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■ RESEARCH ARTICLE

Microbial Fuel Cell that Degrades Different Concentrations of Phenol in 5% Salinity and 1.6% Salinity Sewage

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ABSTRACT: With the development of the chemical industry, more and more high-salinity sewage that contains the pollutant phenol. The research in this paper tested Microbial Fuel Cell (MFC) phenol degradation with a focus on the *Staphylococcus caprae* strain SHBCC D10058 ATCC 35538 as the degrading bacteria. In conditions with a 5% salinity medium and 100mg/L initial *phenol* concentration, after three days of degradation, 46.25% phenol remained. The phenol concentration was initially 220mg/L; after three days of degradation in a 5% salinity condition, 64.74% phenol remained. 65.93% of phenol remained in the medium with an initial phenol concentration of 300mg/L after three days' degradation. When the Microbial Fuel Cell was utilized with only polycarbonate brine (1.6%salinity, 4.07mg/L phenol), the cell had a maximum power output of 30.070mW/m², and after three days of degradation, 32.31% phenol remained.

KEYWORDS: Energy: Chemical; Microbial Fuel Cells; *Staphylococcus caprae*; Phenol degrading; salinity.

■ Introduction

Phenol is an important raw material in producing some resins, bactericides, and preservatives. Due to the development of the chemical industry, more and more phenol has been used, causing more phenol-containing sewage to be produced, including high-salinity phenol-containing sewage. Moreover, because phenol has huge negative effects on humans, such as a strong corrosive effect on skin and mucous membranes, it can damage the central nervous system, liver, and kidney functions. In addition, phenol causes serious environmental harm and can lead to water and air pollution. For example, based on the research of water quality analysis of industrial phenol-containing wastewater: the fish that lived in low phenol-containing water (0.1-0.2mg/L) will have smelly meat, and the fish will die in water containing approximately 70.5mg/L phenol, also, use phenol containing water for irrigation will cause the death of the crop.[1] Furthermore, sewage with high salinity has additional requirements for its disposal process. Many industry products, such as polycarbonates, generate high-salinity sewage as a byproduct that contains phenol.

Polycarbonate is an important plastic with high transparency and strength. It is widely used in many processes, including producing automobile parts, optical elements, CDs, and Shatterproof glass.[2] Due to the methods used to produce polycarbonate, high-salinity sewage with phenol will be produced. Although most methylene chloride, triethylamine, and tert-butylphenol in sewage will be removed through the wastewater treatment process, such as washing and steam stripping, some phenol still remains.[3]

Nowadays, many phenol degradation methods exist, such as chemical, physical, and biological methods. However, there are many limitations to them. Many chemical processes degrade phenol quickly but will consume a large amount of energy, such as research that used an electrochemistry method to degrade phenol by breaking down water to produce hydroxide ions and using the hydroxide ions to oxidize phenol,[4] research that used a photochemistry method to degrade phenol by UV light irradiation,[5] and research that strengthening the degradation of phenol by adding catalysts to the high-voltage pulse discharge reactor.[6] Moreover, as to the physical adsorption approach, this method can remove phenol from sewage to a large extent, with research showing the use of microporous resin to dispose of and filtrate wastewater from polycarbonate production.[3] However, the production of resin also produces sewage.

In contrast, biological degradation methods, such as degrading bacteria and microorganisms to degrade phenol wastewater, do not occupy much area or consume a lot of resources. For examples, research about the identification and isolation of a yeast strain that can tolerate highly concentrated salt and degrade phenol[7], research about isolated and identified *Rhodococcus strain* JDD1H[8] and research about a phenol-degrading Staphylococcus strain.[9] However, even though these biological methods are effective decisions for degrading high-salinity sewage with phenol, there still have other better methods, that can both break down phenol and also generate electricity as an additional benefit. Therefore, another biological degradation method: Microbial fuel cell (MFC), is a better choice because it can simultaneously generate electricity and degrade pollutants in sewage. For example, the research about power generation from phenol degradation by using a Microbial Fuel Cell[10] and research about updates and synergistic effects of hybrid systems on microbial fuel cell degrading landfill leachate.[11] Also, MFC has been regarded as a new type of green power. There has been researching on co-metabolism, which showed enhanced phenol degradation and bioelectricity generation in the microbial fuel cell.

DOI: 10.36838/v5i3.3

Currently, there is little research about using MFC to degrade high-salinity phenol-containing sewage, such as sewage produced by producing polycarbonate with a method of interfacial condensation mainly containing salt, phenol, and bisphenol A. Consequently, the primary purpose of this article is to fill this gap by testing the power output and degradation ratio of MFC in different phenol concentrations in a 5% salinity environment. Moreover, the experiment will also test the feasibility of using MFC to treat sewage produced by polycarbonate production.

The MFC used in this paper is a dual-chamber MFC, with both the pollutant and degrading bacteria in the anode. Moreover, the electrodes of this MFC are made of copper plate and carbon felt. The most critical component of the MFC in this research is the degrading bacteria because it allows the MFC to have the ability to degrade phenol in a high salinity environment and fill the research gap. The degrading bacteria used in this research paper is the *Staphylococcus caprae* strain SHBCC D10058 ATCC 35538, a specific Staphylococcus that can degrade phenol in high salinity environments. Among the phenol-degrading bacteria currently found,[12] only very little research has used *Staphylococcus caprae* as the degrading bacteria in the MFC. Therefore, this research mainly focused on culturing *Staphylococcus caprae* in the MFC, testing its phenol degradation efficiency and electricity-generating efficiency in different phenol concentrations within a 5% salinity environment. Moreover, the feasibility of using the MFC to degrade polycarbonate brine will also be tested.

Materials and Methods

Experimental Setup and Microorganisms:

The MFC used in the experiment was a dual-chamber one. It was mainly made of glass, plastic, and rubber and had a proton exchange membrane (PEM) to divide the two chambers. The maximum volume of both two chambers was 600 cm^3, and the scaled volume was 400 cm^3. The mode of the dual-chamber MFC is shown in Figure 1. When testing the MFC's power and degradation ratio, the chamber's working volume with the anode was 500 cm^3, and the cathode side was 400 cm^3. When testing the feasibility, the biofilm cultivation step used 500 cm^3 fluid medium. After that period, all media in the chamber was removed, and 250 cm^3 of polycarbonate brine was poured in. In addition, the anode side was sealed and viewed as an anaerobic environment during the work and biofilm cultivation period. The cathode side was exposed to air, forming an aerobic environment. During work, wires were connected with electrodes on both sides with a 1000Ω resistor. Moreover, the data collector also linked wires on both sides to collect the voltage data.

PEM, set in the middle of the MFC, was washed after each experiment to remove the impurities and other remaining substances. The membrane was first soaked in a 1000 cm^3 solution of 3% hydrogen peroxide at 60°C for 1 hour. Second, the membrane was soaked in 1000 cm^3 distilled water at 75°C for 1 hour, next it was soaked in a 1000 cm^3 solution of 0.5 mol/L sulfuric acid at 70°C for 1 hour and finally soaked in 1000 cm^3 distilled water at 75°C for 1 hour. In addition, after after this process, the PEM was scrubbed with alcohol and washed with sterilized distilled water before using it in MFC.

The phenol-degrading microorganisms used in this research were mainly the *Staphylococcus caprae* strain ATCC 35538, bought from RE Bio Co., Ltd. The *Staphylococcus caprae* strain was activated and cultivated with the following medium: 20 g/L glucose, 15 g/L peptones, 50g NaCl/L, 0.5 g/L yeast extract, and 0.5 g/L beef extra. Moreover, based on the research about the effects of carbon and nitrogen sources on Staphylococcus JY02's growth and phenol degrading ability[13], the ratio and type of carbon and nitrogen sources in the fluid medium could promote the biofilm's growth and the degradation ability of the bacteria. Also, based on the research related to a kind of phenol-degrading bacteria, Staphylococcus caprae strain CL,[14] the fluid medium used in the MFC was prepared as follows:10 g/L $MgSO_4 \cdot H_2O$, 0.2 g/L $CaCl_2 \cdot 2H_2O$, 3.5 g/L KCl, 2.5 g/L tryptone, 50 g/L NaCl, 10 g/L yeast extract, 18.15 g/L Na_2HPO_4, and 1.2 g/L KH_2PO_4. The salinity concentration of the medium was 5%, and the pH was 7.0 (examined by an electronic pH meter, which from INESA). The reason for choosing and testing the MFC's degrading ability under 5% salinity was based on much research about the use of Staphylococcus to degrade phenol in high salinity environment, such as research about Staphylococcus JY02[13] and an analysis about the isolation and biodegradation characteristic of halophilic phenol degrading strain.[14] Due to the research, Staphylococcus has been shown to be highly adaptable to high salinity environments, so using it to degrade high salinity phenol sewage can better take advantage of Staphylococcus. Moreover, the *Staphylococcus caprae* strain was cultivated on a digital display speed multi-purpose oscillator from Jintan Qu Xicheng XINRUI Instrument Factory, model HY-4A. On the aspect of pH value, in most of the works of literature, *Staphylococcus caprae* is cultivated at a pH of around 7.2 and temperature of around 32°C, such as in research about the effects of carbon and nitrogen sources on Staphylococcus JY02's growth and phenol degrading ability,[13] and the research found a strain of *Staphylococcus caprae* that can degrade phenol.[9]

A phosphate buffer was used on the cathode side of the MFC. The composition of the 1000 cm^3 buffer was as follows: 1.2 g KH_2PO_4, 18.15 g Na_2HPO_4, 40 g NaCl, and 1 g KCl with the pH adjusted to 7.4.

Electrodes are also important for the experiment, and the following steps were use to make electrodes used in the experiment: first, we used carbon felt to cover one copperplate. Then we used copper wire and glue to fix them. The size of the carbon felt was 9 cm x 9 cm, 81 cm^2 and the size of the copper plate was 9 cm x 2.5 cm, 22.5 cm^2. During the experiments testing for phenol degradation, all parts of the carbon felt and copper plate in the anode were submerged in the medium and buffer. In the feasibility experiment, only the bottom 4 cm of the electrode will be submerged. Therefore, the functioning carbon felt area is 36 cm^2, and the functioning copper plate area is 10 cm^2. In addition, the resistance of the electrodes was measured by the multimeter, and the resistance of each electrode was approximately 96Ω.

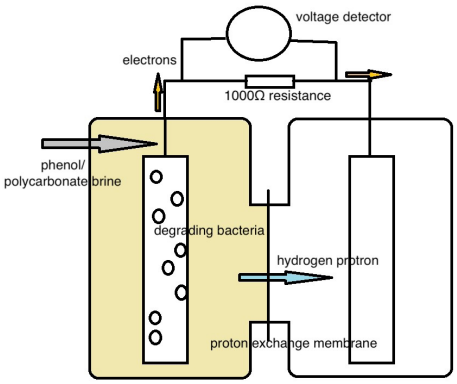

Figure 1: Mode graph of dual-chamber MFC. (Software: Paint).

Experiment Method:

Biofilm cultivation and starting-up of the experiment:

After sterilizing (at MPa 0.15 for 20 mins) and assembling, the MFC must cultivate a biofilm first and then degrade phenol from the high-salinity-containing sewage. In the cultivation step, 500 cm^3 of fluid medium will be added to the anode chamber, and 400 cm^3 buffer will be added to the cathode chamber. During this step, the MFC will be set at 36.5°C for four days to cultivate a biofilm.

After four days of cultivation, phenol was added, and the phenol concentrations on the anode side were set to 100 mg/L, 220 mg/L, and 300 mg/L. The reason for choosing those three concentrations was to test the high-concentration phenol degradation ability, the relationship between phenol concentration and both degradation ratios, electricity generation ability of the *Staphylococcus caprae* strain chosen in the experiment. Also, those phenol concentrations chosen are referenced by other research regarding phenol degrading bacteria, such as research about the different carbon source and nitrogen source's effect on Staphylococcus strain JY02,[13] research about the characteristic of *Candia tropicalis* for phenol degradation[15], and a Staphylococcus strain that can degrading phenol.[7] Moreover, because MFC's electricity generation principle will negatively affect the bacteria, the phenol concentrations chosen in this experiment are lower than those above. In the feasibility experiment, all media on the anode side were removed, and 250 cm^3 of sewage was added. The ratio of polycarbonate brine was set based on research that used microporous resin to dispose of and filter wastewater from polycarbonate production.[3] The sewage composition was: 35.2 mg/L bisphenol A, 4.07 mg/L phenol, 10 g/L Na_2CO_2 and 16.6 g/L NaCl with a pH of 11.2.

After adding phenol or sewage into the MFC, the degradation period begins. During this period, the cell was still set at 36.5°C, which was set to three days. During that time, the data collector recorded the cell's voltage per second. After three days, the concentration of phenol was recorded.

Electrochemical Measurement and Calculations:

The method used to detect the phenol concentration in the experiment was the 4-aminoantipyrine method. For this method, ammonia buffer, 4-aminoantipyrin solution, and 8% potassium ferricyanide solution were used, and the proportion of the buffer was 20 g NH4Cl in 100 cm^3 ammonia. The pH of this buffer was 10. The method's first step was adding a 0.5 cm3 buffer to the test solution. The second step was to shake the vessel and add 1 cm^3 of 2% 4-aminoantipyrin solution. Third, the shaking process was repeated, and 1 cm^3 of 8% potassium ferricyanide solution was added. After these three steps, the container was left to stand for 10 mins, and a UV spectrophotometer (from OPTOP, model 752, multimeter, UNI-T, model UT980D) was used to measure the sample from the anode chamber of the MFC at 510 nm. Moreover, the correlation curve of phenol and absorbance at 510 nm was made to compare and measure the concentration change of phenol during the experiment.

The phenol concentration was measured at the end of the experiment, and before taking samples from the MFC, the cell was stirred to ensure a uniform concentration. In each experiment, two samples were taken from the cell and put in the centrifuge (from JOANLAB) at 5000 r/min for 5 mins. Then, 200 µl of the supernatant was taken from the sample, and 4800 µl of distilled water was added to the 200 µl supernatant. After that, the 4-aminoantipyrine method was used to measure the phenol concentration in the 5 cm^3 sample, and the absorbance results were under 510nm.[16] The following equation calculated the degradation rate of phenol:

$$1 - \frac{\text{final phenol concentration}}{\text{initial phenol concentration}}$$

Each electrode's resistance and surface area was measured and calculated for the electric data. The data collector (from NATIONAL INSTRUMENTS, model NI USB-6008) was used to record the MFC's voltage. The Current Density and Power Density were also calculated. In addition, the graphs of Voltage versus Time, Current Density versus Time, and Power Density versus Time were recorded. The equation for Power Density and Current Density utilized are:

$$\text{Power Density} = \frac{V^2}{A \times R}$$

$$\text{Current Density} = \frac{V}{A \times R}$$

In the equations, A means the area of the anode, and R means resistance.

Result and Discussion

The degrading efficiency of each group:

The standard curve of the relation of phenol concentration and absorbance under 510 nm, which was measured by the 4-aminoantipyrine method, is shown in Figure 2. And the actual absorbance values shown by the stander curve are from 0 mg/L phenol to 40 mg/L phenol, for the reason that the chosen phenol testing method will dilute the testing sample to 1/25 of the origin concentration. For this reason, when we tested samples from 0 mg/L to 1000 mg/L, the absorbance value it showed was equal with phenol from concentrations of 0mg/L to 40 mg/L. The result of the degradation rate is shown in Figure 3. Under the conditions containing 100 mg/L phenol, 5% salinity, which is the same for all three groups, and three days of degradation, the degrading phenol percentage of the MFC was 53.75%; for 220 mg/L phenol, the degrading phenol percentage was 35.26%; for 300 mg/L phenol, the degrading phenol percentage was 34.075%. The result of this research was the same as other research related to phenol-degrading bacteria, *Staphylococcus caprae* strain CL.[14] For

DOI: 10.36838/v5i3.3

with high phenol concentrations, the degrading ability of the bacteria was inhibited, while in low phenol concentration environments, the phenol degrading ability of the bacteria was better. Also, compared with other research about the use of Staphylococcus to degrade phenol, such as the research on the Staphylococcus strain JY02[13] and research about phenol degrading Staphylococcus strain,[9] the degrading ratio in this research is lower, which may be because the electricity generation principle of MFC brings adverse effects to the bacteria. Compared with other MFC research, such as research about the impact of phenol concentration and external resistance to a single-chamber microbial fuel cell[17], and research on the production of electricity from phenol using a microbial fuel cell[18], both have a higher degrading phenol percentage than the degrading ratio in this research, which is probably due to the high salinity environment inhibiting the degrading ability of the bacteria, based on research on another phenol degrading Staphylococcus strain.[9] In the polycarbonate brine degrading experiment, the degrading percentage of phenol was nearly 67.69%. This result shows that the high pH containing bisphenol A affected the phenol degrading ability of *Staphylococcus caprae* strain SHBCC D10058 ATCC 35538. Moreover, the relatively high degrading ratio of this *Staphylococcus caprae* strain shows that it can be a choice for degrading bacteria when using the MFC method to dispose of polycarbonate brine. In addition, because the *Staphylococcus caprae* strain used in the research cannot degrade bisphenol A, it needs to work in conjunction with another bisphenol A degrading bacteria, such as the bacteria in research of bacteria-mediated bisphenol A degradation.[19]

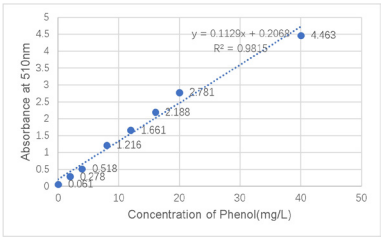

Figure 2: Phenol concentration-Absorbance graph.

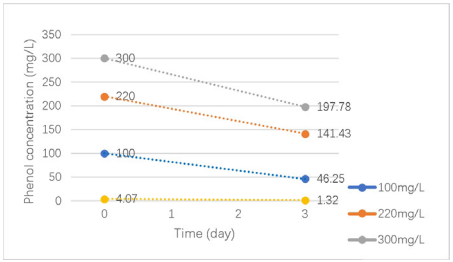

Figure 3: Phenol degrading rate.

The electricity generation efficiency of each group:

The voltage and electricity generation results in each experimental group are shown in Figure 4. The graph shows that when the salinity concentration is constant, the electricity-generating ability of the MFC decreases when the phenol concentration increases. Moreover, in all three groups with different phenol concentrations, the highest voltage appeared during the first 4 hours. In the 100 mg/L group, the highest voltage was 56.7 mV, and the voltage during the experiment period fluctuated between 53.2 mV and 29.7 mV; for the 220 mg/L groups, the highest voltage was 27.6 mV, with the voltage fluctuating between 27.5 mV and 27.4 mV; the 300 mg/L experiment had 17.2 mV as its highest voltage and fluctuated between 10.6 mV and 4.3 mV. And after the first 4 hours, the voltage generated by all three groups was relatively constant. In contrast, the electricity generation trend in the polycarbonate brine experimental group decreased at first, which might be because the high pH environment in polycarbonate brine damages the bacteria. Therefore, the voltage of the cell increased after the first 8 hours, and the highest voltage appeared during the last 4 hours, which was 101.9 mV, and fluctuated between 99.0 mV and 97.1 mV. In addition, based on the voltage data and the phenol degrading data of the MFC, the result shows that the electricity generation ability of the cell has a positive relationship with the degrading phenol percentage. The higher the degrading phenol ratio, the more electricity is generated, similar to many other MFC studies. The results are also comparable with other MFC experiments, such as research about micro-oxygen bioanode in mix-cultured MFCs[20] and research on phenol-degrading anode biofilm with high coulombic efficiency in graphite electrodes microbial fuel cell.[21] In both of those studies, the voltage generated by the MFC first increased and then decreased at the end of the degrading process. But the result shown by this research is different from theirs, which may be due to the high concentration of phenol inhibiting the activity of the bacteria,[9] and this causes the bacteria to degrade with a low ratio continually. Also, the result of the polycarbonate brine degrading group may be because the low pH damaged the bacteria and caused it to need more than three days to finish degrading. In conclusion, the electricity generation ability of *Staphylococcus caprae* strain SHBCC D10058 ATCC 35538 is weak in a high phenol concentration environment and relatively robust in a low phenol concentration environment. The experimental result has a certain practical value in applications, such as degrading polycarbonate brine.

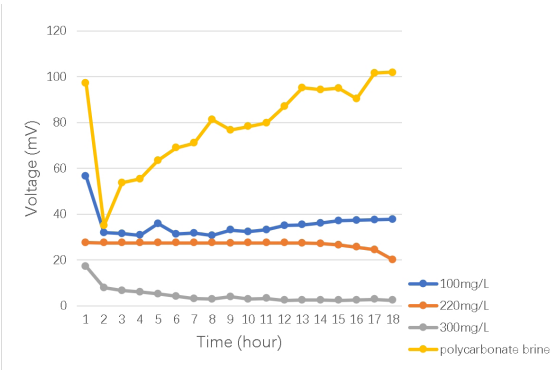

Figure 4: Voltage-Time graph of each experiment group.

The power density and current density of each group:

The result of power density and current density, which are two related values, is shown in Figures 5 and 6. The maximum power density of the experiment groups is 4.132 mW/m^2 in the 100 mg/L group, 0.982 mW/m^2 in the 220 mg/L group, and 0.382 mW/m^2 in the 300 mg/L group. Regarding the

maximum current density, the results are 72.897 mA/m^2 in the 100 mg/L group, 35.539 mA/m^2 in the 220 mg/L group, and 21.168 mA/m^2 in the 300 mg/L group. Based on the data, the trend of both power density and current density is the same for the reason that they are both calculated from the voltage data and the electrodes they used are the same, so identical with the voltage, they are high in first 4 hours and then keep constant. In the polycarbonate brine experimental group, because there is only 250 cm^3 of polycarbonate brine in the anode chamber, the functional surface area of the electrode in this experimental group was smaller than in others. And the maximum power output in this group was 30.070 mW/m^2, and the maximum current density was 294.971 mA/m^2. It is the highest power density in the experiment compared with the other three groups. Moreover, this result also showed that *Staphylococcus caprae* strain SHBCC D10058 ATCC 35538 has stronger electricity generation ability in an environment with low phenol concentration than in an environment with high phenol concentration. In addition, comparing this power density and current density data with many other experiments, the results are similar, as when compared with the research about the production of electricity from phenol using a microbial fuel cell[18], the power density values from both the three different phenol concentrations degrading groups are lower than the power density from experiment group that uses both phenol and glucose as fuel in that research, but the power density from the polycarbonate brine degrading group is higher than that; compare with the research of the effect of phenol concentrations and external resistance in a single-chamber microbial fuel cell.[17] Also, the maximum current density of three different phenol concentrations degrading groups is lower than the maximum current density in that research. The maximum current density in the polycarbonate brine degrading group is slightly higher.

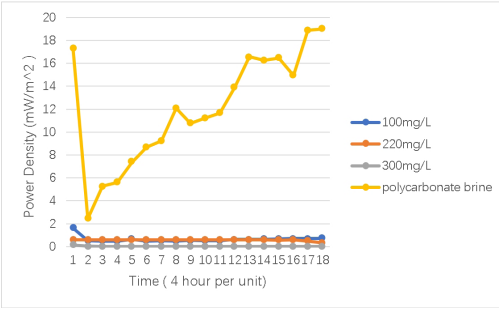

Figure 5: Power density-Time graph of each experiment group.

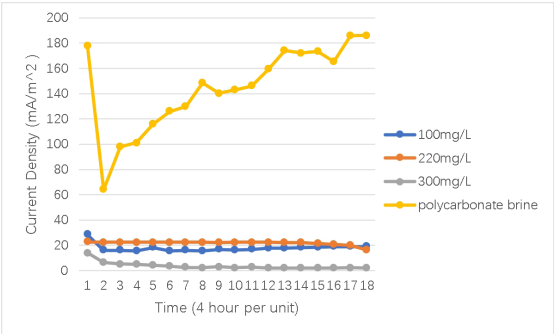

Figure 6: Current density-Time graph of each experiment group.

Conclusion

Based on the experiments above, Staphylococcus caprae strain SHBCC D10058 ATCC 35538 has specific phenol degrading abilities under high-salinity conditions in the MFC. Moreover, under the 5% salinity conditions, there is a positive relationship between the *Staphylococcus caprae* strain's degrading ability and electricity-generation ability and a negative relationship between those two and the phenol concentration. Both are high in low phenol concentrations and low in high phenol concentrations. This may be because the high concentrate phenol inhibits the activity of the bacteria and causes a low degrading ratio and electricity generation ability. Also, the experimental results showed that the *Staphylococcus caprae* strain SHBCC D10058 ATCC 35538 could survive, degrade phenol, and generate electricity in polycarbonate brine at a pH of 11.2. Still, the high pH also causes a negative effect on the bacteria and causes the electricity generated by the cell to be low at the beginning stage of degradation. Moreover, the abilities described above regarding the *Staphylococcus caprae* strain in experiments are more potent when degrading polycarbonate brine in the MFC than degrading high-concentration phenol with a fluid medium. That means the *Staphylococcus caprae* strain SHBCC D10058 ATCC 35538 might be a viable choice for degrading bacteria when using the MFC method for disposal of the sewage from polycarbonate production, which has already passed through some wastewater treatment processes.

However, *Staphylococcus caprae* could not degrade bisphenol A, which means if the MFC is used to dispose of polycarbonate brine, another salt-tolerant bacterium must also be used for the cell to degrade the bisphenol A. Because of that, further research can focus on finding other bisphenol A degrading halobacteria to work together with *Staphylococcus caprae*. Moreover, further research can also focus on reducing the experiment's limitations, such as using a new method to detect phenol concentration more accurately, using a data collector to detect and collect the cell's power, or testing a more extensive phenol concentration range.

Acknowledgments

Firstly, thanks to Beijing Royal School, which supported and funded our research.

Secondly, thanks to the tutors who provided help and guidance in our research, Ms. Zhang and Ms. Wang.

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