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Electricity production by *Ochrobactrum*-related strain CD-1 and Pb^{2+} removal in dual-chamber microbial fuel cell

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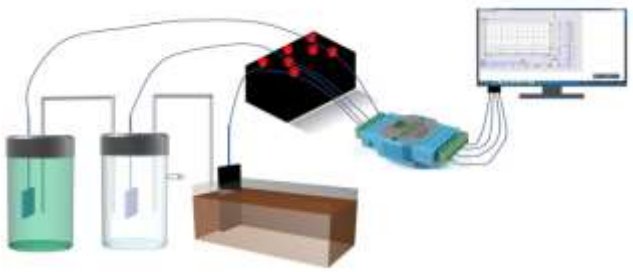
Graphical abstract



Abstract

Dual-chamber microbial fuel cell (MFC) was constructed to treat heavy metal wastewater (Pb^{2+} solution). The results showed the maximum voltage, the maximum electric power density and Pb^{2+} removal were 9.6 mV, 371.0 mV/m^2 and 52.3% respectively. The effects of different cathode chamber environments on the production potential (V, volts) and Pb^{2+} removal rate of strained CD-1 were investigated. The results show that when the cathode chamber is soil, the maximum voltage is 8.88mV, and when the cathode chamber is solution, the maximum voltage is 12.11mV. The removal efficiency of heavy metals polluted from near to far from the electrode plate in the soil is 68.9%, 62.1%, 57.5%, and the removal efficiency of heavy metals in the solution is 67.46%. It can be concluded that when the soil is the cathode chamber. The removal rate of local heavy metals was the highest. When the solution was used as a cathode chamber, the average removal rate of heavy metals was the highest. The bacteria, named strain CD-1, as the electricity production bacteria (EPB) used in the dual chamber microbial fuel cell (MFC), was isolated from activate sludge and identified on its morphology, physicochemical properties and phylogenetic positions. The results indicated that strain CD-1 was Gram-negative, rod. Oxidase and catalase reactions were positive. Strain CD-1 could use gluconate, lactose, D-fructose, L-Arabinose, D-arabinose, acetate, propionate, butyrate, lactate, sucrose and glucose, not use melibiose, galactose, N-acetylglucosamine, ethanol and glycerol.16S rRNA gene sequences analysis showed that strain CD-1 was most related to *Ochrobactrum* sp. (AJ245941) with the homology similarities of 98%. Based on the above results, strain CD-1 was belonged to the genus of *Ochrobactrum* and seemed to represent a novel species.

Keywords: Dual-chamber microbial fuel cell (MFC), heavy metal, potential generation, *Ochrobactrum*

1. Introduction

Unlike heavy metals, which are highly toxic and carcinogenic to many life forms including humans, most organic pollutants are biodegradable by microorganisms metal ions do not degrade into harmless end products. At present, global Pb pollution is increasing year by year, and lead can easily accumulate in animals and humans through the food chain, leading to blood lead. Therefore, efficient lead removal from sewage and soil is ecologically very important. Heavy metals in sewage and soil can be removed by physical and chemical methods. However, these methods cause secondary pollution and may not be cost-effective (Lourie *et al*., 2010). Many reports have shown that bacteria and fungi can biosorb heavy metals Cd(II), Au(III), and Pt(IV) (Rehman *et al*., 2010; Sochor *et al*., 2011; Lin *et al*., 2011; Lin *et al*., 2009; Li *et al*., 2011). Direct exposure of bacteria to heavy metals resulted in lower biosorption of severe toxicity of heavy metals. Furthermore, although physicochemical techniques can remove heavy metals more efficiently, the high cost and secondary pollution limit their practical applications. Therefore, it is very important to develop new technologies for heavy metal removal that are efficient, economical, and non-secondary pollution.

MFC is a new technology that can be applied to the remediation of heavy metals in soil (Tsipa *et al*., 2021). It can use microorganisms to convert the chemical energy of organic matter into electrical energy, and the internal electric field generated can directional migration to remove heavy metal ions. At present, MFC has achieved certain results in the application of water and soil heavy metal removal and recovery. ZHAO (Li *et al*., 2017) set up two constructed wetland microbial fuel cell (CW-MFC) devices, the experimental group (EG, adding 5 mg/L Pb(II)) and the control group (CG), the results showed that the

voltage of EG (343.16 ±12.14 mV) was significantly higher than the voltage of CG (295.49±13.91 mV), and the removal rate of Pb(II) reached (84.86 ± 3%). HABIBUL *et al*., (2016) operated the dual-chamber soil MFC for about 143 days and 108 days to remove Cd and Pb in heavy metal-contaminated soil, respectively, with removal rates of 31.0% and 44.1%, respectively. At this stage, there are many studies on the removal of heavy metal pollutants in water by microbial fuel cell technology, and some considerable results have been achieved. In the field of soil remediation, the main remediation technologies include physical remediation, chemical remediation and biological remediation. The microbial remediation technology in bioremediation technology is easy to operate, has the advantages of non-pollution, renewability, and low cost. If the resource of microorganisms can be effectively utilized and other external conditions are combined to remove pollutants from the soil environment, it will be a waste of heavy metals. Ideal measure for soil remediation. In this study, in order to avoid electron loss due to the mixing of electron acceptor and electron donor solutions, we constructed a two-chamber MFC. Our current study is the first joint removal of Pb^{2+} using MFC to combine pollutants and power generation, avoiding the direct exposure of bacteria to high concentrations of heavy metals, and obtaining a high removal rate of heavy metals.

2. Materials and methods

2.1. Construction of microbial fuel cells

Microbial fuel cells (MFCs) were constructed using transparent polyacrylic. The MFCs had two electrode compartments. The two compartments were separated by a salt bridge (diameter 0.2 centimeter; length 30 centimeter) which was used for cation exchange. The salt bridge was prepared by filling melt agar-KCl (agar, 3 g/liter; KCl, 240 g/liter) into a polyacrylic tube. The anode compartment was loaded with bacterial growth media (BGM) aseptically prepared by adding (per liter): 1g sodium acetate, 0.31g NH4Cl, 0.13g KCl, 3.75g Na2HPO4·12H2O and 4.9g NaH2PO4·H2O for culturing freshly prepared bacterial suspension. The cathode chamber is selected according to different experimental purposes. Both anode and cathode of MFCs were graphite felt (Electrosynthesis, E. Amherst, NY, USA). Both electrodes were cleaned and stored in HCl (0.1 M) and thoroughly rinsed in deionized water prior to use (Hitchens, 1989). Potential (V, volt) and current (I, ampere) of the MFCs were continuously recorded every minute by linking the MFCs to the serial communication port via a 32-channel data acquisition system (Model RDAM-4017). A changeable resistor (Model J2361; scale 0-9999Ω) was connected between anode and cathode for adjusting potential and current produced by MFCs. Data were recorded digitally on a Lenovo compatible personal computer using a control software (Forcecontrot 6.1). This software was to calculate the changes of potential and current with time. The power density was calculated according to the formula: $P= V^2/RA$, where V is the voltage, R is the external resistance, and A is the surface area of the electrode.

2.2. Selection of the best carbon source

On the basis of 2.1 device, the optimal carbon source conditions are explored. The cathode chamber was set as 2 l Pb (NO3)2 solution and the concentration of Pb in the solution was 2500 m g/l. Isometric pre-cultured bacterial cells (20 ml) were incubated in 2 liter of medium amended with sodium acetate (1 g/liter), sodium citrate (1 g/liter) and glucose (1 g/liter) in the anodic chamber respectively. Three sets of MFCs were constructed and placed in an incubator to maintain temperature (35 ℃). The anode and cathode chamber consists of containers of the same size （Cylindrical structure, the base radius is 4cm and the height is 15cm）. A graphite plate of 5 cm×5 cm×0.5 cm is built in and connected to 1000Ω resistance box by wire. The data information is uploaded to the computer by data acquisition system. Both the initial and final concentration of lead ion (Pb^{2+}) in cathodic solution were quantified by Atomic absorption spectrophotometer (Model-TAS986). The removal efficiency of Pb^{2+} was calculated with the following formula: r=[(Ci-Cf)/Ci] %, where r is removal efficiency (%); Ci is initial concentration(mg/l); Cf is final concentration (mg/l). The concentrations of cells in three anode chambers were estimated by reading optical density (OD660). All the experiments were done in triplicate and the average values were used in this study (Figure 1).

Figure 1. Schematic diagram of experimental set-up

2.3. Effect of different cathode chamber environment on potential (V, Volt) generation and pb generation

On the basis of the 2.1 device, different cathode chamber environments were changed. The bacterial cell culture method of 2.2 and the optimal carbon source condition (sodium acetate) obtained from the research were adopted in the anode chamber to construct the double-cathode chamber microbial fuel cell. Cathode chamber M1 is lead nitrate polluted water solution, cathode chamber M2 is lead nitrate polluted soil, the content of lead ion in both cathodes is 500mg. The cathode chamber M2 is a rectangular structure(25×10×10cm) with a 10×10 ×0.5cm graphite plate. The removal efficiency of Pb^{2+} in cathode chamber was measured after operation. The M1 lead ion determination method is the same as in Section 2.2. The water sample to be tested is named S4. The "S" shaped sampling method was used to collect soil samples from three locations near (1 ± 1 cm), in the middle (10 ± 1 cm) and far (19 ± 1 cm) from the cathode plate in M2 cathode chamber, which were named as S1, S2 and S3, respectively. The microwave digestion method (Chen *et*

al., 2013) was used to pretreat the soil to be tested, and the Pb^{2+} in the soil digestion solution was determined by flame atomic absorption spectrophotometry-flame prion absorption and division photometry (general analysis TAS986F) (Figure 2).

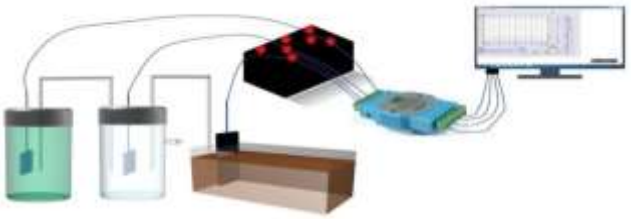


Figure 2. Schematic diagram of experimental set-up

2.4. Isolation and identification of EPB for MFCs

The EPB was isolated from the activate sludge from a local wastewater treatment plant. The activate sludge sample was serially diluted and plated on Zobell marine agar (ZMA) medium (ZoBell, 1941) amended with 500mg/l Pb(NO3)2. Bacterial colonies appeared on the ZMA plates were further streak on mineral salt medium (MSM) amended with varying concentrations of Pb^{2+} (500-2500 mg/l). MSM contained the following ingredients (per liter): 0.5g KH2PO4, 1.91g K2HPO4, 1.5g (NH4)2SO4, 0.5g NaCl, 0.1g CaCl2·2H2O, 0.2g MgSO4·7H2O and 0.05g FeCl3·6H2O (pH 7.0). The colony showing the largest area at the highest lead level was selected as the representative bacterial strain designated CD-1 temporarily for our following experiment.

2.5. Phenotypic characterization of the EPB

Both the cell morphology and MFC electrode enriched with the isolate were examined by scanning electron microscope (SEM) (Hitachi, S-4800) after the specimen was coated with gold (Park *et al*., 2001). Gram-stain for the isolate was tested as previously described (Lu *et al*., 2008). The physichemical properties were examined according to Bergey´s Manual of Determinatice Bacteriology (Breed *et al*., 1957).

2.6. Phylogenetic position analysis

Genomic DNA of strain CD-1 was extracted with TIANamp Bacteria DNA DP302 Kit (Beijing Tiangen Biotech) according to its manuscripts. A 16S rRNA gene fragment of the extracted DNA was amplified by PCR using the primers PF5´-AGA GTT TGA TCC TGG CTC AG-3´and PR 5´-GGY TAC CTT GTT ACG ACT T-3´. PCR reactions contained 100 ng of genomic DNA, each primer at a concentration of 0.4 µM, each dNTP at a concentration of 200µM. The following thermal profile of 3 min at 94 ℃, 32 cycles of 45 s at 95℃, 45 s at 55℃, and extension for 10 min at 72 ℃ was performed using Applied Biosystems (Model: 2720 thermal cycle). The electrophoresis, the purification of PCR products were essential as described previously (Thompson *et al*., 1997).

The 16S rRNA sequences were determined by the automatic DNA sequencer (ABI Prism Model 3700, CA, USA) using the primer of PF as mentioned above. The sequence determined from strain CD-1 was compared to the most closely related sequence retrieved from GenBank database using the BLAST program. All the obtained sequences were aligned using the CLUSTAL X program (Saitou and Nei, 1987). The neighbor-joining phylogenetic tree was constructed using the Mega.5 program (Sharma *et al*., 2002; Tamura *et al*., 2011; Hitchens, 1989). A bootstrap analysis was based on 1000 resamplings.

The 16S rRNA sequence for strain CD-1 has been deposited in DNA Data Bank of Japan (DDBJ) under accession number AB971024.

3. Results

3.1. Physiochemical characterization of strain CD-1

The bacterial colonies appeared on the ZMA plates were further tested for their tolerance on Pb^{2+} by streaking them on MSM medium amended with series concentrations of nitrate lead (500-2500mg/l). The maximum tolerance for the isolates on nitrate lead inoculated on MSM agar plates was 1500mg/l. The purified colony with the largest area was selected and named strain CD-1 for our study. The scanning photos of strain CD-1 were shown as Figure 3(a, b, c). Figure 3(a) and Figure 3(b) were the photos of strain CD-1 cultured in MSM agar medium with no nitrate lead amended and nitrate lead amended (Pb 2+ concentration was 1500 mg/l) respectively. Figure 3(c) was the photo of strain CD-1 attaching on the surface of anode graphite after 5 days' incubation in BGM medium in the anode chamber (arrows points). Strain CD-1 was gram-negative rod (0.45 to 0.55 µm long and 1.15 to 1.27 µm wide) (Figure 3(a)). Oxidase and catalase reactions were positive. Other physiochemical properties of strain CD-1were summarized in Table 1. The strain CD-1 grew on BGM medium supplemented with gluconate, lactose, D-fructose, L-Arabinose, D-arabinose, acetate, propionate, butyrate, lactate, sucrose and glucose. However, strain CD-1 did not use melibiose, galactose, N-acetylglucosamine, ethanol and glycerol (Table 1).

Table 1. Physiological and morphological characteristics of strain CD-1

Characteristic	Strain CD-1
Cell shape	Rod
Carbon sources and electron donors	
Gluconate	+
Lactose	+
Melibiose	-
Galactose	-
D-Fructose	+
L-Arabinose	+
Turanose	W
N-Acetylglucosamine	-
D-Arabinose	+
Acetate	+
Propionate	+
Butyrate	+
Lactate	+
Ethanol	-
Glycerol	-
Glucose	+

Sucrose	+

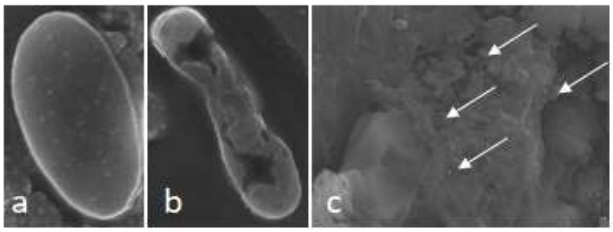


Figure 3. Scanning electron micrograph of cells of strain CD-1 (× 100000) grown in ZMA medium without lead nitrate amended (a) and with nitrate lead amended (b); Cells of strain CD-1 attached on the graphite anode surface (×5000) after 5 days' incubation in BGM medium (c)

3.2. Potential development and power density changes from MFC

The effects of different carbon sources for strain CD-1 on potential generation were tested in the present study. The pre-culture suspension of strain CD-1 (20 ml) was cultured with fresh medium (2 liter) amended with sodium acetate (1 g/liter), sodium citrate (1 g/liter) and glucose (1g/liter) respectively in anode compartment under open circuit conditions. The operational states, including outer resistance (1000Ω) are the same for the above three parallel experiments. The potential development with time was shown as Figure 4. Figure 4 showed the maximum potential was obtained after 12 hr operation (9.6 mV) for strain CD-1 using sodium acetate as its carbon source and 20 hr (7.5 mV), 36 hr (2.4 mV) for strain CD-1 using sodium citrate and glucose respectively. The area of graphite cathode (50×50 mm in dimension) was 0.0025m2. The power density was calculated according to the formula of P= V2/RA mentioned previously and shown in Figure 5. Figure 5 indicated that the power density was increased sharply from 4.2 to 371.0 mW/m2 after 6 hr operation for sodium acetate as carbon source and 12 hr operation (from 2.4 to 225.1 mW/m2) for sodium citrate as carbon source. However, the power density showed a long lag phase and increased slowly after about 30 hr operation when glucose was used as carbon source for strain CD-1.

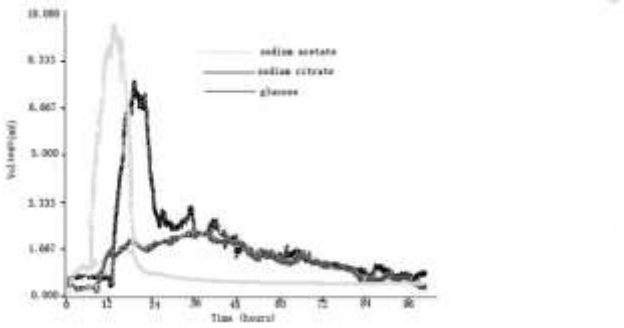


Figure 4. Voltages obtained by the outer resistance (100 Ohm) using various substrates of acetate, citrate and glucose.

3.3. Phylogenetic positions analysis of strain CD-1

The nearly completed 16S rRNA gene sequence (1,441 nucleotide, GeneBank accession number AB971024) of strain CD-1 and the sequences obtained from GenBank were used to constructed phylogenetic tree (Figure 6). The phylogenetic tree indicated that strain CD-1 was fallen in the group of genus Ochrobactrum. Strain CD-1 exhibited the highest 16S rRNA gene similarity of 98% with Ochrobactrum sp. (AJ245941) and was belonged to genus Ochrobactrum.

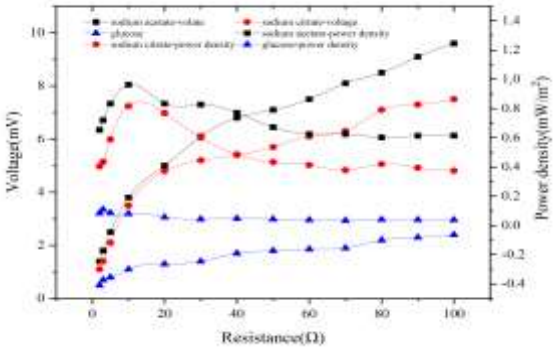


Figure 5. Power density and voltage changes with different carbon sources in medium

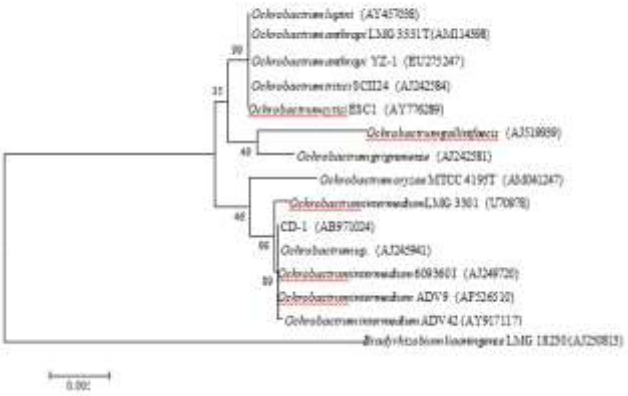


Figure 6. Phylogenetic tree of strain CD-1and closely related species based on their 16S rRNA sequences. The neighbor-joining method was use to constructed this phylogenetic tree. Bootstrap values obtained with 1000 repetitions are indicated at the nodes. Bar 0.005 substitutions per nucleotide position. Accession numbers from GenBank are in parentheses

3.4. The removal of Pb^{2+} by MFCs with different carbon sources

Effect of different carbon sources on removal of Pb^{2+} was determined with acetate, citrate and glucose in anode medium. The initial concentration of Pb^{2+} in medium was 2500 mg/l. The initial and final concentrations of Pb^{2+} in cathode liquid were measured using Atomic absorption spectrophotometer (Model-TAS986). After 3 days' incubation, the removal efficiency of Pb^{2+} were 52.3%, 37.5% and 19.2% for acetate, citrate and glucose respectively. The electron accepted by heavy metal (Pb^{2+}) in cathodic chamber were from metabolism reaction participated by bacterial strain CD-1. In order to examine the effect of carbon sources on bacterial metabolism, we measured the optical density (OD660) of strain CD-1 using different carbon sources. The initial values of OD660 for the culture amended 20 ml pre-culture in three anodic chambers were almost same, 0.068 for acetate, 0.071 for

citrate and 0.059 for glucose. The maximum OD660 values of cells appeared at 12 hr incubation (OD660, 0.74) for using sodium acetate as carbon source, 20 hr (OD660, 0.31) for sodium citrate and 36 hr (OD660, 0.15) for glucose respectively.

3.5. Effect of different cathode chambers on the generation potential (V, volt) and removal of Pb^{2+} from strain CD-1

In this study, the effects of different cathode chamber environments on the production potential (V, volts) and Pb^{2+} removal rate of strained CD-1 were investigated. The change curve of voltage with time is shown in Figure 7. The two groups of curves showed an overall upward trend, the voltage of M1 was significantly higher than that of M2, the maximum voltage of M1 reached 12.11mV, and the maximum voltage of M2 reached 8.88mV. It can be seen from Figure 8 that when the M2 soil is used as the cathode chamber, the removal efficiency of heavy metals at the local position is the highest, and the removal efficiency of Pb at the position S1 is the highest at 68.9%, and the removal rates at the other positions (S2 and S3) are slightly lower, 62.1% and 57.5% respectively , the average removal efficiency was 62.8%; the average removal efficiency of heavy metals was the highest when the M1 solution was used as the cathode chamber, and the removal rate was 67.46%, but it was still lower than that of S1.

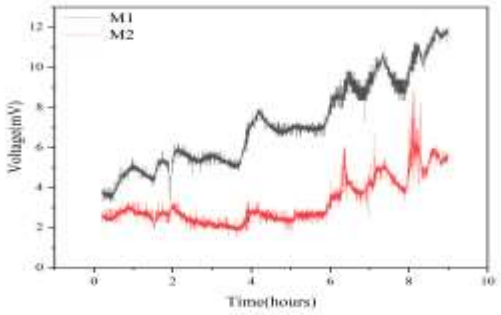


Figure 7. Voltage curve of different cathode chamber environment

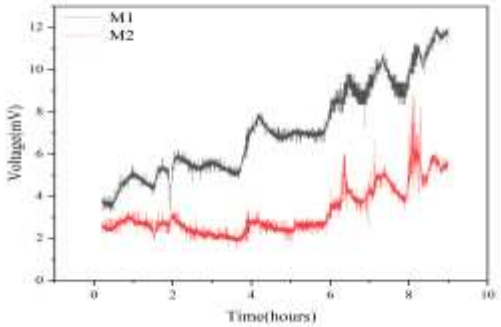


Figure 8. Heavy metal removal efficiency diagram

3.6. Contribution of Bioelectrochemical Reduction to Pb^{2+} Removal in MFCs

There are two mechanisms for Pb^{2+} removal in MFCs, for example, cathodic bioelectrochemical reduction and direct reduction by Pb-reducing microorganisms (Figure 9).

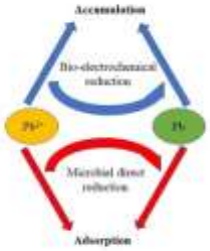


Figure 9. Proposed processes for Pb^{2+} removal in MFCs

H^+ is consumed in the cathode chamber, and OH- is consumed in the anode chamber, so the pH value of the cathode chamber rises, the pH value of the anode chamber decreases, and the soil becomes acidic. The positively charged H^+ migrates to the cathode under the action of an electric field, and OH- moves to the anode, so that the soil pH gradually increases from the anode to the cathode. The mobility of hydrogen ions is 1.8 times that of hydroxide ions, so the entire soil system is acidic. In the acidic region, lead in the soil exists in the soil system in the form of ions, which is conducive to the migration and removal of lead ions. Lead ions have a higher redox potential, and cathode microorganisms are more likely to reduce high-valent lead ions to electron acceptors, thereby accelerating the transfer of electrons between the cathode and anode (Figure 10).

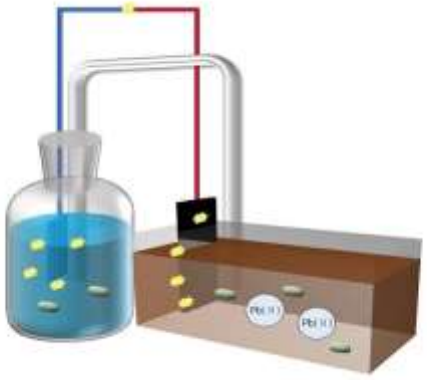


Figure 10. Experimental mechanism diagram

4. Discussion

Sheng *et al*. (2008) reported that the endophytic bacteria Pseudomonas fluorescens G10 and Mycobacterium sp. G16 reduced pb toxicity in Brassica napus. MI-NA SHHIN *et al*. (2012) reported that endophytic bacteria, Bacillus sp. MN3-4 exhibited pb adsorption of 65.8%. The higher pb removal rates was observed by culturing Bacillus sp. MN3-4 in the medium supplied with 1500 mg/l of Pb^{2+}. Compared with the above report, the Pb removal efficiency in our study by Dual-chamber MFC was little lower, only 52.3%. However, the initial concentration of Pb^{2+} in cathodic chamber was 2500 mg/l in our study and much higher than that of 1500 mg/l, the highest resistant

for Bacillus sp. MN3-4 reported by MI-NA SHIN *et al* (2012). It seemed that the directed toxicity of Pb^{2+} on bacteria inhibited bacteria metabolism and led to the lower tolerance on Pb^{2+} for Bacillus sp. MN3-4 than strain CD-1 used in our study. A SEM microgram of strain CD-1 is present in Figure 3(c). SEM revealed that strain CD-1 extracellularly accumulated lead. The extracellular sequestration could be due to the exopolymers produced by strain CD-1. The reaction between the expolymers and metal ions is thought to involve specific ionic interactions. Figure 3(c) indicated the toxicity of ion lead on strain CD-1 grown in MSM agar medium with 1500 mg/l of Pb^{2+}.

By using the Dual-chamber MFC, we demonstrated that heavy metal, lead ion (Pb2+) could be efficiently reduced by accepting electrons generated through decomposition of acetate, citrate and glucose by strain CD-1. Compared to citrate and glucose, acetate seemed to be much suitable for strain CD-1 to generate electron (Figures 4 and 5). Hyung *et al*. (2002) also demonstrated the importance of carbon sources of acetate and glucose in potential development. In a typical microbial fuel, the current is generated when the electrons from the oxidation of the substrate by microorganisms are available to the electrode and is dependent on the bacterial cell growth and on the electrode surface area (Figure 3(b)) (Kim *et al*., 1999; Kim *et al*., 1999; Kim *et al*., 1999). The final OD660 values (acetate, 0.74; citrate, 0.31 and glucose,0.15) obtained by culturing bacterial strain CD-1 for 3 days proved the accordance of larger oxidation capability from microorganism with much more potential generated (Figures 4 and 5) in this study.

C. Jayashree *et al*. (2014) has demonstrated that Ochrobactrum sp. RA1 (NCBI accession no KJ408266) could generate the maximum power density of 362 mW/m2 in a dual chambered MFC, phenol as their sole carbon source. In our study, the maximum power density of 371.0 mW/m2 was observer by Ochrobactrum repbskd strain CD-1(NCBI accession no AB971024) along with a higher removal rates of Pb2+(52.3%), sodium acetate as the carbon source. It suggested electrons generated by oxidizing substrates were easier for strain CD-1(oxidizing sodium acetate) than Ochrobactrum sp. RA1 (NCBI accession no KJ408266) (oxidizing phenol). In conclusion, Ochrobactrum-related strain CD-1(AB971024) isolated from activate sludge could be used to remove heavy metal Pb2+ from liquid environment by oxidizing carbon sources (such as acetate, citrate and glucose) to generate electrons. In addition, the accordance of potential generation with the capability of oxidizing substrates by bacteria has also been demonstrated in this study.

Through double-cathode chamber experiments, we investigated the effects of different cathode chamber environments on the production potential (V, volt) and Pb2+ removal rate of strained CD-1. The experimental results show that the local heavy metal removal efficiency is the highest when the Pb(NO3)2 contaminated soil is used as the cathode chamber, reaching 68.9%; the average removal efficiency of heavy metals is higher when the Pb(NO3)2 solution is used as the cathode chamber, reaching 67.46%. At this stage, there are many studies on the use of microbial fuel cells to treat wastewater, but there are few studies on soil. The above experimental results show that under the same external conditions, the removal efficiency of microbial fuels in local parts of the soil is higher than that of wastewater solutions. Microbial fuel cells can be used as new means of removing heavy metal ions in soil.

Acknowledgments

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