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# Phenol-acclimated activated sludge and *Ralstonia eutropha* in a microbial fuel cell for removal of olive oil from mill wastewater

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**Abstract**−The fuel acclimation process offers flexibility in microbial fuel cell (MFC) power generation behavior. Different concentrations (50-200 mg/L) of phenol were used for adapting the activated sludge (AS), obtained from a local petroleum wastewater treatment plant and *Ralstonia eutropha* pure culture. Anodic biomass capable of oxidizing phenol substrate, using either AS inoculum microbial consortium or *R. eutropha* in the MFC system, has been a reflection of growth supportive functionality of phenol and 150 mg/L as initial concentration was used in the experiments. For both types of inocula. The results of phenol and COD removals obtained for closed system configuration were compared with those under open circuit condition. The current production by AS and *R. eutropha* was improved through phenol acclimation process. The highest power density (PD) using either AS or *R. eutropha* was 11 and 5.8 $mW/m^2$, respectively. In terms of using olive oil mill wastewater as the anodic substrate, the behavior of phenol-acclimated *R. eutropha* was better than that of the synthetic type of wastewater, and the PD value was 7.8 $mW/m^2$.

Keywords: Activated Sludge, Anodic Biomass, Olive Oil Mill Wastewater, Phenol Acclimation, *Ralstonia eutropha*

## INTRODUCTION

Wastewaters (WWs) generated in industries all contain considerable amount of organic compounds, and for treating the WWs, different physical, chemical, and biological technologies are available (ion exchange, chemical precipitation, adsorption, reverse osmosis, coagulation, flocculation) where in each case extensive studies based on theoretical/practical principles have been presented in the literature [1,2]. Paying attention to the quality of the effluent also plays a role in this subject; and to relieve from the burden of the WW release to the environment, the WW must meet certain requirements to select clean and sustainable WW treatment. In maintaining healthy ecosystems and human health, one encounters a complex task and the criteria presented in the literature have been found to be based on choosing by advantage the approach (CBA) developed on basis of the researches reported by Arroyo et al. [3].

Efficiency of some technologies, such as microbial fuel cell (MFC) by coupling to other WW methods, has increased considerably. For instance, the design of two stage MFC and upflow anaerobic sludge blanket (UASB) followed by use of the immobilized biological aerated filter (IBAF) was found to be an effective approach for removal of partial ammonia. The major part of organic compounds in palm oil mill effluent (POME) in the first stage and major part of ammonia was released in the IBAF stage, while the excess organics were also removed in this stage [4]. In these types of integrated approaches, the target pollutant, instead of being removed completely, can be used in anode compartment of the MFC system. The acclimated microbial cells actively catalyze oxidative cleavage of chemical bonds of the contaminant and the energy which is biologically produced upon utilization of the organics by the microbes ultimately is convertible to clean electricity. In fact, the characteristic of the grown bacterial cells as biofilm on anode electrode is to contain cells which are electroactively able to carry out a thermodynamically favorable process in the form of the net anodic-cathodic reaction (negative value for the free- energy change corresponds to positiveness of the redox potential-Nernst equation). Spontaneity of the current production could be matched to the capable microbes in achieving a high rate of electron transfer [5]. Thermodynamic-kinetic relationship based on Marcus theory shows that free energy change as the thermodynamic quantity can be used to obtain the rate constant of the redox reaction (focusing on transition state theory/activation energy as related to the Arrhenius equation) [6,7].

The ability of the electrode to express high power output encounters several limitations in the case of two-dimensional anode electrodes such as low surface area, high internal resistance, and high mass transfer effects [8]. Results of focusing on advances in material science in recent years show development of the three-dimensional electrode; and in addition to carbon-based anodes, cases such as metal-based, carbon composite, and surface-modified anode have all been introduced. The reports show that these materials play a significant role in the performance of the MFCs (single and double chamber with or without air cathode) [9].

Although MFCs mostly rely on the performance of abiotic air-cathode, the anodic electrons and their movement to oxygen acceptor in cathode electrode have shown to be facilitated by use of transition metals such as iron where the redox states of iron for instance change at relatively high rate. And in these reductions of Fe (III) to Fe (II) and the reoxidation of Fe (II) to Fe (III), the electrons would safely deliver to oxygen as the ultimate acceptor of electrons [10]. By participation of microbial cells in these types of

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reduction-reoxidation processes (such as biological manganese oxidation) the role of biocathode in MFC performance has also been described and the relevant cycle determined experimentally [10,11]. MFC system can also be handled in the absence of oxygen in cathode electrode. The movement of electrons in these cases has been explained in terms of anaerobic biocathodes, and regarding the level of redox potential, cathodic nitrate reduction is comparable to oxygen [10,11]. Studies on sediment type MFC showed that with the use of denitrifying bacteria, reduction of nitrate to $N_2$ gas would be possible [12]. MFCs performance in terms of organic compound production using WW, such as brewery WW, has generated considerable interest on application of microalgae which grow well in nutrient-rich WW (brewery WW which has high phosphorous and nitrogen content) [13]. Thus one is able to obtain the biomass produced in the redox reactions and at the same time, the substance of the interest (such as lipids) can be extracted where the possibility of using these lipidic materials for production of biodiesel, for instance, is an interesting subject in area of biofuels production [13].

Presence of phenols in effluent of petroleum refineries, petrochemical production, and several other industries is dominant. With respect to water quality criteria, phenol has been placed in the list of toxic chemicals (US Environmental Agency (EPA)), and according to the World Health Organization (WHO), the maximum allowable level of phenol in drinking water is 1 mg/L [14]. Among various methods available for removal of phenol, biodegradation has found to be in favor of the ecosystem [14]. *Pseudomonadaceae* as the bacterial family is one major component of the AS of the most WW worldwide [15]. Most members of *Pseudomonas* species have many biochemical functions in common with *R. eutropha* [16,17]. The presence of *Pseudomonas* has also been reported in anodic biofilm in the MFC system operated using olive oil mill wastewater (OMWW), which was inoculated with the AS obtained from the WW generated in yeast production plant [18]. We have experienced capacity of *R. eutropha* in biodegradation of OMWW phenolics [19,20]. Comparison between MFC behaviors in response to the mixed culture of the AS obtained from a local petroleum WW plant and *R. eutropha* pure culture provides some useful information. Presence of *Pseudomonas* species in the obtained samples was confirmed [21]. Using phenol containing synthetic wastewater appeared to be a reasonable approach in this comparison. Further experiments in the present work were directed to study MFC performance upon using OMWW substrate and phenol-acclimated *R. eutropha*.

## EXPERIMENTAL

### 1. Preparation of Phenol-acclimated Bacterial Culture

The *R. eutropha* (PTCC 1615) used in the present study was purchased from Iranian Research Organization of Science and Technology (IROST). Slant nutrient agar was used for growing and maintaining bacterium in which sub-culturing was performed by transferring appropriate amount of cells into the nutrient broth (30 °C for 24 h). The aerobically grown bacterial cells were transferred to a 250 ml conical flask, containing 100 ml mineral salt medium (MSM), which consisted of the following ingredients: $KH_2PO_4$ 1 g, $K_2HPO_4$ l g, $(NH_4)_2SO_4$ 1 g, and $MgSO_4{\cdot}4H_2O$ 0.05 g, dissolved in 1 L distilled water. The phenol was added to the MSM as the carbon source. The prepared cultures were incubated in an incubator shaker (120 rpm, 30 °C, WISE cube WIS-20R). Phenol at the 50-200 mg/L as the initial concentration was used for the acclimation process, and complete consumption of phenol by the test bacterium within reasonable time was used as an index of the phenol acclimation.

The activated sludge (AS) used in the present study was collected from the aeration tank of a petroleum wastewater treatment plant (COD at 300-600 mg/L and phenol at 15-45 mg/L) in Arak, central state, Iran.

### 2. The Construction and Operation of MFC

Experiments were implemented using an electrochemical bioreactor, which is made of Pyrex, as a double chamber unit with aqueous air cathode; Fig. 1 indicates the vessel schematic diagram. Height and diameter of each cylindrical chamber was 8.5 cm and 14 cm, respectively. The total volume of the MFC unit was 800 ml including 100 ml headspace. The chambers were separated by a proton exchange membrane (PEM as Nafion 117, DuPont, USA) with 15.9 $cm^2$ surface area. Also, graphite electrode plates (9×4 cm with 0.5 mm thickness) were placed in anode and cathode chambers and parallel to each other with a 15 cm distance between them. Copper wire was used for the connecting and establishing electrical circuit (Fig. 1). Providing maximal passage for hydrogen ions in catholyte is so important due to the high porosity of PEM. Before placing it in the MFC system, the PEM was treated considering the following three steps, with each step lasting for one hour [22]: mix and boil in $H_2O_2$ solution (30%) followed by mixing and boiling in deionized water (pH 7), and in $H_2SO_4$ solution (0.5 M). The electrodes also were treated considering the following protocol (duration of each step was twenty-four hours) [22]: soaking in HCL solution (1 M), followed by soaking in NaOH solution (1 M), and soaking in deionized water.

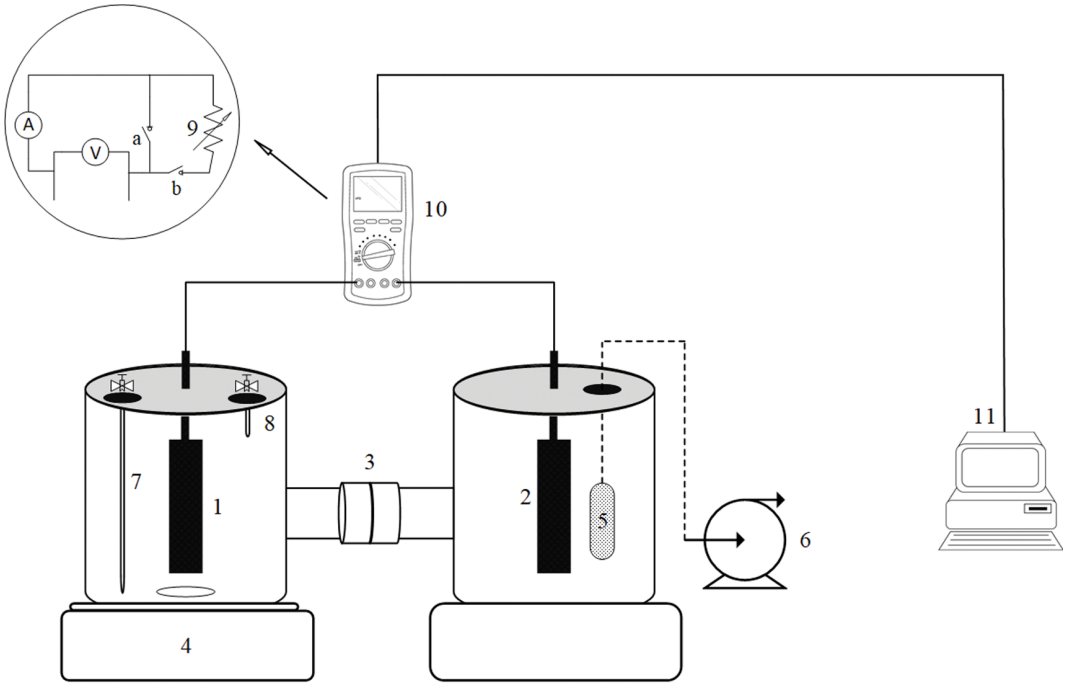
The anaerobic condition for the anode chamber was provided by flushing the chamber periodically with nitrogen gas for 15 min. Phosphate buffer solution (0.1 M, pH 7) was used to fill the cathode chamber and air cathode condition was provided with use of the compressed air. The MFC operation was started by adding the MSM to the anode chamber, and this was followed by the medium inoculation with phenol-acclimated AS (0.2 g/100 ml) or *R. eutropha* (10% v/v). For the culture growth, phenol was added to the anode chamber as the carbon source (phenol containing synthetic type of wastewater). The experiments were performed at room temperature, and the change processes were determined by measuring phenol and COD concentrations.

### 3. Measurements and Calculations

Current and voltage were measured using a multimeter (MT-1860 ProsKit Co, Taiwan), and recorded on a PC. The external load was applied using a resistor box to measure current variations under closed system configuration (MT-RB-MEGATEK, Taiwan). Power was calculated according to the following equation:

$$P=I\times V \quad (1)$$

where the terms P: power (W), I: current (A), and V: acquired voltage (V). Polarization curve was obtained upon the MFC system polarization, in which it was possible to determine power density (PD) generation with considering the anode electrode area



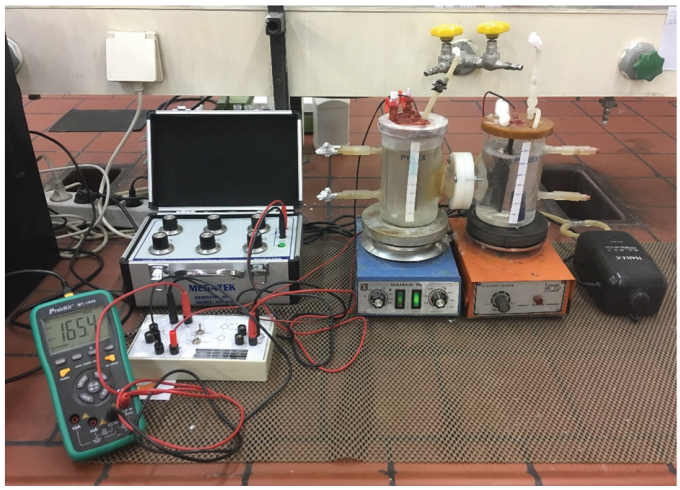



**Fig. 1. Schematic diagram of two-chamber MFC used in the present study. specification of the multimeter (10) are as follows: switches "a" and "b" were off in order to study MFC system under OCV condition. The system was under closed circuit configuration when switch "a" was on and switch "b" was off. When switch "a" was off and switch "b" was on, the system was ready to study effect of different external resistances. The test system picture is also shown.**

1. Anode electrode
2. Cathode electrode
3. Proton exchange membrane (Nafion)
4. Magnetic stirrer
5. Air stone
6. Aquarium pump
7. Port for N2 gas and sampling
8. Air exit port
9. Resistor box
10. Multimeter
11. Personalized computer (data acquisation system)

($A_{anode}$), and by monitoring the output voltage at various external resistances ranging from 5 to 30,000 ohm. The current density (CD) was calculated using following equation:

$$CD=I/A_{anode} \quad (2)$$

The MFC internal resistance was measured from the linear portion slope of the polarization curve.

Chemical oxygen demand (COD) was measured using Merck reagents following manufacturer instructions. Phenol concentration was analyzed using Folin-Ciocalteu reagent as described by Box [23].

Samples were taken from anodic compartment and measurements were performed two times; the mean value±standard deviation was reported in each case.

## RESULTS AND DISCUSSION

### 1. MFC Power Behavior

1-1. Phenol Acclimation and Anodic Metabolism

The fuel cell was designed to receive synthetic type of wastewa-

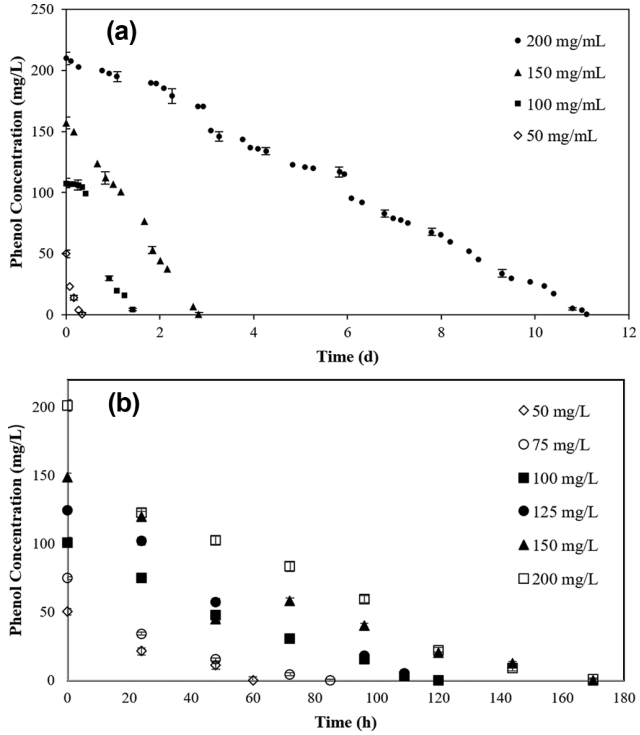



**Fig. 2. Phenol acclimation process applied in the MFC system in the present study with *R. eutropha* (a) and activated sludge (b) as the anodic inocula.**

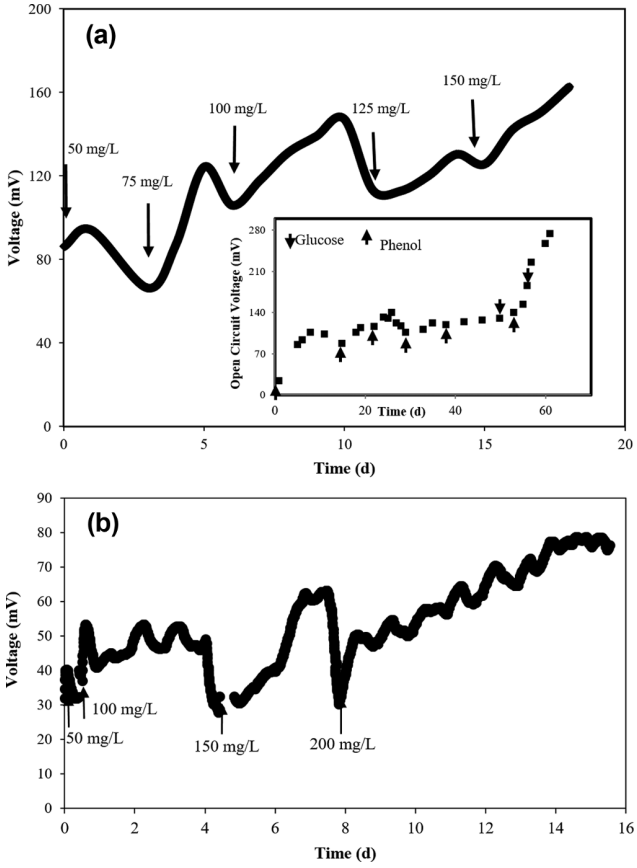
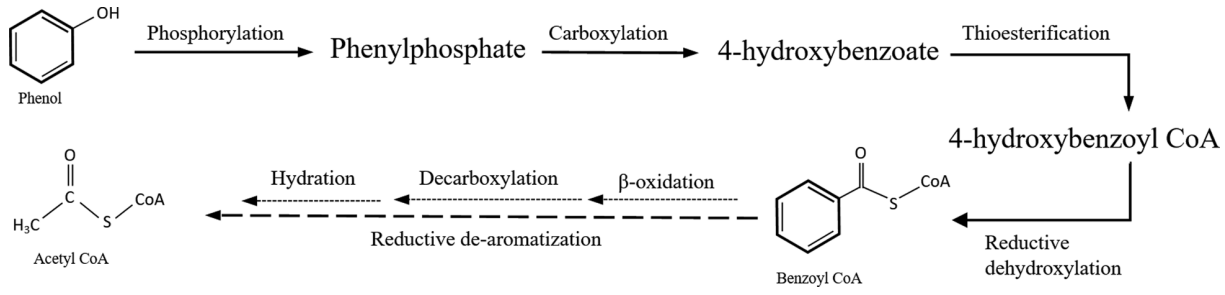



**Fig. 3. Variation of voltage under open circuit condition measured during the MFC operation using aerobic catholyte - the arrows show the time of phenol feeding duration: the activated sludge (a) and *R. eutropha* (b) as the anodic inocula.**

ter containing phenol at increasing initial concentrations in the range of 50-200 mg/L. In this way it was possible to measure the MFC behavior in response to inhibitory effect of phenol on the involved microbial systems. There is a direct connection between the metabolic activities processes in microbes and their adaptation phase to fuel substrate molecules; attempts thus were made to obtain phenol-acclimated microbial consortia for both of the activated sludge and *R. eutropha*. Fig. 2 illustrates the results of phenol degradation by *R.eutropha*; and the inoculum sludge *R. eutropha* was very efficient and able to consume 150 mg/L of phenol within 72 h, while the bacterium behavior to 200 mg/L of phenol was drastically changed. This growth inhibition effect of phenol at high concentration may relate to the presence of intermediates such as catechol, as the phenol derivative which cannot be easily utilized by the bacterium (Fig. 2(a)). A summary of the reactions involved in anaerobic biodegradation of phenol is shown in Scheme 1 where the starting point is the phosphorylation of phenol with use of phenylphosphate synthase followed by carboxylation of the aromatic ring to yield 4-hydroxybenzoate. Thioesterification then gives 4-hydroxybenzoyl CoA and reductive dehydroxylation yields benzoyl CoA, which through a series of the further enzymatic reactions, the formation of acetyl CoA would be the expected metabolite which plays a role in central metabolism of fuel molecules [24].

Synthesis of the operative enzymes in this type of molecular pathway of course is pre-requisite for the efficiency of the relevant metabolic activities (i.e., emphasis should be on importance of the length of adaptation phase) [24]. The MFC behavior in terms of




**Scheme 1. A summary of the reactions involved in biodegradation of phenol under anaerobic condition [24].**

phenol consumption by the AS inoculum can be interpreted efficiently; Fig. 2(b) shows that the 150 mg/L removal was completed within 150 h, which may indicate the *R. eutropha* presence in the AS inoculum. Presence of *Pseudomonas* sp. in the AS inoculum in the present study was confirmed and these two bacterial species (*Pseudomonas* sp. and *R. eutropha*) share common enzymatic behavior in terms of hydrocarbons biodegradation [15,16].

1-2. Dependence of Voltage Generation on the Phenol-acclimated Inoculum

Anodic metabolism activity in treating the synthetic wastewater was examined (AS inoculum) by applying OCV condition. As Fig. 3 indicates, the voltage generation in the MFC electrical circuit reached 100 mV after 6-7 days in response to feeding anode chamber with phenol (100 mg/L). Potential acts as driving force for the redox reaction, and the time needed to record the generation of voltage in the absence of external load in fact reflects microbial cells capacity in anodic release of electrons of the fuel substrate. According to the results shown in Fig. 3, the AS microbes were not able to maintain the oxidative activity for longer time than seven days and phenol feeding program was applied to extend duration of the voltage production. The anodic biofilm (in terms of MLSS content) showed 65% decrease in this repeated feeding program. The results of the preliminary experiments showed that periodical use of glucose at the time of phenol inhibition was beneficial, and this apparently relieved the system's inhibition temporarily (glucose is a readily utilizable substrate by all microbes). The MFC system responded favorably and the undesirable biofilm reduction was controlled; as expected, the voltage generation showed 85% increase (inset in Fig. 3(a)). Biofilm formation on the anode electrode is under the influence of several interrelated factors, as microbes approach and contact with the electrode, metabolic activities of the microbial cells being directed towards producing more reduced forms of coenzymes (such as NADH) which are carriers of the high energetic electrons of anodic substrate (high ratio of $NADH/NAD^+$, for instance). The released energy of electrons from their entrance into the designed circuit of the MFC reactor completes conversion of biochemical (ATP) to electrical forms of energy. Part of the completion is the oxidation of the reduced form of coenzymes in the

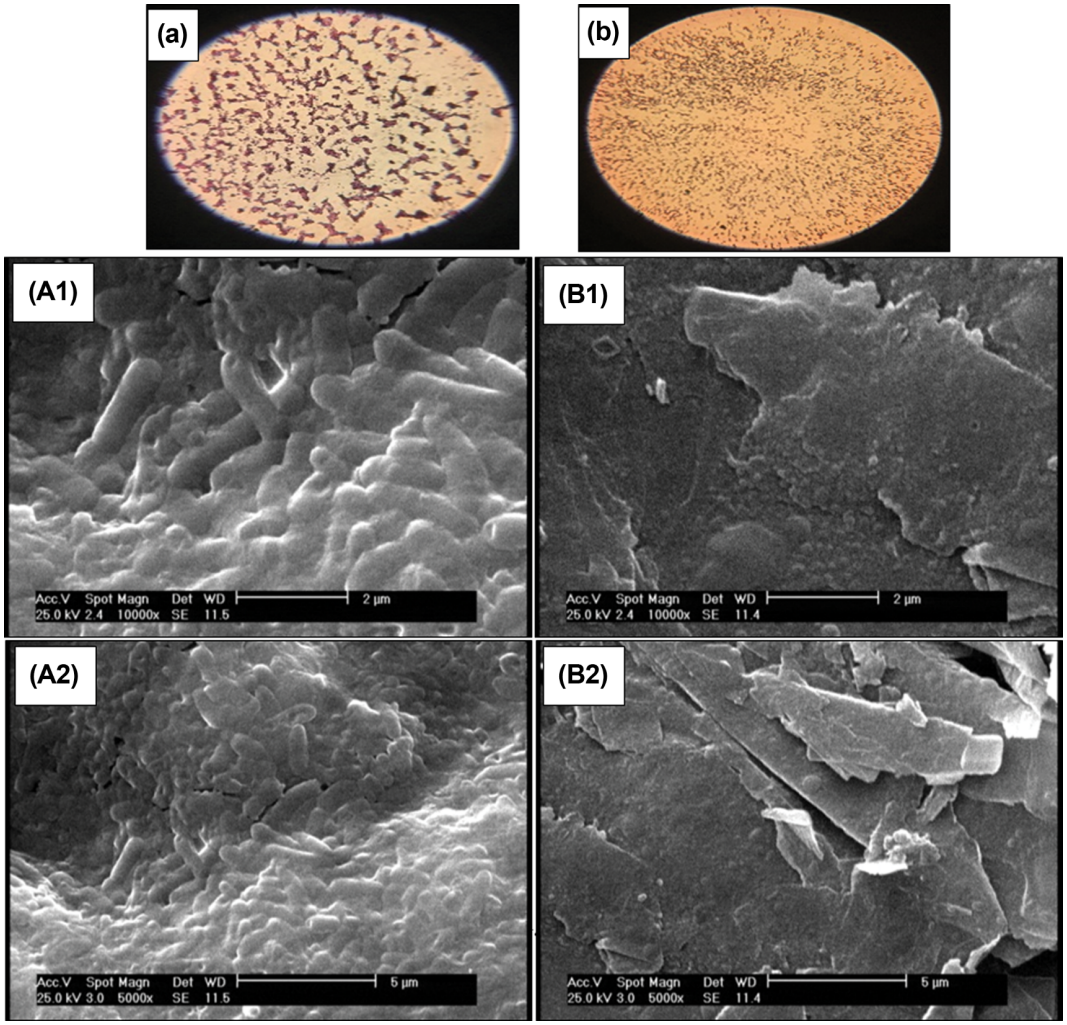



**Fig. 4. SEM images of the anode electron surface following *R. eutropha* growth using phenol substrate as the electron donor ((A1), (A2)) and the electron surface without the bacterial growth ((B1), (B2)). The images were taken at different magnification (at 5,000× and 10,000×). The view which is seen in the inset shows the Gram staining of *R. eutropha* (a) and its presence in the anolyte (b).**

presence of $O_2$ at the cathode electrode; this is provided by chemical energy to support growth of microbes as related to anodic biomass.

Phenol feeding strategy used for the AS inoculum sludge acclimation was also implemented for *R. eutropha*; Fig. 3(b) indicates the results of OCV condition. Fluctuations after 200 mg/L feeding of phenol were high, and by considering relative stable voltage seen in the inset of Fig. 3(a), a decision was made to use 150 mg/L phenol in all experiments.

Importance of the relationship between microbial cells and anode electrode was defined for *Geobacter sulfurreducens* (isolated from marine sediment), which is producer of highest current density in MFC system where the conductivity of this pure cell culture was found to play a crucial role in this regard [25,26]. SEM images presented in Fig. 4 show the massive growth of *R. eutropha* on the anode electrode surface. Despite this observation, the current production was not high, so a reasonable approach is to measure the biofilm conductivity. The Gram staining procedure was used to check the purity of *R. eutropha* biofilm (inset in Fig. 4).

1-3. Closed System Configuration

With use of a resistor box containing a range of resistances and by applying these variable external loads on the connected electrodes, the MFC system performance using *R. eutropha* and AS inoculum was determined (Fig. 5). In the case of AS inoculum, the phenol and COD removal efficiency obtained under closed circuit condition was higher than that obtained in the absence of external resistance (1,000 ohm versus OCV condition). Results are in agreement with Elakkiya et al., in which the AS obtained from dairy wastewater performed differently under OCV condition and aerobic anode compared to the system's behavior with the anaerobic anode [27]. The results reported in the literature show the influential effect of external resistances on the bacterial community structure formed as the biofilm on the anode electrode surface [28]. In the present study and in the case of the AS inoculum containing *Pseudomonas* species, the expectation was to see facilitation of the electrons transportation due to excreted redox mediator synthesize by this type of bacterium (i.e., *P. aeruginosa*) [29]. The AS performance for COD and phenol removals under closed circuit condition after 50 h showed the preference of the AS in the removals compared with those for *R. eutropha* (68% versus 27% for phenol removal and 64% versus 10% for COD removal, Fig. 5). Phenol acclimation process positively affected *R. eutropha* performance in the MFC system where phenol removal, which was low at about 12-27% during 50 h, increased to 90% after 250 h (Fig. 5). This can partially be attributed to the anodic biofilm conductivity of the pure cell culture as has been mentioned for *Geobacter sulfurreducens* [26].

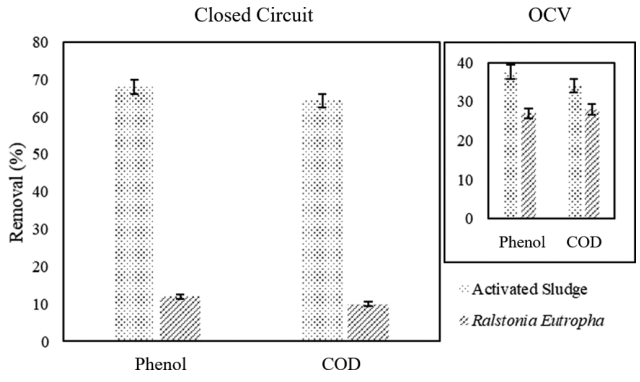



**Fig. 5. COD and phenol removals during MFC operation under open circuit and closed configurations (1,000 ohm) time period division for monitoring the AS inoculum behavior was 48 h while performance of *R. eutropha* was monitored for 250 h.**

**2. Polarization Studies**

Fig. 6 shows the polarization curve in which the voltage at highest level (130 mV) was at zero current. Redox potential is driving force in a bioelectrochemical reaction which occurs at the metallic anode electrode and aqueous solution of anolyte interface (AS or *R. eutropha* inoculum plays a catalytic role in the bioconversion of the substrate "either phenol or the corresponding COD"). The energy barrier for the reaction needs to be controlled through the voltage generation process, but electron transfer activation energy varies as a function of the applied voltage. Thus, the voltage shifts from the equilibrium, and the polarization curve follows a decreasing trend; the decrease of voltage at higher levels of current relates to the internal resistances mainly in the form of ionic and electronic in electrodes and electrolytes [30]. Exchange of the produced metabolites and charges (see Scheme 1 for anaerobic biodegradation of phenol) through electrodes does not follow a desirable pattern in the bioconversion process at a higher current of the po-

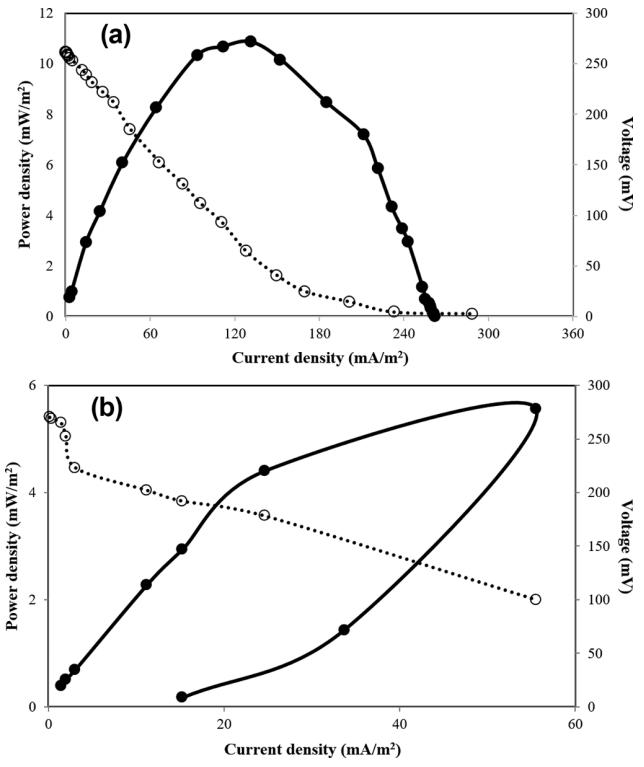



**Fig. 6. Polarization behavior of the MFC operation in the present study where the power curve shows the power as the function of the current (closed circuit). Importance of the ohmic loss relative to the activation and charge transfer resistances also is shown as the anodic polarization curve (open circuit): the activated sludge (a), and *R. eutropha* (b) were used as the anodic inocula.**

larization curve; this mass transfer resistance corresponds to the higher decrease of voltage generated at higher current. The data of ohmic resistance region in the polarization curve were used to quantify the internal resistance by measuring the line slope as it is seen in Fig. 6(a). The deviation from linearity in this type of polarization curve shows the importance of non-ohmic resistance, which has not been observed in the present study [30]. It has been attempted to draw electrons from bacterial membrane and direct them in order to better control the membrane-bounds catalytic reactions, by using electron mediators such as the ferricyanide. Fig. 6(a) shows the MFC behavior upon the addition of ferricyanide to the cathode chamber. Use of ferricyanide as an electron mediator was practiced in the present study, where reduction of $K_4Fe(CN)_6$ to ferrocyanide ($K_3Fe(CN_6)$) in the cathode chamber through electrons and $H^+$ generated in the anode compartment facilitated the cathode reaction in the form of $O_2$ reduction. Fig. 6(a) illustrates the results of the polarization curve for the AS performance, which indicates the highest power density 11 mW/m$^2$ with 100 mA/m$^2$ under 1,000 ohm as the external resistance. Fig. 6(b) shows the result for *R. eutropha*, which are 5.8 mW/m$^2$ with 58 mA/m$^2$. Lower trend of power density seen in the polarization curve may be the result of lower availability of phenol substrate. Apparently, phenol oxidization by *R. eutropha* proceeded satisfactorily. This behavior was also reported by Wu et al., where with use of the composite anode configuration (flat mash or small diameter graphite fiber brushes) a different polarization curve was obtained [31].

**3. Performance of Anodic OMWW Substrate**

Based on the COD content of the phenol containing synthetic wastewater, the OMWW sample was diluted (545 mg/L) and used as the anodic substrate in the present study. The phenol-conditioned *R. eutropha* biodegradative capacity was determined such that COD level of the inoculated substrate during the MFC operation was reduced to 48% after 250 h (Fig. 7(a)). The recorded voltage under OCV condition was about 230 mV after 24 h; the MFC system was able to keep this voltage for 90 h (Fig. 7(b)). One consideration in reducing the negative effect of low conductivity of the real WWs was to support the idea of mixing electrolyte solution [30]. Although OMWW is a good source of organic compound (thus having considerable amount of the energy stored in the relevant chemical bonds), the presence of the inorganic salts in this real WW positively affects the electrical conductivity of the anodic solution of the electrolyte [31]. Potential of improvement of MFC operation in handling OMWW appears to be considerable when one combines the electrolyte conductivity behavior with conductivity of the anodic biomass of the pure cell culture (such as *R. eutropha*).

Current production of 381 mV at 1,000 ohm external resistance was the result of studying the air cathode performance in single chamber MFC (SCMFC) using OMWW substrate inoculated with wastewater obtained from yeast production treatment plant [18]. Raising some points in the reported findings on SCMFC appears to be necessary. For instance, in addition to the aforementioned inoculation subjects, the OMWW as the substrate was conditioned at first with sodium acetate or glucose. In another SCMFC study [33], the OMWW was diluted with domestic wastewater (DW) fourteen times and the anodic biomass showed to have *Geobacter* sp. as a well known synthesizer of electron mediator proteins (e.g.,

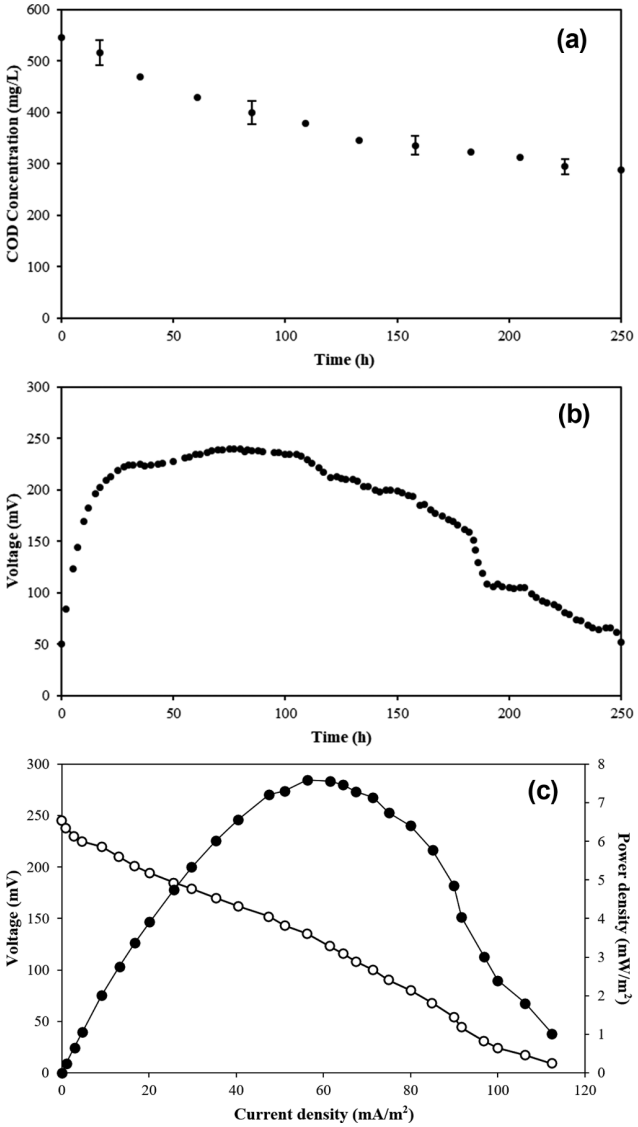



**Fig. 7. Variation of OMWW COD concentrations (a). Variation of voltage under open circuit condition measured during the MFC operation using OMWW as the anodic substrate which has been inoculated with *R. eutropha* (b). The polarization curve for the anodic OMWW substrate inoculated with *R. eutropha* is also shown (c).**

electrogenic bacteria). Further phylogenetic analyses on the anodic biomass showed the presence of *Cupriavidens* sp. (currently known name for *R. eutropha* sp.). The results of the present study on the OMWW in comparison with the aforementioned findings are in a fair position where 60% voltage reported by Sciarria et al., using OMWW+DW as the substrate was obtained in the present study (230 mV versus 380 mV) [33]. Removals of COD and total phenolic in that study were as similar levels to that of the present work, but removal of phenolic in the Bermek et al. study was 49%. Further note in the present study was to plot a polarization curve, and Fig. 7(c) shows the result, where power density is about 7.8 mW/m$^2$. The PD value is slightly better than that for *R. eutropha* behavior shown in Fig. 6(b).

## CONCLUSIONS

By coupling phenol oxidizing anodic biomass containing either AS or *R. eutropha,* pure culture to the oxygen reducing functionality of the cathode electrode in the MFC system, phenol removal and power generation behaviors were examined using the synthetic type of wastewater. By undertaking phenol acclimation process, the current production by AS and *R. eutropha* was improved by both. Presence of *Pseudomonas* species in the AS sample was confirmed where this bacterium is known to be good phenol degrader but anaerobic biodegradation exerts some metabolic limitations. To reduce the extent of the growth inhibition of phenol (and/or its derivatives is shown in Scheme 1), phenol periodically was replaced by glucose (in the case of AS inoculum) and this practice positively affected the process. More work is needed to show the enzymatic behavior of the AS in response to phenol substrate under the experimental conditions of the present work. Conductivity of the anodic biomass in the case of *R. eutropha* pure culture is likely to contribute to the MFC behavior, and in the case of OMWW further improvement is expected. Of course, more work is needed to show the importance of this factor.

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