
Biological Resource

Immobilized *Euglena* Cells (*Euglena* Beads) are Useful for Laboratory Exercises on Photosynthesis at the Secondary Level

Tsutomu NAGASAWA^{1,2)}, Nobuyasu KATAYAMA^{1,3)*}

¹⁾ *Tokyo Gakugei University*, ²⁾ *Kamakura City Onari Junior High School*,

³⁾ *Tokyo Institute of Biology Education, Japan*

(Received: 01 December 2020; Accepted for publication: 25 November 2021;
Communicating editor: K. H. Chu)

Euglena is a common photosynthetic protist, useful as teaching material in biology at secondary and tertiary levels, because it has some unique features of interest to students. *Euglena* can be cultured in an inorganic medium (HYPONEX solution) without contamination by heterotrophic microorganisms, rendering it suitable for experiments on photosynthesis. In this study, we immobilized *Euglena* cells in calcium alginate gel beads (“*Euglena* beads”), facilitating their repeated use in experiments on photosynthesis. In this immobilized state, *Euglena* could propagate and reproduce in 0.1% HYPONEX solution and gradually turned green under the culture conditions. The *Euglena* beads were demonstrated to be a useful substitute for aquatic plants, such as *Elodea* and *Cabomba*, in the qualitative experiments in which a pH indicator was used to detect photosynthetic CO₂ consumption. *Euglena* beads cultured for 30 days had sufficient photosynthetic activity to allow measurement of photosynthetic rates within 30 minutes.

Keywords: *calcium alginate gel beads, Euglena, immobilization, laboratory exercise, photosynthesis, secondary school biology.*

* **Author for correspondence:** E-mail: katayama@u-gakugei.ac.jp

INTRODUCTION

As land dwellers, we are familiar with terrestrial plants as photosynthetic organisms and, at primary and secondary levels, photosynthesis is usually taught using them as examples. At the secondary level, even when students study photosynthesis in aquatic ecosystems, aquatic plants, such as *Elodea* and *Cabomba* (Crawford, 2005; Adams *et al.*, 2012), have usually been employed. However, in aquatic ecosystems algae are also important primary producers (Sze, 1997). Therefore, practical classes using algae are important for secondary students in understanding global ecosystems. Hull (1966) indicated that cultured unicellular microalgae can be used in

laboratory exercises of plant physiology, including photosynthesis. However, in practice, this has not often been adopted.

Usually, the isolation and pure culture of microalgae is demanding for student experiments, and a convenient method for culturing microalgae in the school laboratory setting (*e.g.*, Hull, 1966) would be valuable. However, most species are not easy to be cultured, with *Euglena* being an exception for which simple methods have been developed (*e.g.*, Flinn Scientific, see Website list). Koizumi and Mikami (1976) proposed a simplified culture method for *Euglena*, using only HYPONEX powder, which is available at any gardening shop. *Euglena* is a common photo-

synthetic protist with some well-known unique features making it useful as teaching material in biology. It is now familiar to people because it has been used as a nutrient supplement, as an ingredient in foods, and for producing biodiesel and jet fuel. Therefore, the use of this alga for biology laboratory exercises may stimulate students' interest.

In this study, we aimed to use *Euglena* to inform students that not only higher aquatic plants, but also microalgae, are major producers in freshwater ecosystems. However, there are problems with using *Euglena* cell suspension for detecting/measuring photosynthesis. A major problem is that when cell suspensions are used, it is difficult to harvest the cells for reuse, meaning that we must prepare a large amount of *Euglena* suspension for each experiment. To solve this problem, we introduced an immobilization technique, which allows us to use the same *Euglena* cells repeatedly. Tamponnet *et al.* (1985) showed that *Euglena* cells immobilized in a calcium alginate matrix maintained photosynthetic activity and ultrastructural integrity. After immobilizing *Euglena* cells in calcium alginate gel beads, we examined whether the immobilized *Euglena* could reproduce and whether such "*Euglena* beads" could be used for the qualitative and quantitative experiments commonly carried out in secondary schools in Japan.

MATERIALS AND METHODS

Euglena cell culture

For culturing *Euglena* cells, we used a 0.1% solution of HYPONEX powder (N:K:P = 6:10:5, HYPONEX Corporation), hereafter referred as "Hyponex medium" as described by Koizumi and Mikami (1976) (See Note¹). Cells of *Euglena gracilis* Klebs from Mikami's laboratory at the Miyagi University of Education, were cultured in flasks containing 250 cm³ Hyponex medium in an incubator at 20°C under a light intensity of 40 -

50 μmol/m²/s with a 12:12 h light:dark photoperiod. At 7-week intervals, a 2.5 cm³ cell suspension was inoculated into 250 cm³ of fresh Hyponex medium.

Immobilization of *Euglena* cells and culturing *Euglena* beads

Euglena cells were immobilized by Matsuda's (1994) method. To 120 cm³ of mature (7-week-old) cell suspension, 1.5 g of sodium alginate (Wako Pure Chemicals) was added, and the solution was stirred gently until the powders were dissolved completely. It was dripped from a pipette (bore size approximately 2 mm diameter) into cool 5% calcium chloride solution with gentle stirring. As a result, about 1800 *Euglena* beads were made from 120 cm³ of mixture. After standing in 5% calcium chloride solution for 30 minutes, the *Euglena* beads were washed three times with distilled water. The beads were then put into flasks containing 200 cm³ of Hyponex medium and cultured in a incubator.

After a day, they were transferred to fresh Hyponex medium including 0.03 M KHCO₃ and exposed to high intensity light (200 μmol/m²/s) for two hours in order to promote photosynthesis, and then transferred to 200 cm³ of fresh Hyponex medium before returned to the incubator. This high-intensity-light treatment was repeated every 10 days during the experiment.

Calcium alginate gel beads lacking *Euglena* cells, but otherwise identical, were used as a control.

Qualitative experiment on photosynthesis using *Euglena* beads

In qualitative experiments on photosynthesis commonly carried out in junior high school science classes in Japan (Yamazaki and Tahara, 1998), the *Euglena* beads were used instead of water plants. Bromothymol blue (BTB), a pH indicator, was used for detecting photosynthetic CO₂ consumption (See Note²). To 200 cm³ of 1 mM KHCO₃ solution, 5 cm³ of BTB stock solu-

tion (0.04 w/v%) was added. At this point, the color of this alkaline mixture solution was blue. Then, exhaled air was blown in for about 30 seconds until the mixture turned green (“BTB solution”).

About 300 *Euglena* beads or control beads, cultured for more than 30 days, were washed twice with BTB solution and kept in 200 cm³ BTB solution for about two hours to equalize the pH inside the beads. The beads were then transferred into respective 20 cm³ vials of BTB solution and the vials sealed, with care to avoid air bubbles. For each of three samples, the *Euglena* beads, the control beads, and the blank test (BTB solution only), three vials were placed in high-wall Petri dishes with 20°C water, placed on an OHP (FUJIX EZ-2) and illuminated for 60 minutes. Sheets of paraffin paper between the Petri dishes and the OHP glass deck were adjusted to light intensity of 300 - 350 μmol/m²/s (Fig. 1). The vials were lightly shaken by hand once every five minutes. The color of the BTB solution was observed at 15-minute intervals by placing the vials on white paper.

Measurements of the photosynthetic and respiration rates of Euglena beads

The photosynthetic O₂ production and the respiratory O₂ consumption of cultured *Euglena* beads were measured with the Productmeter (Yokohama *et al.*, 1980). The amount of O₂ was calculated according to the method of Yokohama *et al.* (1986).

About 150 each of the *Euglena* beads and the control beads were put into the reaction vessel and the compensation vessel of the Productmeter, respectively. To each vessel, 7.5 cm³ of Hyponex medium and 0.5 cm³ of 0.5 M KHCO₃ solution were added, giving a final concentration of KHCO₃ of 0.03 M. The vessels of the Productmeter were immersed in a water bath with temperature controlled by a circulator (COOLNIT CL-150F, TAITEC) and mechanically shaken

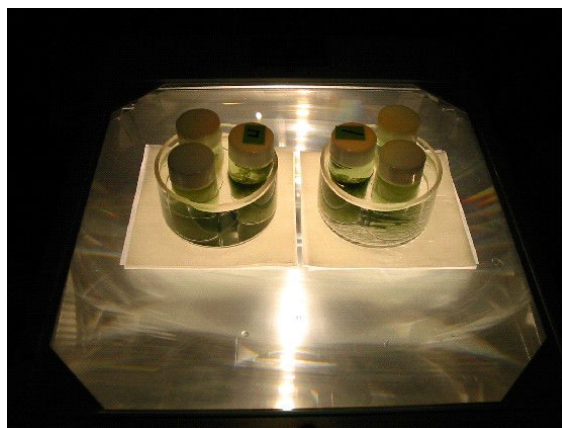


Figure 1: Vials immersed in 20°C water and illuminated by an OHP

continuously. The light source was a slide projector (S-300, ELMO), with the intensity adjusted by measuring the photon flux density with a photometer (MEMORY SENSOR MES-101, Koito Industry).

After each measurement, the *Euglena* beads and the control beads were washed twice with fresh Hyponex medium prior to reuse. After a series of measurements, the beads were put into flasks containing 200 cm³ of Hyponex medium and cultured in the incubator until required for further experiments.

RESULTS

Euglena can reproduce in an immobilized state

Under the culture conditions, *Euglena* beads (Fig. 2) gradually turned green. Thirty days after starting the culture, their green color became deeper (Fig. 3). Microscopic observation confirmed that the cells had formed many colonies (Figs. 4 and 5). The beads did not disintegrate at least for two months, and few *Euglena* cells were lost from the beads during the culture period.

Qualitative experiment on photosynthesis using Euglena beads

Figure 6 shows *Euglena* beads (left), control beads (center) and a blank test (right). Under illumination, the color of the BTB solution in the

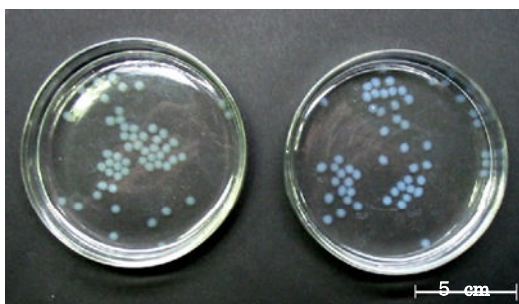


Figure 2: *Euglena* beads (left) and control beads (right) before culturing

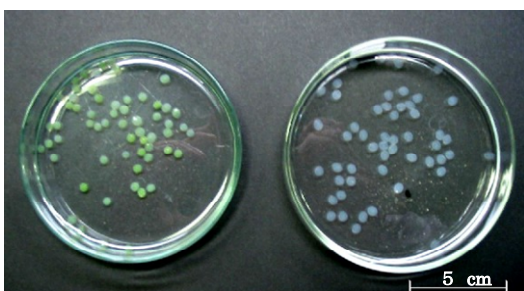


Figure 3: *Euglena* beads (left) and control beads (right) cultured for 30 days

vial containing the *Euglena* beads changed from green to blue-green within 30 minutes (Fig. 6B), and to blue within 45 minutes (Fig. 6C). Control beads and the blank did not change color. The change from green to blue indicates the consumption of dissolved CO₂ in the BTB solution (See Note²), confirming that the *Euglena* cells in the beads consumed CO₂ through photosynthesis.

Changes in the photosynthetic oxygen production of *Euglena* beads during culturing

The change in the photosynthetic oxygen



Figure 4: A micrograph of *Euglena* beads before culturing (×10)

Some black globules seen in the beads are bubbles. The *Euglena* cells are smaller brown grains.

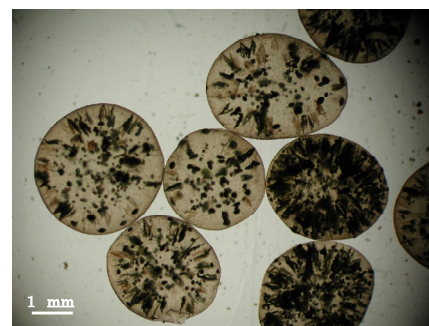


Figure 5: A micrograph of *Euglena* beads cultured for 30 days (×10)

Many colonies of *Euglena* cells are formed in the gel matrix of the beads.

production of *Euglena* beads (about 150 beads) in culture was examined. Initially, the photosynthetic oxygen production was 10.0±3.9 μl O₂/h. Twenty days later it increased by almost eight times. Furthermore, the photosynthetic oxygen production of 30-day-old *Euglena* beads was nearly two times higher than that of 20-day-old



Figure 6: Changes in the color of the BTB solution

A: At the beginning, B: Under light illumination for 30 minutes, C: Under light illumination for 45 minutes. *Euglena* beads are on the left, control beads are in the center, and a blank test is on the right.

ones (Fig. 7). This increase in oxygen production in culture resulted from the reproduction of *Euglena* cells in the gel beads.

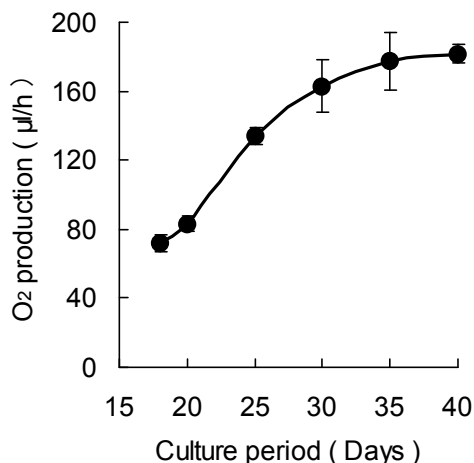


Figure 7: Changes in the photosynthetic oxygen production of *Euglena* beads in culture

The measurements were done under the light intensity of 200 µmol/m²/s at 30°C. The *Euglena* beads (approx. 150) were used repeatedly for measurement.

Photosynthetic curve of *Euglena* beads

The photosynthesis-light curve (Fig. 8) and the photosynthesis-temperature and respiration-temperature curves (Fig. 9) were obtained by using 30-day-old *Euglena* beads. Light saturation at 30°C occurred at around 300 µmol/m²/s (Fig. 8). The optimum temperature for photosynthesis at the light intensity of 200 µmol/m²/s was at around 20 - 30°C (Fig. 9).

DISCUSSION

Euglena cells possess many features that make them useful in biology education. These include: 1) they can be observed under a low-power microscope; 2) they can swim by means of a flagellum and execute phototactic responses; 3) they can change cell shape; 4) they have chlorophyll *a* and *b*, like green plants, though its major photosynthetic product is not starch; 5) under inappropriate growth conditions they can encyst, while under appropriate growth

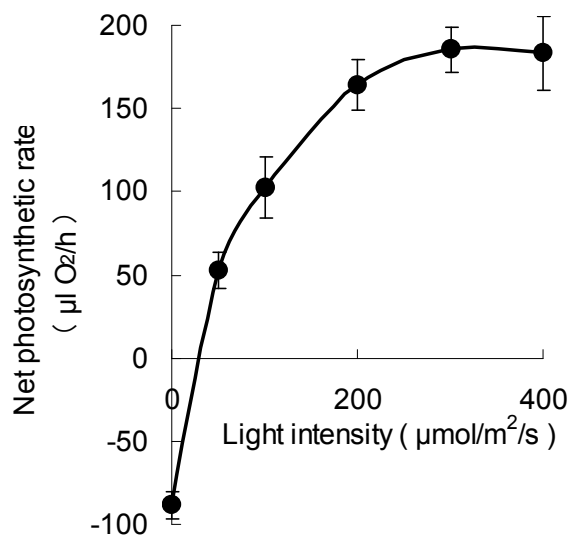


Figure 8: Photosynthesis-light curve of *Euglena* beads

The measurements were carried out at 30 °C. The *Euglena* beads (approx. 150) were used repeatedly for measurement.

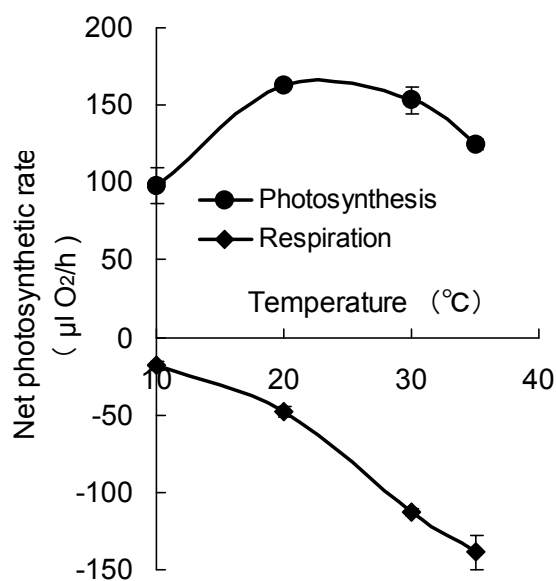


Figure 9: The photosynthesis-temperature curve and respiration-temperature curve of *Euglena* beads

The measurements of photosynthesis were carried out at a light intensity of 200 µmol/m²/s. The measurements of respiration were carried out in dark conditions. The *Euglena* beads (approx. 150) were used repeatedly for measurement.

conditions they can reproduce rapidly and form water-blooms; and 6) they sometimes accumulate huge amounts of carotenoid pigments and become reddish. Therefore, this microalga can be used for a variety of student exercises in biology, not only for microscopic observation, but also for experiments on phototaxis (Espey, see Website list) and population growth (Oswald and Kwiatkowski, 2011). It also has the potential to be used for environmental monitoring in environmental education (Danilov and Ekelund, 2001; Ahmed and Häder, 2010). However, no report on the use of this organism for a secondary student laboratory exercise on photosynthesis has been published. This is possibly because of its motility and tendency to be contaminated by bacteria in liquid culture (See Note¹).

In the present study, to facilitate and simplify experiments on *Euglena* photosynthesis in secondary schools, *Euglena* cells were immobilized in calcium alginate beads as described by Matsuda (1994), a method similar to those of CAROLINA Biological Supplier Co. and SAPS: Science and Plants in School (See respective websites). Immobilization techniques of immobile microalgae were developed in the 1980s and are now used for a wide variety of biotechnological applications (Kaparapu, 2017). Some freshwater green algae, such as *Scenedesmus* (Chevalier and Noüe, 1985), *Botryococcus* (Baillez *et al.*, 1986) and *Chlorella* (Llangovan *et al.*, 1998), and marine microalgae (Hertzberg and Jensen, 1989; Moreno-Garrido *et al.*, 2005) retain their photosynthetic activities in calcium alginate or κ -carrageenan beads. The use of microalgae (indefinite species) immobilized in calcium alginate beads, or “algal balls,” in an experiment on photosynthesis in student laboratory classes has already been proposed (CAROLINA Biological Supplier Co. and SAPS). Similarly, Eldridge (2004) used immobilized *Scenedesmus quadricauda*, and Crawford (2006) and Andrews *et al.*

(2015) followed Eldridge’s method in their practical studies at schools.

In the present paper, we show that the immobilization seems effective for a motile microalga, such as *Euglena*, permitting uncomplicated measurement of photosynthetic activity in biology laboratory classes at the secondary level. Our results indicate the following three advantages for such studies: 1) the beads can be used for experiments repeatedly so a large quantity of culture does not need to be prepared; 2) the experiments can be done under different conditions with the use of the same beads; 3) in a qualitative experiment, photosynthesis can be observed simply by watching the color of a pH indicator solution.

In the qualitative experiment, the use of *Euglena* beads allows us to detect the changes in color of the BTB solution more easily in comparison with the use of free algal cells, because the *Euglena* cells are contained by the gel beads, and we can observe the color of the solution easily without any contamination of the *Euglena* cells (Fig. 6). When the *Euglena* beads cultured 30 days or more were used, the photosynthetic CO₂ consumption by *Euglena* cells could be detected within 30 minutes by examining the color of the BTB solution. So, this experiment can be carried out in one school hour of secondary schools.

In the quantitative experiment, when a 30-day-old *Euglena* beads culture was used, the amount of oxygen evolved from the *Euglena* beads is high enough for measuring their photosynthetic oxygen production with the Productmeter within 30 minutes under the conditions described above. Therefore, the experiment is possible to be accomplished within a one period of secondary schools.

In order to use the *Euglena* beads for these quantitative and qualitative experiments, we had to culture them for more than 20 days. If we

could use an algal culture of high cell density for the immobilization procedure, we would be able to use the algal beads for photosynthesis experiments immediately after making them. However, we could not obtain such a high cell density because of the slow growth rate of *Euglena* cells under the culture conditions of Koizumi and Mikami (1976) suggested. Although immotile microalgae can be collected from suspension culture by low-speed centrifugation, we could not adopt this method for *Euglena* since the precipitated *Euglena* cells were dispersed into supernatant immediately. Therefore, further studies are required to test the concentration and the kind of HYPONEX for culture medium as well as the other culture conditions, such as temperature and light intensity.

Chevalier and Noüe (1985) used immobilized *Scenedesmus* cells for sewage treatment repeatedly in their laboratory tests. *Euglena* can reproduce well in eutrophic waters such as sewage and frequently lead to algal blooms, as does *Scenedesmus*. *Euglena* was reported to be useful for removing nutrients and heavy metals from sewage (Ahmed and Häder, 2010), so that immobilized *Euglena* cells can possibly be used for sewage treatment at municipal scale. In schools, the *Euglena* beads can be used as teaching material, not only in biology, but also in environmental education programs that focus on algal blooms caused by eutrophication and in studies of sewage treatment.

Note¹: A standard culture method for *Euglena* is shown on the website of Flinn Scientific (*cf.* its website). The culture media mentioned in this website are not difficult to prepare, but the preparation of Hyponex medium is much easier. We, however, did not adopt the culture media provided by Flinn Scientific, because these culture media contain organic substances and, on the website, bac-

terial contamination is mentioned. When we adopt such culture media, it always requires a strict sterilization practice to avoid bacterial contamination. Yet such practice is often difficult in the general biology laboratory setting in secondary schools. If bacterial contamination occurs, we cannot measure the correct photosynthetic rate and respiration rate of *Euglena* beads.

Note²: At around pH 7, the color of a diluted BTB solution is green. It turns yellow in acid conditions and blue in alkaline. When a BTB stock solution is mixed with 1 mM KHCO₃ solution (an alkaline solution), the color of this mixture is blue. When exhaled air is blown into the mixture, CO₂ in the exhaled air dissolves into the mixture, thereby acidifying the mixture and turning its color green. As the dissolved CO₂ in the BTB solution is consumed by photosynthesis, this raises the pH of the solution gradually and changes its color back to blue. The detectable pH range of BTB is 5.6 – 8.2, and some other pH indicators which have a similar detectable range can be substituted for BTB (*cf.* SAPS website).

ACKNOWLEDGEMENT

We are very grateful to Professor Emeritus Kazuyuki Mikami of the Miyagi University of Education, who provided us with cultured *Euglena* cells and gave us valuable advice on culturing *Euglena*, and to Professor Emeritus Peter Tyler of Deakin University, Australia, who helped us to edit the manuscript.

REFERENCES

- Adams, A., Moore, G., Rutherford, A., Stewart, F., Crawford, K. and Beaumont, P. (2012) *Cabomba* – an exocharmic plant! *School Science Review* **93**(344): 9-12.
- Ahmed, H. and Häder, D.-P. (2010) Rapid

- ecotoxicological bioassay of nickel and cadmium using motility and photosynthetic parameters of *Euglena gracilis*. *Environmental and Experimental Botany* **69**: 68–75.
- Andrews, K., Beaumont, P. and Crawford, K. (2015) Measurement of limiting factors in photosynthesis. *School Science Review* **96** (356): 31-35.
- Bailliez, C., Largeau, C., Berkaloff, C. and Casadevall, E. (1986) Immobilization of *Botryococcus braunii* in alginate: influence on chlorophyll content, photosynthetic activity and degeneration during batch cultures. *Applied Microbiology and Biotechnology* **23**(5): 361-366.
- Crawford, K. (2005) *Cabomba* – a reliable alternative to *Elodea*? *SSERC Bulletin* **215**: 10–12.
- Crawford, K. (2006) SAPS photosynthesis kit: The use of algal balls to investigate photosynthesis. *SSERC Bulletin* **219**: 2-5.
- Chevalier, P. and Noüe, J. (1985) Wastewater nutrient removal with microalgae immobilized in carrageenan. *Enzyme and Microbial Technology* **7**(12): 621-624.
- Danilov, R. A. and Ekelund, N. G. A. (2001) Using the green flagellate *Euglena gracilis* Klebs as physiological dosimeter: is a long-term bioassay more significant than a short-term one? *Turkish Journal of Botany* **25**: 43–44.
- Eldridge, D. (2004) A novel approach to photosynthesis practicals. *School Science Review*, **85**(312): 37–45.
- Hertzberg, S. and Jensen, A. (1989) Studies of alginate-immobilized marine microalgae. *Botanica Marina* **32**: 267-273.
- Hull, R. (1966) The use of unicellular algae in the biology classroom. *The American Biology Teacher* **28**(2): 120-121.
- Kaparapu, J. (2017) Microalgal immobilization techniques. *Journal of Algal Biomass Utilization* **8**(1): 64-70.
- Koizumi, S. and Mikami, K. (1976) Some assignments of biology education and some organisms of teaching materials – mostly the protozoan –. *Annual Reports from the Research Institute for Science Education* **12**: 3-8. (in Japanese)
- Llangovan, K., Cañizares-Villanueva, R. O., González Moreno, S. and Voltolina, D. (1998) Effect of cadmium and zinc on respiration and photosynthesis in suspended and immobilized cultures of *Chlorella vulgaris* and *Scenedesmus actus*. *Bulletin of Environmental Contamination and Toxicology* **60**: 936-943.
- Matsuda, H. (1994) Experiments of respiration with immobilized yeast. *Japanese Journal of Biological Education* **34**(4): 292-297. (in Japanese)
- Moreno-Garrido, I., Campana, O., Lubián, L. M., and Blasco, J. (2005) Calcium alginate immobilized marine microalgae: Experiments on growth and short-term heavy metal accumulation. *Marine Pollution Bulletin* **51**(8): 823-829.
- Oswald, C. and Kwiatkowski, S. (2011) Population growth in *Euglena*: A student-designed investigation combining ecology, cell biology, & quantitative analysis. *The American Biology Teacher* **73**(8): 469-473.
- Sze, P. (1997) *A Biology of the Algae*. 3rd ed. McGraw-Hill. pp.16-18.
- Tamponnet, C., Coslantino, F., Barbotin, J. N. and Calvayrac, R. (1985) Cytological and physiological behaviour of *Euglena gracilis* cells entrapped in a calcium alginate gel. *Physiologia Plantarum* **63**(3): 277–283.
- Yamazaki, J. and Tahara, Y. (1998) Let's make a "Photosynthesis pendant!" *The Heredity Supplemental Issue* **10**: 14-15. (in Japanese)
- Yokohama, Y., Katayama, N. and Furuya, K. (1980) Simplification of gasmetry for meas-

uring respiration and photosynthesis. *In*: Imahori, K., Kille, R. A. and Koshida, Y. (eds.) *Proceedings of the Eighth Biennial Conference of Asian Association for Biology Education, 1980 (Osaka)*, pp.159-165.

Yokohama, Y., Katayama, N. and Furuya, K.

(1986) An improved type of "Productmeter," a differential gas-volumeter, and its application to measuring photosynthesis of seaweeds. *The Japanese Journal of Phycology* **34**:37-42. (in Japanese with English abstract)

WEBSITES

CAROLINA Biological Supplier Co.: Using algae beads as a model for photosynthesis

<https://www.carolina.com/teacher-resources/Interactive/essentials-algae-beads/tr40904.tr> <accessed: Oct. 20, 2021>

Flinn Scientific: Culturing *Euglena*

<https://www.flinnsci.com/culturing-euglena/dc10578/> <accessed: Oct. 20, 2021>

SAPS (Science and Plants in School): Photosynthesis with algal balls

http://www.saps.org.uk/secondary/teaching-resources/235?dm_i=19FH,189MY,6Q22VI,45OSG,1 <accessed: Oct. 20, 2021>

Espey, M: *Euglena*, the phototaxic protozoa (Winning Experiment Procedures from the NIH LAB Challenge)

<https://www.drugabuse.gov/euglena-phototaxic-protozoa> <accessed: Oct. 20, 2021>