



An “Artificial Leaf” that can Generate Oxygen and Electricity

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Abstract

Oxygen is essential for all lives on earth. It generally takes too long for seedlings to mature enough to produce significant oxygen through photosynthesis. Meanwhile, portable electricity is crucial for everyday human activities, but chemical batteries can be environmentally unfriendly. To mediate both problems simultaneously, we proposed to develop an artificial leaf using chlorella that can rapidly produce oxygen and electricity at a low cost. We used chlorella, a type of single-cell algae, as the photosynthetic element in the artificial leaf. Our experiments showed that 2.0% sodium alginate and 5.0% barium chloride hydrogel had the best water retention and air permeability properties, making it most suitable as the substrate for the artificial leaf. When chlorella was combined with this substrate, the oxygen output reached 10.87 ± 0.56 mg/L, 98.9% of the maximum production after six hours. The recorded maximum voltage of one piece of the artificial leaf containing copper and magnesium metal conductors was 1.285 ± 0.13 volts. This study demonstrated the feasibility of producing a low-cost chlorella-based artificial leaf that generated electricity comparable to an AA battery, suggesting its potential to power a simple device.

Introduction

Oxygen is essential for respiratory reaction, energy production, and the survival of humans and animals. Through photosynthesis, plants generate oxygen from carbon dioxide, providing humans and animals with oxygen and greening the environment. However, it takes a significant amount of time for a seed to mature into a tree that can produce a meaningful amount of oxygen. On the other hand, microalgae such as *Chlorella sorokiniana*, a species of freshwater, single-cellular microalgae, can proliferate and thrive in simple environments. The chlorophyll-rich microalgae can perform photosynthesis requiring only CO₂, sunlight, water, and trace minerals.¹ This property allows microalgae to capture CO₂ and produce O₂. In fact, microalgae generate over 75.0% of oxygen needed for animals and humans on a global scale.²

In addition to oxygen, electricity is also an essential part of modern life. However, chemical batteries can be environmentally unfriendly. Therefore, we began considering using artificial means to create a substitute that could produce electricity simultaneously.³ In addition to generating comparable oxygen, it is known that most algae are capable of photosynthesis and can create electric currents during the process.⁴ By combining algae with the concept of an artificial leaf, we can green the environment and turn CO₂ into oxygen. Purifying air quality while generating power will enable us to fulfill the vision of a green and pollution-free lifestyle. In this study, the chlorella was used to create an artificial leaf to investigate its potential to generate oxygen and electricity.

Methods

1. *Cultivation of chlorella for artificial leaf*

1.1 *Chlorella Cultivation*

Chlorella sorokiniana was cultivated in a solution supplemented with 2.7% commercial fertilizer (Hyponex No. 4), formulated at 25.0% total nitrogen, where 4.5% was nitrate nitrogen, 5.0% phosphoric anhydride, and 20.0% potassium oxide. The same dose of fertilizer was added every three days. Other cultivation conditions were: 1) light intensity of 100 µmol photons m⁻² s⁻¹, 2) 12 hours of light-dark cycles, and 3) temperature at 25°C. The solution was continuously supplemented with air by an air pump and air stone and stirred on an agitator. Water pH was measured, and algae watercolor was observed

daily. If the pH value was too acidic, air pumping time was increased to eliminate the accumulated nitrogen. If the value was too basic, phosphates were added until the pH value was neutralized. The absorbance spectrum and optical density of the chlorella culture medium were measured every 20 nm from 400 nm to 700 nm with a spectrophotometer to determine the peak value. The final chlorella culture for artificial leaf assembly was harvested with the determined absorbance peak. (Thermo Scientific GENESYS 20 Spectrophotometer).

1.2 Bacteria collection and bacteriostatic agent selection

Environmental bacteria were collected from the bottom of a shoe with a sterile wet cotton swab. The swab was then smeared on an LB agar plate. The plate was incubated at 37°C for 16 hours. A single bacterial colony was selected and transferred to 5 mL of LB broth. It was incubated at 37°C for 12-16 hours with continuous shaking at 200 rpm until the liquid culture reached 0.3 to 0.4 absorbance unit at OD600 (Thermo Scientific GENESYS 20 Spectrophotometer).

Fifteen mL of 0.5% soft agar LB medium at 40°C was prepared with a mixture of 200 µL of the bacteria culture. The unsolidified LB agar medium contained the collected bacteria and was poured on top of hard LB agar plates to form the doubled-layered bacteria plate for the testing of different bacteriostatic agents. Boric acid, tannic acid, and chitosan solutions were individually prepared at 0.0%, 0.625%, 1.25%, 2.5%, and 5.0% in pure sterilized water. Each of the three double-layer bacteria plates was drilled with five holes and subsequently filled with either 150 µL of boric acid, tannic acid, or chitosan solutions. Each hole contained one given concentration listed above.

All the doubled-layered bacteria plates were incubated at 37°C overnight. The bacteriostatic ring size was characterized

2. Determining the optimal concentrations of sodium alginate and divalent ions for the artificial leaf substrate

2.1 Artificial leaf gel water retention test

Sodium alginate solutions at 0.5%, 1.0%, 1.5%, and 2.0% were added with 5.0% calcium chloride and barium chloride aqueous solution respectively. The solidified gel

was collected after 10 minutes. Each gel was placed indoors at a well-ventilated location and weighted once every 12 hours to assess the water retention effect for a total of 48 hours.

2.2 Artificial leaf gel air permeability test

Each gel was prepared as described in 2.1. The solidified gel was placed in an incubator at 60°C to dry till the weight remained consistent with a thin-film appearance. After drying, the thin-film gel was placed in a 50 mL sterile centrifuge tube and topped off with doubled-distilled water. The centrifuge tubes were sealed with transparent tape. The sodium alginate gel was weighed once every 12 hours to observe the air permeability effect of the gel for a total of 48 hours.

3. Determining the oxygen production capability of the chlorella artificial leaf

3.1 Artificial leaf model fabrication

One hundred grams of model powder was weighed and mixed with 125 mL of water in a plastic tub to form the molding paste. A piece of leaf was pressed into the molding paste. After the paste solidified, the leaf was removed.

3.2 Artificial leaf fabrication

Fifty milliliters of 2.0% sodium alginate solution and 15 mL of chlorella solution at the concentration of 3×10^8 cells/mL were mixed well and poured into the leaf mold. Calcium chloride and barium chloride solutions at 5.0% concentration were sprayed on the solution of the sodium alginate and chlorella mixture, respectively. The gel was removed from the mold after gel formation.

3.3 Artificial leaf oxygen measurement

The artificial leaf was placed in 500 mL of pure water for the dissolved oxygen to be measured by a dissolved oxygen meter daily.

4. Determining the voltage generated in the artificial leaf medium

Metal plates in the following combinations, Zinc/ Copper, Zinc/ Aluminum, Zinc/ Magnesium, Copper/ Aluminum, Copper/ Magnesium, and Aluminum/ Magnesium, were placed in the chlorella/ sodium alginate solution at the concentration of 3×10^8 cells/mL to

determine the voltage generation at room temperature. Two metal plates were placed in the sodium alginate solution with enough length of wire retained. The wires were connected to a multi-purpose meter to determine the voltage generation.

After determining the metal plates that generated the highest voltage, the two plates were placed in 15 mL of chlorella/sodium alginate solution at the concentration of 3×10^8 cells/mL, and 5.0% barium chloride aqueous solution was sprayed into the solution for gel formation. The hardened gel was removed from the leaf mold. The voltage generated in the leaf was then measured with a multi-purpose meter.

Results and Discussion

1. Determining the absorbance peak of growing chlorella culture

The absorption spectrum of a given substance is the function of the wavelength of incident light. The composition of the substance determines the location of the absorption peak.⁵ The absorption spectrum of the chlorella culture medium was measured at 20 nm increments from 400 nm to 700 nm. The ocular density was recorded.

(A)

Table 1. Chlorella Concentration Spectrophotometer Test

Wavelengths (nm)	400	420	440	460	480	500	520	540
OD value	0.312	0.296	0.292	0.306	0.284	0.280	0.274	0.256
Wavelengths (nm)	560	580	600	620	640	660	680	700
OD value	0.239	0.224	0.212	0.200	0.186	0.180	0.190	0.189

(B)

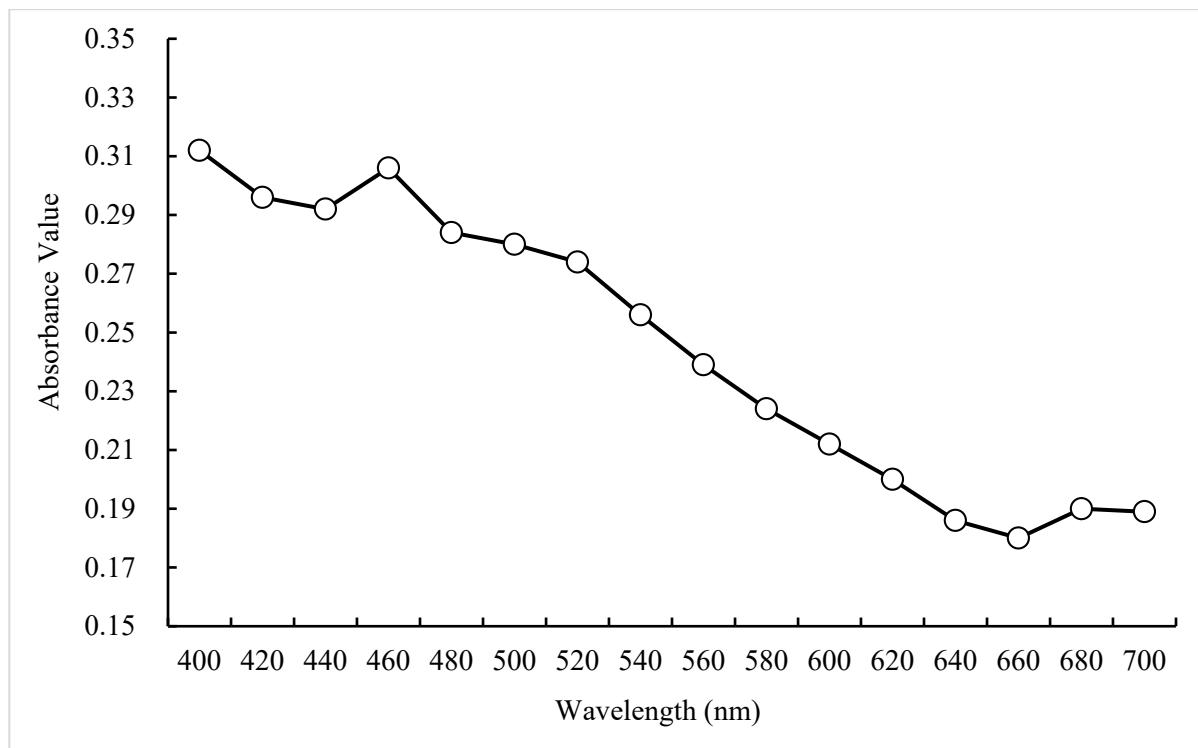


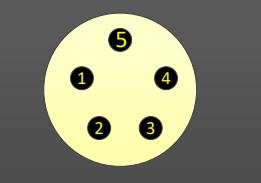
Figure 1: The absorbance spectrum chlorella culture medium. **(A)** The measured ocular density at each incremental wavelength. **(B)** Plot graph of the measured ocular density.

The experimental results indicated that the maximum absorbance was 0.312 at the wavelength of 400 nm, which was used in the subsequent experiments to measure the growth conditions of chlorella. All chlorella used to form the artificial leaves were harvested at the determined density.

2. *Selecting bacteriostatic agent as the preservative for artificial leaf*

Boric acid, tannic acid, and chitosan solutions were individually prepared at 0.0%, 0.625%, 1.25%, 2.5%, and 5.0% in pure sterilized water and were added onto the surface layer of the double-layer bacteria plate. After overnight incubation, the bacteriostatic zone size was measured. This experiment was performed in triplicate. (Bacteriostatic ring diameter: cm)

(A)

Schematic illustration	Boric acid bacteriostasis	Tannic acid bacteriostasis	Chitosan bacteriostasis
			

*The photos are author's original images.

(B)

Table 2. Bacteriostatic Ring Size of Boric Acid, Tannic Acid, Chitosan

Solution Concentration (%) (Zone number on the plate)	Bacteriostatic Ring (Diameter: cm)		
	Boric Acid	Tannic Acid	Chitosan
0.000 (1)	0.0	0.0	0.0
0.625 (2)	0.9	0.0	0.0
1.250 (3)	1.2	0.8	0.0
2.500 (4)	1.5	0.9	0.0
5.000 (5)	2.1	1.2	0.0

(C)

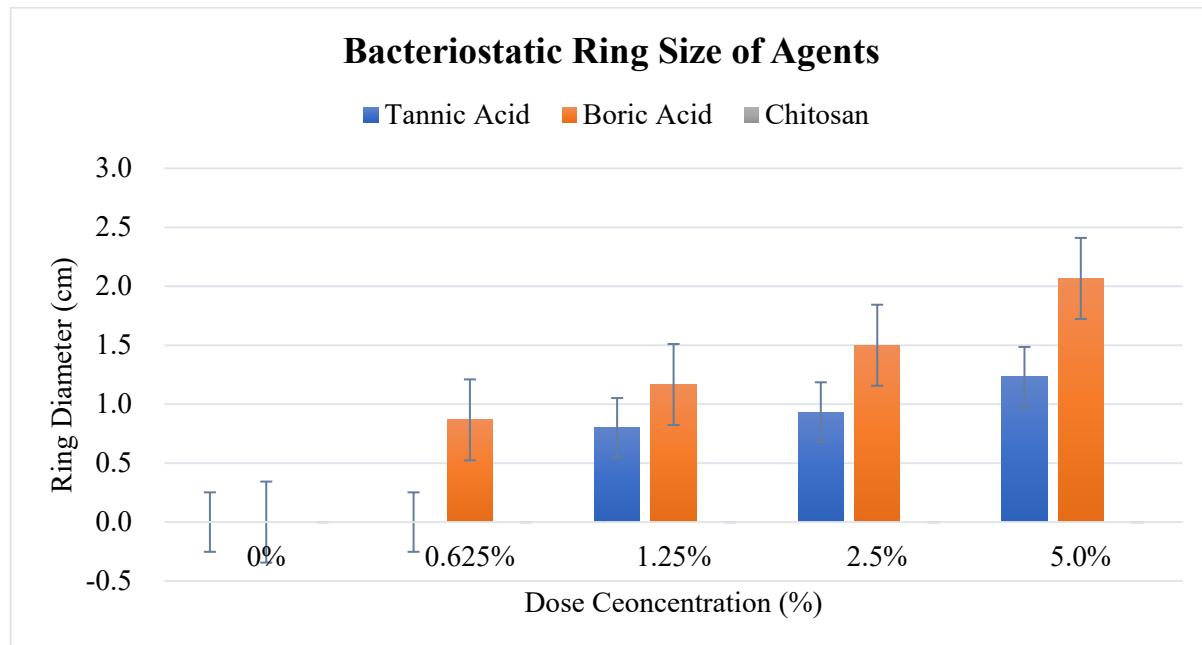


Figure 2: Bacteriostatic Effect of boric acid, tannic acid and chitosan. (A) Photo of each of the tested plates. (B) The measured bacteriostatic zone size of each tested agent in one representative experiment. (C) Bar graph of the bacteriostatic zone size of boric acid treated plates, N = 3.

The artificial leaf designed in this study was made of totally natural materials and could retain moisture to a certain degree. This medium is likely to harbor bacteria and mildew. Therefore, three different natural bacteriostatic and fungal static agents were first tested for their suitability to be incorporated into the artificial leaf medium. In brief, tannic acid is a common chemical substance in the plant world and is related to plants' growth and pest resistance.⁶ Boric acid is widely present in nature, and seawater contains boric acid. Boric acid can be used for disinfection or the treatment of minor cuts and burns and has been shown to have antibacterial properties.⁷ Chitosan is composed of chitin deacetylated (CH_3CO^-), which is optimal for the inhibitions of *Staphylococcus aureus* and *Salmonella* with the effect of requiring only 0.5 mg/ml to inhibit 50% of growth.⁸⁻⁹ For *Streptococcus*, a concentration of 5.0 mg/ml is required to exhibit the inhibitory effect.¹⁰

Chitosan did not show to have any bacteriostatic effects. Tannic acid's bacteriostatic effect was inferior to boric acid at all tested concentrations (Figure 2 A, B). Boric acid at 2.5% was the lowest concentration that was able to afford an evident bacteriostatic ring (Figure 2 A, B, C). Therefore, this concentration was used as the concentration of the bacteriostatic agent to be added to the boric acid aqueous solution to be prepared for the subsequent experiments.

3. Determining the optimal concentrations of sodium alginate and divalent ions for the artificial leaf substrate

Different sodium alginate concentrations were combined with 5.0% calcium chloride or 5.0% barium chloride to determine the optimal water retention and air permeation condition for chlorella growth. The experiment was conducted at 20°C and under 70.0% humidity. Each experimental group was conducted in triplicate.

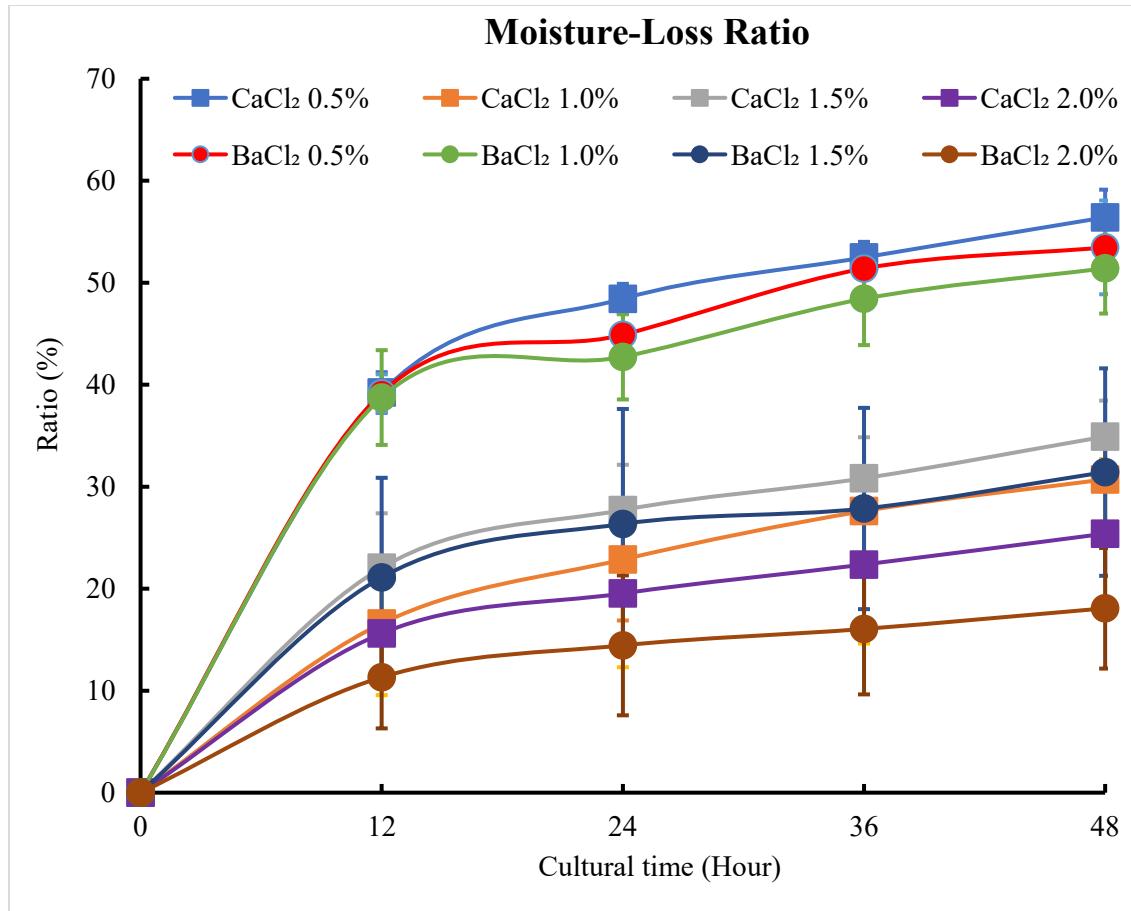


Figure 3: Moisture-loss ratio graph of different sodium alginate hydrogels made with varying concentrations of sodium alginate and 5.0% calcium chloride or 5.0% barium chloride, N = 3.

The water retention experiment revealed that the moisture-loss ratio of sodium alginate gel was negatively correlated with the sodium alginate concentration (Figure 3). Further, there was no difference in the moisture-loss ratio, whether it was divalent calcium chloride or barium chloride. After the cultivation of 48 hours, the water diffusion inhibition rates reached $25.40 \pm 7.31\%$ and $18.08 \pm 5.91\%$, respectively. The groups with the lowest water retention were the calcium and barium chloride groups added with 0.5% sodium alginate. After the cultivation of 48 hours, the water diffusion inhibition rates reached $56.41 \pm 2.72\%$ and $53.46 \pm 4.60\%$, respectively.

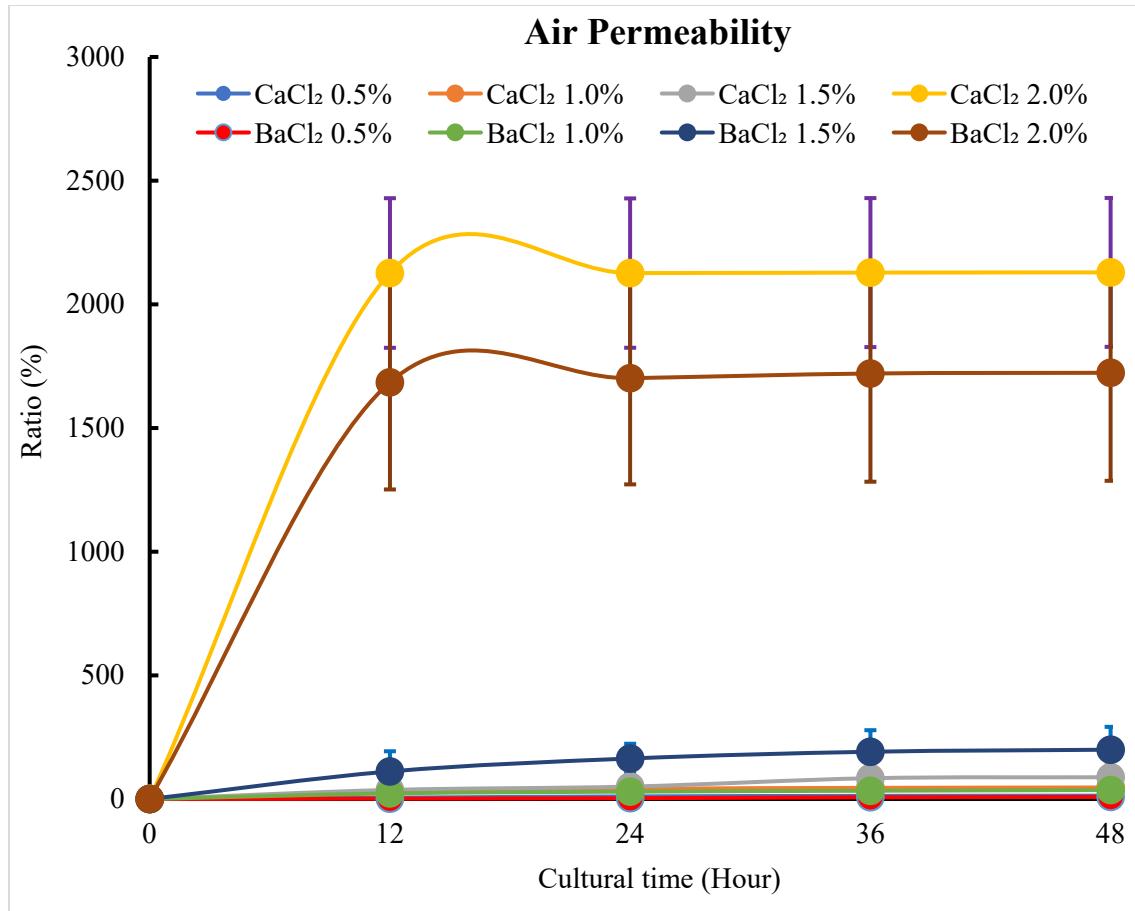


Figure 4: Air permeability graph of different sodium alginate hydrogels made with varying concentrations of sodium alginate and 5.0% calcium chloride or 5.0% barium chloride, N = 3.

Sodium alginate at 2.0% in combination with calcium chloride and barium chloride groups showed the best effect with a similar trend of air permeability. The air permeability ratio reached its peak after 12 hours and slowly declined afterward (Figure 4). The calcium chloride group showed the highest air permeability ratio reaching $2129.1 \pm 300.96\%$ at 48 hours of the experiment, followed by the barium chloride group reaching $1723.12 \pm 436.72\%$ at 48 hours of the experiment. Sodium alginate at concentrations below 2.0% had very low permeability ratios. For instance, the barium chloride group added with 0.5% sodium alginate showed an air permeability ratio of only $8.42 \pm 5.59\%$.

Sodium alginate ($\text{C}_6\text{H}_7\text{O}_6\text{Na}$)_n is a polysaccharide-based biological polymer from the marine organism brown algae and is formed from the sodium salts of alginic acid.¹¹ When sodium alginate encounters divalent cations such as Ca^{2+} and Ba^{2+} , the divalent

cations will replace the monovalent sodium ion (Na^+) and seize the intermolecular carboxylate ion (COO^-) for the sodium alginate solution to transform from the solution state into the durable as well as elastic calcium alginate gel state.¹² Sodium alginate hydrogel can hold more than 98.0% of water, allowing it to provide an aqueous environment for chlorella growth.¹³

Lower concentrations of calcium and barium chloride were tested during the initial optimization stage to determine the appropriate rigidity of the gel when combined with sodium alginate. We observed that bivalent ions at 5% provided the best texture for casting the gel substrate with sodium alginate. The combined result from moisture loss and air permeability tests showed sodium alginate at 2.0% was the most optimal concentration as the base media for the artificial leaf. Since hydrogel with 2.0% sodium alginate and 5.0% barium chloride performed better in retaining water, and 2.0% sodium alginate and 5.0% calcium chloride performed better in air permeability. The oxygen production capability test would determine the final composition of the artificial leaf substrate.

4. Determining the oxygen production capability of the chlorella artificial leaf

The artificial leaf substrate was cast using 2.0% sodium alginate and divalent ionic (5.0% calcium chloride and 5.0% barium chloride) compounds. The oxygen production capability of chlorella in artificial leaf substrate was measured afterward. The experiment was performed at 22°C, with each experimental group performed in triplicate.

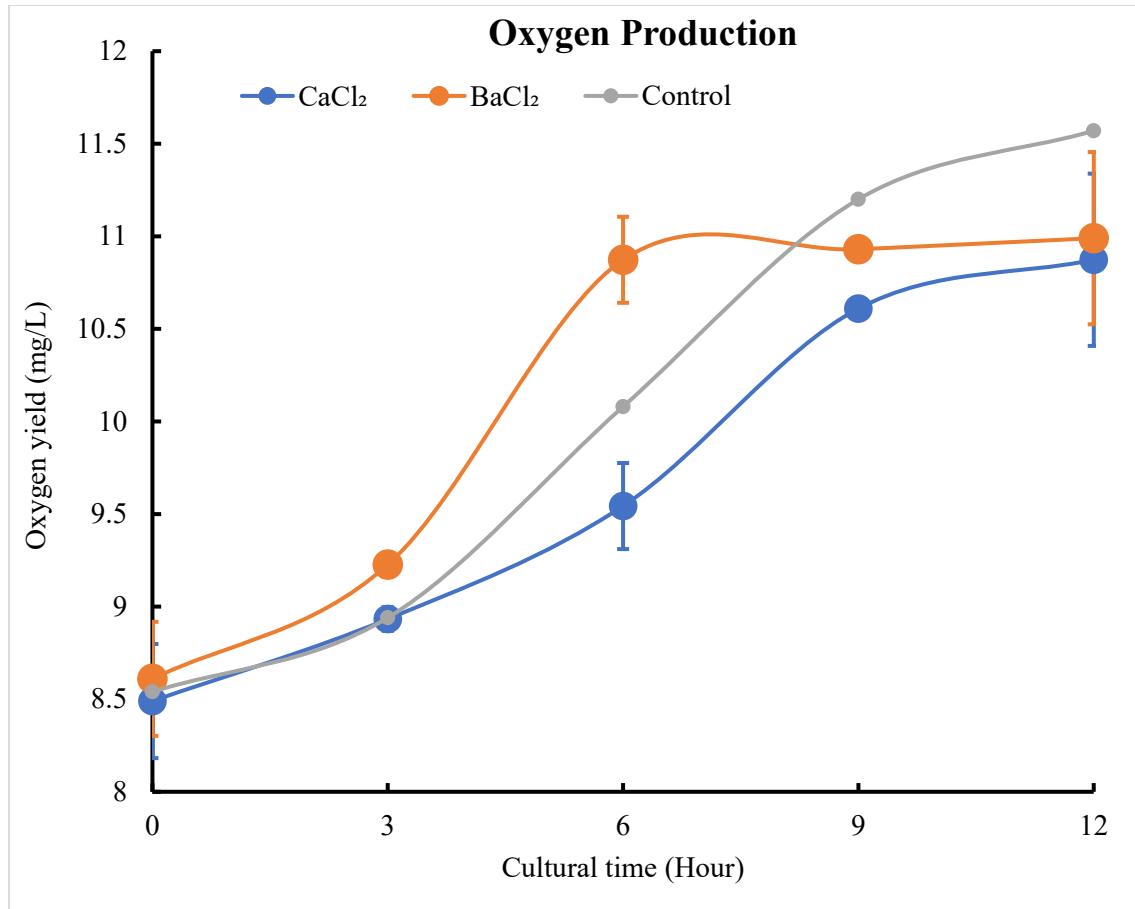


Figure 5: Oxygen production graph of different sodium alginate hydrogels made with sodium alginate at 2.0% and 5.0% calcium chloride or 5.0% barium chloride, N = 3.

The results of oxygen production capabilities of various chlorella artificial leaf substrates (5.0% barium chloride and 5.0% calcium chloride) revealed that barium chloride-containing gel allowed the chlorella to produce oxygen faster (Figure 5). The oxygen yield reached 10.87 ± 0.56 mg/L at 6 hours and 10.99 ± 0.15 mg/L at 12 hours. On the other hand, calcium chloride-containing gel showed a steady rise, reaching the peak at 12 hours with an oxygen production capability of 10.87 ± 0.47 mg/L. Based on this result, it was determined that barium chloride at 5.0% and sodium alginate at 2.0% was the best substrate combination as the final artificial leaf substrate.

It is to be noted that the oxygen production capabilities could be further increased if chlorella cultivation was optimized to increase the content of chlorella in the artificial leaf.

5. Determining the voltage generated in the artificial leaf medium

Copper, zinc, aluminum, and magnesium metal plates were placed in the chlorella/sodium alginate solution to determine the voltage generation. The experiment was performed at room temperature, with each metal plate combination performed in triplicate.

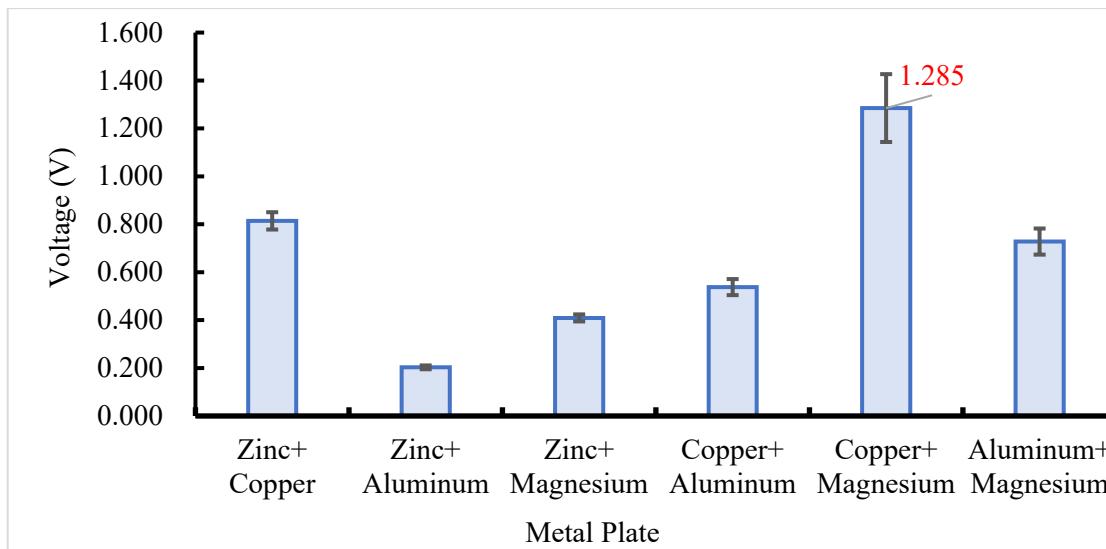


Figure 6: Voltage generated with different metal plate setups, N = 3.

Copper and magnesium plates generated the highest average voltage at 1.285 ± 0.130 volts (Figure 6). This metal combination was then embedded in the artificial leaf. The voltage was recorded at 1.320 volts (Figure 7). The voltage generated in this proof-of-concept artificial leaf was comparable to a commercial AA battery (1.5 volts).¹⁴ It is possible to boost the total voltage output by connecting multiple artificial leaves in series. This showed the potential for it to power simple electrical devices, such as low-voltage LED lights.



Figure 7: Voltage measured with copper and magnesium plates embedded in the artificial leaf. *The photo is author's original image.

Conclusion

In conclusion, our experiment established the fundamental parameters to fabricate a hydrogel containing chlorella that can produce oxygen and generate electricity simultaneously. We created a bacteriostatic hydrogel base using 2.5% boric acid, 2.0% sodium alginate, and 5.0% barium chloride. This combined matrix also minimized water loss and allowed the suspended chlorella to produce oxygen at 10.99 ± 0.15 mg/L at 12 hours. The artificial leaf also generated a maximum voltage of 1.320 V using copper and magnesium metal plates as the conductors. The major advantage of the described artificial leaf is that it uses all-natural components that can be broken down in nature and are environmentally friendly.

Future directions include further optimization of microalgae growth and density to maximize oxygen and electricity output, increasing the electric current, prototyping an artificial leaf battery, and testing to power electrical devices. Overall, this research has demonstrated the proof-of-principle of harnessing oxygen and electricity from the chlorella artificial leaf.

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