

GUAR GUM HYDROGEL MICROBIAL FUEL CELL USING B.MEGATERIUM

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Abstract

The main factors of a microbial fuel cell (MFC) are the anode and the microorganisms around it that produce electrons. This research focuses on increasing the efficiency of a MFC in plant-MFC (PMFC) or aquarium-like conditions by inoculating microorganisms that help produce electrons to the soil. Guar gum was used as a base structure as it can culture microorganisms and create a hydrogel by cross-linking with borax.[1,2] Bacillus megaterium was added to the hydrogel as it can survive under the basic conditions of borax while producing electrons.[3] Glucose was added as well to help culture the bacteria. Then, the change of voltage was observed along with the growth of B.megaterium. A compound consisting of 0.5% glucose, 1% Guar gum, 10% Borax, and B.megaterium culture medium was created with a volume ratio of 1:25:1:1 in each order. This compound will be named GGB-B. GGB-B was able to keep its shape as a hydrogel along with a voltage increase of 250mV compared to control due to the stable growth of B.megaterium. 100g of GGB-B was placed inside a beaker and was covered with aquarium soil. Two goldfishes (Carassius auratus) were added. Voltage, pH, and the health of goldfishes were observed for 9 days. The result was approximately a 150mV increase in voltage compared to control (tap water). pH decrease was about 1.3, showing a pH of 6.12. There was no change in the goldfishes, and the GGB-B under the aquarium soil fused with the soil as it disintegrated. Therefore, when electron-producing microorganisms such as B.megaterium are added to guar gum, the microorganisms would slowly spread over the soil, thereby increasing the efficiency of MFC. This could also apply to PMFC and aquarium-like conditions as well.

Keywords: Guar gum hydrogel, microbial fuel cell, b.megaterium.

Introduction

A microbial fuel cell (MFC) is functioned when microorganisms culture by consuming glucose or other similar nutrients. During this process, microorganisms in the anode produce electrons [1]. Therefore, the role of microorganisms present in the anode is critical to the fuel cell, and those who have this role are mostly anaerobic [1]. These anaerobic microorganisms require a mud-like environment to culture. Since it is difficult to make MFCs under this condition, the use of aerobic microorganisms is required. Also, when a microorganism that can improve the fuel cell's efficiency is inserted into the soil, the efficiency might fall over time as the microorganism leave the soil.

Therefore, to culture a microorganism to soil, it must not leave the soil over time. To fulfill this purpose, Guar gum hydrogel was used. Guar gum reacts with Borate ions to crosslink into a hydrogel form which not only allows it to be B.megaterium's carrier [2,3] but also allows

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it to be a nutrient source for microorganisms as Guar itself is composed of polysaccharide connections of mannose and galactos [4].

B.megaterium, an aerobic microorganism which is gram positive, was selected to be cultured into the Guar hydrogel. *B.megaterium* produces acetate to release electrons at the anode [5], *B.megaterium* was chosen as it can survive under the alkali conditions of Borax which is treated to Guar and therefore can be cultured in guar gum hydrogel [1,6].

In this experiment, *B.megaterium* will be cultured in Guar gum hydrogel to find out if it could be applied to soil under water conditions such as PMFCs or Aquariums and to find out possible methods of increasing the efficiency of the MFC thereby improving the voltage.

Method

The culture of B.megaterium in Guar Gum

The efficiency of Guar gum for *B.megaterium* culture and the growth of *B.megaterium* under Guar gum + Borax ion hydrogel condition was checked. 1% Guar gum (UHV-66, Haji dossa nutralum ltd from Pakistan) was used after being sterilized inside an Autoclave at 121 °C, for 15minutes. Then, 1% Guar gum solution with volumes of each 50 μ L, 100 μ L, 150 μ L was added to 1mL NB (Nutrient Broth). *B.megaterium* (KTCT 1098) 100 μ L was inoculated and was cultured under 30°C for 2 days inside an incubator.

The cultured solution's absorbance was measured using a Spectrophotometer (UV-Vis spectrum Jenway 7305) from 630nm wavelength. Then, the effect of Borax (Duksan, Sodium tetraborate, Decahydrate), which is used when turning guar gum into hydrogel, on *B.* was checked. 100 μ L of *B.megaterium* culture medium was added to NB 1mL and 5% Borax solution of each 40 μ L and 50 μ L was added. Absorbance was measured.

Culture of B.megaterium in guar gum hydrogel

B.megaterium was successfully cultured in both 1% Guar gum solution and 5% Boax solution and therefore a hydrogel was composed using the same concentrations to culture *B.megaterium*. 1mL *B.megaterium* was added to 25 mL of 1% Guar gum solution and 5% Borax 1mL was treated to create a hydrogel. 500 μ L of this hydrogel was dropped into NA(Nutrient Agar) to confirm successful culturing under hydrogel conditions.

Then, 5% Boarx solution was added to 1% Guar gum to create a hydrogel and *B.megaterium* 1mL was inoculated to check if *B.megaterium* was cultured by spreading out.

The oxidation of B.megaterium

Production of electrons by *B.megaterium* on the anode was tested. 1% Agar and NaCl was binded inside a plastic tube to create a salt bridge. This salt bridge was connected between two conical tubes. On one side, *B.megaterium* solution was added and NB solution was added to the other. Cu, Zn electrodes were added like the picture below and voltage was measured. Then, Cu and Zn was each placed into NB solution and *B.megaterium* solution in an opposite manner to measure the voltage. Both electrodes were placed inside the *B.megaterium* solution to measure voltage.

The difference of B.megaterium growth on dependence to glucose treatment on guar gum hydrogel

When glucose is treated to guar gum hydrogel, *B.megaterium* can grow faster thereby improving the voltage. However, when 3g of Glucose was treated to 20 mL 1% Guar gum solution, the cross-link reaction did not occur. This was because Glucose reacted with some of the Borate ions bonded to Guar gum which lead to the breaking of crosslinks [7]. Therefore, glucose concentration was manipulated to find a certain concentration of glucose that could maintain a certain viscosity and voltage.

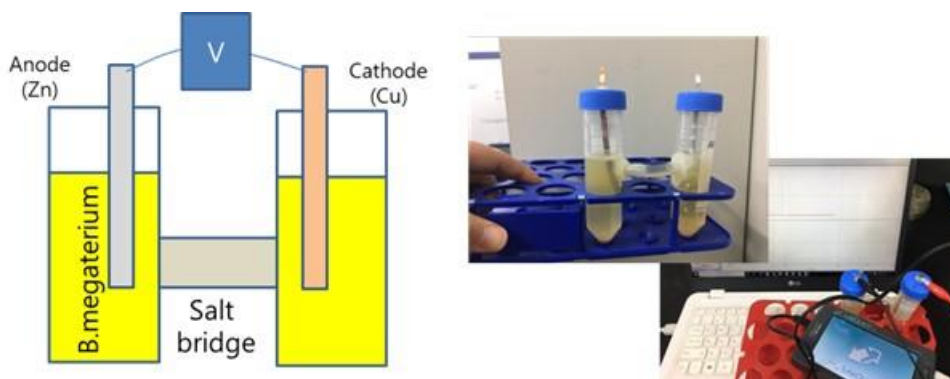


Fig. 1. Voltage measured using salt bridge

Glucose was added to 25 mL of 1% Guar gum solution to make solutions of concentrations 0%, 0.2%, 0.5%, and 1%, Then, 1mL B.megaterium was inoculated and 1 mL 5% Borax was added. The complex of Guar gum, Glucose, Borax, and B.megaterium will now be mentioned as GGB-B from now on. The complex was cultured inside an incubator for 2 days and voltage, viscosity, and pH was measured.

In this experiment, B.megaterium released acetate using glucose and guar gum was hydrolysed [8]. Therefore, the complex lost viscosity and turned into a liquid form. In order to raise the viscosity, Glucose solutions of concentrations 0%, 0.2%, 0.5%, 1% was added to Borax solutions of concentrations 5% and 10 % and the spreading of the GGB-B hydrogels were measured over 5 minutes. The voltage for each case was measured to look for the most appropriate concentration of Borax and glucose. Along to this, samples of each hydrogel was taken using loops and then streaked onto NA culture mediums to check for the growth of B.megaterium.

The variation of GGB-B voltage, pH, and viscosity depending on temperature and buffer treatment

GGB-B was created with 10% Borax solution and 0.5% solution, which showed appropriate spreading and voltage accordingly to the previous experiment. Then, to prevent GGB-B's pH turning acidic, buffer was suggested to be treated. The buffer was created by mixing 0.2 M $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (Duksan) and 0.2M. $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (Sigma) to create solutions each of pH 7, and 8. Then, 1mL each of pH 7 and pH 8 solution was added to GGB-B. The complex was cultured under temperatures of 37°C and room temperature (about 20°C). pH and voltage was measured.

Appliance to aquariums

The possibility of applying GGB-B to aquarium-like conditions was confirmed.

The ratio of GGB-B was 1% Guar gum: 0.5% Glucose: 10% Borax: B.megaterium culture medium = 25:1:1:1 and a total of 100mL was created. The created 100g GGB-B was placed into a beaker and aquarium soil (Neo soil compact, Aquario) 200g was added on it. Finally, two goldfishes and 1L of water was added with an oxygen supply. Voltage, pH, COD, DO, and the survival of goldfishes were checked from day 1 to day 9.

To prevent water contact with the Zn anode and only allow contact with the GGB-B containing soil, the anode was placed inside a conical tube and the anode was only exposed for the height of the soil.

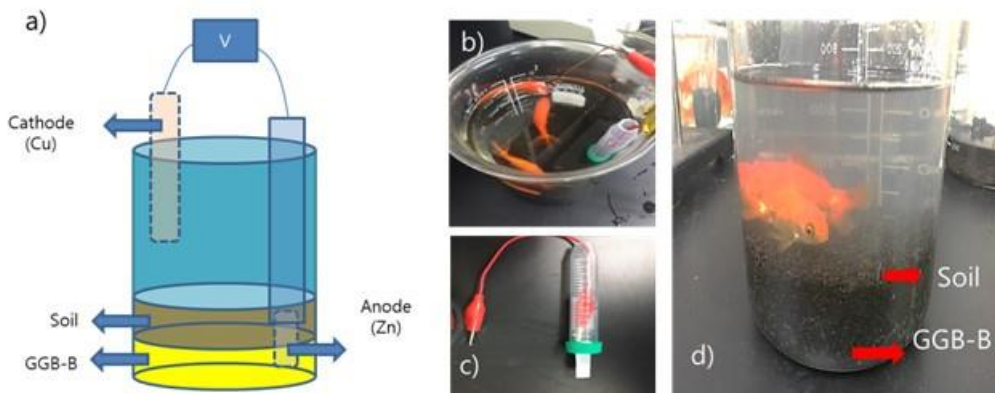


Fig. 2. GGB-B and goldfish treatment a) connection between GGB-B and anode, b) goldfish observation; c) Anode used in experiment d) after three days

Results and Discussion

The change of B.megaterium under Guar gum and borax conditions

Guar gum’s role as a nutrient for B.megaterium growth was checked, as well as B.megaterium’s survival under alkali Borax. As a result, the increase of guar amount by 50 μL resulted in B.megaterium’s absorbance increase. At 150 μL of guar, the absorbance increased by 5 times compared to control

When borax’s influence on B.megaterium growth was checked, there was only minimal change in absorbance depending on borax’s presence.

Therefore, a conclusion that B.megaterium can be cultured under guar gum Borax hydrogel condition was derived.

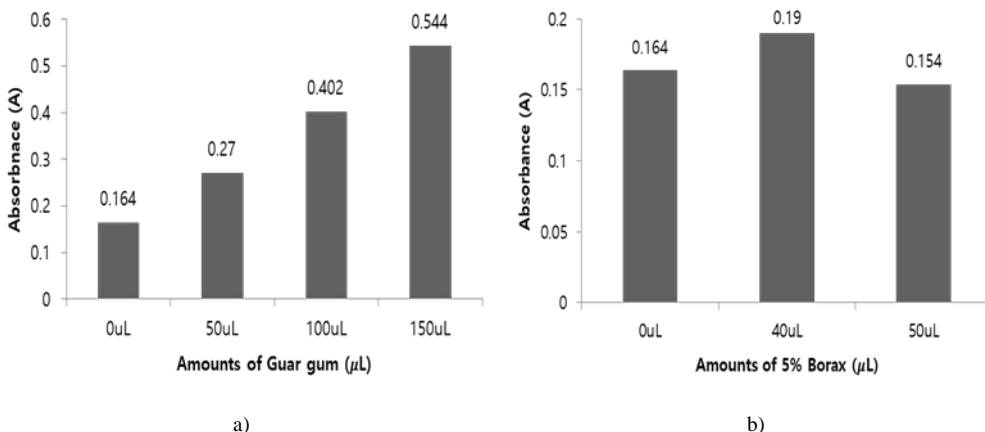


Fig. 3. Absorbance of: a) B.Megaterium in Guar gum; b) B.megaterium in Borax

The change of B.megaterium under Guar gum – Borax hydrogel conditions

It was checked if B.megaterium could be cultured when a hydrogel was created by adding B.megaterium to Guar gum and Borax was mixed. First, when a hydrogel was created with guar gum and borax and B.megaterium was inoculated, the spreading of bacteria was observed as in Fig. a). Also, when guar gum, B.megaterium, and Borax was mixed and treated on NA culture medium, it could be observed that B.megaterium grew around the speraded region. Therefore, the culture of B.megaterium by mixing guar gum and borax was proved to be possible.

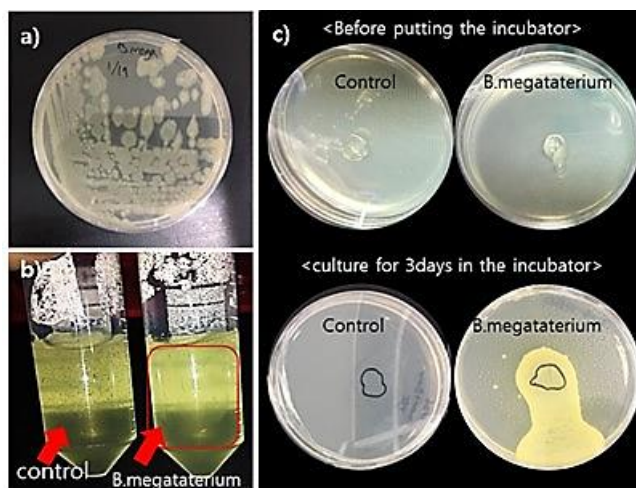


Fig. 4. Growth of *B. megaterium* a) *B.megaterium* colony; b) *B.megaterium* in Guar hydrogel; c) *B.megaterium* cultured in Guar hydrogel

The voltage change due to oxidation of B.megaterium

To check if *B.megaterium* produces electrons, the Zn electrode was added to *B.megaterium*, and the Cu electrode was added to NB and voltage was measured. As a result, placing Zn electrode in *B.megaterium* and Cu electrode in NB had a higher voltage compared to placing Zn electrode into NB and Cu electrode into *B.megaterium* and placing Zn and Cu electrodes both into *B. Megaterium* each by 0.059V and 0.022V. This result reconfirms that placing the Zn anode in *B. megaterium* causes a rise in voltage.

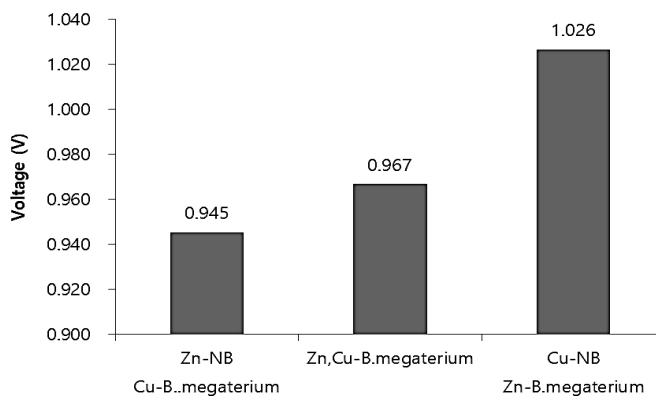


Fig. 5. The change of voltage of the solution with Cu and Zn electrodes added

The change of Guar hydrogel depending on glucose concentration

1mL of 5% Borax and 1mL of *B.megaterium* was added to 25mL 1% Guar gum and Glucose was added each with concentration 0.2% ~ 1%. After 2 days of culturing, the viscosity, pH, and voltage of each GGB-B, a mixture of Guar gum, Glucose, Borax and *B.megaterium*, was measured. The viscosity of GGB-B which was placed inside the incubator was lost. This is because *B.megaterium* releases acetate using glucose which results in the hydrolysis of guar gum, which thereby weakens the crosslink bond between guar gum and borax. The voltage of GGB-B from the incubator increased as the concentration of glucose increased, but pH decreased as glucose concentration increased.

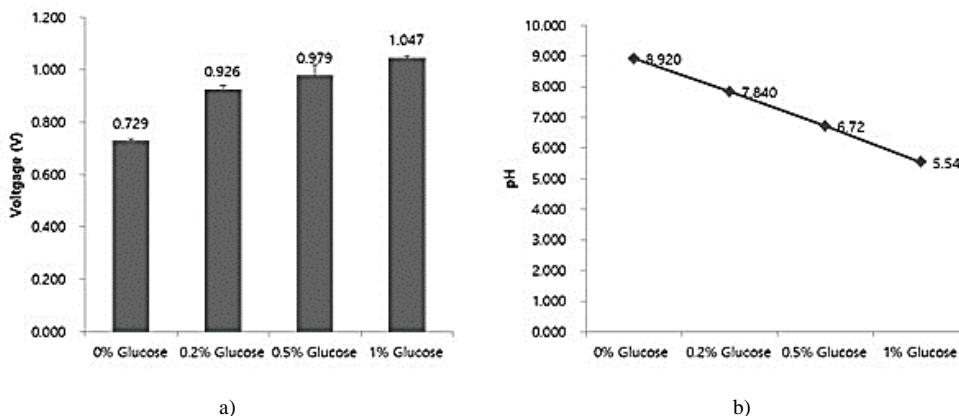


Fig. 6. a) voltage change and b) pH change caused by change of glucose concentration

However, the increase of glucose concentration could result in the weakening of GGB-B crosslink bonds so an experiment was done by trying two Borax concentrations of 5% and 10%. When viscosity was measured by observing the spread of GGB-B on a petri dish over 5 minutes, the GGB-Bs that kept their original shape best were GGB-B composed of 10% Borax and 0.2% Glucose and GGB-B composed of 10% Borax and 0.5% Glucose.

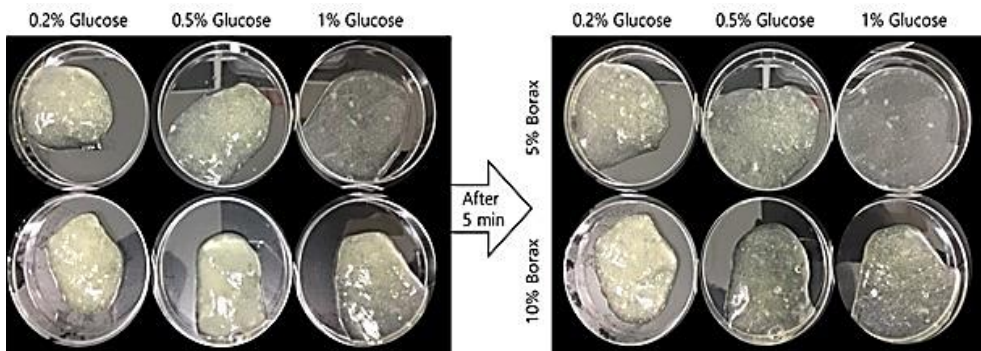


Fig. 7. Change of viscosity of Guar gum hydrogel by concentrations of glucose and Borax

When voltage was measured for these GGB-Bs, the voltage was higher 1% Glucose compared to 0.2% and 0.5% Glucose, and 1% Glucose and 5% Borax mixture had higher voltage compared to 1% Glucose and 10% Borax. However, in overall, the voltage did not show great variance.

The change of voltage, pH, and shape of GGB-B depending on temperature and buffer conditions

When *B.megaerium* was cultured at an excessive speed, the viscosity of GGB-B decreased, making it harder to treat inside soil. Therefore, buffer was added to control the pH of GGB-B, and the shape, voltage, and pH of GGB-B was observed under temperatures of 20°C and 37°C.

When the shape was observed 3 days later, GGB-B which were under 0.2% Glucose and 0.5% Glucose + pH 8 buffer conditions which were placed in 37°C, the GGB-B bond was not maintained and it could not be taken out of their conical tubes, like the picture below. The other two GGB-Bs in 37°C did not maintain their shape. The GGB-Bs placed in 20°C kept their shape apart from the GGB which was composed of 0.5% Glucose. What is peculiar about

the 0.5% Glucose GGB-B is that every GGB-B apart from this 0.5% Glucose GGB-B released water. The reason seemed to be buffer causing more bonds within GGB-B which released water inside.

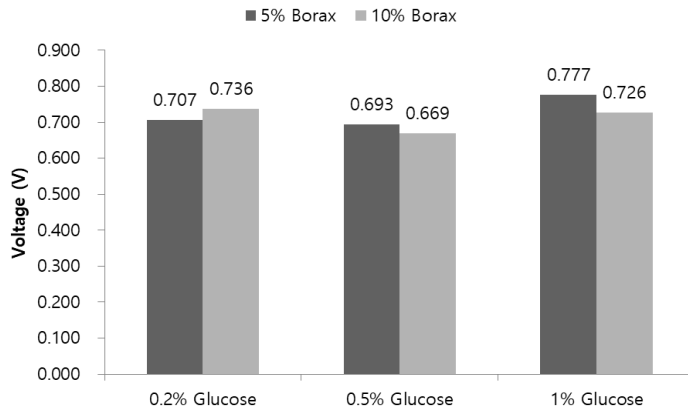


Fig. 8. Hydrogel voltage change by glucose and Borax concentration

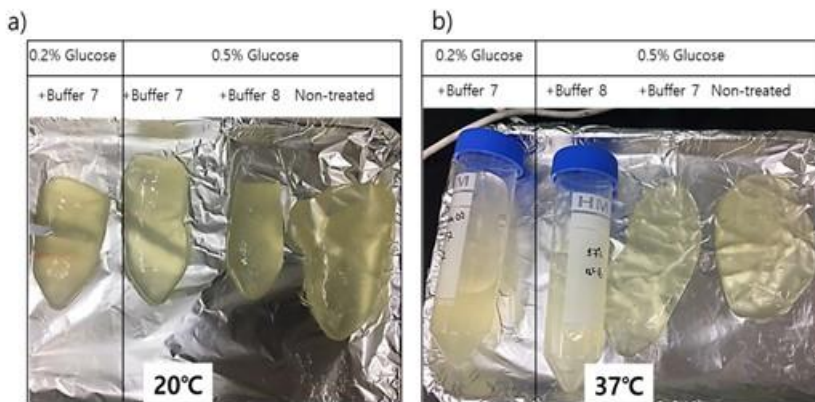


Fig. 9. Change of GGB-B: a) 20 °C condition b) 37 °C condition

The change of GGB-B was observed under water sediment conditions. GGB-B was cultured for 3 days under 20°C and placed inside a conical tube with 20mL water and the amount of water absorbed by GGB-B and shape of GGB-B was observed. As a result, 0.5% Glucose GGB-B did not absorb water but on other three conditions, GGB-B absorbed 3mL ~ 3.5mL of water. It seems that the water released by the buffer was absorbed back again.

The voltage change of GGB-B was highest when cultured under 37°C for 5 days. The second highest was when cultured under 20°C for 5 days, and 0.5% Glucose GGB-B was measured higher compared to other conditions.

pH was opposite to voltage; it was lowest when GGB-B was cultured under 37°C for 5 days, and highest when cultured under 20°C for 3 and 5 days.

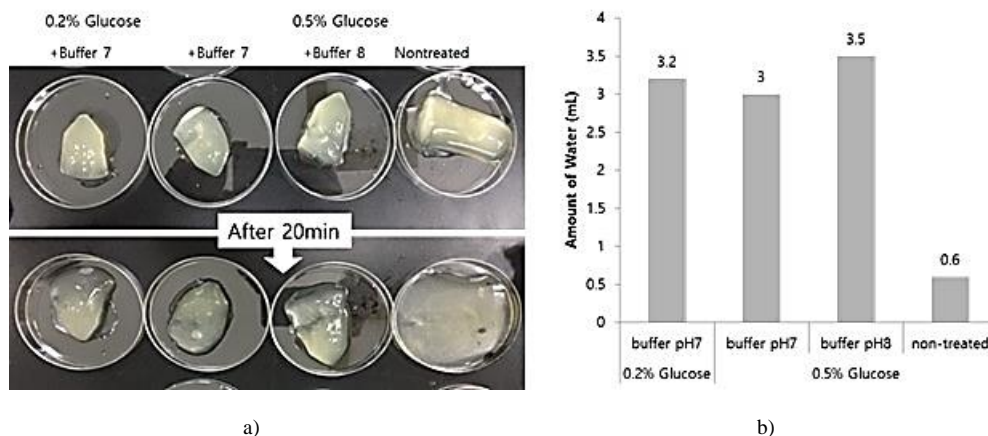


Fig. 10. Buffer-treated GGB-B: a) viscosity change; b) water absorbance

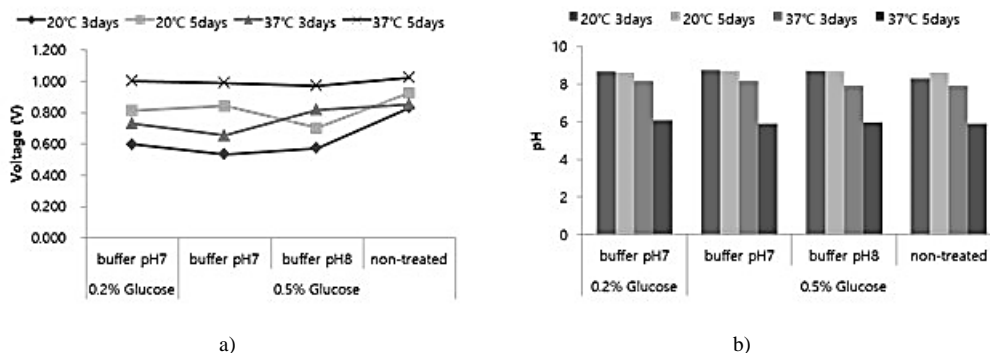


Fig. 11. GGB-B by different buffer and temperature conditions: a) voltage change; b) pH change

Therefore, GGB-B that maintained its shape along with high voltage, high pH and water maintenance was 0.5% Glucose GGB-B without buffer under 20°C. In aquarium conditions, the water temperature does not exceed 20°C, so following experiments were done under this condition.

The changes of voltage, water quality, and goldfishes in a GGB-B treated aquarium

After observing the beaker for 9 days after treating GGB-B, the dissolved GGB-B under the aquarium soil left the soil and made the water opaque. However, there was no special change in the goldfishes.

The DO of water quality was similar to the control due to the use of oxygen supply by 8~10ppm, and COD was higher than control by 4ppm as 10ppm due to GGB-B dissolving under aquarium soil.

The voltage increased constantly in the beaker treated with GGB-B and goldfishes from 1.007V to 1.101V after 9 days, and when compared to control, there was a difference of 0.097V~0.191V as shown in fig 12. The beaker with only GGB-B showed no voltage change until day 2, but voltage increased after 7 days and the voltage change was similar to that of with goldfish.

While the control had a pH of 7.42~7.62, the beaker with GGB-B's pH turned weak acid, and GGB-B with goldfish had a slightly lower pH. The water quality pH change seems to be due to *B.megaterium* releasing acetate by dissolving Glucose and Guar gum, and this acetate hydrolysing Guar gum, thereby accelerating the dissolving of GGB-B.

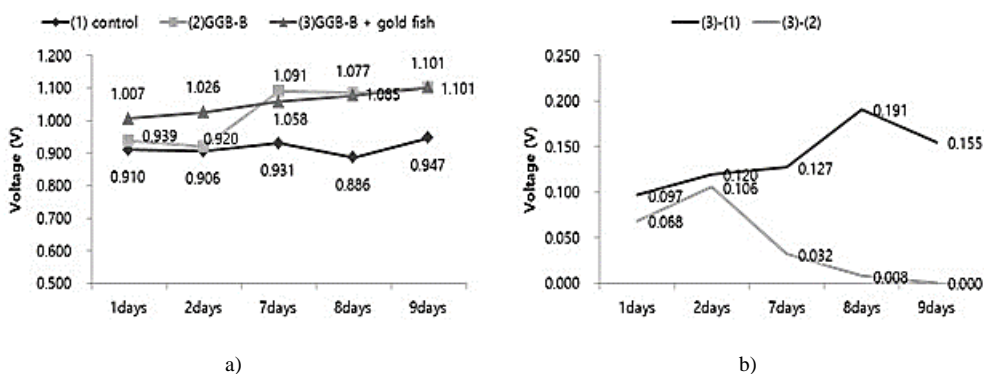


Fig. 12. Voltage change: a) GGB-B and GGB-B+goldfish; b) control sample

Table 1. pH change in GGB-B and GGB-B + gold fish

	(1) control	(2) GGB-B	(3) GGB-B + gold fish
1 days	7.62	6.93	5.98
2 days	7.57	6.54	5.88
7 days	7.52	6.83	6.07
8 days	7.47	6.68	6.14
9 days	7.42	6.54	6.57

Overall, when GGB-B is treated to soil, the Glucose and B.megaterium consisting the guar hydrogel causes an increase in voltage and the production of acetate can dissolve guar, which then spreads the voltage-increasing microorganism widely around the sediment, increasing the efficiency of microbial fuel cells such as PMFC.

Conclusion

Guar gum and hydrogel was utilized as a way to increase efficiency of microbial fuel cells in soils of PMFC or Aquarium conditions by adding bacteria. When 1% guar gum solution was added to B.megaterium, the absorbance increased, and the bacteria was well-cultured in 5% borax solution which is crosslinked to Guar gum in hydrogel state as well. When B.megaterium was inoculated into Guar gum hydrogel, B.megaterium was successfully cultured around the hydrogel again.

To help the culture of B.megaterium, Glucose was added to Guar gum to create a GGB-B (Guar gum+Glucose +Borax+B.megaterium) complex. Glucose can react with borate ions which are cross linked to Guar gum and therefore can make the bonds inside the hydrogel softer. However, under high concentrations, the bonds can be broken completely. Also, when GGB-B was incubated under 20°C and 37°C, GGB-B under 37°C did not maintain its shape as a solid. This is because the acetate released by B.megaterium hydrolyses guar gum. Under 20°C, B.megaterium was grown at a slower speed, which helped the hydrogel maintain its shape.

Therefore using the conditions where the shape was maintained with an appropriate level of voltage of 0.975 V, (1% Guar gum 25mL, 0.5% Glucose 1mL, Borax 1mL, B.megaterium 1mL) GGB-B was created and then treated to aquarium soil. After measuring the voltage for 9 days, compared to 2 days, the voltage increased by about 0.094V, and 0.097V ~ 0.191V compared to control. pH was slightly acidic compared to control, and GGB-B slowly dissolved causing the water to be less transparent.

In conclusion, microorganisms that increase battery efficiency can be treated to soil under water using GGB-B, and the dissolving of GGB-B can help the spread of microorganisms over

to soil itself. By manipulating the concentration of Guar gum and Glucose, the dissolving rate of GGB-B is also manipulatable which can help inoculating microorganisms to the soil at a certain rate, which can be applied to PMFC or sediments in aquarium conditions.

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