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作品名稱　Development of MBR, CO2 absorption ball

得獎獎項　一等獎

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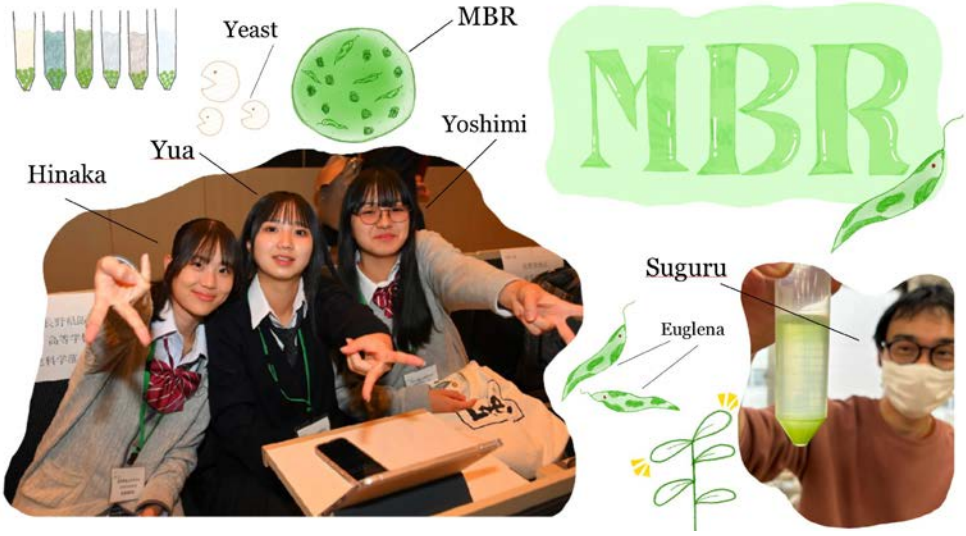

Development of MBR, CO_2 absorption ball

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Abstract

We invented the Midori（which means green）Bioreactor (MBR), beads of euglena and other microalgae fixed in calcium alginate that absorbs carbon dioxide (CO_2). We examined the effect of 19 different solutions and two different organisms on MBR cultivation. Surprisingly, when the MBR was supplied with carbon dioxide or cultured with yeast, they became drastically darker green. Chromatography revealed this green color to be that of microalgae such as green algae or Euglena because chlorophyll *a* and chlorophyll *b* were detected. Under sunlight, MBR absorbed CO_2 and the absorption rate was 1.5 L CO_2/day/1L of MBR. Furthermore, when we put MBR in the water tank, they increased the amount of dissolved oxygen without polluting the environment. These results indicate that MBR can absorb CO_2 by photosynthesizing without leaking out the inside microalgae.

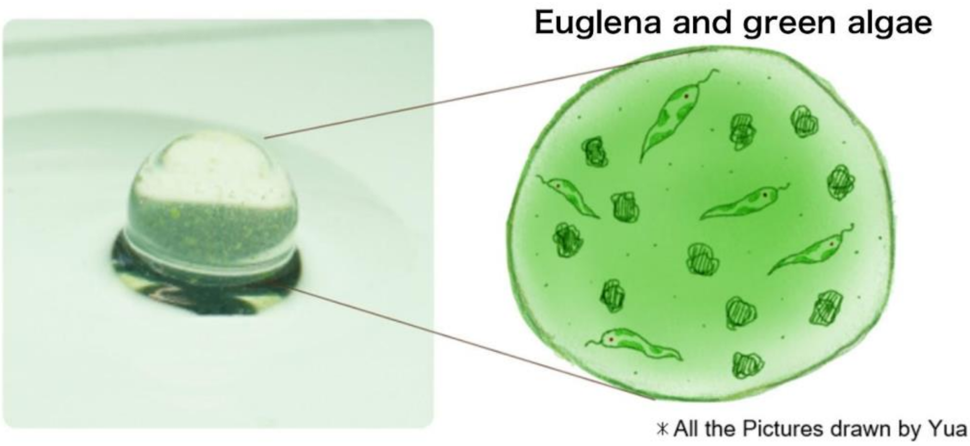


Fig.1 MBR, Midori Bioreactor

Introduction

Global warming is caused by increasing CO_2[1] and to decrease CO_2 is essential. We wanted to use chloroplasts for photosynthesis, because activating photosynthesis can reduce CO_2, increase oxygen, and also provide energy. However, it is impossible to incubate chloroplasts. So we decided to use green algae such as Euglena (Midori Mushi in Japanese), because they have chloroplasts. To make fuel from Euglena, cultivation and extraction methods are being studied[2]. We fixed Euglena in calcium alginate and got the balls that can photosynthesize. Fixing them in that ball could protect living organisms and prevent pollution of the external environment (Fig.2). There are few examples of bioreactors with Euglena fixed in calcium alginate. In this study, we examined **biochemical characterization of MBR incubation** and photosynthetic ability of MBR. Water quality tests were also performed to reveal the effects of MBR on the environment and living things (Fig.3).

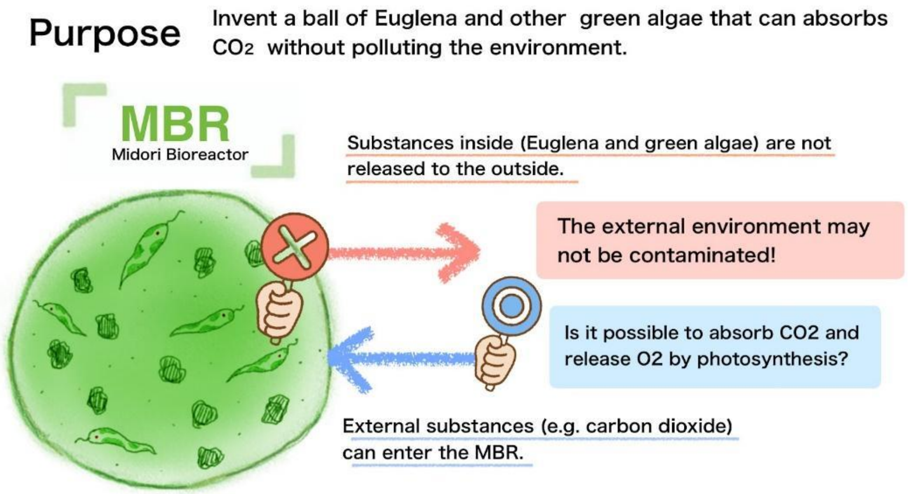


Fig.2 Research Purpose

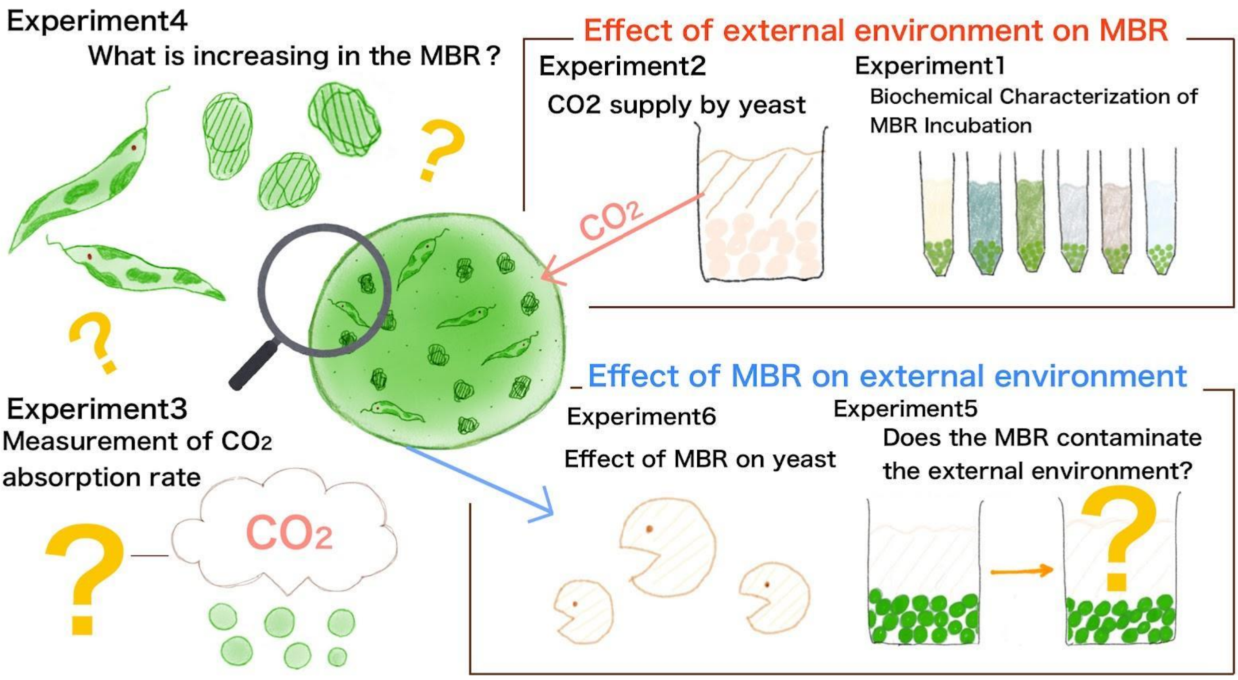
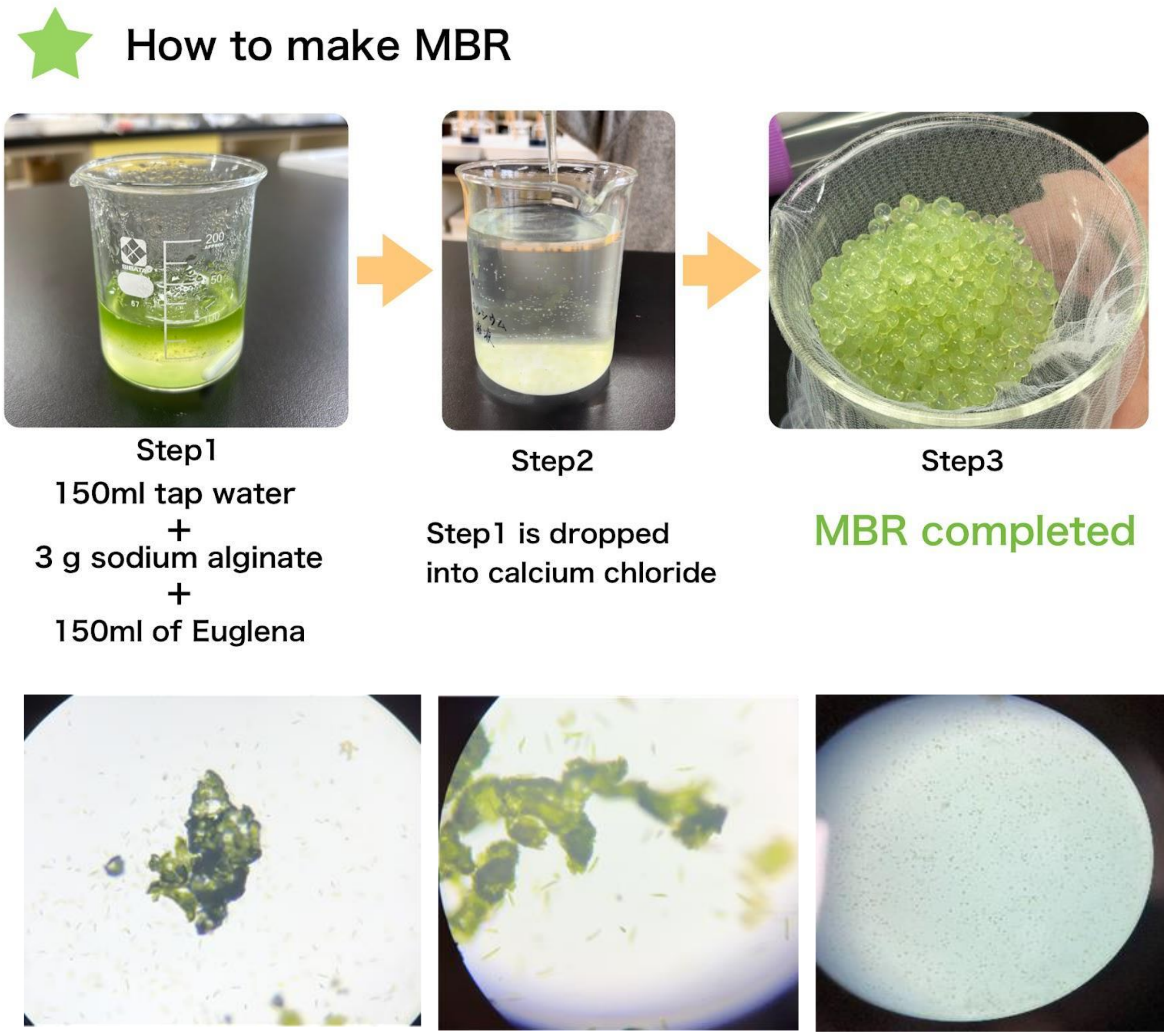


Fig.3 Experimental Plans

Materials and methods

How to make MBR (Midori Bioreactor)

3 g of sodium alginate in 150 ml of water and 150 ml of the Euglena（Midorimushi, Nagoyaka Co., Ltd） were mixed in beaker and dropped into the 1 % calcium chloride using Komagome Pipette (Fig.4). We named these balls MBR (Midori Bioreactor), in which green algae such as Euglena are fixed. The fixed organisms were checked under a microscope. All the experiments were carried out using this MBR. Yeast（Dry Yeast, Nissin Foods Co., Lt d.） was used to incubate MBR and to make Yeast Bioriactor.



The fixed organisms were checked under a microscope

Fig.4 How to make MBR

Biochemical Characterizations of MBR Incubation 【Experiment 1】
Effect of Chemicals and Living things on MBR

To reveal the way to culture MBR efficiently and the environment in which MBR is available, we incubated MBR with chemicals. Referring to previous studies[3)], MBR was cultured in 19 different solutions, sugars, acids, bases, metal ions and other organic matter at 10 mM (Fig.5C). Furthermore, to examine the effect of organisms on MBR, MBR was cultured in yeast or Euglena. We judged the green color of MBR visually. The green color similar that of the MBR cultured in water was used as the standard. The MBR was darker green than that was judged as activation, and lost them or turned pale was judged to be inhibition. As a control experiment, we cultured Euglena not fixed in MBR. We mixed 1 mL of the 100 mM solution with 9 mL of the Euglena to a final concentration of 10 mM (Fig.5B). After 7 days, absorbance was measured and the relative values were used to judge green. We made the concentration at 10 mM because even the acid with the greatest degree of ionization is pH 2, which allows Euglena to live[3)]. Therefore, we thought we could observe the effect of the substance itself.

Can MBR absorb CO_2? 【Experiment 2】
CO2 Supply Experiment to MBR
To test whether CO_2 makes MBR green darker, CO_2 was supplied to MBR from an Erlenmeyer flask containing 10% glucose and yeast bioreactor, Yeast BR(Fig.6). Yeast BR is yeast-trapped calcium alginate balls that have proved that yeast survival is increased and CO_2 is generated for at least 20 days in previous studies[4)].

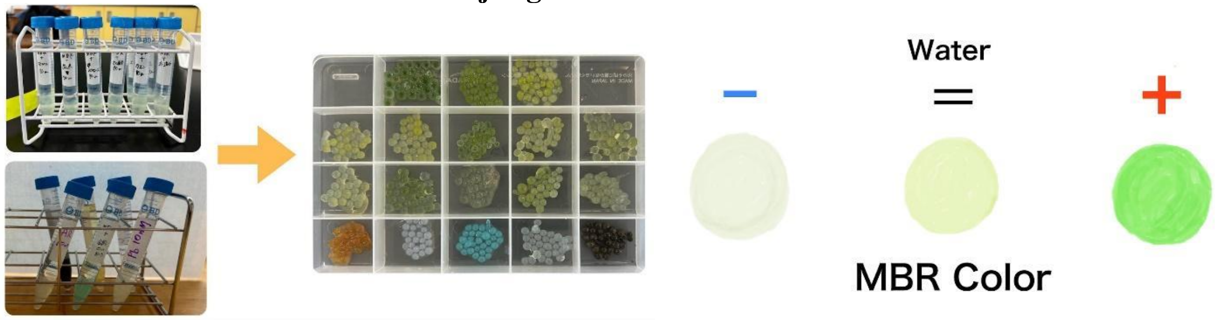
How much CO2 does MBR absorb? 【Experiment 3】
Measurement of MBR CO_2 absorption
To reveal whether MBR really absorbs CO_2, the CO_2 concentration of MBR was measured by a CO_2 concentration meter (Go Direct carbon dioxide sensor GDX-CO2) under sunlight. We used about 30 mL of MBR (400 grains) in 300 mL bottle. MBR cultured with yeast and made green darker was used.

What is the identity of Midori inside MBR? 【Experiment 4】
Thin-layer chromatography to detect photosynthetic pigment.
To identify the MIDORI that grew in the MBR, we performed Thin-layer chromatography[5)]. The type of photosynthetic pigment was judged from the color and Rf value[6)].
Optical microscope observation of the contents inside the MBR
We sliced MBR thinly with a razor blade and the contents of the MBR were observed under an optical microscope (600x).

How does MBR affect the external environment? 【Experiment 5】
Water Quality Test
To examine the effect of MBR on the external environment, we performed Water Quality Test. ①150 mL MBR + 350 mL water, ②150 mL BR + 350 mL water (BR: calcium alginate with nothing fixed), ③150 mL Euglena + 350 mL water, and ④control (500 mL water) were left at room temperature. Water was used from a tank in which killifish were kept. After 22 days, the concentration of each substance was checked. The river water test kit（TZ-RW-3, Kyoritsu Chemical Laboratory） to measure COD, ammonium nitrogen (NH_4^+), nitrite nitrogen (NO_2^-) nitrate nitrogen(NO_3^-) and phosphate phosphorus (PO_4^{3-}) . pH test papers(S75-3524, NaRiKa) were used. Concentration of NH_4^+ was measured with Digital Pack Test Ammonia DPM2-NH4 (Kyoritsu Rikagaku Kenkyusho) and dissolved oxygen levels were measured with Dissolved Oxygen Meter PDO-519（FUSO）.

How does MBR affect organisms? 【Experiment 6】
Culturing yeast with MBR
To examine the effect of MBR on external organisms, we cultured yeast with MBR. The yeast was used for alcoholic fermentation at 40°C with 10% glucose. The CO_2 produced was collected using the water displacement method, and we judged whether the yeast is alive or not from the amount of gas produced. In this case, we used yeast because it is easily available and usable as an organism. We also examined whether MBR increases the amount of dissolved oxygen in the presence of yeast and Euglena.

A. Incubation of MBR and how to judge MBR color



B. Cultivation of euglena

C. Chemicals and Living things used for MBR cultivation

Living things	Euglena、 Yeast
Sugar	Glucose($C_6H_{12}O_6$), Sucrose($C_{12}H_{22}O_{11}$), Mannitol($C_6H_{14}O_6$)
Acid	Acetic acid(CH_3COOH), Citric acid($C_3H_4(OH)(COOH)_3$) Oxalic acid($(COOH)_2$), Hydrochloric acid(HCl)
Base	Sodium hydroxide($NaOH$), Ammonia(NH_3)
Salt	Sodium bicarbonate($NaHCO_3$), Sodium chloride($NaCl$)
metal ion	$AgNO_3$、$Cu(NO_3)_2$、$Fe(NO_3)_2$、$Zn(NO_3)_2$、$Pb(NO_3)_2$、$Al(NO_3)_3$
Other organic matters	Urea（$CO(NH_2)_2$、 Ethanol(C_2H_5OH)

Fig.5: Incubation of MBR

MBR was incubated in various solutions at room temperature(15～35°C).

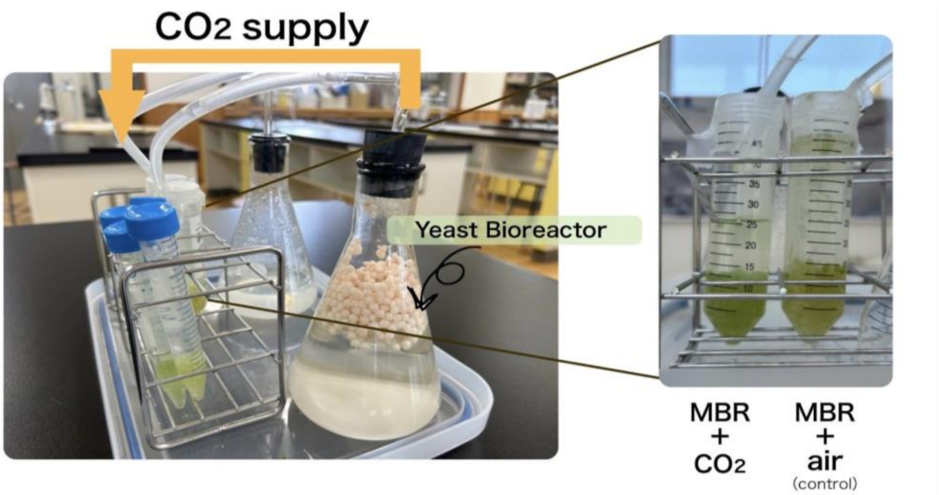


Fig.6 CO2 supply to MBR from yeast BR

CO_2 was supplied to MBR by Yeast Bioreactor. Every few days, 10% glucose was replaced to ensure continued CO_2 generation. As a control experiment, air was blown into the MBR from an Erlenmeyer flask without yeast BR (air).

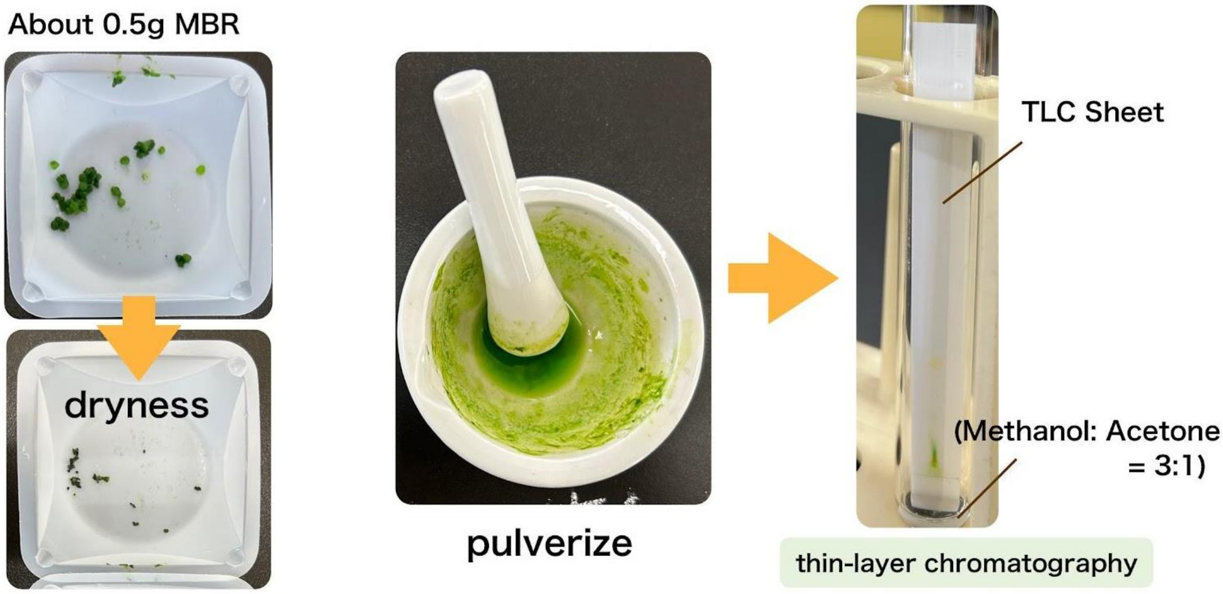


Fig.7　Detection of photosynthetic pigments by thin-layer chromatography
0.5 g of dried MBR was grinded and used for thin-layer chromatography. MIDORI that grew in the MBR was identified based on the type of photosynthetic pigment. The Rf value is calculated by dividing the distance travelled by the component by the distance travelled by the solvent. TLC sheets, development solution (methanol:acetone = 3:1), and extraction solution (petroleum ether:acetone = 7:3) were used.

Results and Discussion

MBR's greenness changed depending on the solution【Experiment 1】
Sugar (glucose) as a carbon source and ammonia as a nitrogen source activated growth as expected. In addition, sucrose, mannitol, ethanol, and acetic acid also activated growth. According to recent studies[7)], Acetic acid and ethanol give plants drought tolerance. Therefore, these may also be beneficial for the growth of green algae such as Euglena. On the other hand, oxalic acid, hydrochloric acid, sodium hydroxide, and six metal ions inhibited growth (Fig.8). The colors of the MBR were changed to the same colors as the added metal ions, such as black, blue, or yellow. These results suggest that MBR can absorb and use components of external substances.Surprisingly, MBR became greener cultured with yeast rapidly. On the other hand, MBR cultured with Euglena had no effect (Fig.9).Why did MBR cultured with yeast become the darkest green? CO2 is released by yeast respiration. So photosynthesis in MBR was promoted by CO2. The MBR cultured with Euglena didn't become dark color because the Euglena outside the MBR also photosynthesize and absorb CO2, thus reducing the amount of CO2 available for MIDORI in the MBR.

CO_2 made MBR darker green 【Experiment 2】
We prepared MBR with a CO_2 supply or with an air supply. MBR supplied with CO_2 became darker green than those supplied with air (Fig.9). This result suggests that CO_2 can facilitate the growth of Euglena in MBR by promoting photosynthesis. The darker green of MBR in experiment 1 may be due to CO2 emission by yeast. However, this green color isn't as dark as MBR culture with yeast. Something in the yeast probably affects MIDORI in MBR. Green color varies by MBR. There may be microalgae that tend to increase within the MBR. We would like to compare the contents of the

MBR absorbed CO2 【Experiment 3】

We measured CO_2 concentration under sunlight to know whether MBR can absorbs CO_2 by photosynthesizing. Interestingly CO_2 concentration started to decrease when MBR was exposed to sunlight. CO_2 concentration decreased in test tubes with MBR, whereas the CO_2 concentration did not change in test tubes without MBR (Fig.11). This result suggests that MBR can absorb CO_2 by photosynthesis. Calculations indicate that a 1.0 L MBR can absorb about 1.5 L of CO_2 per day. To make MBR reduce CO_2 more efficiently, we should clarify the relationship between light and CO2 absorption. We will investigate CO_2 absorption under dark and light conditions. We would also like to investigate the effects of light (sunlight and fluorescent light) and temperature, and the difference in CO_2 absorption rate between water and air.

Chlorophyll *a* and *b* were detected in the MBR and many green cells in MBR were identified from the morphological characteristics【Experiment 4】

At least five photosynthetic pigments were detected in the MBR. The second green pigment from the top (Rf value 0.525) and the third yellow-green pigment from the top (Rf value 0.455) were determined to be chlorophyll *a* and chlorophyll *b,* respectively (Fig.12). Optical microscope observation revealed green spherical cells inside the MBR. These results indicate that the identity of MIDORI that grew in the MBR was green algae such as Euglena. From these results we concluded that the green in the MBR are Euglena and green algae because green algae are the only microalgae that possess both chlorophyll *a* and *b*[5]. Because the sufficient amounts of chlorophyll in the MBR was detected by thin layer chromatography, it may be possible to extract other useful substances[8] such as plastics and biofuel from the MBR. MBR makes it easier to recover and concentrate cultured Euglena[9], because organisms in MBR can't escape. We were also interested in photosynthetic products within the MBR. To check for the presence of starch, iodine solution was dripped into the MBR, but it was difficult to see the color change (data not shown).

MBR increased the amount of dissolved oxygen in water without polluting the external environment.【Experiment 5】

The water with MBR was clear, but the water with Euglena was green. The dissolved oxygen (DO) of the water with the MBR was 15.0 mg/L, 1.9 times higher than the water 8.0 mg/L. Also, the water with MBR, the concentrations of ammonium, nitrite, nitrate, and phosphate ions were low (Fig.13 ①). On the other hand, the water with the Euglena, DO was high but polluted (Fig.13③). There is a possibility that pollutants are adsorbed in the MBR, because ammonium ions do not leak from the MBR. We would like to investigate the concentration of ammonium ions in the MBR in the future. Dissolved oxygen levels during yeast cultures without MBR decreased to 2.0 mg/L, but yeast cultured with MBR was 4.0 mg/L (Fig.14). These results reveal that oxygen is generated from the MBR even if there are organisms outside.

Gas production of yeast cultured with MBR was high【experiment 6】

Gas production was 1.2 times higher in yeast cultured with the MBR than in yeast without the MBR (Fig.15). So yeast may grow well by culturing with MBR because of the following reasons. Oxygen emitted from the MBR (Fig.14) was supplied to the yeast and increased yeast survival. Yeast division has accelerated, then yeast increased or grew to a large size, resulting in increased respiration rate. As the yeast grew by oxygen, the Pasteur effect led to the development of Mitochondria and increased respiration. In the future, we would like to clarify that MBR is safe by studying the effects on organisms other than yeast, such as killifish and crayfish.

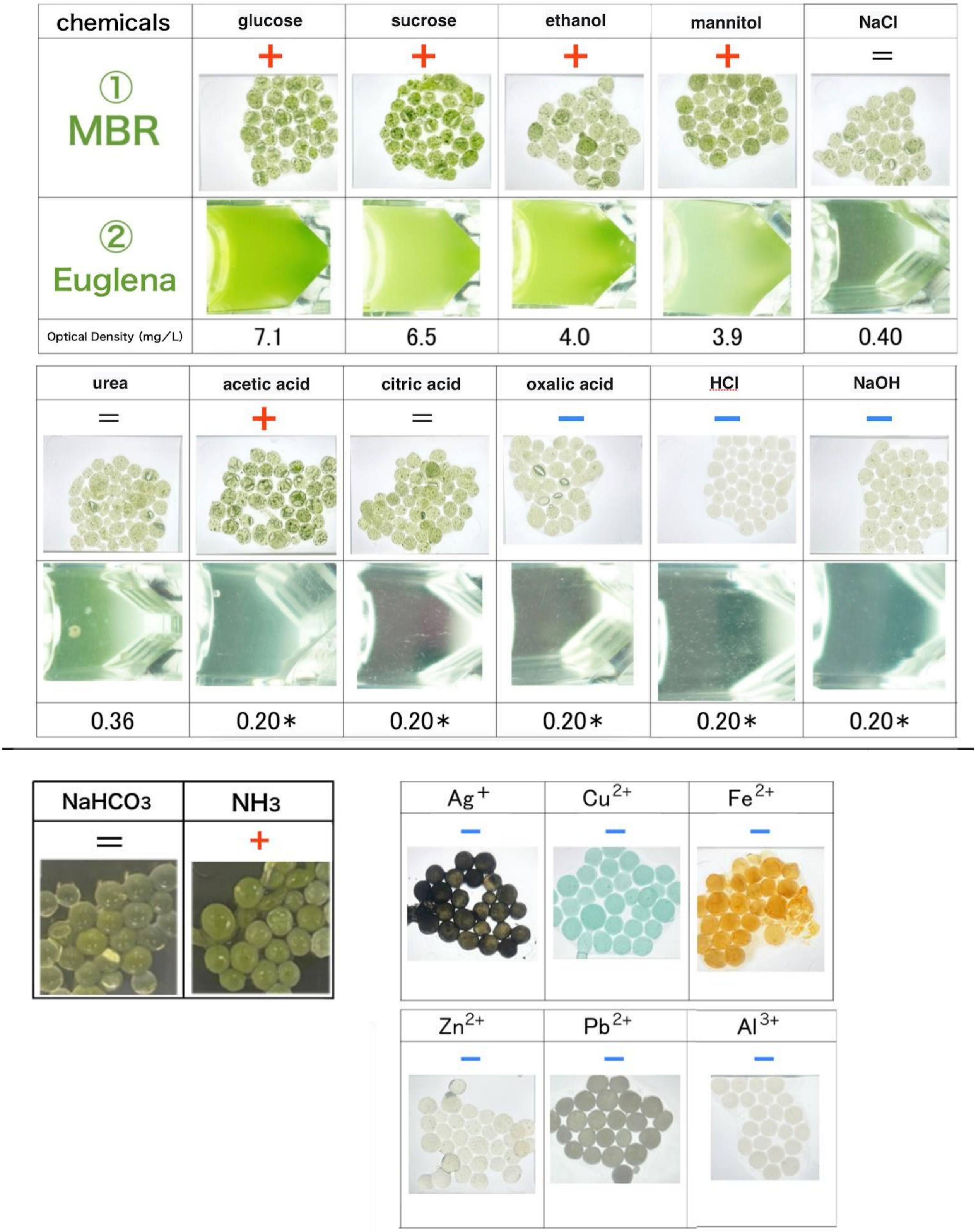
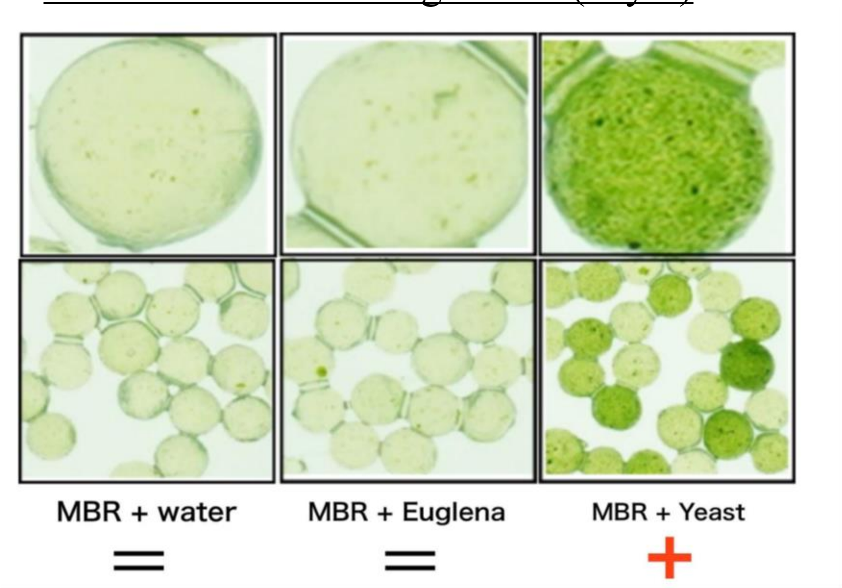


Fig 8: Effects of various solutions on the MBR

MBR was cultured in 19 different solutions at 10 mM and two different organisms. We judged the green color of MBR visually. 0.20* means that absorbance is less than 0.20.

MBR cultured with organisms (day 7)



MBR cultured with yeast (day 14)

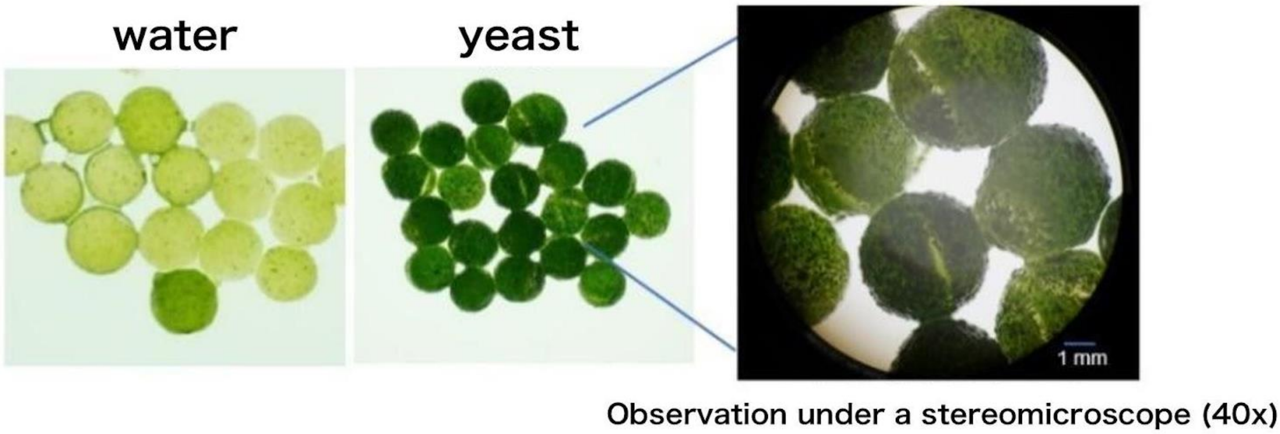


Fig 9: Effect of external organisms on the MBR
MBR and yeast or Euglena were mixed in water and cultured in the same test tube.

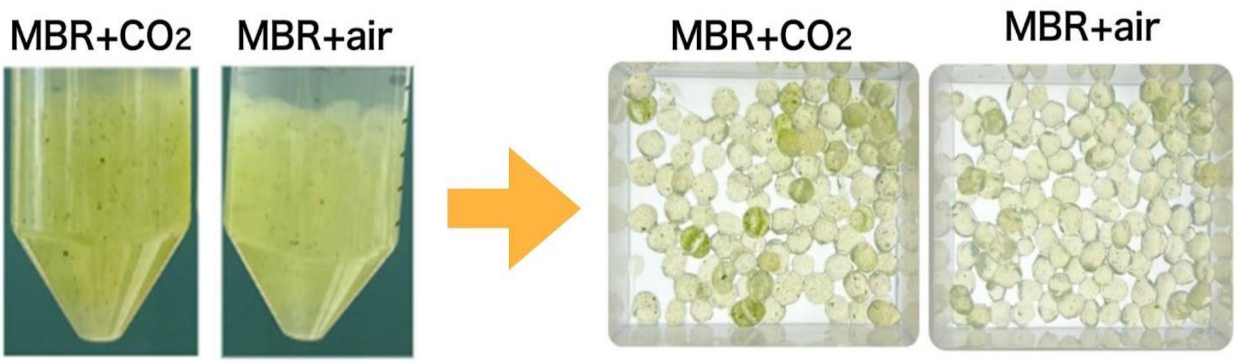
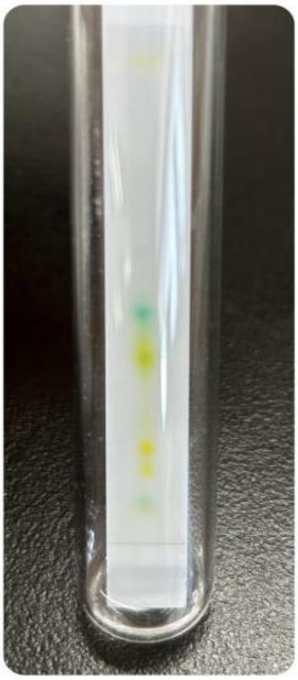


Fig.10: MBR with CO_2 supplied for 10 days.

	0 second	After 100 seconds	Absorption
1st	370ppm	202ppm	**168ppm**
2nd	388ppm	222ppm	**166ppm**
3rd	524ppm	354ppm	**170ppm**

Fig.11 Change in CO_2 concentration
400 grains of MBR (about 30 mL) were used in a 300 mL container. Measurements were taken under sunlight. Go Direct CO_2 sensor GDX-CO2 was used to measure the concentration of CO_2.

Photosynthetic pigments in MBR



photosynthetic pigment	Color	Rf value
feofficine	Gary	0.61
chlorophyll a	Green	0.525
chlorophyll b	Yellow green	0.455
violaxanthin	Yellow	0.246
neoxanthin	Yellow	0.126

Green cells in MBR

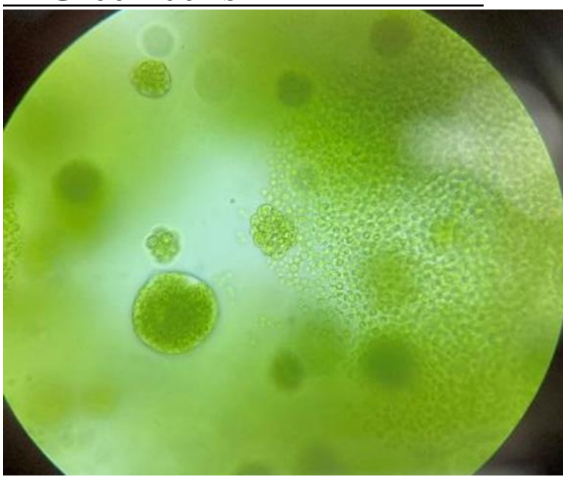
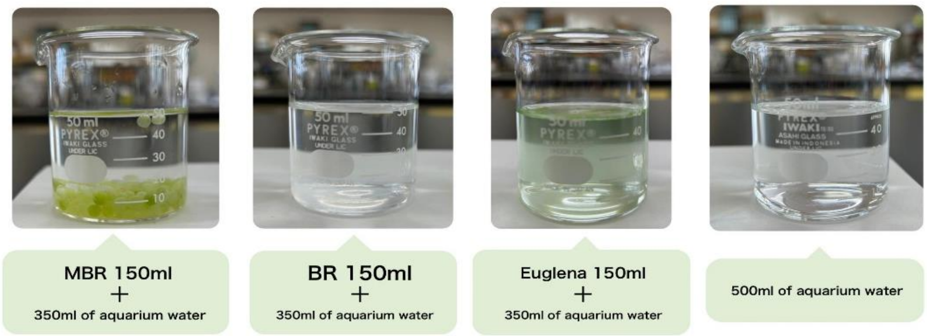


Fig.12 Identification of organisms grown in the MBR
Thinly sliced MBR was observed under an optical microscope (600x)



	1. MBR	2. BR	3. Euglena	4. Aquarium water(Control)
DO (mg／L)	15.0mg/L	7.6mg/L	13.2mg/L	8.0mg/L
NH_4^+ (mg/L)	0.2	0.2	58.5	0.2
pH	5～7	5～7	5～7	5～7
NO_2^- (mg/L)	0.02	0.02	1	0,02
NO_3^- (mg/L)	0,2	0,2	10	0,2
PO_4^{3-} (mg/L)	0,2	0,2	2	0,05

Fig.13 Effect of MBR on water quality
Photographs were taken by transferring to a 50 mL beaker. Water quality tests results after 22 days.

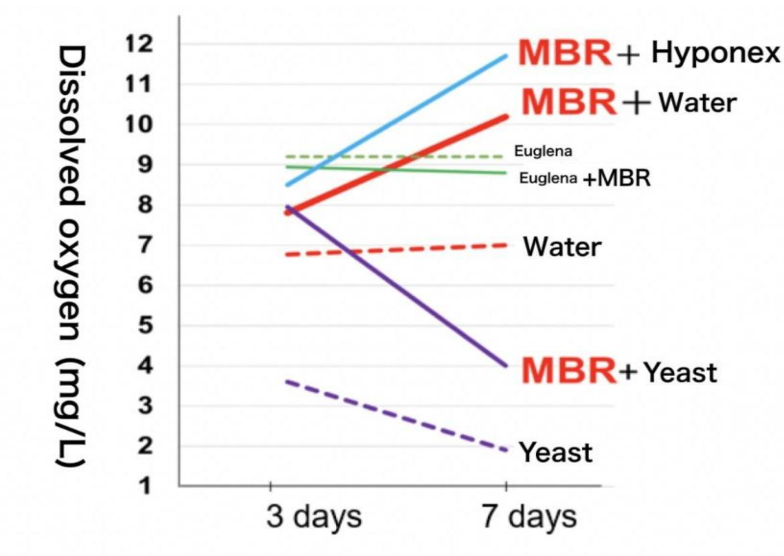


Fig.14 MBR increased dissolved oxygen content

Fig.15 Alcoholic fermentation using yeast cultured with MBR
We cultured yeast with MBR and used the yeast for alcoholic fermentation, and the amount of gas production was examined.

Conclusion

In this study, we revealed biochemical properties of MBR incubation and established how to make MBR, CO_2-absorbing balls (Fig.16). In the MBR, green algae and Euglena can perform photosynthesis and increase without leaking to the outside. We would like to check if MBR can absorb where emit large amounts of CO_2 in the future. For example, thermal power plants and wineries. MBR generates oxygen and can increase the amount of dissolved oxygen in water without polluting the environment, potentially increasing the survival rate of creatures. We can use MBR in water and other applications.

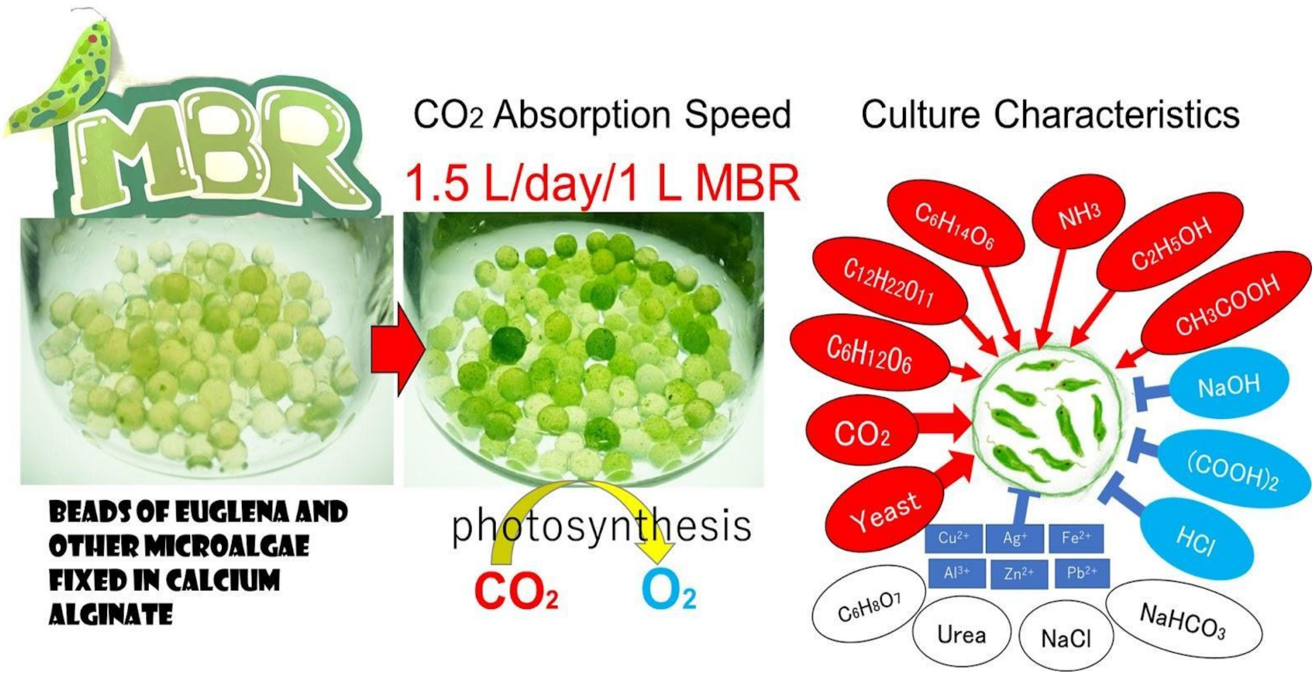


Fig. 16: Summary of this study
We invented the Green Bioreactor (MBR), beads of euglena and other microalgae were trapped in calcium alginate that absorbs carbon dioxide and clarified the culture characteristics.

Future Plan

We should consider how to utilize MBR after use because MBR is thought to have a use-by date. Since chlorophyll in MBR is an organic nitrogen compound, they could be used as fertilizer if the MBR are crushed or otherwise buried in the soil. We will examine if MBR can increase plant growth efficiency (Fig.17).

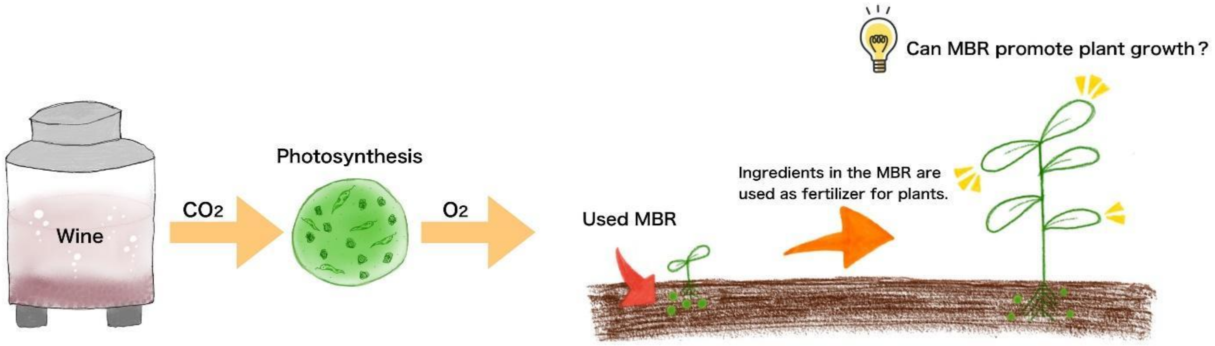


Fig.17 Future Plan
We want to use MBR at thermal power plants and wineries and test the effect of MBR application to plants.

Acknowledgements

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【評語】080017

Advantages:

1. This research has novelty, many possible applications, and potential for commercialization.
2. The MBR can absorb CO_2 by photosynthesizing without leaking out the inside microalgae, useful for converting CO_2 to O_2.

Comments and suggestions:

1. For further applications, it is suggested to evaluate methods for scaling up MBR production.
2. Can MBR be regenerated or reactivated after three weeks?
3. What is the life time of MBR in an open or a close water system? I assumed the CO_2 in the air will be absorbed and O_2 will be emitted. Will the organisms inside MBP eventually die? How about in a coculture system?
4. If they put MBP in a fish tank, will the fishes become healthier because more O_2 in the water.