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Electrosynthesis of acetate from inorganic carbon (HCO_3^-) with simultaneous hydrogen production and Cd(II) removal in multifunctional microbial electrosynthesis systems (MES)

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Abstract

The simultaneous production of acetate from bicarbonate (from CO_2 sequestration) and hydrogen gas, with concomitant removal of Cd(II) heavy metal in water is demonstrated in multifunctional metallurgical microbial electrosynthesis systems (MES) incorporating Cd(II) tolerant electrochemically active bacteria (EAB) (*Ochrobactrum* sp X1, *Pseudomonas* sp X3, *Pseudomonas delhiensis* X5, and *Ochrobactrum anthropi* X7). Strain X5 favored the production of acetate, while X7 preferred the production of hydrogen. The rate of Cd(II) removal by all EAB (1.20 – 1.32 mg/L/h), and the rates of acetate production by X5 (29.4 mg/L/d) and hydrogen evolution by X7 (0.0187 $m^3/m^3/d$) increased in the presence of a circuital current. The production of acetate and hydrogen was regulated by the release of extracellular polymeric substances (EPS), which also exhibited invariable catalytic activity toward the reduction of Cd(II) to Cd(0). The intracellular activities of glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and dehydrogenase were altered by the circuital current and Cd(II) concentration, and these regulated the products distribution. Such understanding enables the targeted manipulation of the MES operational conditions that favor the production of acetate from CO_2 sequestration with simultaneous hydrogen production and removal/recovery of Cd(II) from metal-contaminated and organics-barren waters.

Keywords: microbial electrosynthesis system; extracellular polymer substance; acetate production; Cd(II) removal; hydrogen production

1 Introduction

Carbon dioxide (CO_2) is a greenhouse gas and an attractive feedstock for biocatalysis. Many acetogenic bacteria can catalyze a variety of redox reactions and are competent in the formation of acetate from the H_2-dependent reduction of CO_2 [1]. The microbial metabolisms of CO_2 can be realized in microbial electrosynthesis systems (MES), in the presence of an applied electrical current, utilizing hydrogen-producing cathodes interfaced with electrochemically active bacteria (EAB) [2]. Similarly, the indirect conversion of CO_2 (via hydrogen) in MES, enables the bioconversion of CO_2 to a variety of chemicals, such as acetate [3-5].

In addition to the sequestration and utilization of CO_2 to control the greenhouse effect, another environmentally significant problem is the removal and/or recovery of heavy metals from wastewater, including Cd(II), a highly hazardous heavy metal often present in industrial organics-barren wastewaters from chemical manufacturing, mining, extractive metallurgy, nuclear, and other industries [6-7]. For this purpose, bioelectrochemical systems utilizing mixed or pure EAB cultures fed by bicarbonate have been shown to be effective methods for the reduction and recovery of Cd(II) [7-11]. Therefore, it can be envisaged that appropriately designed multifunctional MES using self-regenerative EAB should be able to convert inorganic carbon from CO_2 sequestration to building block organics (e.g., acetate) while simultaneously reducing Cd(II) from wastewaters [12]. Such multifunctional systems may be developed to be attractive and sustainable metallurgical methods for the production of biofuels and biomaterials from industrial waste. EAB generally adopt special metabolic pathways which release extracellular polymeric substances (EPS), as a protective mechanism from the harmful effects of Cd(II) [13-14]. Since EPS are composed of variable degrees of saccharides and proteins with negative charge, EPS can electrostatically bind the positively charged Cd(II) ions, in addition to the adsorption of the ions on the functional groups on the bacterial cell envelopes [13-14].

In this study, the simultaneous production of acetate from bicarbonate (from CO_2 sequestration)

and hydrogen gas, with concomitant removal of Cd(II) heavy metal in water is demonstrated in multifunctional metallurgical MES incorporating Cd(II) tolerant electrochemically active bacteria. Pure cultures of four well characterized gram-negative EAB including *Ochrobactrum* sp X1, *Pseudomonas* sp X3, *Pseudomonas delhiensis* X5, and *Ochrobactrum anthropi* X7 were selected since they can efficiently remove Cd(II) with a variable degrees of intracellular distribution of different forms of cadmium [7,9-10]. However, the simultaneous production of acetate from inorganic carbon and of hydrogen by these strains has not been shown. Using single strains allows an understanding of the physiological activities of the bacteria at the electrode surface, whereas comparing the bacterial behaviors is beneficial for understanding the impact of each bacterium on the MES products. The EPS release mechanism from the EAB under metal stress is investigated since this is expected to regulate the rate of reduction of Cd(II) from the wastewater. The impact of electrochemically active cytochromes in the EPS that allow extracellular electron transfer are also examined, since these may further enhance the rate of products formation, similarly to nitrate removal and CH_4 production [15-16].

The antioxidative activity of glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) in the bacteria are expected to be altered by either cadmium sequestration or by scavenging $O_2^{\cdot -}$ induced by Cd(II), as a defense against the harmful effect of Cd(II) [17-18], while the dehydrogenase activity relates to the biocatalytic ability for hydrogen evolution [19]. These intracellular physiological activities are expected to be altered in the presence of Cd(II) and in the presence of a circuital current in the proposed multifunctional metallurgical MES. Therefore, the EPS components and the activities of SOD, CAT, GSH and dehydrogenase in these bacteria were systematically investigated in this study, in response to changes in the initial Cd(II) concentration and in the circuital current. These activities and EPS components were further correlated to the response of the bacteria in the proposed multifunctional metallurgical MES for the microbial electrosynthesis of acetate from organic carbon, with simultaneous hydrogen evolution and Cd(II) removal/recovery.

2 Materials and methods

2.1 Reactor assembly

Duplicate two-chamber reactors comprising cylindrical chambers, 2.0 cm long, 3.0 cm in diameter and 14 mL each, as previously described [8,10-11], were used in all experiments. The cathode electrodes were three-dimensional material of graphite felts (Sanye Co., Beijing) [20-21] whereas the anode electrodes were carbon rods. The anodic and cathodic chambers were separated by cation exchange membranes (CEM, Ultrex CMI-7000, Membranes International, Glen Rock, NJ; projected surface area: 7.1 cm^2) since preliminary results showed negligible transfer of anions (e.g., acetate, phosphate, and bicarbonate) and similar migration of Cd(II) through the CEM, compared to the use of an anion exchange membrane (AEM, Ultrex AMI-7001, Membranes International, Glen Rock, NJ) (see Supporting Information and Fig. S1). All reactors were covered with aluminum foil to exclude ambient light.

2.2 EAB inoculation and reactor operation

The four EAB (X1, X3, X5 and X7), thriving at low nutrients under anaerobic conditions, were isolated from cathodes previously exposed to Cd(II) fed with bicarbonate as carbon source. They were harvested after 24 h incubation in an anaerobic and sterile medium (pH = 5.8) as previously described [10]. The cells were then concentrated and washed 3 times in sterile physiological saline solutions. The cathodes of each reactor were slowly inoculated with the isolates (10^8 CFU/mL) of each of the four EAB with the catholyte composed of NH_4HCO_3 (0.15 g/L), $KHCO_3$ (0.20 g/L), KH_2PO_4 (0.012 g/L); $MgSO_4 \cdot 7H_2O$ (2.0 mg/L), vitamins 0.6 mL/L and mineral 0.6 mL/L. The anolyte was a buffer solution made of NaH_2PO_4-$NaHPO_4$ (5.0 mM). The inoculation and solution replacements were performed in an anaerobic glovebox (YQX-II, Xinmiao, Shanghai). The initial Cd(II) concentration in the catholyte was varied in the range from 0 to 60 mg/L, and the initial pHs and solution conductivities in the electrolytes of both chambers were invariably adjusted to be 5.8

and 5.8 mS/cm, respectively [22]. A constant cathodic potential of –700 mV versus standard hydrogen electrode (SHE) (VSP, BioLogic) was applied to the reactors based on the range of cathodic potentials (– 800 to –600 mV) reported in literature [23], and the circuital current was automatically monitored. The reactors were operated in fed-batch mode at room temperature (25 ± 3 ºC). Three duplicate reactors were used in all experiments.

Three reactors were used as controls: one was operated without the inoculum (abiotic control); the second was inoculated but operated under open circuit (OC) conditions to examine acetate production and Cd(II) removal in the absence of a circuital current and to confirm that hydrogen production was only produced in the presence of a circuital current (CC); and the third was operated in the absence of Cd(II) to monitor the variation of the production rates of acetate, hydrogen, extracellular proteins and exopolysaccharides from the system in the presence of Cd(II).

2.3 Sampling, analysis and characterization

Dissolved EPS were extracted as reported by Kang et al. [24]. Briefly, the graphite felt from the reactors was submerged into a physiological saline solution and then sonicated on ice three times for 10 s intervals at power level 3 (Branson Sonifier 250, Danbury, USA). The electrode was removed from the solution then rinsed with an additional 3 mL of physiological saline solution and discarded. The mixtures were then sonicated with an intensity of 2.7 W·cm^2 and a frequency of 40 kHz on ice for 10 min. The mixtures comprising the combined EAB biofilm and EPS were either analyzed by cyclic voltammetry (CV), as described in later sections, or immediately centrifuged at 11000 × g and at 4 ºC for 20 min. The precipitates were collected and used for assessing the physiological activities, as described in later sections, whereas the supernatants were filtered through a 0.22 μm acetate cellulose membrane. The nucleic acid in the filtrate was negligible, implying that cell lysis did not occur during the EPS extraction [25]. The filtrate, constituted primarily by EPS, was also analyzed by CV as described in later sections. The exopolysaccharides in the filtrate were assayed by the phenol-sulfuric acid method [26], whereas extracellular proteins were quantified by the Bradford

assay (Bio-Rad, Hercules, CA) using bovine serum albumin as a calibration standard. The biomass immobilized on the cathodes and in suspension in the catholyte was measured as previously described [27].

Three-dimensional fluorescence excitation-emission matrix (FEEM) spectroscopy (F-7000, Hitachi, Japan) equipped with 1.0 cm quartz cell and a thermostat bath was used for quantifying and characterizing the EPS in the filtrate, as previously shown [24]. The intracellular physiological activities, dehydrogenase, SOD, CAT and GSH in the harvested and washed biofilms and in the planktonic cells were assessed as previously described [19,28-29]. The results were expressed per gram cell dry weight (mg/g DW). The procedure was repeated at least three times for each strain and Cd(II) loading condition.

The bioelectrochemical behaviors of the cathodic biofilms and EPS alone were examined by CV in a three-electrode configuration with a potentiostat (Bio-Logic VMP3) at scanned potentials of –1.3 – +0.3 V (vs. SHE) and a scan rate of 1.0 mV/s [20,22].

The elemental composition of the cadmium adsorbed on the surface of the EAB was analyzed by X-ray photoelectron spectroscopy (XPS; Kratos AXIS Ultra DLD). The formation of minor organics such as formate, acetate, propionate, ethanol and 2-oxobutryate were determined using an Aminex HP-87H column on a Shimadzu LC-ADVP HPLC equipped with a UV detector and a mobile phase of 0.005 M H_2SO_4 with a detection limit of ca. 5 μM acetate, formate, propionate, ethanol and 2-oxobutryate [30].

2.4 Calculation

The removal/recovery rate of Cd(II) (mg/L/h), the conversion of inorganic carbon (%), the acetate production rate in the catholyte (mg/L/d), the rate of extracellular proteins (ng/L/h) and exopolysaccharides (ng/L/h), the hydrogen production rate (m^3 H_2/m^3 reactor/d), and the cathodic electrons for hydrogen evolution (η_{H2}), acetate production ($\eta_{acetate}$) and Cd(II) removal were calculated as previously described [8,21,31] as shown in SI.

One-way ANOVA in SPSS 19.0 was used to analyze the statistical differences among the data, and all of the data indicated significance levels of $p < 0.05$.

3 Results and discussion

3.1 System performance

The MES performance was initially evaluated at constant Cd(II) concentration of 20 mg/L. Significantly higher Cd(II) removal rates [1.20 ± 0.03 (X5) – 1.32 ± 0.02 (X1) mg/L/h at 12 h; 0.38 ± 0.01 (X3) – 0.42 ± 0.01 (X1) mg/L/h at 48 h] (Fig. 1A) and hydrogen production [0.0015 ± 0.0001 (X1) – 0.0187 ± 0.0018 (X7) m^3/m^3/d at 12 h; 0.0019 ± 0.0001 (X1) – 0.0210 ± 0.0005 (X7) m^3/m^3/d at 24 h] (Fig. 1B) were observed in the presence of EAB in comparison to the controls systems in the absence of circuital current, the abiotic controls, or in the absence of Cd(II). Conversely, very low hydrogen production was observed in the control experiments performed with only EPS with or without Cd(II) (Fig. S2). Therefore, the combined effect of EAB catalysis and circuital current were essential to establish high rates of Cd(II) removal and/or hydrogen production. Such effect has also been observed with mixed cultures or pure EAB for the removal of Cr(VI), Cd(II), Co(II) or Cu(II) [9-10,21] and in other systems for hydrogen evolution in the absence of metals [32]. The presence of Cd(II) ions substantially enhanced the production of hydrogen by strain X7, which was also reflected by almost one order of magnitude increase in the energy content, whereas only minor effects were observed with strain X3 and a slight inhibition was observed with X1 and X5 (Fig. 1B; Table S1). The similarity of hydrogen production in the abiotic controls with or without Cd(II), clearly excluded any impact of Cd on the production of hydrogen [33]. The rate of hydrogen production by X7 was up to 4 order of magnitude higher than the rate observed by the well-known *Geobacter sulfurreducens* ($0.026 \pm 0.003 \times 10^{-4}$ m^3/m^3/d) in a MES at a potential of – 0.6 V (vs. SHE) in the absence of Cd(II) [34]. Since the circuital current did not significantly varied among the EAB (Fig. 1D), it may be deduced that Cd(II) may regulate the production of hydrogen by affecting the EAB physiological metabolism, including the EPS amount and composition and the SOD, CAT, GSH and

dehydrogenase activities in these bacteria. This conclusion is further supported in the subsequent sections.

The production of acetate [12.2 ± 1.7 (X1) – 29.4 ± 3.1 (X5) mg/L/d at 12 h; 11.6 ± 0.4 (X3) – 15.1 ± 1.2 (X5) mg/L/d at 48 h] was also significant in the presence of both EAB and circuital current, while acetate was not produced in the abiotic controls (Fig. 1C) and in the OC experiments (data not shown). Accordingly, each EAB exhibited different extents of appreciable acetate accumulation under prolonged operational time, which contrasted with the absence of acetate production in the abiotic controls (Fig. S3). These results collectively confirm that the inorganic carbon reduction process needed to be guided by the bioelectrocatalytic activities of the EAB. The acetate production rate was within the wide range of 1.1 – 197 mg/L/d obtained in the absence of Cd(II) with other pure cultures of *Sporomusa ovata*, *Clostridium ljungdahlii*, *Acetoanaerobium* or *Moorella* in MES at potentials of –0.7 – –0.4 V vs. SHE [35-36]. The acetate production rate were also higher than the rates observed with *Stenotrophomonas* sp. WH-11, *Ochrobactrum* sp. WH-13, *Castellaniella* sp. WH-14, *Sinomicrobium oceani* WH-15 or *Metallosphaera yellowstonensis* (1.68 – 8.64 mg/L/d) [37-39], but lower than the rates obtained with mixed cultures (18.7 – 1584 mg/L/d) in the absence of Cd(II) with a bicarbonate feed (2.0 – 4.0 g/L) or continuously feeding pure CO_2, at cathode potentials in the range from –1100 to –600 mV [23,40-42]. Specifically, strain X5 achieved the highest rate of acetate production (Fig. 1C) although the production of hydrogen was significantly lower than X7 (Fig. 1B), implying the suitability of this Cd(II)-tolerant X5 strain as an acetate producer. The presence of Cd(II) diverted a higher fraction of cathodic electrons towards hydrogen evolution (η_{H2}) with X7 or acetate production ($\eta_{acetate}$) with X5 (Table S1), consistent with the results shown in Fig. 1B and C. Therefore, X5 and X7 EAB displayed a different utilization mechanism of the cathodic electrons.

Under CC conditions the rates of production of extracellular proteins (Fig. 1E) and exopolysaccharides (Fig. 1G) were several folds higher than under OC conditions (Fig. 1F and H),

which suggests a stimulating effect of circuital current on the production of EPS. Such effect has also been reported in the removal of nitrate in mixed cultures in the absence of Cd(II) [15]. In addition, the regulating role exerted by Cd(II) ions and evidenced by the enhanced extracellular proteins released by strains X3 and X7, was reflected by higher production of hydrogen in these EAB (Fig. 1B), although such effect was not observed with strains X1 and X5 (Fig. 1E). In consequence, the stress conditions imposed by the presence of Cd(II) on strains X3 and X7 ions may have stimulated the production of hydrogen through the release of a higher amount of extracellular proteins. The rates of exopolysaccharides released by the EAB under a circuital current, however, were invariably stimulated by the Cd(II) stress (Fig. 1G) and were higher than the rates observed under OC conditions (Fig. 1H). Under the OC conditions, the presence of Cd(II) improved the release of extracellular proteins by strain X7 (Fig. 1F), similar to the release of extracellular proteins observed with conventional *Pseudomonas* sp., *Escherichia coli* or anaerobic mixed cultures under Cd(II), Cu(II), Cr(VI) or Ag(I) stress conditions [14,24].

Here Fig. 1

The presence of a circuital current stimulated the EAB to efficiently metabolize inorganic carbon (no Cd(II): 92 – 98%; Cd(II) presence: 79 – 100%; operation time: 48 h) (Fig. 2A), compared to the OC conditions (25 – 31%) (Fig. 2B). The specific metabolism of these Cd(II)-tolerant EAB towards the production of acetate in this metallurgical MES was further evidenced by the absence of reaction by-products in both the headspace (absence of methane) and in the catholyte solution (absence of other minor organic acids including formate, propionate, ethanol and 2-oxobutryate) with or without Cd(II). The absence of reaction by-products gives these Cd(II)-tolerant EAB a potential advantage over many other acetate producers, which require further separation processing steps to separate acetate from the reaction by-products [5].

Here Fig. 2

3.2 Impact of Cd(II) concentration

The impact of Cd(II) concentration on system performance and EPS components was evaluated in the range from 0 to 60 mg/L to cover a wide range of industrial waters. An increase in the initial Cd(II) concentration invariably enhanced its removal rate (Fig. 3A), illustrating significant stress tolerance of these EAB towards Cd(II), likewise other mixed cultures for Cd(II) removal [8]. Strain X5 showed an increase in the rate of acetate production as the concentration of Cd(II) increased up to 60 mg/L (Fig. 3B), while a decrease was observed with the other three strains.

Strain X7 achieved appreciable higher rates of hydrogen production, varying the Cd(II) concentrations from 10 to 40 mg/L (Fig. 3C) and achieved the highest rate at 20 mg/L. The results show that the robustness of strain X7, as a hydrogen producer at an optimal concentration of Cd(II) and of strain X5 as an efficient acetate producer under highly Cd(II) stress conditions.

Here Fig. 3

Compared to the OC conditions (Fig. 4B) or in the absence of Cd(II) (Fig. 4A), the presence of a circuital current favored the release of extracellular proteins in all strains, although the release by X1, X3 and X5 strains was negatively impacted by increasing Cd(II) concentrations (Fig. 4A and B). Strain X7 on the other hand, secreted the greatest amount of extracellular proteins at a Cd(II) concentration of 20 mg/L, after which decreased, under both CC (Fig. 4A) and OC (Fig. 4B) conditions. The amount of extracellular proteins secreted by strain X7 under CC conditions correlated to the rate of hydrogen production. The greatest values were observed at the same initial concentration of Cd(II) (Fig. 3C), implying a regulating effect of Cd(II) to the amount of extracellular proteins and, therefore, to the rate of hydrogen evolution. The higher rate of extracellular proteins released by all EAB in the presence of circuital currents also correlates with the electron transfer proteins secreted by the electroactive *Shewanella* sp. in the absence of Cd(II) [16].

The release of exopolysaccharides was invariably positively related to the Cd(II) concentration under CC conditions (Fig. 4C), and the amount were greater than those under OC conditions (Fig. 4D). Considering the slight change observed on the release of extracellular proteins under OC

conditions (Fig. 4B), the strains may have developed a protective mechanism under increasing Cd(II) stress conditions, by preferentially releasing exopolysaccharides rather than extracellular proteins, in agreement with results obtained with conventional *Pseudomonas* sp. EJ01 under Cd(II) stress [14] and with other bacteria under Au(III) stress conditions [24].

Here Fig. 4

FEEM spectroscopy displayed the EPS components released by the EAB under Cd(II) stress conditions (Fig. 5), and the associated relative percentages (Fig. 6). These results varied significantly among the EAB species, and depended on circuital current and Cd(II) concentration. The presence of a circuital current with strains X1, X3 and X5 and in comparison to OC controls, invariably stimulated the release of a higher amount of soluble microbial byproduct-like matter in series IV (Ex / Em = 250 – 450 nm / 260 – 380 nm) at all Cd(II) concentrations investigated (Figs. 5A – L, 6A – C and S4). The impact of the electrochemically active cytochromes in the EPS matrix has been associated with extracellular electron transfer processes [16] and the role of these species under Cd(II) stress conditions cannot be excluded. Conversely, protein (tyrosine)-like matters in series I (Ex / Em = 200 – 250 nm / 260 – 320 nm) secreted by the three EAB was completely suppressed under CC rather than OC conditions (Figs. 5A – L and 6A – C).

Varying the Cd(II) concentration, at 20 mg/L it invariably induced the release of protein (tryptophan)-like materials in series II (Ex / Em = 200 – 240 nm / 330 – 370 nm) and polycarboxylate-type humic acid (fulvic acid in ultraviolet region)-like matters in series III (Ex / Em = 200 – 240 nm / 380 – 500 nm) for X1, X3 and X5 strains (Fig. 5B, F and J; Fig. 6A – C). Such species may be responsible in the regulation of the bacterial metabolism for acetate and hydrogen productions, since a peak in these species observed at 20 mg/L Cd(II) coincided with either a high rate of acetate production (X5 strain) or a peak in hydrogen evolution (X3 and X7 strains) (Fig. 3C and D).

Exceptionally, strain X7 always released a higher amount of protein (tryptophan)-like matters in

series II (Ex / Em = 200 – 240 nm / 330 – 370 nm), soluble microbial byproduct-like materials in series IV (Ex / Em = 250 – 450 nm / 260 – 380 nm), and polycarboxylate-type humic acid (fulvic acid in visible region)-like matters in series V (Ex / Em = 250 – 450 nm / 380 – 550 nm) under CC rather than OC conditions (Fig. 5M – P; Fig. 6D). In addition, this strain secreted a higher amount of protein (tryptophan)-like matters in series II (Ex / Em = 200 – 240 nm / 330 – 370 nm) and protein (tyrosine)-like matters in series I (Ex / Em = 200 – 250 nm / 260 – 320 nm) at higher Cd(II) concentrations. The diversity of the components released by strain X7 under Cd(II) stress conditions and in the presence of a circuital current, may have contributed to the appreciable higher rate of hydrogen produced by this strain in the metallurgical MES (Fig. 3C).

Here Fig. 5

Here Fig. 6

CV analysis of the EAB and abiotic cathodes did not show the presence of oxidation-reduction peaks (Fig. 7A, F, K and P). In contrast, one strong oxidation-reduction peak appeared on all the EAB cathodes after spiking with 10 mg/L Cd(II) (Fig. 7B, G, L and Q). The catalytic action of the EAB biofilms was consistent with that observed with mixed cultures using Cr(VI), Cu(II) or Cd(II) as electron acceptors [8-9]. Accordingly, EPS alone exhibited a similar oxidation-reduction peak to the EAB films, confirming similar catalytic activities of the EPS. This result corroborated with other studies that showed EPS functioning as reducing agents for Ag(I) ions to nanoparticles Ag(0) [24]. An increase in Cd(II) concentration from 20 mg/L to 60 mg/L enlarged the oxidation-reduction peaks for all EAB biofilms and for the associated EPS (Fig. 7C – E; H – J; M – O; R – T), coinciding with the increase in exopolysaccharides (Fig. 4C), reflecting the role of exopolysaccharides in extracellular electron transfer under increasing Cd(II) stress conditions.

Here Fig. 7

While the presence of an electron acceptor such as Cd(II) clearly benefited the electron transfer from the cathode, the reason for this beneficial effect is not well established [5,12,43].

With regard to the cathodic electrons used for acetate production, hydrogen evolution and Cd(II) removal, it is difficult to exactly quantify the amounts of electrons used by each product. Although, electron acceptors (HCO_3^-, H^+, or Cd(II)) are beneficial for the electron transfer process from the cathode [46], it has yet to be demonstrated that electron transfer to these species are also associated to cell respiration. In fact there are a variety of other possible mechanisms by which bacterial cells might catalyze the reduction of Cd(II), and the production of acetate and hydrogen [47]. Very recently, the energy acquisition by *Shewanella oneidensis* MR-1 in an aerobic environment lacking exogenous organic carbon sources for oxygen reduction sources has been demonstrated by coupling an oxidation electrode cathode with the reduction of oxygen [44, 48]. This respiratory process may be important to explain the physiological behavior of the Cd(II)-tolerant EAB in the present cathodic energy-limited environments and in their cellular conversion of the cathodic electrons for the microbial synthesis of acetate, despite that direct proofs about such electron transfer for Cd(II) reduction and for cell respiration are still needed.

3.3 Intracellular physiological activities

The dehydrogenase activity in the biofilms (Fig. 8A) was observed to be higher than in the planktonic cells (Fig. 8B), which is consistent with the rates of hydrogen production (Fig. 3C) as a function of the EAB strain and the Cd(II) concentration, and in agreement with that by the diverse acetogenic microorganisms in the absence of Cd(II) [35].

Compared to the OC controls (Fig. 8D, F and H), the presence of a circuit current always diminished the GSH (Fig. 8C), CAT (Fig. 8E) and SOD (Fig. 8G) activities in both the EAB biofilms and in the planktonic cells, implying similar responses of these differently located EAB to the Cd(II) toxicity. At each Cd(II) concentration and in the presence of circuital current, lower GSH, CAT and SOD biofilms activities (Fig. 8C, E and G) than in the planktonic cells (Fig. 8D, F and H) and negligible changes in the OC controls, demonstrated a more robust response of the biofilms to the Cd(II) toxicity than the planktonic cells. Strain X3 from the cathode biofilms (Fig. 8C, E and G) and

in the catholyte (Fig. 8D, F and H) in most cases presented apparent higher GSH and CAT activities than the other strains, regardless of the presence of circuital current, presumably ascribed to its intrinsic characters.

Here Fig. 8

3.4 XPS analysis

XPS analysis on the EAB films collected from the cathodes after the passage of a circuital current, reported the exclusive presence of Cd(0) (Fig. 9A, C, E and G; Table S2), while under OC conditions only Cd(II) was observed (Fig. 9B, D, F and H; Table S2). Therefore, Cd(II) was reduced to Cd(0) on these EAB in the presence of a circuital current. Since Cd(II) was also reduced by EPS alone (Fig. 7), both EPS and the EAB biofilms contributed to the reduction of Cd(II) ions. In contrast, only chemical adsorption of Cd(II) on the electrodes was observed in the absence of both circuital current and EAB (Fig. 9J), in agreement with results observed with other metals [45].

The net Cd(0) production with strain X3 was 0.055 ± 0.004 mg, while with strain X5 was 0.063 ± 0.006 mg, both of which were lower than 0.091 ± 0.008 mg observed in the abiotic controls (Table S2; Fig. 9I). Conversely, greater amounts of Cd(0) were produced by strains X1 (0.121 ± 0.003 mg) and X7 (0.099 ± 0.005 mg) than the abiotic controls (Table S2), emphasizing their higher catalytic activity for Cd(0) formation.

Here Fig. 9

4 Conclusions

In this study we have demonstrated a sustainable process for the microbial electrosynthesis of acetate from bicarbonate with simultaneous hydrogen evolution and Cd(II) removal in a multifunctional metallurgical MES with tailored EAB. Such multifunctional MES may enable new prospects for the efficient conversion of CO_2, from a broad range of sources, to C1 feedstocks, with simultaneous generation of a clean source of energy and heavy metals removal/recovery. The presence of a circuital current and Cd(II) ions induced the EAB to release greater amounts of EPS

with a higher compositional diversity, that increased the catalytic reduction rate of Cd(II) to Cd(0), in addition to yielding greater amounts of reaction products: acetate from X5 and hydrogen from X7.

The products formed under stress conditions, induced by the presence of Cd(II) and in the presence of a circuital current, were related to the EAB intracellular GSH, CAT, SOD and hydrogenase physiological activities, and to the amount and composition of released EPS. Although the production of acetate in this study [11.6 ± 0.4 – 29.4 ± 3.1 mg/L/d] was lower than in other reports carried out with mixed cultures in the absence of Cd(II) at more negative cathode potentials (e.g., –1100 mV) and at higher inorganic carbon concentration (e.g., 2.0 – 4.0 g/L) [23,36,38,40-41], this study has shown the diverse behavior of EAB for yielding greater amounts of either acetate (X5) or hydrogen (X7), with simultaneous Cd(II) removal under cruel heavy metal stress conditions. This aspect should allow the tailoring of multifunctional MES towards the desired synthesis products in metal rich environments.

Further studies should also focus on the identification of the cadmium-related genetic regulatory networks in such EAB, which govern their function predictions [5,12], on the design of the MES and on the biocompatibility of the electrode materials used as biocathodes, which increase the system performance. In addition, it is increasingly important to develop and characterize a continuous treatment processes in order to improve the system efficiency and large-scale performance of such MES. Currently, at least 3.0 kWh/kg acetate with over 85% of energy was consumed for acetate separation in conventional MES [31]. Further studies on these aspects are warranted.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Fig. 1 Comparison of (A) Cd(II) removal, (B) hydrogen production, (C) acetate production, (D) circuital current, (E and F) extracellular proteins, and (G and H) exopolysaccharides in MES with different EAB biofilms at various operational times, under closed circuital (CC) or open circuital (OC) conditions (initial Cd(II): 20 mg/L).

Fig. 2 Distribution of total carbon under closed circuital (CC) (A) or open circuital (OC) (B) conditions with or without stressed Cd(II) as a function of operation time (initial Cd(II): 20 mg/L).

Fig. 3 (A) Cd(II) removal, (B) acetate production, and (C) hydrogen production as a function of initial Cd(II) concentration (operation time: 24 h).

Fig. 4 Extracellular protein (A and B) and exopolysaccharide (C and D) released by the EAB under various initial Cd(II) concentrations, and closed circuital (CC) (A and C) or open circuital (OC) (B and D) conditions (operation time: 24 h).

Fig. 5 Fluorescence excitation emission matrix (FEEM) contours of extracellular polymer substances produced by (A, B, C and D) X1, (E, F, G and H) X3, (I, J, K and L) X5, and (M, N, O and P) X7 strains, at an initial Cd(II) concentration of (A, E, I and M) 0 mg/L, (B, F, J and N) 20 mg/L, (C, G, K and O) 40 mg/L, or (D, H, L and P) 60 mg/L under closed circuital conditions.

Fig. 6 Comparison of integral of fluorescence area of extracellular polymer substances produced by (A) X1, (B) X3, (C) X5, and (D) X7 at various Cd(II) concentrations and under closed circuital

(CC) or open circuital (OC) conditions.

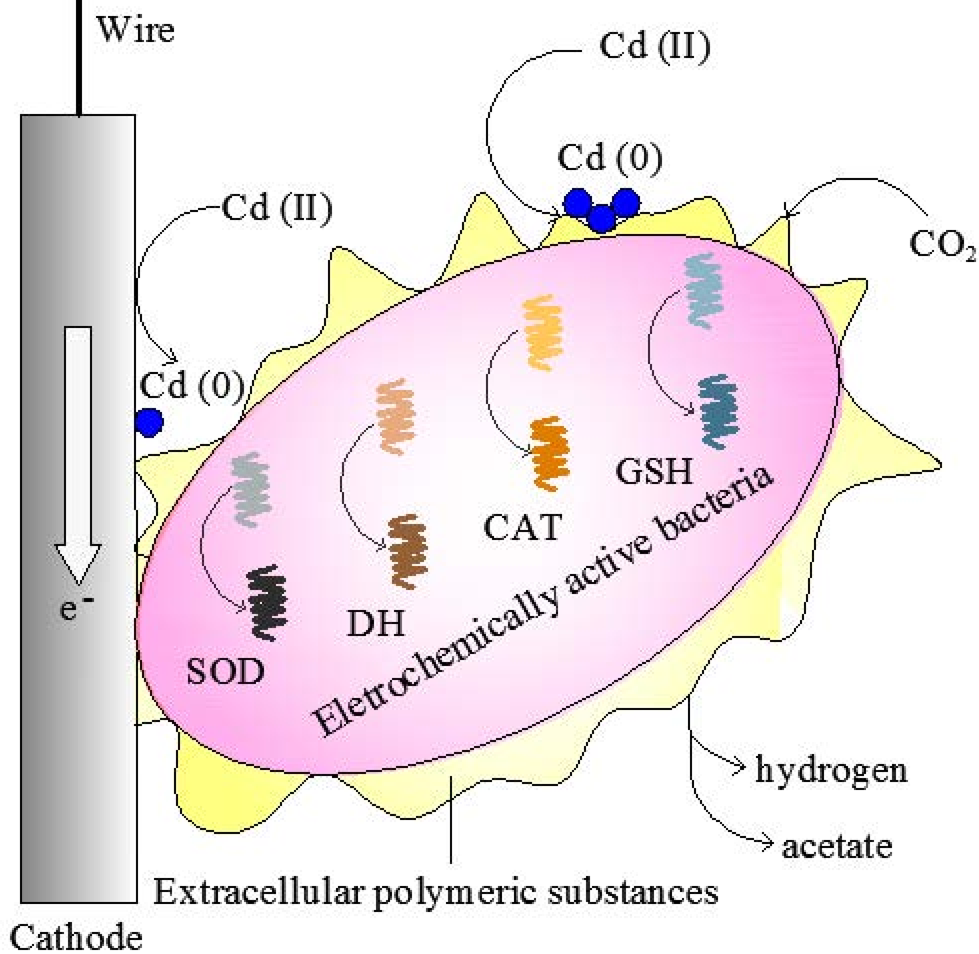
Fig. 7 CV tests on the cathodes catalyzed by (A, B, C, D and E) X1, (F, G, H, I and J) X3, (K, L, M, N and O) X5 or (P, Q, R, S and T) X7 at an initial Cd(II) of (A, F, K and P) 0 mg/L, (B, G, L and Q) 10 mg/L, (C, H, M and R) 20 mg/L, (D, I, N and S) 40 mg/L, or (E, J, O and T) 60 mg/L.

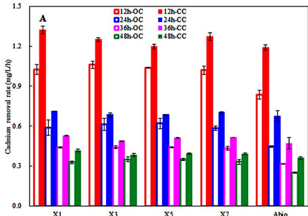
Fig. 8 Activities of dehydrogenase (A and B), glutathione (GSH) (C and D), catalase (CAT) (E and F), and superoxide dismutase (SOD) (G and H) in the biofilms (A, C, E and G) and planktonic cells (B, D, F and H) under closed circuital (CC) or open circuital (OC) conditions.

Fig. 9 XPS spectra of cadmium precipitates on either the EAB of (A and B) X1, (C and D) X3, (E and F) X5, and (G and H) X7, or (I and J) abiotic controls under closed circuital (A, C, E, G and I) or open circuital (B, D, F, H and J) conditions (initial Cd(II): 20 mg/L, 20 batch cycles).

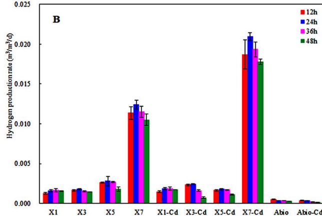
Highlights

- → Cd(II)-tolerant electrochemically active bacteria (EAB) favor H_2 or acetate production;
- → EAB release extracellular polymeric substances (EPS) to increase products production;
- → Products distribution correlates with intracellular physiological activities;
- → EPS released exhibits catalytic activity toward the reduction of Cd(II) to Cd(0);
- → Circuital current and Cd(II) stress induce EAB to release higher EPS amounts.

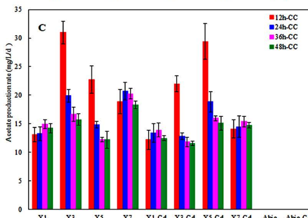

Wire
Cd (II)
Cd (0)
Cd (II)
CO2
Cd (0)
GSH
CAT
DH
SOD
Eletrochemically active bacteria
e-
hydrogen
acetate
Extracellular polymeric substances
Cathode



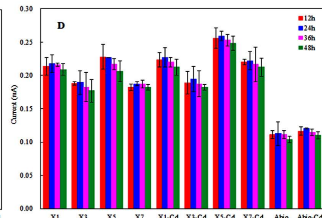
A
12h-OC
12h-CC
24h-OC
24h-CC
36h-OC
36h-CC
48h-OC
48h-CC
Cadmium removal rate (mg/L/h)
1.5
1.2
0.9
0.6
0.3
0.0
X1
X3
X5
X7
Abio



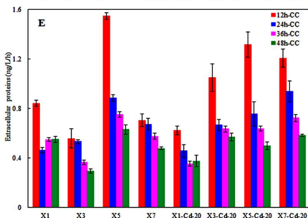
B
12h
24h
36h
48h
Hydrogen production rate (m3/m3/d)
0.025
0.020
0.015
0.010
0.005
0.000
X1
X3
X5
X7
X1-Cd
X3-Cd
X5-Cd
X7-Cd
Abio
Abio-Cd



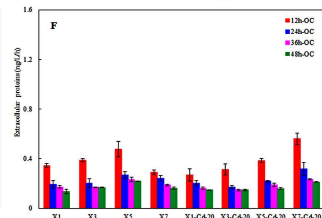
C
12h-CC
24h-CC
36h-CC
48h-CC
Acetate production rate (mg/L/d)
35
30
25
20
15
10
5
0
X1
X3
X5
X7
X1-Cd
X3-Cd
X5-Cd
X7-Cd
Abio
Abio-Cd



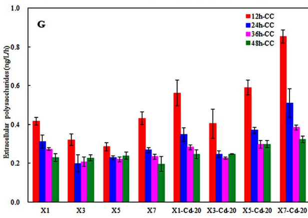
D
12h
24h
36h
48h
Current (mA)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
X1
X3
X5
X7
X1-Cd
X3-Cd
X5-Cd
X7-Cd
Abio
Abio-Cd



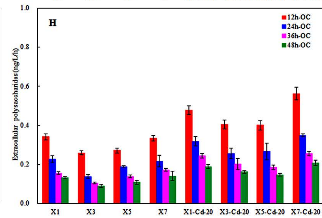
E
12h-CC
24h-CC
36h-CC
48h-CC
Extracellular proteins (ng/L/h)
1.6
1.2
0.8
0.4
0
X1
X3
X5
X7
X1-Cd-20
X3-Cd-20
X5-Cd-20
X7-Cd-20



F
12h-OC
24h-OC
36h-OC
48h-OC
Extracellular proteins (ng/L/h)
1.6
1.2
0.8
0.4
0
X1
X3
X5
X7
X1-Cd-20
X3-Cd-20
X5-Cd-20
X7-Cd-20



G
12h-CC
24h-CC
36h-CC
48h-CC
Extracellular polysaccharides (ng/L/h)
1.0
0.8
0.6
0.4
0.2
0.0
X1
X3
X5
X7
X1-Cd-20
X3-Cd-20
X5-Cd-20
X7-Cd-20



H
12h-OC
24h-OC
36h-OC
48h-OC
Extracellular polysaccharides (ng/L/h)
1.0
0.8
0.6
0.4
0.2
0.0
X1
X3
X5
X7
X1-Cd-20
X3-Cd-20
X5-Cd-20
X7-Cd-20

A

□ the rest ■ others ■ acetate ■ extracellular polysaccaride ■ extracellular protein ■ biomass

Total carbon (mM)

4.0
3.0
2.0
1.0
0.0

X1 X3 X5 X7 X1-Cd X3-Cd X5-Cd X7-Cd
12 h

X1 X3 X5 X7 X1-Cd X3-Cd X5-Cd X7-Cd
24 h

X1 X3 X5 X7 X1-Cd X3-Cd X5-Cd X7-Cd
36 h

X1 X3 X5 X7 X1-Cd X3-Cd X5-Cd X7-Cd
48 h

B

□ the rest ■ others ■ acetate ■ extracellular polysaccaride ■ extracellular protein ■ biomass

Total carbon (mM)

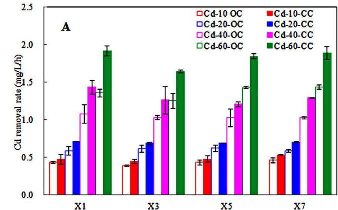
4.0
3.0
2.0
1.0
0.0

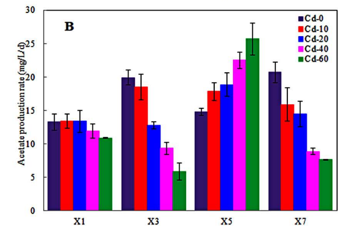
X1 X3 X5 X7 X1-Cd X3-Cd X5-Cd X7-Cd
12 h

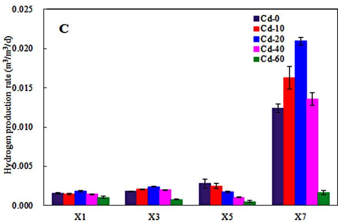
X1 X3 X5 X7 X1-Cd X3-Cd X5-Cd X7-Cd
24 h

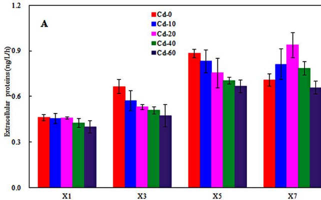
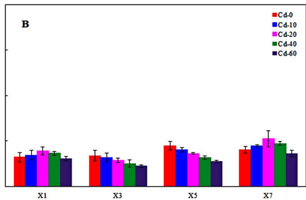
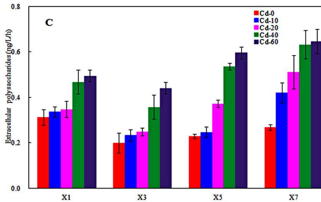
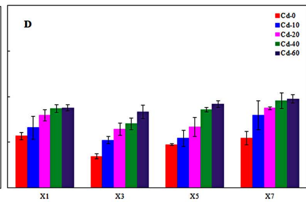
X1 X3 X5 X7 X1-Cd X3-Cd X5-Cd X7-Cd
36 h

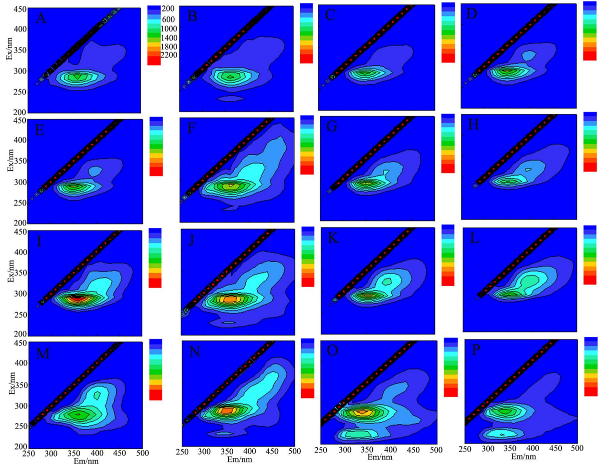
X1 X3 X5 X7 X1-Cd X3-Cd X5-Cd X7-Cd
48 h

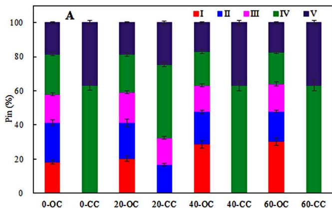

A
Cd-10 OC
Cd-10-CC
Cd-20-OC
Cd-20-CC
Cd-40-OC
Cd-40-CC
Cd-60-OC
Cd-60-CC
Cd removal rate (mg/L/h)
2.5
2.0
1.5
1.0
0.5
0.0
X1
X3
X5
X7

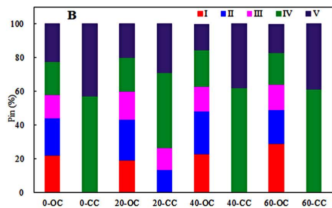

B
Cd-0
Cd-10
Cd-20
Cd-40
Cd-60
Acetate production rate (mg/L/d)
30
25
20
15
10
5
0
X1
X3
X5
X7

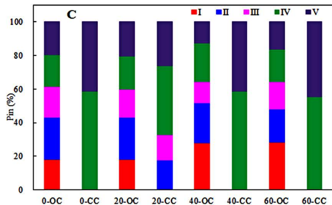

C
Cd-0
Cd-10
Cd-20
Cd-40
Cd-60
Hydrogen production rate (m3/m3/d)
0.025
0.020
0.015
0.010
0.005
0.000
X1
X3
X5
X7

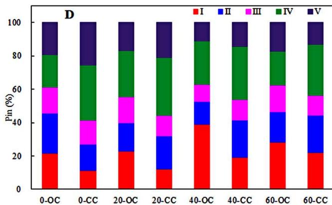

A
Extracellular proteins (ng/L/h)
1.2
0.9
0.6
0.3
0.0
Cd-0
Cd-10
Cd-20
Cd-40
Cd-60
X1
X3
X5
X7

B
Cd-0
Cd-10
Cd-20
Cd-40
Cd-60
X1
X3
X5
X7

C
Extracellular polysaccharides (ng/L/h)
0.8
0.6
0.4
0.2
0.0
Cd-0
Cd-10
Cd-20
Cd-40
Cd-60
X1
X3
X5
X7

D
Cd-0
Cd-10
Cd-20
Cd-40
Cd-60
X1
X3
X5
X7

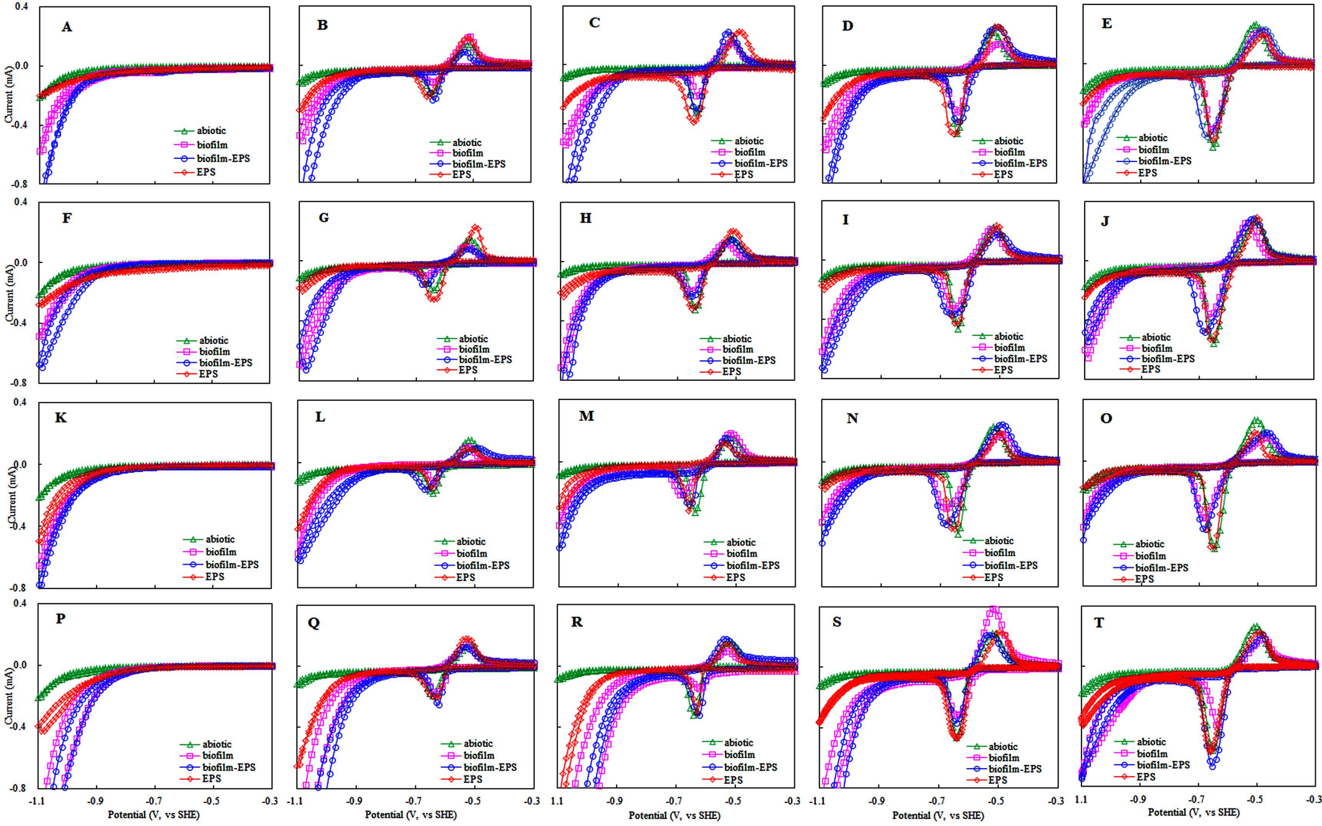

A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
200
600
1000
1400
1800
2200
Ex/nm
Em/nm
450
400
350
300
250
200
250
300
350
400
450
500

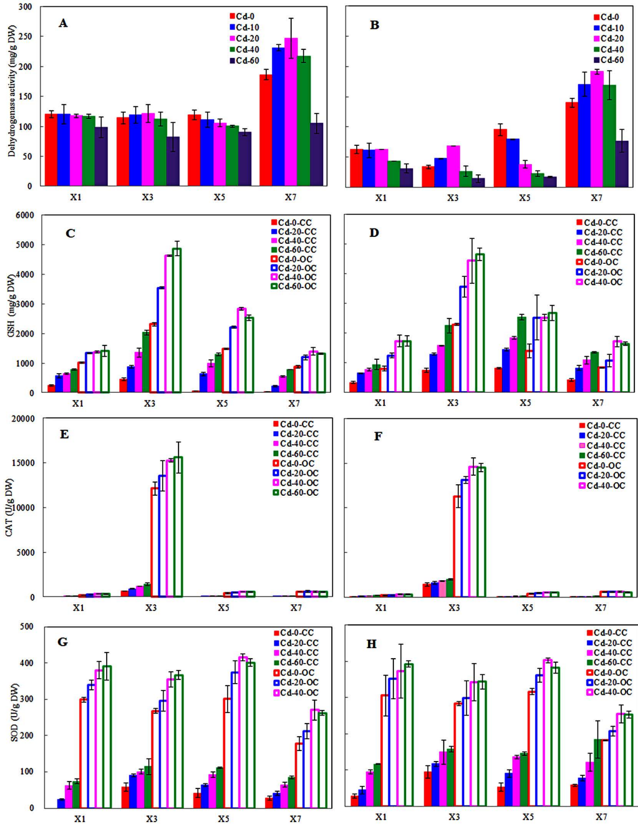

A
I
II
III
IV
V
Pin (%)
0
20
40
60
80
100
0-OC
0-CC
20-OC
20-CC
40-OC
40-CC
60-OC
60-CC

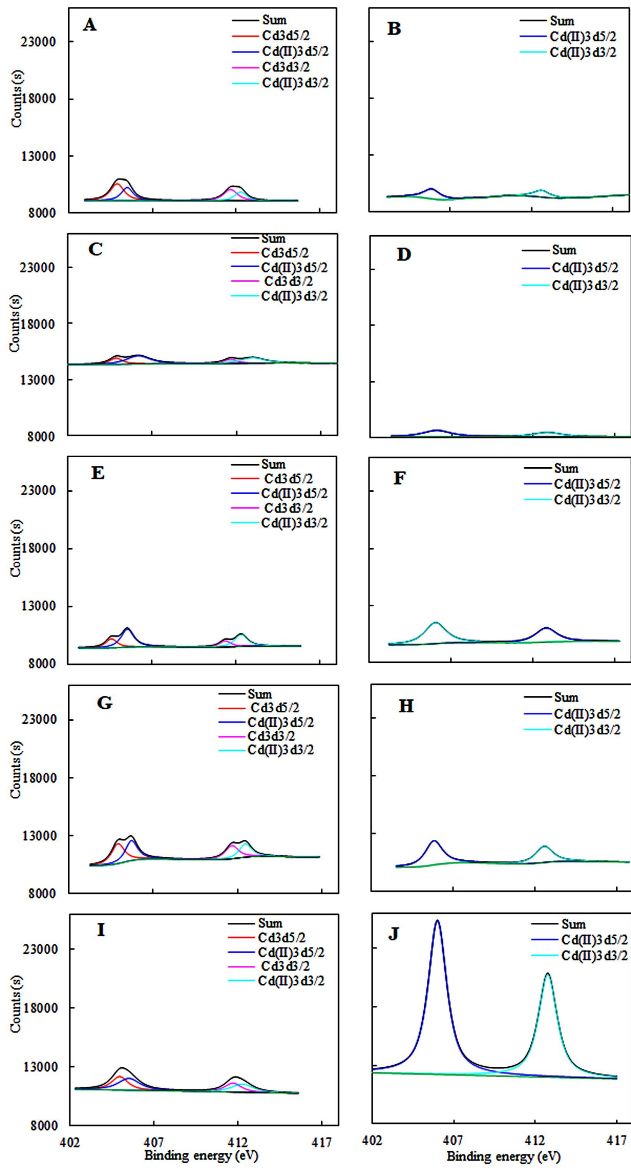

B
I
II
III
IV
V
Pin (%)
0
20
40
60
80
100
0-OC
0-CC
20-OC
20-CC
40-OC
40-CC
60-OC
60-CC


C
I
II
III
IV
V
Pin (%)
0
20
40
60
80
100
0-OC
0-CC
20-OC
20-CC
40-OC
40-CC
60-OC
60-CC


D
I
II
III
IV
V
Pin (%)
0
20
40
60
80
100
0-OC
0-CC
20-OC
20-CC
40-OC
40-CC
60-OC
60-CC


A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
abiotic
biofilm
biofilm-EPS
EPS
Current (mA)
0.4
0.0
-0.4
-0.8
Potential (V, vs SHE)
-1.1
-0.9
-0.7
-0.5
-0.3


A
B
C
D
E
F
G
H
Dehydrogenase activity (mg/g DW)
GSH (mg/g DW)
CAT (U/g DW)
SOD (U/g DW)
Cd-0
Cd-10
Cd-20
Cd-40
Cd-60
Cd-0-CC
Cd-20-CC
Cd-40-CC
Cd-60-CC
Cd-0-OC
Cd-20-OC
Cd-40-OC
Cd-60-OC
X1
X3
X5
X7


A
B
C
D
E
F
G
H
I
J
Sum
Cd3d5/2
Cd(II)3d5/2
Cd3d3/2
Cd(II)3d3/2
Counts(s)
8000
13000
18000
23000
402
407
412
417
Binding energy (eV)