Assessment of environmental maintenance, reproduction of pristine zones along coastal areas using a productmeter and its application in research and education

Chika Suzuki (Center for Science, Technology and Innovation Policy Studies, Kyushu University, suzuki@sti.kyushu-u.ac.jp)
Akira Kurashima (Graduate School of Bioresources, Mie University, kurasima@bio.mie-u.ac.jp)

Abstract
The productmeter is a tool used to measure the volume change in gas while maintaining a fixed concentration of gas in a container, which in turn, compresses the sample. In this study, we used an improved and more complex productmeter than previously designed for research and education. We performed measurements of daytime photosynthesis in *Ulva pertusa* using the productmeter in order to examine its potential use as a teaching tool in university programs (higher education) and to study environmental maintenance, reproduction, and creation in coastal areas. By performing continuous measurements during algal cultivation using a productmeter, we aimed to obtain data on time, and discover applications suitable for biological experiments in teacher training courses using long-term continuous measurements, as well as for use in research experiments. We conducted measurements at 30 ℃ on the 1st day, 25 ℃ the 2nd day, 20 ℃ the 3rd day, and 15 ℃ on the 4th day, in order to determine the optimum water temperature. These measurements also revealed that the level of photosynthesis at different temperatures, with the greatest rates measured at 30 ℃ and the lowest at 15 ℃.

Key words
productmeter, *Ulva pertusa*, photosynthesis, teaching tool, outreach

1. Introduction

The Marine Master Plan recently created in Japan (April 26, 2013, cabinet decision), included a new clause, requiring actions to enhance and improve marine education and general understanding of the ocean. Changes were made in February of 2015 to the Seto Inland Sea environmental conservation basic plan, based on the Seto Inland Sea environmental conservation special measures law. This conservation plan was entitled, "Viewpoints of environmental maintenance, reproduction of the area along the shore, and the creation", and various points related to this theme focused on conventional measures were included, to maintain the natural state and water quality of the Seto Inland Sea. According to Suzuki et al. (2013), it is difficult to find opportunities to study the marine environment in its natural state within the current education programs. Additionally, there has been a focus worldwide on the use of coastal environments ranging from seaweed forests to organisms that represent highly important resources. It is clear that even if completed stepwise, there is a need for the government to improve marine-related education currently available at elementary, secondary, and higher education levels, and discuss measures to systematically link them (Basic Plan on Ocean Policy, 2013).

2. Equipment

2.1 The history of the role of the productmeter in education

We often utilize an infrared gas analyzer to measure carbon dioxide (CO_2) absorption using the infrared spectrum. Pulse amplitude modulation (PAM) and oxygen electrodes are devices used frequently to measure photosynthesis in terrestrial plants and seaweeds. However, we cannot use them in high school and junior high school classes because they are too expensive. In addition, the commonly used pressure gauge and Winkler method have certain limitations because the protocols are complicated, and it is difficult to obtain data as the time available in each class is usually limited to only an hour.

The productmeter is a device that measures changes in oxygen levels, which are linked to photosynthesis. This instrument is similar to the differential gas volumeter developed by Yokohama and Ichimura (1969) (Figures 1, 2). Seaweeds uptake inorganic carbon from seawater through photosynthesis and release oxygen. Inorganic carbon in seawater accounts for approximately 45 ml/L, which is an extremely high percentage compared to the amount of atmospheric CO_2

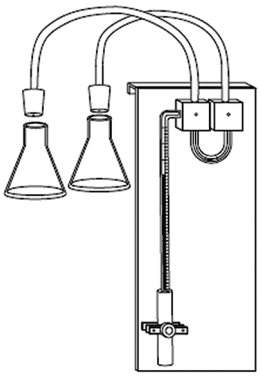

Figure 1: Schematic diagram of the body of the productmeter developed in this study

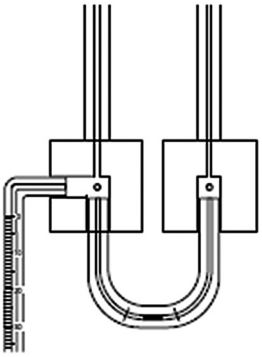

Figure 2: Section of the productmeter responsible for measurements

(0.03 %). Therefore, the majority of CO_2 that seaweeds absorb through photosynthesis is obtained from seawater. However, when seawater is saturated with oxygen, oxygen from seaweeds is released into the air; thereby it does not get dissolved in seawater. Therefore, if seaweed is kept in a container with seawater and air, shaken, and maintained at a constant temperature, oxygen released into the air can be measured using the productmeter, thereby facilitating the determination of the photosynthetic rate.

Since the main parts of the original productmeter were originally made of glass, the previous version of this instrument required careful handling. In addition, petrofabric construction and operation were simplified, which was facilitated by Yokohama et al. (1981; 1986), and ensured rapid measurements were effectively used in the short time period allotted in high school classes (Figure 3). The main parts of the instrument consist of petrofabric, which is a combination of a glass tube and a rubber block using plastic. Furthermore, in the initial model, we used a glass stopcock to block outside air from the interior of the device; however, the operation was later facilitated with a complex plastic tape (Yokohama et al., 1986). Since the productmeter measures increases and decreases in oxygen directly (Figure 3), we wanted to ensure intuitive and easy operation compared to other apparatus. Finally, it is suitable for instructing students in the class about photosynthetic measurements since it is more affordable to construct this device than PAM or oxygen electrodes, and infrared gas analyzers.

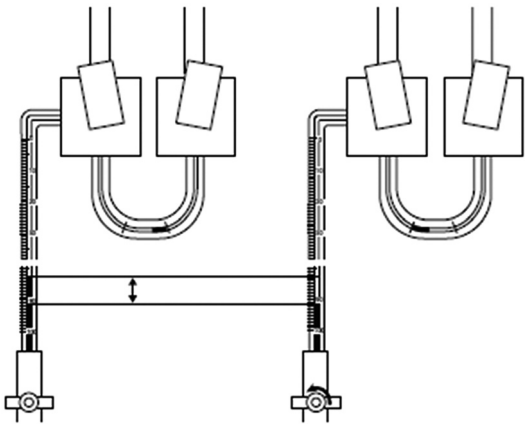

Figure 3: Schematic diagram of the measurement principle utilized by the productmeter

Notes: To measure photosynthesis rates, the left edge of the droplet is matched with the left line, while to measure respiration, it is matched with the right line. The increase or decrease in oxygen content is determined depending on the amount of droplets that move.

2.2 Measurement of algal photosynthesis using the productmeter

Photosynthesis is the most important physiological process in plants, and it is discussed in detail in biology textbooks for secondary education. To determine the photosynthetic rate, it is necessary to measure either the change in O_2 concentration or the change in absorbed CO_2. However, it is difficult to complete these measurements using the school equipment currently available.

For terrestrial plants, using organic differentiation measurements, analyzing a leaf cut from a plant is more likely to produce varying results than those obtained using the whole plant. Therefore, in order to accurately assess the photosynthetic ability of a single leaf, it is necessary to measure the photosynthesis of the whole plant. Although it is relatively easy to obtain this measurement for terrestrial plants, no suitable tools were developed to date for photosynthesis experiments in general education.

In contrast, in algae, including seaweeds, all parts of the plant body equally perform photosynthesis, as well as absorption of nutrient salts and moisture. Therefore, it is possible to measure photosynthesis even if only a small portion of the plant is used, facilitating extreme ease in handling the specimens studied. In addition, CO_2 is rarely the limiting factor in photosynthesis because there is more inorganic carbon present in seawater than in air. Therefore, for the practical demonstration of measurement of the photosynthetic rate among students, seaweed is a more suitable material than terrestrial plants.

2.3 Important factors in the measurement of photosynthesis

In senior high school classes, a conventional productmeter in a small tank is used. A more complex productmeter for higher education would be more effective since it can facilitate accurate measurements by adjusting the water droplet. In order to measure many samples simultaneously, the apparatus was upgraded, and 4 or 6 samples can now be processed together.

Since the instrument can process a larger number of samples, the skill of the operator using the machine is important. Additionally, it is now possible to double-check mea-

surements if 2 replicates of samples are available, which has been described by Suzuki et al. (2013). In any productmeter measurement, the basic operation methods are the same for various productmeters. In order to measure samples accurately, various aspects of the experiment should be regulated, including the quantity of light as well as the length of the light/dark cycle of in relation to natural conditions, especially for algae. For example, in order to complete measurements

Figure 4: Measuring portions of a more complex productmeter

Notes: Measuring portions of a more complex productmeter, in which six samples can be simultaneously analyzed. Basic design of the instrument followed that determined by Suzuki et al. (2013).

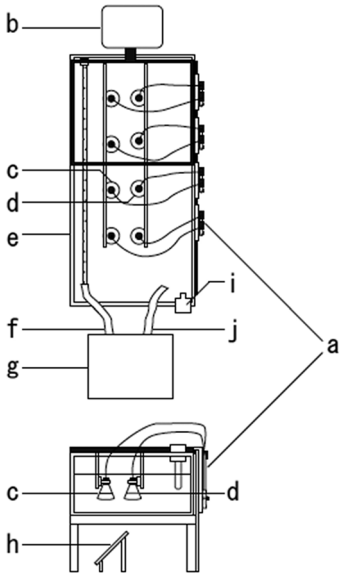

Figure 5: Schematic of the general arrangement of a more complex productmeter

Notes: Schematic of the general arrangement of a more complex productmeter2004 to simultaneously measure changes in four samples (Kurashima, 2004). The letter designations are defined as follows: a (productmeter), b (shaker), c (reaction vessel), d (compensation vessel), e (water bath), f (freeing port), g (temperature controller), h (mirror), i (current motor), j (sluice gate).

for a continuous daylong experiment, it is essential to measure the quantity of light as well as the outdoor light/dark cycle in relation to the algal biorhythm. Thus, if 10:00 A.M. is set as the starting measurement time, it is necessary to adjust the measurement in relation to the quantity of zero light (respirometry), measured at sunset. The productmeter is easily influenced by changes in temperature when measuring large quantities of gas. If the temperature fluctuates during measurement, a straight line is not obtained when the measurements data are plotted. In order to precisely perform the measurement, a regular darkroom with an air conditioner and ventilating fan is needed to avoid changes in ambient room temperature (Kurashima, 2004 partial modification). The objective of the present study was to design a device for application in higher education, and daylong measurements were obtained mainly using a more complex productmeter (Figures 4, 5).

3. Materials and methods

In the study by Suzuki et al. (2013), the principal objective was to compare rates and characteristics of photosynthesis in *Ulva pertusa* and *Ulva meridionalis*. However, in the present study, *U. pertusa* was analyzed since it is characterized by a higher photosynthetic rate. We cultured this species and then performed continuous all daylong measurements. In order to calculate the ideal sample volume, we used a strong light intensity of 400 µmol/m^2/s and conducted experiments over the course of 20-30 minutes, in order to keep ensure the liquid remained in the middle of the scale, as was done previously (Kurashima, 2004), with some adjustments. In the preliminary experiment, the ideal sample of *U. pertusa* was found to be approximately 5 cm^2. We conducted 3 experiments using 5-cm^2 sections of *U. pertusa* laminae. Cork bowlers were used to cut 5 × 1 cm^2 samples from the laminae. We adjusted the water temperature to 30 °C on the first day, 25 °C on the second day, 20 °C on the third day, and 15 °C on the fourth day. In addition, we ensured that the water temperature for *U. pertusa* was adjusted to mimic the seawater temperature off the coast of Japan. We used a public holiday to practice maintaining the water at the correct temperature, and avoiding potential spore emissions produced from the sudden change in temperature between the third day and the fourth day. For educational purposes, we started the measurement on Monday and completed it on Friday. Data obtained on Wednesday was carefully examined.

4. Results and conclusion

4.1 Data handling and results

To assess the raw data, measured values of optical power and photosynthesis (per cm^2) for *U. pertusa* were used to approximate the photosynthesis-irradiance curve with a hyperbolic tangent (photosynthesis speed = $P_{max} \times tanh\ (I / Ik)$

– *R*). Next, changes in optical power over the course of each day were calculated from an approximate formula with a sine curve (optical power at time $t = I_{max} \times (\sin (\pi \times t / D)$ 1.4) to determine optical power at specific water depths from early morning to midnight. Finally, changes in optical power over each day were substituted into the formula for the photosynthesis-irradiance curve to assess changes in O_2 generation rates over the course of one day. Students measured the following: (1) Irradiance (*I*), (2) Photosynthesis (per cm^2), (3) maximum rate of gross photosynthesis (P_{max}), (4) Saturation irradiance (*Ik*), (5) Maximum quantity of light per day (I_{max}), (6) Sunshine duration (*D*), (7) Depth (*d*), (8) Absorption coefficient (*k*) to calculate the daily quantity of oxygen produced from algae per 1 cm^2. We recommend using a worksheet (Figure 6) to process the data automatically.

In comparison to the daylong experiment introduced previously (Suzuki et al., 2013), the culture process was omitted to conclude on a certain day. However, in the present study, we accounted for algal growth by lengthening the folia cut by cork bowlers with tweezers and mounted them with a division. It was necessary to photograph this after the measurement was obtained each day from the exact top of the leaves, to facilitate accurate determination of growth. We then calculated the total area measured after each experiment, using image-processing software. In this study, we conducted measurements at 30 ℃ on the 1st day, 25 ℃ the 2nd day, 20 ℃ the 3rd day, and 15 ℃ on the 4th day in order to determine the optimum water temperature for growth. These measurements also revealed the changes in the rate of photosynthesis at different temperatures, with the highest rate measured at 30℃ and the lowest at 15℃.

4.2 Conclusion

Based on the method proposed by Suzuki et al. (2013), we established specific and accurate procedures to test daylong measurements and compare various photosynthesis characteristics of *Ulva pertusa*. Therefore, we expect that the method and instrument developed in this study can be utilized in the experiments for students. By performing continuous measurements during algal cultivation using a productmeter, we aimed to obtain real time data, and discover applications suitable for biological experiments in either teacher training courses using long-term continuous measurements, or research. The productmeter is used in the physiological study of many different types of seaweeds as well as non-marine life. This is not limited to an educational use, and has been used in a variety of studies.

Previous studies have used measurements of growth to clarify a level, distribution of seaweed, seasonal prevalence, in relation to photosynthesis, as well as the temperature and quantity of lite to the quality of photosynthesis (Yokohama, 1973a; 1973b; 1973c; Kageyama and Yokohama, 1974; Mizusawa et al., 1978; Murase et al., 1989; Sakanishi et al., 1989; Murase et al., 1994; Maegawa and Sugiyama, 1995; Kurashima et al., 1996; 2003; Serisawa et al., 2001; Murakami et al., 2004). In particular, Sakanishi et al. (1988), developed a technique

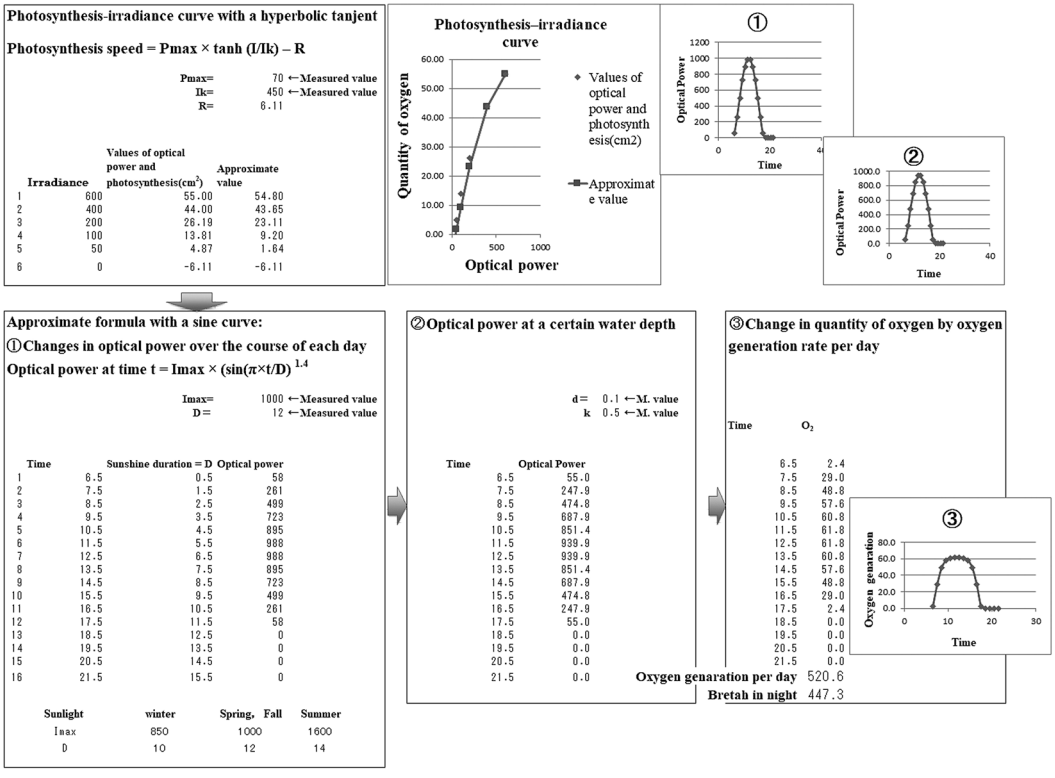

Figure 6: Scheme of table that can be used to track measurements and calculate daily rates of oxygen generation

to measure photosynthesis of kombu using folia, to perform tectonic analysis of large algae and seaweeds.

Maegawa et al. (1987; 1988) investigated *Eisenia bicyclis* and *Ecklonia cava*, Murase et al. (2000) studied *Sargassum macrocarpum*, and Abe et al. (2003; 2008) focused on *Zostera marina* and *Zostera japonica*;. these authors clarified that the distribution pattern of individual marine products large size plant depended on the photoenvironment in the community, which could be measured with a productmeter.

In these studies, photosynthesis was measured across a wide variety of large marine plants, by assessing a characteristic shared by green algae, red algae, seagrass (including the brown algae such as kombu), bladder wrack using the productmeter. However, measurements of plant photosynthesis, except for in the case of seagrass and seaweed, can be measured by the productmeter. Previous researchers performed measurements by improving the containers used for sampling. Maegawa et al. (1988), investigated respiration in *Haliotis diversicolor aquatilis* and *Toxopneustis pileolus*, *Scorpanodes guamensis*, and Yokohama et al. (1989) analyzed photosynthesis in *Fungia fungites*, *Galaxea fascicularis*, as well as *Montipora aequituberculat*. In addition, Nakamura (2003) and Nakamura et al. (2003) measured the photosynthetic rate of *Acropora pruinosa*, which is a temperate-zone coral, and clarified the influence of different temperatures on tropical corals.

Since petrofabric facilitated the manufacture of a simple productmeter, training on photosynthesis measuring equipment has become easier. However, we ensured the measurements were easy because the instrument is simple and can be used to measure photosynthesis and respiration in various plants. Further studies on the measurement of photosynthetic rates obtained over the course of an entire day using a productmeter would facilitate the estimation of the rate of algal growth and overall productivity of algal beds, which would be beneficial for environmental maintenance, reproduction, and creation of pristine zones along coastal areas.

Acknowledgements

Funding was provided by the "Women Researcher Promotion Program" research skill-up grant (Chika Suzuki).

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(Received: March 23, 2020; Accepted: March 31, 2020)