of IRB were investigated. After 43 days' operation, the COD removal in R1 was 94.5% while it was only 86.7% in R2 and 80.5% in R3 (Fig. 1A). Meanwhile, reactor R1 presented the highest efficiency for the color removal among the three reactors (Fig. 1B). Compared with R3, the higher performance of R2 might be attributed to the effects of redox reactions of MEC. It is known that the microbes on the anodic biofilm can oxidize small molecular VFAs, which help to enhance the degradation of VFAs and COD removal. Cathode serving as electron donor was also favorable for the reduction of azo dyes. During this period, the reduced Fe(II) was maintained between 18.6 and 23.7 mg/L, indicating that the activity of IRB was maintained well. Accompanied by the Fe(III) reduction, some small molecule VFAs were consumed by IRB.

As shown in Fig. 1B, the current between the electrodes of R1 increased gradually from 10.5 ± 1.2 to 27.3 ± 2.3 mA. It showed a higher value than that of R2, indicating that the anodic reaction
might be enhanced via the addition of Fe(III). Additionally, the enhanced transfer of the electrons to cathode was conducive to the reduction of azo dye. However, the generation of Fe²⁺ might be a potential reason for the increased current. To clarify it, an equal amount of Fe(II) (in the form of FeCl₂) to the effluent of R1 was added to the influent of R2. Despite this, the current of R2 had no significant changes with the same effluent Fe²⁺ to R1. It could be assumed that some functional electrochemically active IRB was enriched to enhance the electron transfer reactions between the electrodes which finally improved the current. Considering the enrichment of electro-active strains, which were important to determine the performance of the MEC combined anaerobic reactor. The IRB strains from anodic biofilm of R1 were investigated as follows through the approach of isolation and characterization.

### 3.2. Isolation and identification of IRB strain

After 51 days' operation and enrichment, the suspension of anodic biofilm of R1 was used for the isolation of IRB. During the isolated process, five bacterial strains with Fe(III) reducing ability were obtained. An IRB strain, designated as XB, presented the highest Fe(III) reducing efficiency (about 92%, initial 2 mmol/L Fe(III)-citrate) which was selected and investigated as a typical strain in this experiment (Fig. 2A). As shown in Fig. S1, the colony morphology of XB on the petri plate was white, round and smooth. The strain XB is Gram-negative, short rod, 1.1–1.6 μm long and 0.6–0.8 in width (Fig. S1A). The thickness of XB cell membrane was 27–37 nm and the diameter of XB pilus was 14–15 nm (Fig. S1B and C). To identify the strain XB, 16S rDNA genes of XB were amplified and sequenced. A phylogenetic tree based on the neighbor-joining method was constructed. From Fig. 2B, the sequence of 16S rDNA of XB was compared with those of reference organisms obtained from GenBank data libraries. The phylogenetic relationship revealed that strain XB had the close relationship with strains of A. hydrophila. Thus, the strain XB was identified as a member of A. hydrophila sp. strain, named as A. hydrophila. XB (GenBank accession no. KCS07819).

### 3.3. Fe(III) reduction by A. hydrophila XB

#### 3.3.1. Effects of electron donor on Fe(III) reduction of A. hydrophila XB

Five different carbon sources were selected as electron donors for Fe(III) reduction of A. hydrophila XB. As shown in Table 1, glucose, lactate, acetate, propionate, butyrate and lactate could be used by A. hydrophila XB for Fe(III) reduction. Thereinto, two most effective Fe(III) reduction performance were observed with glucose and acetate as carbon sources, which resulted in 10.2 ± 0.8 and 11.3 ± 1.1 μM Fe(III) reduction, respectively. Comparatively, the utilization ratio of propionate and butyrate by A. hydrophila XB for Fe(III) reduction was poor.

It was known that formate as a product of glucose oxidation was usually mineralized in the presence of formate dehydrogenase that might play an important role in the electron transfer from substrate to Fe(III) oxides and/or electrode. Several researches linked the process of Fe(III) reduction with the activity of the respiratory chain [24,25], in which formate dehydrogenase was considered as one of the most key factor. The activity of formate dehydrogenase was studied in this study using electron transport inhibitors in terms of rotenone and capsaicin. As shown in Table 2, the addition of rotenone and capsaicin caused 57.1% and 47.9% inhibition of Fe(III) reduction that was in agreement with the report of Woznica et al. (2003) who attributed it to the inhibition of active sites on the surface of formate dehydrogenase [26].

#### 3.3.2. Effects of pH, temperature and NaCl concentration on Fe(III) reduction of A. hydrophila XB

The effects of pH on microbial Fe(III) reduction of A. hydrophila XB were investigated in the range of 5–10. As shown in Fig. 3A, the optimal pH for the Fe(III) reduction was 7.0. Nevertheless, the Fe(III) reduction was almost completely inhibited at the pH of 5.0 and 10.0.

Changes of temperature also significantly affected the performance of Fe(III) reduction by A. hydrophila XB. From Fig. 3B, the most favorable temperature for the Fe(III) reduction of A. hydrophila XB was 30 °C which was the same as the operational temperature of reactor R1. Indeed, this temperature was also usually used in the practical anaerobic digestion. Comparatively, the different temperature like 20, 40 and 50 °C would decrease the Fe(III) reducing activity of A. hydrophila XB.

Fe(III) reduction capacity of A. hydrophila XB in the presence of 0–5% NaCl was studied. As shown in Fig. 3C, when the NaCl concentration increased from 0% to 1%, the reduced Fe(II) content raised from 5.57 to 7.46 mg/L, suggesting that appropriate addition of salinity might stimulate the mechanism of A. hydrophila XB for Fe(III) reduction. With increasing NaCl concentration from 1% to

<p>| Table 1 |
| Effects of different substrates on the Fe(III) reduction of A. hydrophila XB. The concentration of these substrates are equivalent to 1000 mg/L COD. |</p>
<table>
<thead>
<tr>
<th>substrate</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>Lactate</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(II) (μM)</td>
<td>11.3 ± 1.1</td>
<td>4.8 ± 1.6</td>
<td>3.8 ± 0.9</td>
<td>7 ± 1.7</td>
<td>10.2 ± 0.8</td>
</tr>
</tbody>
</table>
Effects of inhibitors on Fe(III) reduction by *A. hydrophila* XB.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Inhibitor concentration (µM)</th>
<th>Fe(III) reduction (µM)</th>
<th>Inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>9.8 ± 0.6</td>
<td>–</td>
</tr>
<tr>
<td>Rotenone</td>
<td>200</td>
<td>7.1 ± 0.5</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>5.1 ± 0.2</td>
<td>47.9</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>200</td>
<td>8.2 ± 0.5</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>4.2 ± 0.5</td>
<td>57.1</td>
</tr>
</tbody>
</table>

Fig. 3. Effects of (A) pH, (B) Temperature and (C) NaCl concentration on the Fe(III) reduction of *A. hydrophila* XB in 20 h.

5%, Fe(III) reduction content decreased gradually and reached to a lowest level of 0.53 µM in the presence of 5% NaCl. The results indicated that high salinity might inhibit the activity of *A. hydrophila* XB for Fe(III) reduction.

The optimum Fe(III) reducing conditions of *A. hydrophila* XB was similar to the operational conditions of R1, indicating that the operation of R1 for enriching these Fe(III) reducing cultures was easily accepted and available for the scale up in practice due to its relatively broad condition for enrichment.

### 3.4. Electrochemical activity

It is accepted that most of exoelectrogens such as *Geobacter* and *Shewanella* belong to iron reducing bacteria (IRB) as the electron transfer mechanism between IRB and Fe(III) oxides was similar to that between IRB and electrode in terms of direct electron transfer, nano-wire and electron shuttling mediators [9–11]. Besides most studied model microbes of *Shewanella* and *Geobacter*, more electrochemically active bacteria were studied recently such as *Clostridium butyricum* EG3, *Pseudomonas aeruginosa* and *Rhodopseudomonas* DX-1, which were noted to be present mostly in the anodic biofilm of electrochemical reactors [16,17,27,28]. Considering *A. hydrophila* XB isolating from anodic biofilm and its Fe(III) reducing activity, it was speculated that this novel strain might have electroactivity. In this study, the electrochemical activity of *A. hydrophila* XB was determined by cyclic voltammogram (CV). As shown in Fig. S2, the isolate XB was electrochemically active, which could be observed by the apparent redox peak on the CV. The shape of reduction peak was different from oxidizing peak, indicating that the redox reaction of the cell suspension was a quasi reversible reaction. It reported that c-Type cell membrane cytochromes was favorable for the directly electron transfer between bacterial cell and the anode [29], which had been found in *A. hydrophila* ATCC7966. *A. hydrophila* XB had the same genus with *A. hydrophila* ATCC7966 and it was likely to be one of the possible reasons for the electrochemically active of *A. hydrophila* XB.

To further clarify the electrochemical activity of *A. hydrophila* XB, a two-chamber microbial fuel cell (MFC) was operated by inoculating *A. hydrophila* XB in the anode chamber of MFC. After 30 days’ operation and stabilization, the electricity production performance of MFC was improved. After dosing 1000 mg/L acetate as anodic substrate, a sharply increase in power output was noticed and it reached a maximum voltage output of 0.35 V (0.35 mA at 1000 Ω) on 38 h (1.6 days) (Fig. 4). The maximum current density was maintained at 0.46 mA/m² from 1.6 to 4.46 days. Subsequently, the voltage output decreased rapidly below 50 mV. To clarify the functional electrochemically active bacterium, the DGGE analysis of anodic biofilm was conducted. As shown in Fig. 5, only one dominant band was observed, which was identified as *A. hydrophila* XB. It indicated that the enrichment of electrochemically active bacterium *A. hydrophila* XB on the anode was effective for producing electricity.

#### 3.5. Decolorization by *A. hydrophila* XB

It was reported that *A. hydrophila* had high capability for color removal [30,31]. Therefore, the enrichment of *A. hydrophila* in the MEC combined anaerobic reactor (R1) might be a key reason for its high decolorization. However, there was few reports focus on the decolorization by a novel *A. hydrophila* with electroactive activity which was isolated from the anodic biofilm. In this experiment, the performance of *A. hydrophila* XB on decolorization was investigated.

As shown in Fig. 6, three typical azo dyes i.e. Methyl Red, Acid orange II and Reactive brilliant red X-3B were used in this decolorization experiment. The decolorization of Acid orange II by *A. hydrophila* XB reached 90.6% i.e. 90.6 mg/L dye in 6 h at an initial dye concentration of 100 mg/L, which was higher than that of *Enterococcus fecalis* ID8017 which degraded only 66.4 mg/L Acid orange II in the same time period. For the decolorization of Methyl Red, *A. hydrophila* XB shared the similar ability as that of Acid orange II. The decolorization of Reactive brilliant red X-3B was
relatively slow and reached to 92.7% in 14 h. The different decolorization rate might be linked with the various chemical structures and dye class among these three dyes [31,32].

During the operation of R1, even though facing high concentration toxic dyes and its products e.g. aromaticamine, the performance of R1 was not influenced significantly which was likely to be linked with the enrichment of toxicity – adapted strains via the addition of Fe(III). As a model strain, the enrichment of A. hydrophila XB via the addition of Fe(III) oxides was in well agreement with the high decolorization of R1. It was reported that A. hydrophila had a higher tolerance to aromaticamine by a comparative assessment that provided a high performance of color removal [33]. Therefore, it would be a promising approach to enrich more Fe(III)-reducing exoelectrogens with the ability to degrade and tolerate some toxic contaminants in a MEC combined anaerobic via dosing Fe(III) oxides.

4. Conclusions

The addition of Fe(III) hydroxide in a MEC combined anaerobic reactor (R1) has shown a positive effect on the treatment performance in terms of COD removal and decolorization as compared to the reference reactors (R2 and R3). After 51 days’ operation and enrichment, A. hydrophila. XB was isolated from the anodic biofilm of R1 as a typical novel IRB among five isolates, showing high electrochemical activity, Fe(III) reduction and decolorizing efficiency. This study provided a promising approach to enrich electro-active functionally consortium capable of decomposing refractory substances through adding Fe(III) in bio-electrochemical reactors, which was meaningful for the industrial wastewater treatment.

Acknowledgments

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2014.02.102.

References


