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Original Article

The Potential of using Microbial Fuel Cells as a "Quality" Monitor for Ornamental Seawater Aquarium

Jing Ya Hsu [1], Chia-Chun Lee [1], Zhang Ron Jian [1], Jaw-Chyun Chen [2], Chorng-Horng Lin [1*]

[1] Department of Bioresources, Da-Yeh University, DaCun, Taiwan
[2] Department of Medicinal Botanicals and Foods on Health Application, Da-Yeh University, 168 University Road, Dacun, Changhua 51591, Taiwan

Corresponding Author: Chorng-Horng Lin, PhD, Associate Professor, Department of Bioresources, Da-Yeh University, DaCun, Taiwan. Tel: +886-4-8511888, E-mail: clin@mail.dyu.edu.tw

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Abstract

Introduction: Microbial Fuel Cells (MFCs) can be applied as biological sensors for the monitoring of Biochemical Oxygen Demand (BOD), toxic substances, and nutrients in the water. Research on the application of MFC biosensors in ornamental aquariums is relatively limited.

Materials and Methods: In order to carry out this study, we applied a single-chamber and mediator-free sediment MFC in an ornamental seawater aquarium as an economical and straightforward biosensor to monitor water quality. Accordingly, the water quality parameters, including temperature, pH, the Dissolved Oxygen (DO), Oxidation-Reduction Potential (ORP), and the Electrical Conductivity (EC) were analyzed.

Results: After the induction of an artificial die-off environment, the Dissolved Oxygen and Oxidation-Reduction Potential showed decreasing trends, and the Electrical Conductivity showed an increasing trend. The voltage output decreased during the initial die-off stage. Principal Component Analysis (PCA) clusters the individuals of the initial die-off stage at the boundary of the regular individuals. Spearman correlation suggests that electricity generation during the initial die-off stage is positively correlated with ORP.

Conclusions: According to the findings of the present study, it can be stated that the die-off stress causes electrochemical inhibition, resulting in a decrease in electricity generation.

Keywords: Sediment Microbial Fuel Cells, Biosensor, Voltage, Artificial Die-off

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Introduction

Microbial biosensors are analytical devices that use microbial metabolism, biochemical reactions, and sensors to convert analyte concentrations into a measurable signal. At present, microbial sensors have been developed for environmental, food, and biomedical applications analyzing DO, BOD, toxic substances, and microbial communities.[1,2] Among these biosensors, MFCs are mostly used for the detection of BOD and toxic substances.[3]

MFCs are a method of using microorganisms to oxidize organic matter to produce electrical energy. The reduced products of metabolism supply electrons, or the electrons are efficiently transferred to the anode by a natural or artificially added electron transporter. Additionally, the electrons are transferred to the cathode via a resistor. The cathode can be an air cathode or aerated. Protons are derived from the oxidation of organic matter. The produced protons move to the cathode where they combine with electrons and oxygen to form water on the surface of the cathode. A dual-chamber device can prevent oxygen from diffusing to the anode so a proton exchange membrane separates the cathode and anode.[4,5] MFCs can be applied in marine sediments or wastewater treatment; that is, the organic matter provided by sediments or wastewater supplies nutrients for the growth of microorganisms to achieve the dual purpose of biological environment decontamination and energy production.[4,6-8]

There have been several studies on sediment MFCs applied in aquaculture. The performance of sediment MFCs accomplish the Chemical Oxygen Demand (COD) and Total Kjeldahl Nitrogen (TKN) removal of aquaculture pond water, and the COD and TKN removal efficiencies can be 84.4% and 95.3%, respectively.[9] Furthermore, optimal COD removal, Total Nitrogen (TN) removal, and power density can be achieved if the following parameters are maintained: a feed pH of 7.6-8.5, a 90-100 cm distance between electrodes, and an external resistance of 0-52 Ω.[10] The combination of a sediment MFC and a brackish aquaculture tank model can reach 40% COD removal and 52% nitrogen removal efficiency with a current density of 2.3 mA/m^{-2} and a power density of 0.05 mW/m^{-2} in a model aquaculture system.[11] Recently, sediment MFCs have been applied for the remediation of sulfidic sediments around aquaculture operations.[12] Interestingly, an MFC with an anion exchange membrane that cooperates with a Recirculating Aquaculture System (RAS) can efficiently remove nitrogen and provide power.[13] However, research on the application of MFC biosensors in ornamental aquariums is relatively limited.

The total trade in live marine ornamentals has been estimated at approximately 44 million U.S dollars annually,[14] and 10% of the U.S. population has already invested in keeping an aquarium.[15] It is likely that aquarium keeping is not only a hobby but also related to science and art, especially conservational ethics.[15,16] As water quality affects the growth and health of fish, it should be modulated carefully.[17] This study aims to develop an economic MFC biosensor to monitor the water quality of seawater aquariums. Thus, a single-chamber, mediator-free sediment MFC has been applied. Additionally, the voltage, pH, temperature, EC, DO, and ORP were recorded, and the Spearman rank correlation and PCA were obtained in R version 3.4.1.

Materials and Methods

Ornamental Seawater Aquarium with the Construction and Operation of an MFC

The soil used in this study was collected from a wetland (24°02'N, 120°22'E) in Fangyuan Township, Changhua County, Taiwan. Coral sand and artificial seawater were purchased from a local aquarium shop. The median grain sizes of the soil and coral sand were in the range of 0.1-1 mm and 2-5 mm, respectively.

The seawater aquarium contains a single-chamber, mediator-

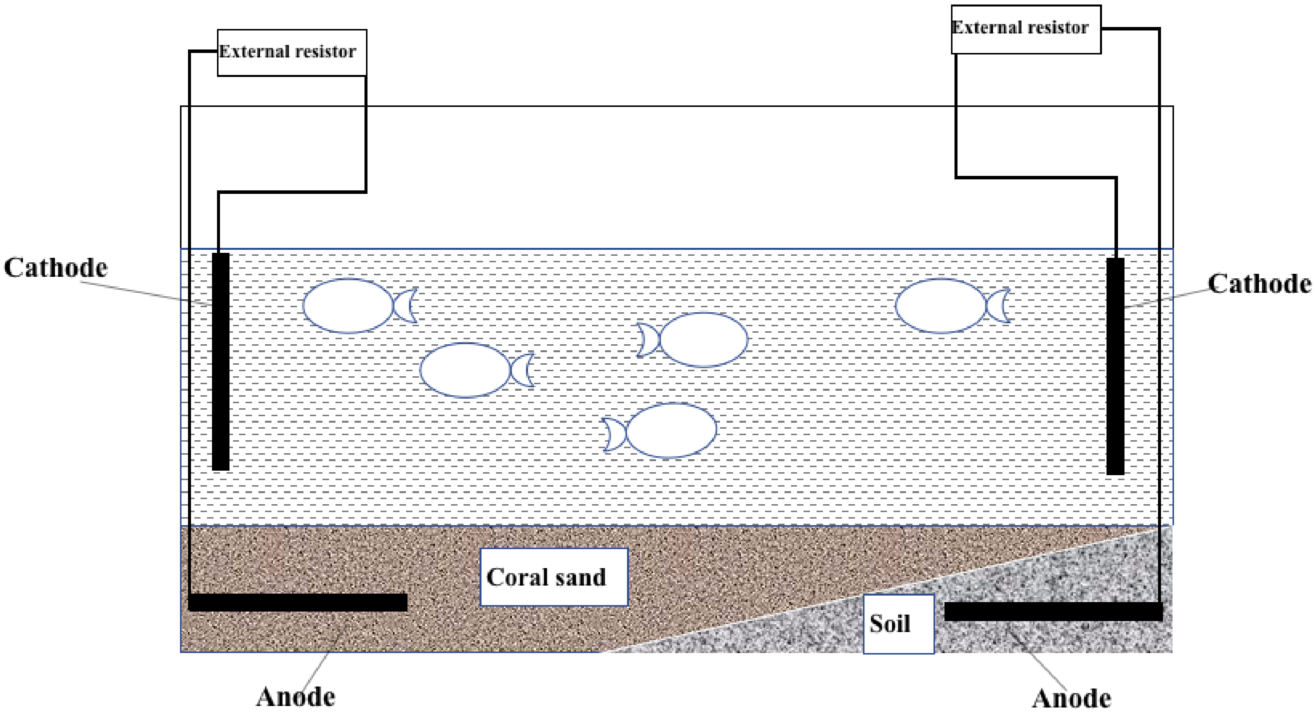

Figure1. A Schematic Aquarium of a Single-Chamber, Sediment-type Microbial Fuel Cell.

free, sediment-type MFC device (Figure 1). The soil and coral sand were placed in an aquarium tank (45 × 22.5 × 27 cm) at a depth of 5 cm. Two woven carbon fiber sheets (12 × 12 cm, Skyline Carbon Co., Taipei, Taiwan) were used as the anode (-) and cathode (+); the anode was buried in the soil or coral sand, and the cathode was allowed to float in the seawater. A 1 kΩ external resistance was applied as the load. There were two resistors selected for consideration. The first was a 300 Ω external resistor, and the voltage output appeared to be low. The second was a 1 kΩ resistor, which we used for other sediment MFC studies to show the maximum power density.[18] The filtration pump power was 4.5 W at a flow rate of 360~480 L/h^{-1}. Until the water quality was stable, yellow-bellied damselfishes (*Pomacentrus coelestis*) were placed in the aquarium. The fish were fed once every two days. Freshwater was added to keep the seawater specific gravity (sg) between 1.020 and 1.024. The voltage was recorded on a dual-channel data collection multimeter, Prova 803 (TES Electrical Electronic Corp., Taipei, Taiwan), and the data were downloaded by an RS-232 interface connection to a computer. The pH, temperature, EC, DO, and ORP were recorded on a multifunctional water detector meter, WA-2017SD (LUTRON ELECTRONIC ENTERPRISE CO., LTD., Taipei, Taiwan). Accordingly, three repetitive experiments were performed. The soil and coral sand were reused after being exposed to sun for three days.

Artificial Die-off Environment

After three weeks of regular feeding, the fish were removed, and ten giant Pacific oysters were chopped and placed in the

Table 1. The Voltage, Temperature, DO, pH, EC, and ORP of Sediment-type MFC in Seawater Aquarium. Three Repetitive Experiments (Expt.) Were Performed. Individual (Indiv.) Indicates Each Measurement

Expt.	Indiv.	Soil (mV)	Coral Sand (mV)	T (°C)	DO (mg/L)	pH	ORP (mV)	EC (mS)
I	1	95	0.7	23	8.4	7.83	139	54.4
	2	108	14	24	8.5	7.72	195	53.8
	3	83.8	14.9	24.8	8.9	7.77	196	53.3
	4	38.2	12.2	25.2	8.2	7.4	209	51.9
	5	27.2	19.7	25.1	9.9	7.89	198	52.8
	6	50.5	173.9	24.1	10.2	7.7	124	52.1
	7	-108	82.3	23.6	12.3	7.8	209	53.2
	8	33.2	27.8	23	13.2	7.88	202	54.2
	9	-214	18.6	23.2	13.6	7.8	217	52.3
	10	24.5	0.2	23.1	14.2	7.88	123	53.3
	11*	0.024	0.012	23.7	13.6	7.82	236	51.8
	12*	0.003	0.012	22.9	13.2	7.63	140	52.8
	13*	-11.8	-0.032	23.4	11.2	7.64	147	52.9
	14**	0.0014	-0.053	23.5	11.2	7.61	-190	53
	15**	0.0007	-0.029	23.5	10.6	7.65	-267	53.3
II	16	2.6	44.5	24	10.5	7.85	209	52.7
	17	3.5	9.4	23.7	12.5	7.8	205	54.6
	18	13	7.5	25.3	12	7.76	209	54.2
	19	20.8	4.6	25.3	11.8	7.9	134	54.3
	20	20	1.7	22.9	11.8	7.85	207	51.7
	21	3.4	2.1	24.1	12.9	7.89	162	52.1
	22	6.8	143	25.2	10.4	7.83	117	53.0
	23	2.8	6.7	24.3	11.2	7.8	220	53.4
	24*	0.522	26.727	25.5	9.6	7.5	190	55.2
	25*	-0.23	-7.6	21.8	7	6.12	117	54.8
	26*	-6	-3.89	25	7.5	9.86	77	55.4
	27**	-0.0022	-21.455	25.1	7.2	7.4	29	55.8
	28**	-0.064	-20.704	24.9	6.3	7.63	-227	57
III	29	8.6	-36	27	3.6	7.76	196	52.6
	30	40.6	-29.9	23.8	3.9	7.85	223	53.8
	31	4.5	-5.3	25	3.8	8.01	224	53.2
	32	4.5	-7	24	4.1	7.98	220	55.5
	33	4.9	-26.5	25	8.8	8.06	230	49.6
	34	10.4	-45.5	25	7.3	8	214	49.5
	35	44.3	-6.2	25	9.8	8.04	224	52.2
	36	49	-27.1	25	9	8.05	122	51.5
	37	5.4	-19.7	25.8	8.2	8.1	198	48.3
	38	20.5	-0.8	25	9.8	8	225	52.4
	39	16.8	-2.1	22.2	13.8	8.04	219	50.2
	40	13.5	-8.5	22.7	12.6	8.07	229	54
	41	2.6	-26.4	22.5	13.4	7.99	247	52.4
	42	3.4	24.6	21.9	12.8	8.01	239	51.3
	43	18.4	21.4	21	10.9	7.46	253	53.8
	44	47.9	59.6	21.5	6.4	8.4	159	47.9
	45*	25	1.4	21.7	7.8	8.25	236	51.6
	46*	28	-14.17	22.9	7.2	8.52	252	48.6
	47*	3.875	-85.97	21	4.5	8.4	120	48.9
	48**	1.257	86.52	25.7	1.8	8.42	-227	49.9
	49**	0.124	25.93	22.5	0.9	8.46	-254	50.1

* Indicates the measurement of the first three days after the artificial die-off initiation.

** Indicates the measurement of the fourth and fifth days after the artificial die-off initiation.

aquarium. Additionally, the filtration pump was removed. The voltage, pH, temperature, EC, DO, and ORP were recorded every day for five days.

Data Analyses

PCA was performed in R version 3.4.1 with the prcomp function.[19] Spearman rank correlation of the variables was developed in R version 3.4.1 with the cor and corrplot functions.[19]

Results and Discussion

In the aquarium, the soil and coral sand, where the anodes were located, were used as the bed. Table 1 lists the voltage outputs and water quality parameters, including temperature (°C), DO (mg/L^{-1}), pH, ORP (mV), and EC (mS). Before the artificial die-off stages, the temperature, DO, pH, ORP, and EC values ranged from 21 to 25.8 °C, 3.6 to 14.2 mg/L^{-1}, 7.4 to 8.4, 122 to 253 mV, and 47.9 to 62 mS, respectively. According to The Fish Database of Taiwan, yellow-bellied damselfish, which are a popular aquarium fish, are widely distributed in the Indo-Pacific region. These fish mainly inhabit the waters near the bottom of gravel beds in lagoons and coastal reefs. Furthermore, these fish can be found in the reefs around Taiwan, especially in the north.[20] In regard to the reef aquarium recommendation, the following water parameters were controlled: the salinity, temperature, and pH were maintained at 1.026 sg, 76-83 °F (24.4-28.3 °C), and 7.8-8.5, respectively; moreover, the ORP was controlled but not recommended.[21] The monitoring and maintenance of water quality in the Coral Reef Exhibition at the Reef HQ

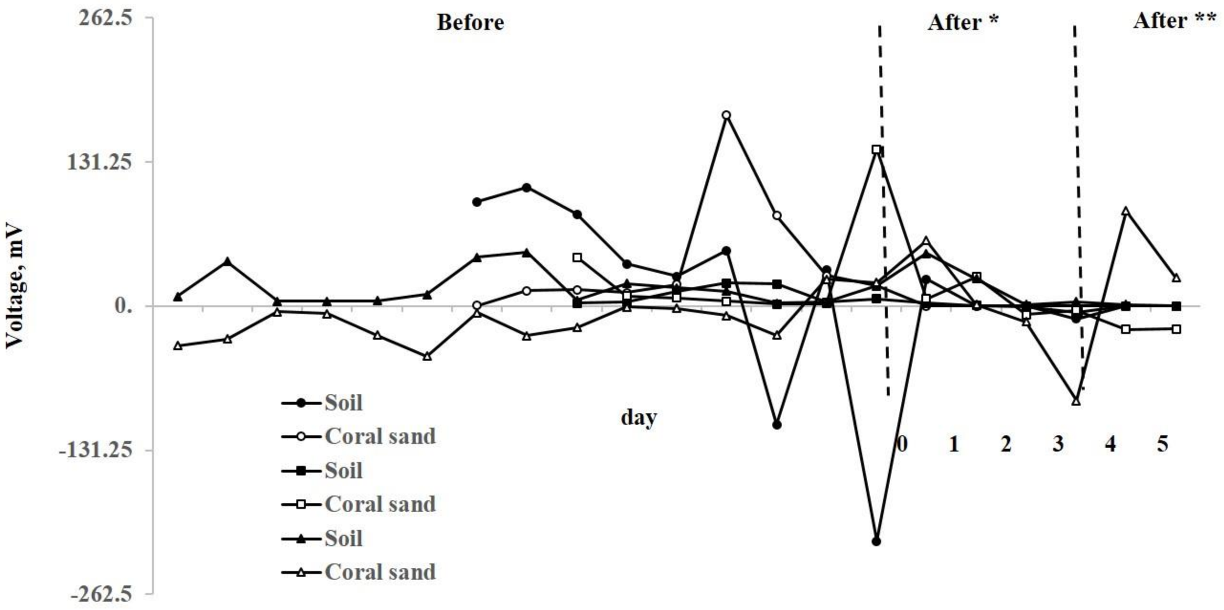

Figure 2. The Voltage Fluctuation of the Sediment-type MFC in Seawater Aquarium as a Function of Time. Three repetitive experiments were performed. Soil and coral sand indicate the sediment where the anodes located. Before, After * and After ** indicate the days before the artificial die-off initiation, the initial die-off stage (day 0, 1, 2, and 3), and the later die-off stage (day 4 and 5) respectively.

Aquarium showed that the temperature, pH, salinity, ORP, and DO were 20-29.5 °C, 7.9-8.2, 33-36 ppt (parts per thousand, 1.0249-1.0271 sg), 300-500 mV, and 90-100%, respectively.[22] In our aquarium, the water parameter showed variation within the acceptable range in which one or two fish died during the non-die-off stage. During the artificial die-off periods, the DO and ORP showed decreasing trends (Table 1) because the filtration pump was turned off and the giant Pacific oysters were chopped and placed in the aquarium. The EC showed an increasing trend after the induction of artificial die-off.

Furthermore, we constructed sediment MFCs in the aquarium. Figure 2 shows the fluctuations in daily voltage output. The voltage output of MFCs showed variation during the experiment but not increasing trends. Interestingly, the voltage outputs showed a consistent decrease in the first three days of the die-off stages. After that, the voltages again showed variation. The energy output circuit could indeed have contributed to the acclimation of certain microorganisms, and *Geobacteraceae* and *Desulfobulbaceae* have been shown predominantly in anodic biofilms and positively correlated with electricity generation efficiency.[6,23-25] Different sediments for MFCs could yield diverse microbial communities resulting in diverse power outputs.[26,27] Bacterial counts could range from 4×10^6 to 2×10^9 g^{-1} dry soil in different soils,[28] 1.3×10^9 g^{-1} (dry weight) in the sediment of coral reefs,[29] and 8 to 10×10^8 g^{-1} at depths of 0-10 cm in the sediment of Checker Reef, Oahu, Hawaii, USA,[30] suggesting that bacterial numbers in coral sand and soils might not vary much. However, the median grain sizes of the soil and coral sand were in the range of 0.1-1 mm and 2-5 mm, respectively. It is likely that more microbes would contact the anode of the MFC with soil sediment, thus producing a higher voltage output. During the initial die-off stage, the stress caused the inhibition of microbial electrochemical activities or the death of certain microbes, resulting in a reduction in the voltage output. Studies on the microbial diversity and potential pathogens in freshwater ornamental fish aquarium water have shown highly diverse microbial communities and sources of potential pathogens.[31] Microbial dynamics analysis in ornamental seawater aquariums needs to be performed for clarification.

We further performed the PCA generated in R version 3.4.1 to analyze the correlation between the variables and the clustering based on similar characteristics. Figure 3 shows the plot of the PCA results, suggesting that the individuals could be separated into three subgroups: the before die-off stage, the initial die-off stage (the first three days), and the later die-off stage (4 days later). The individuals classified in the before die-off stage were grouped in the center of the plot. Individuals classified in the initial die-off stage (the first three days) were located on the boundary or outside of the first subgroups, although they were not grouped near each other. The individuals of the last subset were situated on the left side away from the different subgroups. For individuals 11, 12, and 13 of the initial die-off stage, the result suggested that they had higher DO and ORP values but with lower MFC voltage outputs with soil as the sediment; this result was because they were located in the same direction as the DO and ORP variables but in the opposite direction as the voltage output variable. According to the location of the individual relative to the variables, the

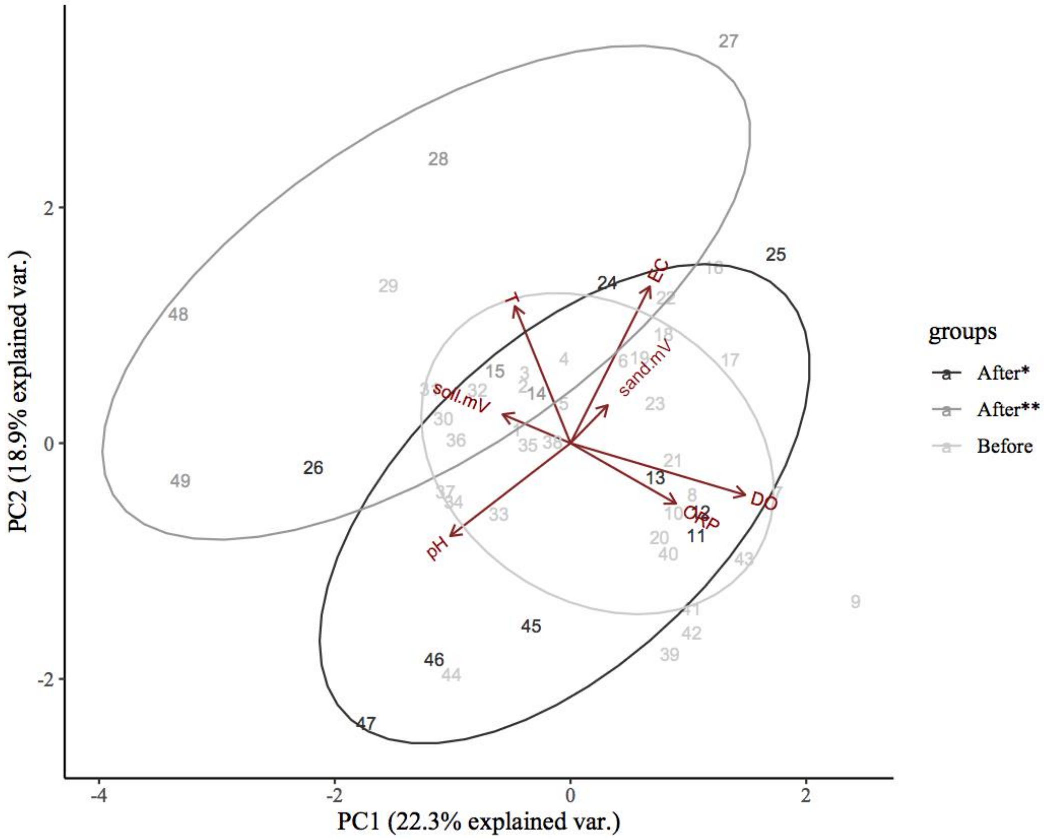

Figure 3. Principal Component Analysis (PCA) Analysis Biplot of the Individuals. The variables are soil (mV), coral sand (mV), temperature (T), DO, pH, EC, and ORP of sediment-type MFC in seawater aquarium. Each number represents the measurement sample as listed in Table 1. Sample groups are indicated by using different colors. Before, After * and After ** indicate the days before the artificial die-off initiation, the initial die-off stage (day 1, 2, and 3), and the later die-off stage (day 4 and 5) respectively.

results suggested that individuals 24 and 25 had relatively high EC values, and individual 26 had a low DO and ORP. Individuals 45, 46, and 47 had somewhat high pH values. It is challenging to identify which variables were relative to those individuals classified in the initial die-off stage, since the individuals scattered into the surroundings of the first subgroup instead of remaining at the same coordination. PCA did group well for the individuals of the later die-off stage, suggesting that they would have higher voltage outputs and lower DO and ORP values.

To analyze the voltage outputs of the MFC correlating to which variables are specified in the initial die-off stage, we performed the Spearman rank correlation of variables, as generated in R version 3.4.1. The results suggested that the ORP was positively correlated with the voltage outputs of the MFC with either soil or coral sand used as the sediment (Figure 4). The DO was positively correlated with the voltage outputs of the MFC with coral sand and negatively correlated with the voltage outputs of the MFC with soil. The EC was highly negatively correlated with the voltage outputs of the MFC with soil. Thus, during the initial die-off stage, the stress caused by the decrease in the concentration of oxygen and the dead bodies of the giant Pacific oysters led to a decrease in ORP and also a reduction in the voltage outputs of the sediment-type MFC in the aquarium (Figure 2).

The water quality parameter affects the performance of MFCs. The ORP is the activity or strength of oxidizers and reducers in regard to their concentration and electrochemical level. In a dual-chamber MFC, the anodic ORP has been recorded to -550 ± 10 mV.[32] Studies on the remediation of simulated malodorous surface water by a columnar air-cathode MFC, which is a single-chamber MFC, showed that the MFCs could raise the ORP from -281.2 mV to -135.7 mV after 24 h. This ORP elevation could be attributed to improved sulfide oxidation and inhibited sulfate reduction.[33] A negative ORP is likely preferred for MFC performance. Rago et al.[34] studied three MFCs with cathodes at different DO concentrations: i) air breathing, ii) water-submerged, and iii) assisted by photosynthetic microorganisms. Results revealed that the air-breathing MFC exhibited the highest maximum current. To improve the cathode reaction, a biocathode was developed in which an electron-consuming bacterial consortium was applied as a cathode catalyst.[35] Interestingly, with increases in the DO concentration, hetero-trophic denitrifying bacterial activity was inhibited, and the proportion of nitrobacteria was enhanced. Nevertheless, the internal resistance of the reactor gradually increased in order to reduce the maximum power density.[36] If photosynthetic

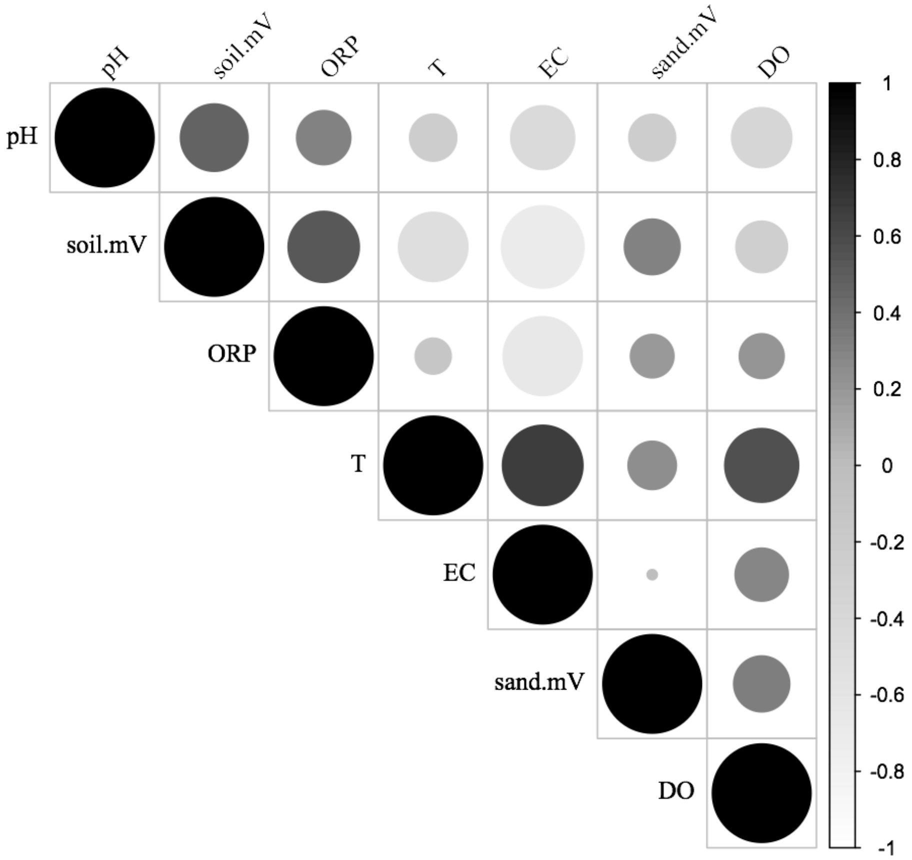

Figure 4. Spearman Rank Correlation Analysis of the Initial Die-off Stage (day 0, 1, 2, and 3) Variables in Sediment-type MFC. Strong correlations are indicated by black squares, whereas weak correlations are indicated by light grey squares. The colors of the scale bar denote the nature of the correlation with 1 indicating perfect positive correlation (black) and -1 indicating perfect negative correlation (white) between two variables.

microorganisms were applied in the biocathode, they would supply a high DO level, up to 16 mg/L^{-1}, which sustained the aerobic microbial community to improve the oxygen reduction reaction through aerobic metabolism.[34] Therefore, oxygen is essential for a cathodic response, which is also vital in an aquarium. However, we constructed a single-chamber, mediator-free MFC with no proton exchange membrane between the anode and cathode; thus, the oxygen supplied by the filtration pump could reduce the anodic reaction and MFC efficiency.

Conclusion

MFCs have been applied as biosensors for the detection of BOD and toxic substances. The microbial communities in aquarium water and sediment should be related to the water quality and the power output efficiency of the MFC. Here, we presented a feasible MFC monitor for ornamental seawater aquariums, showing that the decrease in the voltage output of the sediment MFC was positively correlated with the decrease in the ORP caused by the artificial die-off environment. We believe that the initial die-off stage could suppress the electrochemical reactions of the microbes or cause the death of certain microbes, resulting in the decrease in voltage output. Therefore, if the MFC biosensor is connected to a digital-type potentiometer, or the signal is converted into a red or green light, according to the voltage change, the aquarium keeping is more fun and easier. Further investigation and analysis of microbial dynamics in ornamental aquariums are required to better understand the electrochemical status.

Authors' Contributions

JYH performed experiments and analyzed data. CCL and ZRJ performed experiments. JCC designed the experiments. CHL guided the work and wrote the manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

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