VNU Journal of Science: Natural Sciences and Technology, Vol. 37, No. 3 (2021) 57-65

VNU Journal of Science: Natural Sciences and Technology

Journal homepage: https://js.vnu.edu.vn/NST

Original Article

Voltage Reversal During the Operation of a Sediment Bioelectrochemical System Integrated in a Brackish Aquaculture Model: Causes and Solutions

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Received 15 August 2021
Revised 30 August 2021; Accepted 31 August 2021

Abstract: The sediment bioelectrochemical system (SBES) is expected to become a novel biotechnology with numerous outstanding application potentials, such as *in-situ* bioremediation and pathogen control. Nevertheless, one of the prevalent problems when applying the SBES in practice is the voltage drop or reverse voltage of the system. In this study, we studied the effects of different environmental factors such as light, temperature and air humidity and of internal SBES factors such as sulfate concentration, dissolved oxygen concentration and biocathode on voltage reversal in the SBES. Light, temperature and air humidity did not appear to be associated with voltage reversal. Sulfate concentration in the SBES tank water did not either significantly change during voltage reversals, indicating that sulfate did not compete with the anode for electrons. On the other hand, the change in dissolved oxygen (DO) concentration and biofilm formation on the cathode appeared to be the major factors causing such phenomenon. Therefore, aeration and frequent replacements of the cathode are suggested to overcome the problem, which will help to enhance the practical applicability of the SBES.

Keywords: Power decrease, power density, SMFC electricity generation, voltage drop, voltage reversal.

1. Introduction

A sediment bioelectrochemical system (sediment BES or SBES) is a BES with an anode embedded in the anaerobic sediment and a cathode suspended in the aerobic water column above the anode electrode [1, 2]. Similar to the conventional microbial fuel cells (MFCs), SBESs convert chemical energy to electrical energy with the aid of microorganisms as biocatalysts [3]. Electrochemical organisms residing on the anode, such as those belonging to the genera of *Geobacter* and *Desulfuromonas*, generate electrons by degradation of organic matter and/or oxidation of sulfide and other substances in sediment. These electrons move to cathode due to voltage potential difference between the anode and the cathode and react with oxygen to form water; thereby generating electricity. The

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https://doi.org/10.25073/2588-1140/vnunst.5308

resulting electron flows thus promote remediation of sediment by enhancing physicochemical and microbial metabolic reactions [4, 5].

There are great potentials of BESs as an alternative energy source, novel wastewater treatment processes, or biosensors for oxygen and pollutants. Sajana et al., [6] have successfully built a SBES system integrated into freshwater aquaculture ponds to investigate its waste removing performance in the freshwater environment. Their obtained result showed a good removal efficiency of the system, with 80% COD and 90% total nitrogen removed in fish pond water samples in India [6]. In our previous studies, a SBES also demonstrated its potentials of *in-situ* bioremediation of brackish aquaculture tank models, which had not been reported before. The SBES removed 20-30% more COD of the tank water, compared to the control. After 1 year, the SBES also reduced the amount of sediment in the tank by 40% and thus could remove approximately 40% more COD and approximately 52% more nitrogen from the sediment, compared to the control. Insignificant amounts of nitrite and nitrate were detected, suggesting complete removal of nitrogen by the system [7]. Thus, SBESs may make it possible to remediate water and sediment in aquatic environments without direct external energy supply. Furthermore, SBESs can also be used as biosensors. For example, Cheng et al., [8] developed a marine MFC-biosensor for detecting the acetate concentration (up to 10 mM), which can also be used for real-time measurement of an even lower level of acetate present in seawater. The reliability of sensor signals was confirmed by a linear relationship of the obtained peak voltages with the increasing acetate concentrations. The detection limit for acetate in this study was found to be as low as 5 mM [8]. Therefore, SBESs can offer many applications, especially for sustainable development.

Despite the promising prospect of SBESs, there are still several challenges when applied in practice. Practical applications of BESs in general are restricted by a number of electrochemical and microbiological constraints, fluctuations in conditions, poor stable performance over long term operation, and other operational problems. In field applications, SBESs are exposed to unpredictable fluctuations of the weather, which are expected to change their performances drastically. One of the consequences caused by those operational condition fluctuations may be voltage reversal, as reported with general BESs [9].

It is noteworthy that there has not been any evaluation concerning voltage reversal with SBESs. Therefore, it is important to analyze the factors that may cause this problem in SBESs. This will not only help the practitioners avoid unintended consequences when applying SBESs in practice, but also give some ideas to the SBES researchers about optimizing the system. In this study, after successfully building SBESs integrated in brackish aquaculture models, we observed voltage decreases or sometimes voltage reversals. Thus, we focus on investigating the causes of these phenomena and suggest several solutions to overcome them.

2. Methodology

2.1. Sediment Bioelectrochemical System Construction and Operation

Two rectangular parallelepiped glass tanks (type P, each having the dimensions of 30 cm × 20 cm × 25 cm) were used as pond models in this study. One tank having a sediment bioelectrochemical system (SBES) installed was used as the test tank, while the other tank without SBES served as the control. The SBES installed in the test tank consisted of a sediment anode and a cathode floating on the water surface, and actually used the tank water as the electrolyte. The sediment anode included a 2-cm-thick layer of graphite granules (3-5 mm in diameter) (Xilong Chemical Co., China) and an underlying graphite felt having the dimensions of 15 cm × 7 cm × 0.9 cm (Osaka Gas Chemicals Co., Japan). The cathode was a graphite felt of the same size and type.

Graphite rods were glued to the graphite felts of the anode and the cathode for collecting electrical voltage and connected with copper wires to an external resistor of 10 Ω. The sediment of the test tank was already enriched with an electricity-generating bacterial consortium and that of the control already inoculated with a microbial source from aquaculture ponds in the previous study [7].

In the experiments (described later) to investigate the effect of sulfate, dissolved oxygen and biofilm growth on the cathode, 4 square parallelepiped tanks (type m, each having the dimensions of 11 cm × 11 cm × 6.2 cm) were also used: two as the test tanks, the others as the control. Each test tank was installed with an SBES having a similar cathode yet of a smaller size (5 cm × 5 cm × 0.5 cm) and an anode including a 1.5-cm-thick layer of graphite granule and an underlying graphite felt having the dimensions of 5 cm × 7 cm × 0.5 cm. The other components of the systems were similar to the ones described above.

In the default operation, pre-mixed artificial brackish water (1,5% in salinity, prepared with Marinium Reef Sea salt (Mariscience International Co. Ltd., Thailand) was used to fill each experimental tank. Thus, each rectangular parallelepiped tank has a final water volume of 6 L, and each square parallelepiped tank has that of 0.5 L. Each tank was fed with the shrimp feed GAMMA 6 (TOMBOY Co., Vietnam) at a rate of 0.051 g d^{-1} per tank (for rectangular tanks) and 0.0051 g d^{-1} per tank (for square tanks), equivalent to the daily load of uneaten feed in an actual aquaculture pond with 30-day-old shrimp [10]. The systems were operated at the temperature of 30 ± 2 °C (typical average temperature of brackish aquaculture ponds in Vietnam).

2.2. Experiments to Investigate Potential Factors Affecting Voltage Reversal in the SBES

External factors: the effect of lighting conditions was evaluated by monitoring the voltage of the SBES in two cases: i) When it was covered with a cardboard box (no lighting); and ii) When there was no cover (the control). For the effects of temperature and air humidity, we monitored the room temperature and the room air humidity with a hygrometer (Xiaomi, China) during the periods when voltage reversal occurred.

Internal factors: sulfate concentration and dissolved oxygen (DO) concentration of the water in the test tank (harboring the SBES) were monitored (as described below) during the periods when voltage reversal occurred. In addition, to further test the effect of DO, in some experiments we aerated the water surface (near the cathode) with a fish tank air purger (SOBO, China). We also tested the effect of biocathode by replacing a cathode in use for more than 1 year with a brand-new graphite felt or by sterilizing that in-use cathode with a 70% ethanol solution for 24 hours before installing it back into the SBES. We also tried removing biofilm growth on the cathode by treating it with an Ag nanoparticle solution (comprising 100 mg/L nano-Ag and 1 mM sodium citrate) for 2 hours.

2.3. Sulfate and DO Concentration Analysis

Water samples (approximately 20 mL each, in triplicate) were collected at the middle level of the water body of each tank (vertically 5 cm from the bottom) at the moments of interest. For each sample, we prepared two 25 mL tubes, including one for the blank sample and the other for the actual sample. We added 5 mL of buffer solution A (containing 30 g $MgCl_2.6H_2O$, 5 g $CH_3COONa.3H_2O$, 1g KNO_3 and 20 mL CH_3COOH per 1 liter) to both tubes. 0.5 g $BaCl_2$ was subsequently added to the actual sample tube only and shaken until all components were dissolved. The sulfate concentration in each sample was calculated by reference to a calibration graph plotted from the results obtained with standard solutions containing 0, 5, 10, 20, 30, and 40 µg of sulfate per 25 mL. The standard samples (for plotting the calibration curve) were treated similarly to the actual samples. The sulfate concentration of each sample was determined based on its optical density measured at the wavelength of 420 nm [11].

The DO concentration of the SBES tank water at a time of interest was measured with a DO logger (Hach, USA) by inserting it right to the sampling point.

2.4. COD Analysis and Protein Analysis of Different Cathode Samples

To evaluate the level of biofilm growth on the SBES cathode with time, we analyzed COD and protein contents of the 4 following cathode samples: one used in the SBES for 1 year, one used for 2 months, new graphite felt dipped in the experimental tank for 5 minutes (as if the cathode had just been used) and new graphite felt (the control)). 1 cm^2 of an electrode of interest was cut off and submerged into 5 mL of distilled water before being subjected to sonication for 10 minutes. This causes most of the biofilm to be removed from each cathode sample. Then the suspensions were collected for further analyses.

The COD of each suspension, which reflects the biomass amount in the respective electrode piece, was measured by the closed reflux colorimetric method, using chromate as the oxidant [12]. However, due to the high chloride concentration of the samples, they were pre-treated with HgSO4 [13], as follows: every 10 mL of a sample was mixed with 0.9 g of HgSO4, before being measured by the above-mentioned method.

The protein content in each suspension above was solubilized by adding 1 mL of 2N NaOH to 500 µl of the respective suspension and boiling for 5 minutes. After cooling, 1 mL of 2N HCl was added to neutralize the solution. The total protein concentration was determined by the dye binding method described by Bradford [14] based on a standard curve previously generated from measuring bovine serum albumin standard solutions.

2.5. Other Analyses and Calculations

The voltage between the anode and the cathode of the SBES installed in each test tank was monitored with a real-time digital multimeter (Keithley model 2700, Keithley Instruments Inc., USA). Data were recorded every 5 minutes. The data were processed using standard statistical methods.

3. Results and Discussion

3.1. The Effect of External Factors on the Performance of Sediment Bioelectrochemical System

It was observed that two similar SBESs operated under the same conditions underwent voltage reversal simultaneously (Figure 1). Hence, we assume that electrical generation by SBESs is likely affected by environmental conditions around them. It was also reported that sunny conditions and the water temperature affected SBESs power-generating performance [4, 15]. We, therefore, examined whether voltage reversal here was due to environmental factors such as sunlight, water temperature, and relative humidity.

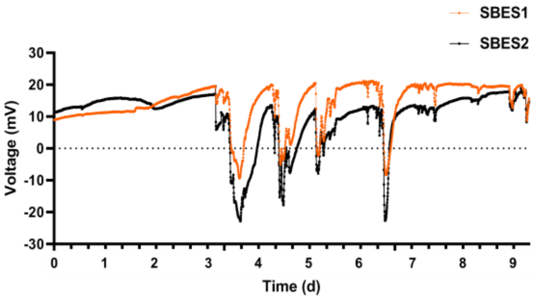

Figure 1. Real-time recorded patterns of the voltages generated by two similar SBESs.

First, the tank that was not exposed to light still experienced repeated reductions in power output at the same times as the other (Figure S1). Thus, light does not seem to be associated with the SBES voltage reversal.

Second, there was no significant temperature change during the decrease in power (Figure S2). Indeed, it was observed with other SBESs that temperatures in the range of 20-35 °C did not significantly affect voltage production over a prolonged period and minor negative effects might occur only at lower temperatures (10 °C) [15]. Therefore, temperature is not the cause of the loss of voltage density.

Third, the change in air humidity was not either related to the change in electricity generation (Figure S3). In detail, while the relative humidity in the room was quite stable at around 40% during the experimental course, on some days there was no fluctuation of voltage, but on some others, the voltage decreased sharply. It has been reported that too high humidity reduced power production [16], but no significant increase of voltage density occurred at the humidity lower than 30%. Possibly, in this study, the day-to-day humidity difference was not large enough to realize a correlation between the humidity and the SBES voltage. In other words, the effect of air humidity on the power generation of the SBES was not observed in this study.

From the above results, it can be concluded that the external factors are almost not the cause of power decreases or voltage reversals observed with the SBES.

3.2. The Effects of Internal Factors on the Performance of Sediment Bioelectrochemical System

When voltage reversal occurs, it means the functions of anode and cathode are reversed. Thus, we assume that electrons are not transferred to the cathode, and instead, there is an alternative electron acceptor at the anode. Meanwhile, the SBESs also produced a H_2S-like smell, and based on the oxidation potential of the oxidants, SO_4^{2-} can be thought to be the electron acceptor at the anode. However, the measurement results of SO_4^{2-} concentration during the fluctuations of the voltage (Figure 2) show that SO_4^{2-} concentrations were approximately equal, at about 0.6 mg/L, when the voltage was at different states: peak, descending, bottom, ascending. Yet, we cannot explain the smell of H_2S. It is possible that H_2S can be produced by some microbes in the sediment but does not interfere much with the electricity generation of the SBES [17].

It is probably that the SBESs experienced simultaneous power reductions due to internal factors indirectly influenced by the environmental factors. In a study by Kubota et al., [4], it was considered that a change in weather would lead to a corresponding DO change [4].

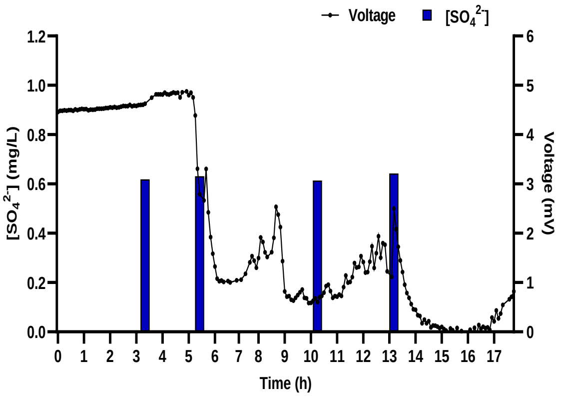

Figure 2. The correlation between the SO_4^{2-} concentration and the voltage of the SBES during one cyclic power reduction.

We therefore investigated the correlation between the generated voltage of two similar SBESs and the DO concentrations in them (Figure 3). With one SBES (SBES1), when the voltage reached the highest level at around 5 mV, the DO concentration in the cathode peaked at about 3.4 mg/L. In contrast, the DO concentration decreased suddenly, to about only 0.025 mg/L, during the period that the voltage decreased or even voltage reversal happened. The exactly same phenomenon occurred with the other SBES (SBES2). Therefore, it appears that the depletion of DO was the major factor associated with the decrease in the performance of the SBES. This means the oxygen-reducing reaction at the cathode is critical to the performance of the SBES, as the dissolved oxygen concentration determines the availability of O_2 at the cathode [18]. Furthermore, the decrease of the cathodic DO may result in an increased internal resistance that leads to power density reduction [19].

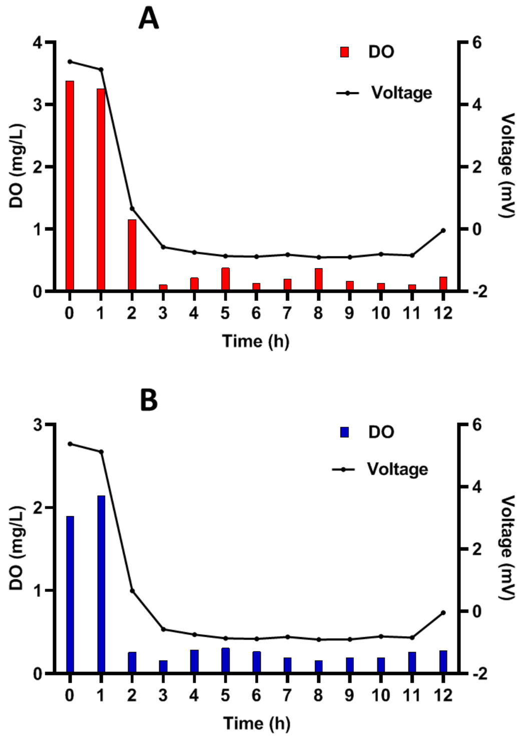

Figure 3. The correlation between the DO concentration and the voltage of two similar SBESs (SEBS1 (A) and SBES2 (B)).

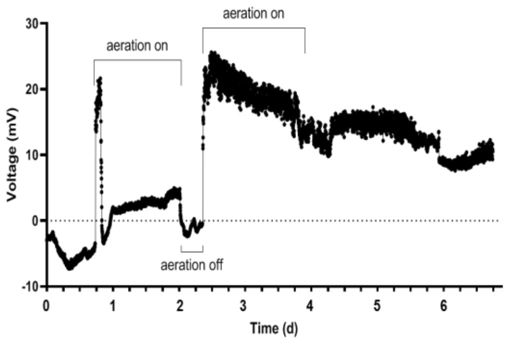

Figure 4. Effect of aeration on the electricity generation of the SBES.

In short, electricity generation by SBESs varied considerably along with changes in DO concentration but not with those in SO_4^{2-} concentration. A reasonable solution to this is aeration. Hence, we carried out an experiment that applied aeration when voltage reversal occurred. Once the SBES was aerated, the voltage gradually increased; and even if the electricity might decrease again, the reduction was also lower than before the aeration (Figure 4).

Based on the above results, we can deduce that the DO depletion causing voltage drop or reversal may have resulted from the overgrowth of numerous aerobic microorganisms on the cathode, which has been exposed to oxygen for a long time. The stability of power generation is mostly dependent on the performance of the cathodes over time, as the anode behavior has been shown to be stable for more than a year [20]. When the SBES is operated for a long time, a biofilm will form on the cathode. Figure 5(A) showed that the protein content on the cathode after one year of operation was about 8 µg/mL, which was significantly higher than that of the cathode after two months of operation. This implies that the cathode biofilm grows with time, which is also demonstrated by the COD measurement results Figure 5(B).

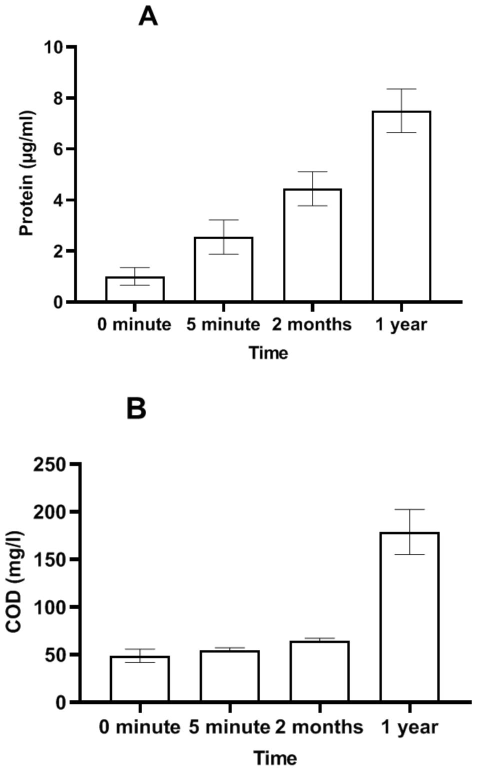

Figure 5. The protein (A) and COD (B) contents in the SBES cathodes at different times of operation.

The protein and COD analysis results also showed that there was much more biofilm on the cathode that had been operated longer, especially compared to a new cathode. However, some protein and COD values obtained with the new cathode (the control) and the new cathode submerged into the SBES water for 5 minutes suggested that there were some components in the graphite and/or in the SBES water that slightly interfered with our measurements. A biofilm can hinder the ion transport to and from the cathode, reducing the hydroxide ions diffusion from the cathode to the anode [20]. It has been recently shown that a microbial biofilm on the cathode hinders the mobility of the OH^- ions released by the oxygen reduction reaction, inducing a strong alkalinization, leading to a large decrease in the cathode open circuit voltage and the performance of BESs [21].

While biofilm formation causes cathode performance to decrease over time, the cathode biofilm does not appear to have any beneficial impact. Thus, biofilm formation has an overall adverse impact on SBESs performance and it has also been shown that biofilm removal improves the performance of SBESs [22, 23]. Therefore, we tested several approaches to reduce such “biofouling” of the cathodes.

We first tried scouring the cathode and treating it with silver nanoparticles but these treatments almost completely did not increase the electricity generation of the SBES (Figure S4). Thus, it may be difficult to remove internal foulants inside the cathode. Moreover, these techniques have only led to small reductions in biofilm formation, and the antimicrobial activity was not constant over time [20].

Therefore, the cathodes of operating SBESs were replaced with new cathodes to determine their impacts on power production (Figure 6). It is interesting that a SBES originally operated with a recently-installed (new) cathode could generate a positive and stable voltage, but when that cathode was replaced with an old cathode (from another SBES producing negative voltage at that time), its voltage decreased (Figure 6, orange curve). Surprisingly, the positive voltage could be restored when the new cathode was used again. On the other hand, another SBES being operated with an old cathode of its own produced a reduced and unstable voltage, but when that cathode was replaced with a new cathode, its voltage dramatically increased to positive levels. Noticeably, such positive levels could be maintained when that SEBS was operated again with its own old cathode yet sterilized with 70% ethanol. All these results strongly support the hypothesis that replacing the cathode would be the best solution for removing the cathode biofilm and overcoming voltage reversal.

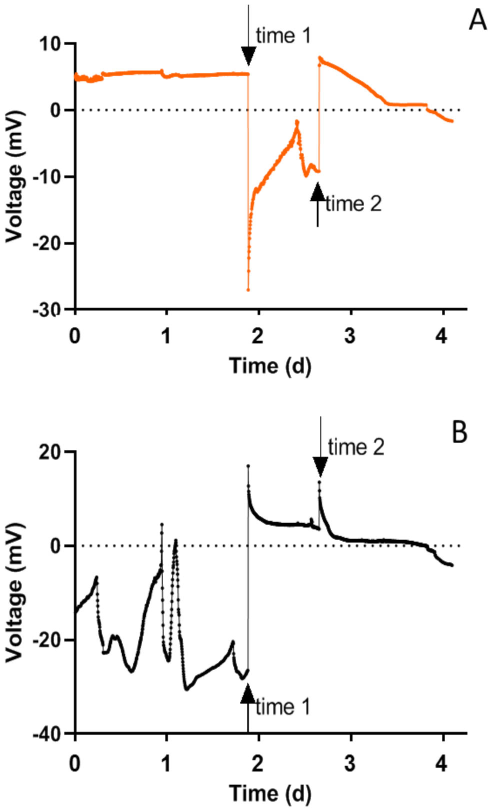

Figure 6. The patterns of the voltages generated by two SBES operated with various cathodes.

Notes: orange curve (A): the pattern of a SBES originally operated with a recently-installed (new) cathode, then with a one-year-old cathode from another SBES from time 1 and with the new cathode again from time 2; black curve (B): the pattern of a SBES originally operated with its own one-year-old cathode, then with brand new cathode from time 1 and with the one-year-old cathode treated with 70% ethanol for 24 h from time 2.

4. Conclusion

Environmental factors did not cause voltage reversal in a sediment bioelectrochemical system (SBES) operated under brackish conditions. The most prominent cause of voltage instability in such a SBES is the change of dissolved oxygen concentration in it, most probably resulted from biofilm formation on the cathode in long terms. To recover the voltage, decrease and reversal caused by dissolved oxygen concentration reduction, aeration should be applied. A more thorough measure is to regularly renew the cathode to overcome the cathode biofilm formation issues. Future studies should examine the causes of dissolved oxygen concentration changes, thereby achieving the stable power generation of the system.

Acknowledgements

This research was funded by the National Foundation for Science and Technology Development (NAFOSTED) under Grant NCUD.04-2019.20.

References

[1] D. R. Bond et al., Electrode-reducing Microorganisms that Harvest Energy from Marine Sediments, Science, Vol. 295, No. 5554, 2002, pp. 483-485.

[2] C. E. Reimers et al., Harvesting Energy from the Marine Sediment-water Interface, Environmental Science and Technology, Vol. 35, No. 1, 2001, pp. 192-195.

[3] P. Aelterman et al., Continuous Electricity Generation at High Voltages and Currents using Stacked Microbial Fuel Cells, Environmental Science and Technology, Vol. 40, No. 10, 2006, pp. 3388-3394.

[4] K. Kubota et al., Operation of Sediment Microbial Fuel Cells in Tokyo Bay, an Extremely Eutrophicated Coastal Sea, Bioresource Technology Reports, Vol. 6, 2019, pp. 39-45.

[5] Y. Sharma, B. Li, The Variation of Power Generation with Organic Substrates in Single-chamber Microbial Fuel Cells (SCMFCs), Bioresource Technology, Vol. 101, No. 6, 2010, pp. 1844-1850.

[6] T. M. Sajana, M. Ghangrekar, A. Mitra, Application of Sediment Microbial Fuel Cell for in Situ Reclamation of Aquaculture Pond Water Quality, Aquacultural Engineering, Vol. 57, 2013, pp. 101-107.

[7] T. H. Pham et al., A Laboratory-scale Study of the Applicability of a Halophilic Sediment Bioelectrochemical System for in Situ Reclamation of Water and Sediment in Brackish Aquaculture Ponds: Establishment, Bacterial Community and Performance Evaluation, Journal of Microbiology and Biotechnology, Vol. 29, No. 7, 2019, pp. 1104.

[8] L. Cheng, S. B. Quek, R. Cord-ruwisch, Hexacyanoferrate-adapted Biofilm Enables the Development of a Microbial Fuel Cell Biosensor to Detect Trace Levels of Assimilable Organic Carbon (AOC) in Oxygenated Seawater, Biotechnology and Bioengineering, Vol. 111, No. 12, 2014, pp. 2412-2420.

[9] S. E. Oh, B. E. Logan, Voltage Reversal During Microbial Fuel Cell Stack Operation, Journal of Power Sources, Vol. 167, No. 1, 2007, pp. 11-17.

[10] B. E. Logan, Microbial Fuel Cells, John Wiley and Sons, 2008.

[11] Å. P. Kolmert, Wikström, K. B. Hallberg, A Fast and Simple Turbidimetric Method for the Determination of Sulfate in Sulfate-reducing Bacterial Cultures, Journal of Microbiological Methods, Vol. 41, No. 3, 2000, pp. 179-184.

[12] A. Eaton et al., Standard Methods for the Examination of Water and Wastewater 20th edn American Public Health Association: Washington, DC, USA.[Google Scholar], 1998.

[13] I. Vyrides, D. Stuckey, A Modified Method for the Determination of Chemical Oxygen Demand (COD) for Samples with High Salinity and Low Organics, Bioresource Technology, Vol. 100, No. 2, 2009, pp. 979-982.

[14] J. B. Hammond, N. J. Kruger, The Bradford Method for Protein Quantitation, in New Protein Techniques, Springer, 1988, Pp. 25-32.

[15] S. W. Hong et al., Experimental Evaluation of Influential Factors for Electricity Harvesting from Sediment using Microbial Fuel Cell, Bioresource Technology, Vol. 100, No. 12, 2009, ppp. 3029-3035.

[16] Y. Ahn, F. Zhang, B. E. Logan, Air Humidity and Water Pressure Effects on the Performance of Air-cathode Microbial Fuel Cell Cathodes, Journal of Power Sources, Vol. 247, 2014, pp. 655-659.

[17] D. E. Holmes et al., Protozoan Grazing Reduces the Current Output of Microbial Fuel Cells, Bioresource Technology, Vol. 193, 2015, pp. 8-14.

[18] Y. Zhang et al., Effect of Dissolved Oxygen Concentration on Nitrogen Removal and Electricity Generation in Self pH-buffer Microbial Fuel Cell, International Journal of Hydrogen Energy, Vol. 45, No. 58, 2020, pp. 34099-34109.

[19] Q. Tao et al., Effect of Dissolved Oxygen on Nitrogen and Phosphorus Removal and Electricity Production in Microbial Fuel Cell, Bioresource Technology, Vol. 164, 2014, pp. 402-407.

[20] R. Rossi et al., In Situ Biofilm Removal from Air Cathodes in Microbial Fuel Cells Treating Domestic Wastewater, Bioresource Technology, Vol. 265, 2018, pp. 200-206.

[21] B. Erable et al., Iron-nicarbazin Derived Platinum Group Metal-free Electrocatalyst in Scalable-size Air-breathing Cathodes for Microbial Fuel Cells, Electrochimica Acta, Vol. 277, 2018, pp. 127-135.

[22] W. Liu et al., Influence of Soluble Microbial Products on the Long-term Stability of Air Cathodes in Microbial Fuel Cells, Electrochimica Acta, Vol. 261, 2018, pp. 557-564.

[23] F. Zhang, D. Pant, B. E. Logan, Long-term Performance of Activated Carbon Air Cathodes with Different Diffusion Layer Porosities in Microbial Fuel Cells, Biosensors and Bioelectronics, Vol. 30, No. 1, 2011, pp. 49-55.