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Development of Bioelectrochemical System for Monitoring the Biodegradation Performance of Activated Sludge

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Abstract In the microbial electrochemical system (MES), the microbial-electrode interactions are often regulated by the metabolic pathway and respiratory activities. To improve the efficiency of MES, there is a need to introduce a microbial community that provides a continuous oxidation of organic substrates with a sustainable current output. Thus, activated sludge was suggested and the rapid evaluation of its biodegradation activity, using cyclic voltammetry, was performed. Stimulation of the metabolic pathway led to the appearance of an oxidation peak current (22 $\mu A/cm^2$, at about 750 mV), whereas the electrochemical signals were originated only from the metabolically active microbes. Cell viability, cultivation time, type, and concentration of the degradable organic substrates have been identified as major regulators for the electrocatalytic performance. From two different microbial communities, the generated electrochemical signal of the aerobic activated sludge was more than twofold higher in converting the degradable organic substrates (glucose, acetate, and succinate at 10 g/L) into oxidation current. On the other hand, the secretion of electroactive metabolite(s) in the extracellular matrix was determined as a source of electrochemical signal. Moreover, the mechanism(s) of the microbe-electrode interactions were demonstrated. Therefore, the current bioelectrochemical system could be used as a platform for monitoring the rate of substrate degradation as well as measuring the metabolic pathway activity.

Keywords Microbial electrochemistry · Electroanalytical studies · Activated sludge · Biodegradation performance measurements

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Introduction

The removal of organic pollutants from wastewater is necessary for the water treatment process [1]. Classical chemical approaches for the removal of organic pollutants from wastewater include extraction, adsorption [2], steam distillation, photochemical degradation [3], and using selective nanocomposite [1, 4, 5]. In recent years, microbial growth on organic substrates was proposed to sustain the biodegradation and removing the organic wastes. In microbial electrochemical system (MES), the use of electrocatalytic activity of microorganisms to liberate electrons from degradable organic substrates [6–8] has exhibited very interesting applications for the rapid assessment of microbial activity [9, 10], as well as for the development of microbial fuel cells (MFCs) [11]. Therefore, through the MES, the concept of biodegradation could be exploited as an efficient strategy for organic waste removal and bioelectricity generation [12, 13].

To obtain electric current from microbial activity through the MES, several bioelectrochemical reactions take place between the metabolically active organisms, organisms which are able to oxidize the organic matters, and the electrode surface (the final electron acceptor). In this case, the current density is a measure for the efficiency of organic substrate degradation [14]. In principle, the extracellular electron transfer processes from electrochemically active organisms toward electrode surface [15–19] is accomplished through two main mechanisms: the first way is the direct electron transfer (DET), which needs a physical contact of a redox active microbial moiety [20], including redox proteins like cytochromes or bacterial nanowires [21, 22] with the electrode surface; the second way is the mediated electron transfer (MET). In such system, the extracellular electron transfer can be mediated using exogenous redox compounds (natural or artificial electron shuttles) [12, 23–25].

To secure a sustainable wastewater treatment and efficient electricity generation, the selection of a microbial community which is capable of degrading the organic substrates with continued rates is a great challenge [12, 26–28].

Hence, for establishing a high performance and reliable MES, the selection of potential microbial communities, i.e., electrochemically active organisms that are able to communicate with the electrode surface via transferring exocellular electrons, is a crucial issue.

Among the most common microbial technologies in wastewater treatment, activated sludge was used as a source of microorganisms [29]. In this regard, the construction of activated sludge-based bioelectrochemical system was achieved [30]. Nevertheless, optimizing the activated sludge-based bioelectrochemical system and understanding the mechanism(s) of the electron transfers are required.

Thus, the main concerns of the current study are as follows: firstly, to study the effective parameters that are regulating the bioelectrocatalytic performance of the activated sludge. Secondly, to provide a rapid evaluation for the biodegradation rate, by following up the relationship between the biodegradation rate and the current output. Finally, to identify the mechanism(s) included in microbe-electrode interaction(s).

To achieve the setting goals, a comprehensive electroanalytical study have been carried out by using cyclic voltammetry, whereas the microbe-electrode interaction was investigated under different microbiological and electrochemical conditions.

Material and Methods

Reagents and Equipments

Glucose, sodium acetate, and sodium succinate dibasic hexahydrate (Fine-Chem Limited and Merck, respectively) were used as degradable substances for the activation of the metabolic pathway of activated sludge. Synthetic graphite (1–2 μm) and paraffin oil were obtained from Aldrich and Fluka, respectively. Sodium phosphate with pH 7 was used for washing the microbial cells and as a supporting electrolyte. Shimadzu spectrophotometer (Shimadzu UV-240, Japan) was used for measurement of the optical density of microbial cell number at 600 nm ($OD_{600\ nm}$).

All electrochemical measurements were performed using a computer-controlled Gamry Potentiostat/Galvanostat/ZRA G750, which is connected to a three-electrode system comprising carbon paste (CP) as a working electrode, Pt disk as an auxiliary electrode, and Ag/AgCl/3 M KCl as a reference electrode.

Preparation of the Activated Sludge for the Bioelectrochemical Measurements

The raw activated sludge was collected from municipal sanitation station in Benha City (Benha, Egypt). The bacterial cells of a certain volume were collected by centrifugation (4000 rpm, for 10 min at room temperature). The cell pellets were thoroughly washed with 0.1 M phosphate buffer. The washed cells were then resuspended and incubated in phosphate buffer containing acetate (10 g/L) for the activation of metabolic pathway. Cyclic voltammetry was used for monitoring the bioelectrochemical performance at different incubation times (e.g., 3 h and overnight).

Electrochemical Measurements

According to the reported procedure [9, 10, 31], the carbon paste electrodes were prepared by thoroughly hand-mixing of 1 g synthetic carbon powder with 0.4 ml paraffin oil in a small hand mortar. The prepared paste was packed into the tip of the electrode assembly with a surface area of 0.5 cm^2. Electrode surface regeneration was performed by polishing with a wet smooth paper till a shiny electrode surface was obtained. Prior to the electrochemical measurements, the working electrode was electrochemically activated in phosphate buffer (0.1 M, pH 7) by ten cyclic scans from 0.2 to 1.0 V (vs Ag/AgCl/3 M KCl) with scan rates of 50 mV/s. Aliquots of the metabolically active cells were incubated for 5 min into the electrochemical cell containing 10 mL of phosphate buffer. Cyclic voltammograms were recorded in the potential range from 0.2 to 1.0 V with scan rates of 50 mV/s without stirring at room temperature.

Selection of Suitable Sludge

To investigate the difference in the electrocatalytic activities of two types of activated sludge aerobic and anaerobic, the metabolic pathway was activated before acquiring the electrochemical signals. In this case, a culture for each type of activated sludge was incubated in phosphate buffer solution containing acetate (10 g/L) for 3 h at 30 °C.

Effects of Carbon Sources on the Electrochemical Behaviors of Activated Sludge

A certain volume from the washed cells was incubated in 24 mL phosphate buffer solution containing the utilizable carbon sources (glucose, acetate, or succinate) for 30 min at 30 °C. The microbial cell population was measured at (OD_{600}) before running the electrochemical experiment.

Statistics

All the presented results are the average of three replicates. The standard errors (±) were not more than 10 % in all the experiments. The illustrated values in the figures are subtracted from the negative controls (phosphate buffer without the microbes).

Results and Discussion

Bioelectrochemistry of Activated Sludge

Several single-strain-based MESs have been used successfully for the detection of cellular and metabolic pathway activity of bacteria and yeasts [19, 32–34]. Although the use of activated sludge as a source of microbes in the MES is promising, bioelectrochemical characteristics of its microbial community are not yet investigated. Therefore, the idea of this work is to provide a rapid evaluation for the bioelectrocatalytic activity of the living microbial cells in the activated sludge matrix. Consequently, the washed microbial cells were incubated with the electrode systems, and the possibility of microbe-electrode interaction was measured before and after the stimulation of the metabolic pathways using degradable organic substrates. The electrochemical signal was obtained only from the metabolically active microbes, whereas irreversible oxidation peak current in the potential range from 700 to 800 mV was obtained. On the other hand, the metabolically inactive cells, microbes without organic matters, did not show electrochemical activity (Fig. 1). Thus, the generated electrical current is attributed to the rate of catabolism.

Hence, the utilization of the carbon sources is not only essential to the microorganisms' viability and growth but also to enable the electrocatalytic activity.

Selection of Suitable Activated Sludge Communities

For most bacteria and fungi, the oxidation of carbon sources is the main store of electrons elaboration. Thus, the electron transport chain, linked to aerobic or anaerobic respiration, is the most important compartment in the living systems. Therefore, the selection of an appropriate activated sludge model as the basis for the construction of MES of both types was recorded before and after the activation of the metabolic pathway. At the same carbon source concentration, the electrocatalytic efficiency of the aerobic sludge was higher, whereas the amount of electrical current generated from that type of sludge was at least twofold more than that obtained from the anaerobic sludge. As shown in Fig. 2, the peak currents generated from the aerobic sludge were 3.13 and 1.25 μA/OD with and without glucose, respectively. However, the current output of the anaerobic sludge was 1.36 and 0.4 μA/OD with or without the glucose, respectively.

These results point to the involvement of the respiratory chain in the electron transfer from the active microorganisms to the electrode surface. Hence, to enable high organic substrate

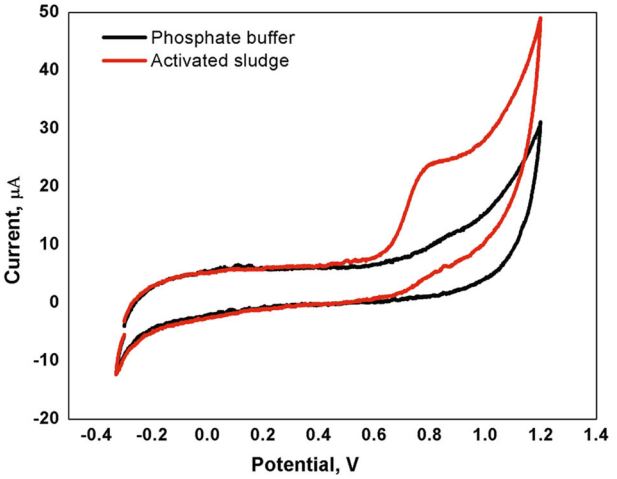


Fig. 1 Cyclic voltammograms of metabolically active aerobic sludge (*red line*) and PB-containing 10 g/L of glucose (*black line*), with scan rate of 50 mV/s. The microbial cell density was of $OD_{600\ nm}$=0.6 (color figure online)

degradation combined with the high electrochemical signals, the effective parameters which regulate the use of the aerobic activated sludge in the microbial electrochemical system needs to be defined.

Influence of Type and Concentration of Carbon Source

As shown in Fig. 2, the aerobic activated sludge exhibited a good performance on converting the organic substrates into electrical current. Consequently, understanding its bioelectrochemical property is very important to improve the electrochemical functions, and hence, it can be used as a good provider for constructing a new microbial fuel cell approach. Thus, the influence of various carbon sources on its bioelectrochemical performance was

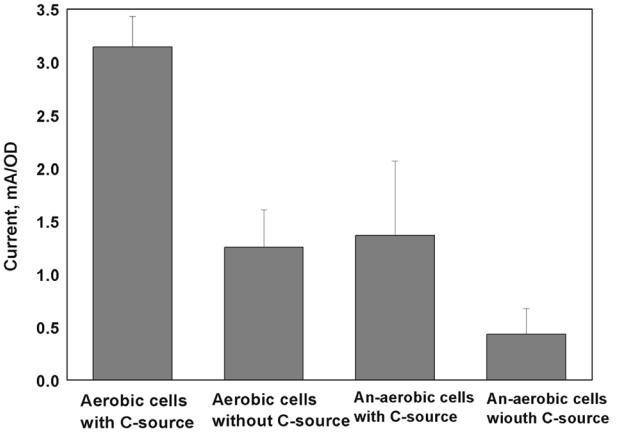


Fig. 2 Bioelectrochemical behavior of two different types of activated sludge, aerobic and anaerobic. The electrochemical signals of both types were obtained before and after metabolic pathway activation. Background currents were subtracted in each case

investigated. In this regard, before measuring the electrochemical signals, three different organic substrates (acetate, glucose, or succinate) were used for the metabolic pathway activation.

Stimulation of the metabolic pathway increased the electrocatalytic activity of the microbial community. Therefore, higher electrochemical signals were obtained when the microbial cells were grown on the organic substrates (10 g/L), in a comparison to the responses of metabolically inactive microbial cells (no carbon source in the microbial culture). However, the highest electrochemical response was obtained when the cells were grown on acetate as the sole source of carbon. As shown in Fig. 3a, acetate produced the highest peak current among the other carbon sources, whereas the peak current values were 2.5, 15, 10, and 7 μA/OD for phosphate buffer, acetate, glucose, and succinate, respectively.

Accordingly, to identify the concentration of the organic substrates that effectively stimulate the metabolic pathway and the bioelectrochemical performance, bacterial cells were grown on different concentrations of acetate or glucose.

Figure 3b shows a gradual increase of the electrical current with increasing the acetate concentration in the microbial cultures. However, in case of utilizing the glucose, the amount of electrical current was dropped when glucose concentration in the microbial culture was greater than 5 g/L.

To explain this phenomenon, the excessive amount of glucose in the microbiological cultures might lead to a redirection of the metabolic pathway from respiration to fermentation [35, 36]. Since the respiratory chain is the main source for the electron transfer, acetate was more favorable for the microbe-electrode interactions. Therefore, acetate at 10 g/L was chosen for the further investigations.

Figure 3 illustrates the variation of the corresponding current values of acetate and glucose with their different concentrations ranging from 1 to 20 g/L. It is clear that the peak current values are 9.6, 15.5, 9.4, and 6.7 μA/OD at glucose concentration of 1, 5, 10, and 20 g/L, respectively (as shown in Fig. 3a), while in case of acetate, the peak current values are 2.4, 5.2, 9, and 15.3 μA/OD at acetate concentration of 1, 5, 10, and 20 g/L, respectively (as shown in Fig. 3b).

Effect of Microbial Cell Density on the Electricity Generation

Controlling the bacterial growth is one of the most important considerations for optimizing the MFC performance. Therefore, the influence of microbial cell density on the production of electrical current has been studied. In this regard, the electrochemical measurements were carried out at different concentrations of metabolically active microbial cell numbers. As a result, a gradual increase in the electrical current was obtained when the cell number was increasing (Fig. 4). However, the exponential growth of the electrical current was observed when the bacterial cell number was greater than $OD_{600\ nm}$ of 0.3. The current density is directly proportional to the electroactive biomass production; therefore, the consumption rates of organic substrates were dependent on the number of viable microbial cells.

Thus, to obtain a significant amount of current output, certain concentration of bacterial cell number is recommended to exist from the beginning in the MES.

Effect of Supporting Electrolyte Type

In the bioelectrochemical systems, in addition to the biological parameters which regulate the electrochemical performance of the activated sludge, there are other factors influencing the electrochemical reactions such as the supporting electrolyte type. Thus, using acetate as a

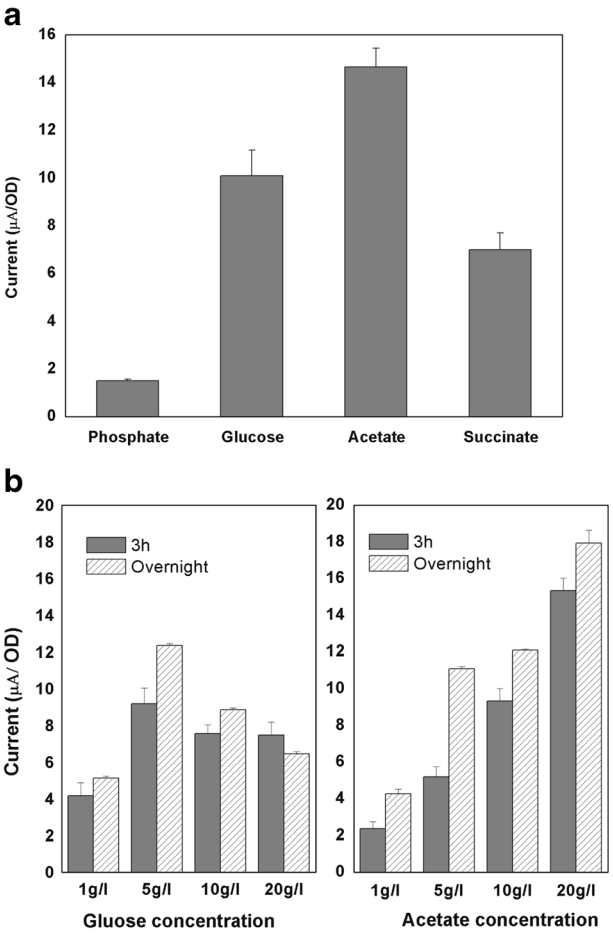


Fig. 3 **a** Effect of various carbon sources on the electrochemical performance of aerobic activated sludge. Cell density was $OD_{600\ nm}$=0.4, and carbon source concentration in each independent culture is 10 g/L, scan rate 50 mV/s. The presented values in the graph are the subtractive values from the individual blanks (each blank consists of carbon source in PB without microbial cells). **b** Effect of glucose and acetate concentrations on the electrical current production of the aerobic activated sludge. Background currents were subtracted in each case

carbon source, the metabolic pathway was activated in different electrolytes; then, the electrochemical response was measured.

Although phosphate buffer is the optimal physiological buffer, the bacterial cells were electrochemically active in most of the tested electrolytes, as shown in Fig. 5. On the other hand, there was no electrochemical response for the microbial cells which were incubated in phthalic acid (as shown in Table 1, Supplementary Information); this might be due to the acidic medium which can affect the cell proliferation. Therefore, phosphate buffer (pH 7) was considered as the optimal electrolyte for the proposed bioelectrochemical system.

Identification of Extracellular Electron Shuttles: Electrochemical Activity of the Supernatant

As shown before, the construction of mediatorless microbial electrochemical systems was possible. The obtained oxidation currents were in the potential range from +600 to +800 mV

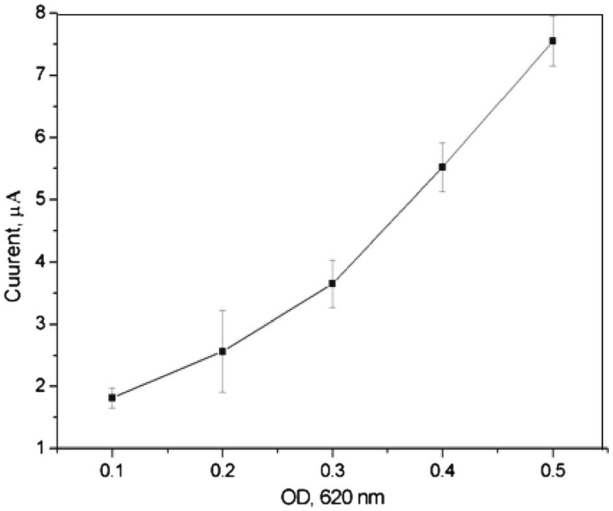


Fig. 4 Relationship between the metabolically active microbial cell density and the electrical current using acetate (10 g/L) as utilizable carbon source. Background currents were subtracted in each case

(vs Ag/AgCl reference electrodes) [19, 32–34]. The faradaic current densities were dependent on the concentrations of the electron transfer agents and on the efficiencies of electron transfer reactions to the electrode, whereas the first are influenced by properties of the cell culture, such as cell density or metabolic activity leading to the accumulation of electron donors. The latter depend on the electrochemical properties of the electron transferring compound and on its steric accessibility, i.e., the physical contact between the respective redox center and the electrode.

Nearly all microorganisms were covered by cytoplasmic membranes with additional layers, such as cell walls, peptidoglycans, or outer membranes, so that microbial electron transfer chains are physically separated from the extracellular environment. Electrons, which are

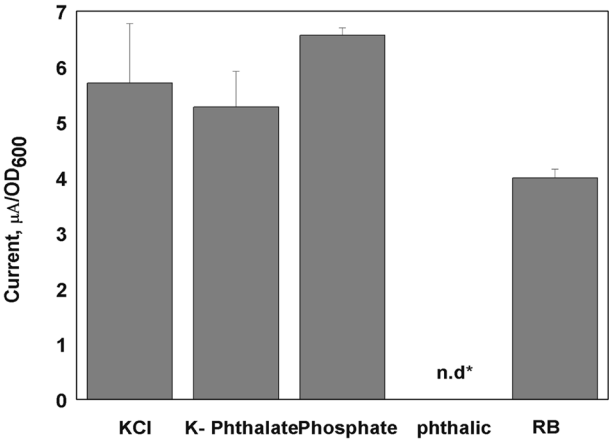


Fig. 5 Effect of type of supporting electrolyte on the electrical current generated from metabolically active cells. The utilized concentration for each electrolyte solution is 0.1 M. pH values were presented in Table 1 (SI). Background currents were subtracted in each case

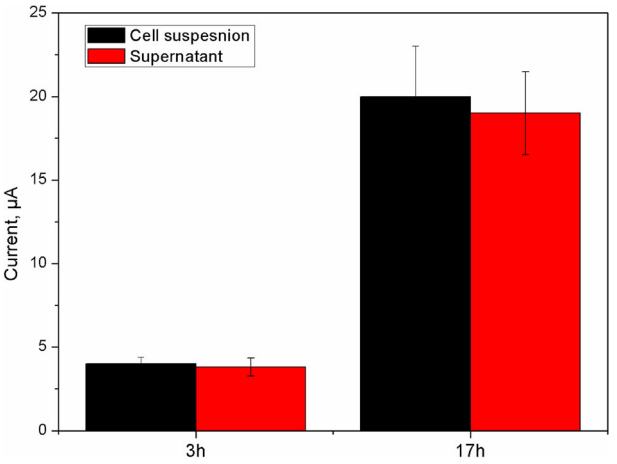
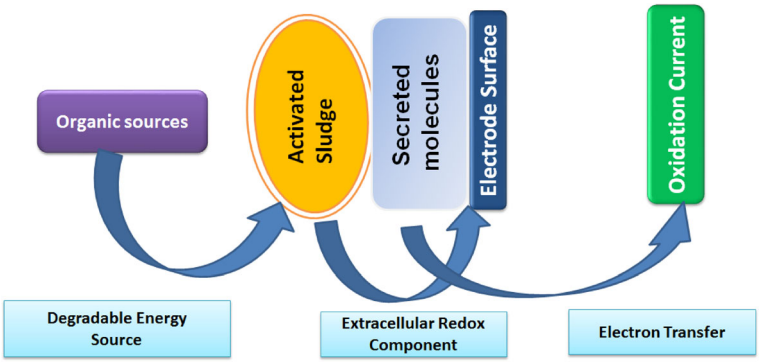


Fig. 6 Bioelectrochemical behaviors of the microbial cell suspension in phosphate buffer containing 10 g/L of acetate (electrolyte), after 3 and 17 h, in comparison with the supernatant, from which the cells were removed by centrifugation after their presence in the electrolyte

transferred to extracellular electron acceptors such as electrodes in technical systems or insoluble metal oxides in natural environments, have to cross these barriers!

Interestingly, bacteria are able to transfer electrons directly to electrodes (mediatorless) either via the presence of the electroactive compounds (*cytochromes* for example) at the outer-cell membrane [37, 38] or via production of conductive nanowires (*Pili*) [21, 22, 39]. Moreover, they are reported to produce and secrete low molecular weight compounds that act as natural-electron shuttles and additionally have quorum sensing properties [24, 25, 40]. Optimization of energy gain from bacterial to bioanode in microbial fuel cells is based on these aspects. Moreover, electron transfer to external electron acceptors, in particular to solid-state materials such as electrodes, is possible even in the presence of oxygen.

However, in our case, the nature of electron transfer from the microbial community of the activated sludge is unknown. Since the mediatorless electron transfer was realized, we focused



Scheme 1 A schematic representation of the proposed mechanisms of the activated sludge-based electrochemical system. Due to the change of cell viability and metabolic activity of the cells, liberating the electrons from the carbon source (microbial catalytic oxidation of organic substances). As a result, the secretion of electrochemically active component(s) into the extracellular environment is detectable

our investigations on the mechanism of the electron transfer from the microbial community to the electrode surface. The dependence of the electrochemical signals on the incubation time with the organic substrates (2 and 17 h) was an indication for the metabolic activity, as shown in Fig. 6. So, the accomplishment of direct contacts with electrodes seems unlikely. Thus, we wondered whether microbial community of the activated sludge may secrete electroactive metabolites with electron transfer properties. As these compounds might be present in the electrolyte solution, thus, the microbial cells were removed by centrifugation, and the cell-free solution (supernatant) was tested for electrochemical activity. Cyclic voltammetry of the supernatant demonstrated an oxidation peak of the same height and position as the peak of the corresponding cell suspension, as shown in Fig. 6. Thus, the electrochemical signal was independent of physical contact between the cells and the electrode. The microbe-electrode interaction in the current system is a self-mediated electron transfer process via secretion of secondary electroactive metabolite(s).

Scheme 1 describes the possible reaction mechanism of the self-mediated electron transfer in the activated sludge-based electrochemical system.

The microbial cells grown on the degradable organic substrates produce extracellular electroactive molecules which are responsible for the self-mediated electron transfer. As a result, the oxidation current is generated.

Conclusion

In order to optimize the bioelectrocatalytic performances of the activated sludge and evaluate its capacity to convert the degradable organic compounds into electrical current, an activated sludge-based bioelectrochemical system was designed. The factors affecting the microbe-electrode communication were investigated. The presence of degradable organic substrates in the microbial cultures was necessary to keep continues electron transfer. Moreover, the electrocatalytic performance of the system showed that the microbial community with high cell number led to a higher substrate conversion rate; hence, greater current density output was achieved. The mechanism of microbe-electrode interaction(s) was attributable to a self-mediated electron transfer via a secreted compound(s).

Thus, the optimized bioelectrochemical approach could be considered as a promising model for the future development of a large-scale platform for the simultaneous wastewater treatment and bioelectricity generation.

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