Journal of Marine Biology & Oceanography

Research Article A SCITECHNOL JOURNAL

Proposal of a Culture Method for Maintaining High Growth of Sterile *Ulva* spp./species (Chlorophyta)

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Received date: April 4, 2022, Manuscript No. JMBO-22-59398;

Editor Assigned date: April 6, 2022, PreQC No. JMBO-22-59398 (PQ);

Reviewed date: April 18, 2022, QC No. JMBO-22-59398;

Revised date: April 28, 2022, Manuscript No. JMBO-22-59398 (R);

Published date: May 5, 2022, DOI: 10.4172/2324 -8661.1000230

Abstract

To determine height growth conditions of sterile *Ulva lactuca*, green algae collected from Tokyo Bay, Japan, were evaluated for efficient culture. A culture tank simulating the sea surface was prepared, and the maximum condition of specific growth rate was verified. This was achieved using the Photosynthetic Photon Flux Density (PPFD) of the bottom of the culture tank as an index-an alternative to leaf area index. In the results, the specific growth rate showed the highest value at the average PPFD of 20.6 μmol m^{-2} s^{-1} for one week on the bottom of the culture tank. The specific growth rate decreased significantly at 4.0 μmol m^{-2} s^{-1}. From these data, the method of controlling the density of sterile *Ulva* using the PPFD of the bottom surface as an index will lead to a culture control method. Consequently, it is possible to maintain a high growth rate of sterile *Ulva* without the complicated leaf area index measurement. Based on these data, we propose a new type of efficient, sterile *Ulva* production system instead of our model that has been advocated using enriched seawater.

Keywords: *Ulva lactuca*; Sterile *Ulva*; Growth rate, Specific growth rate, Photosynthetic photon flux density

Introduction

The eutrophication of warm inner bays progresses along with social activities such as the excessive fertilization of fields [1-3] and the accumulation of food residues on the seabed by sea surface aquaculture [4-6]. This is especially the case in the coastal bay area where the replacement of seawater is small, and there are concerns about the impact on aquatic fisheries such as the occurrence of red tide [7,8].

However, in such areas, the overgrowth of sterile *Ulva* spp. and their seawater remediation function have been reported, especially in shallow water. Furthermore, in Southern Kyushu in Japan, attempts have been made to suppress the accumulation of N and P in the aquaculture area by adjoining a cage for the growth of sterile *Ulva* spp.. As a result, the improvement of marine environmental technology is desirable. As part of this technological development, sterile *Ulva* spp. are being cultured and produced as a functional food and feed for fish and shellfish. This has the potential to reduce N and P levels in the inner bays [9-11]. Furthermore, while biomass-based global warming response technology is drawing attention, sterile *Ulva* have the property of easily maintaining grown organisms, so they not only absorb CO_2 but also fix CO_2. It can also be expected as a biological resource for so-called blue carbon [12,13]. Thus far, we have evaluated the growth rate of sterile *Ulva* spp. for 37 days. This was done using a culture tank on land that simulates the sea; as a result, the production of edible parts of rice is five times that of grains. It has been confirmed that it exhibits an above high growth rate [14]. Furthermore, in order to maintain a high growth rate, it has been suggested that culture control using the Leaf Area Index (LAI) as an index is an important factor for the total leaf/culture area.

Nevertheless, in order to measure LAI, it is necessary to calculate the total area of sterile *Ulva* spp. over time during culture. For this measurement, the following procedure is followed: 1) Collect sterile *Ulva* spp. from the culture tank, 2) Measure and confirm the number of individual sterile *Ulva* spp., 3) Measure the area of each sterile *Ulva* spp. (numerous steps involving measurement), and 4) Calculate the total area measured. Therefore, LAI is an index that is difficult to use easily for efficiently producing sterile *Ulva* spp.. In particular, the index was found to be difficult to apply when assuming the production of large-scale sterile *Ulva* spp.. Consequently, in this paper, we focus on the Photosynthetic Photon Flux Density (PPFD) on the bottom of the culture tank as an alternative index to LAI. In addition, we clarify the relationship between the photosynthetic photon bundle density on the bottom of the culture tank and the proliferation of sterile *Ulva lactuca*. Furthermore, the efficient culture production mode of sterile *U. lactuca* is described, using the photosynthetic photon flux density at the bottom of the culture tank as an index.

Materials and Methods

Selection of *Ulva* species

In this study, we chose to use *U. lactuca*, obtained from the marine park at Yokohama in Tokyo Bay, as this species is known to stably increase in culture.

Measurement of photosynthetic photon flux density on the bottom of the culture tank

The PPFD (μmol m^{-2} s^{-1}) at the bottom of the culture tank of *U. lactuca* was measured three times a day, and the average PPFD was calculated every week.

Cultivation equipment

The main cultivation equipment consisted of a seawater pump, seawater tank, water pump, aeration device, and culture tank. Seawater was pumped by the seawater pump and collected in the tank. From here it was supplied to the culture tank using the water pump. The 42-L culture tank had light-shielding material covering its sides so that sunlight could only enter from the top surface (0.1 m^2), so as to

simulate the light reception situation in the sea. The culture tank was installed in the daylight solar irradiation area on the north side of Imari satellite, Institute of ocean energy, Saga university, Japan.

Culture conditions

The locations of the *U. lactuca* sampling sites in Japan are shown in Figure 1. *U. lactuca* was isolated from Tokyo Bay [8] and the growth rates (g-dry·m^{-2}·d^{-1}) evaluated using the culture tank in Imari (Figure 1).

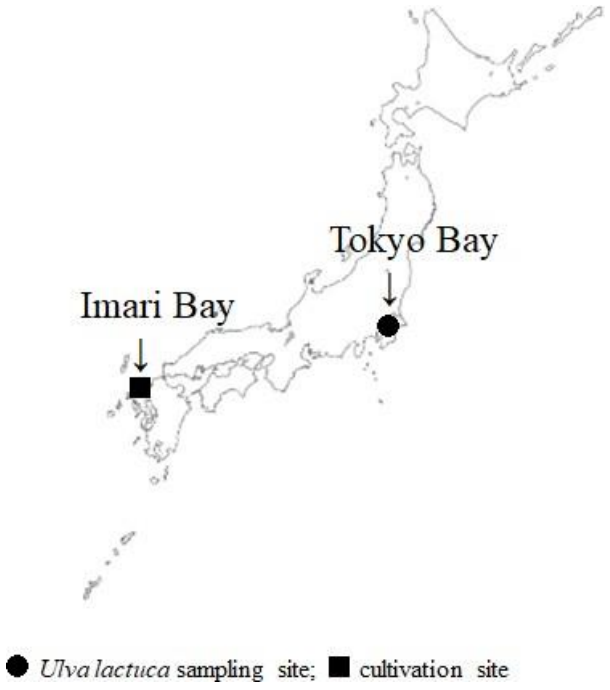

Figure 1: Locations of the *Ulva* spp. sampling sites in Japan.

A total of 254 sheets of φ 48.5 mm *U. lactuca* was added to the culture tank to give an LAI of five. A culture was performed at a seawater flow rate of 2 L/h and an aeration rate of 24 L/h. Because the growth rate of *U. lactuca* has been shown to depend more on the concentration of NO_3-N than PO_4-P [8], PO_4-P was set to a constant value of 0.04 ppm in the culture. However, concentrations of NO_3-N were maintained at 0.8 ppm.

Measurement of growth rate

During the culture period, the wet weight of *U. lactuca* was measured once a day as an index of growth status. The dry growth rate (g-dry·m^{-2}·d^{-1}), meanwhile, was measured after the culture was completed.

Results and Discussion

Culture process for *Ulva lactuca*

The transition of wet weight was measured as an evaluation index of the growth degree of *U. lactuca* in the installed culture tank, with the results shown in Figure 2. The wet weight increased with time, and it was confirmed that it grows smoothly in 0.8ppm-NO_3-N seawater by stirring. Furthermore, in order to grasp the transition of the growth rate more accurately, the specific growth rate was measured, with the results shown in Figure 3. From this measurement, it was found that the specific growth rate became maximum on the seventh day of culture, and then decreased. In this culture environment, the supply of CO_2 by NO_3-N and aeration is sufficient, suggesting it may be light limitation that is a growth factor after seven days (Figures 2 and 3).

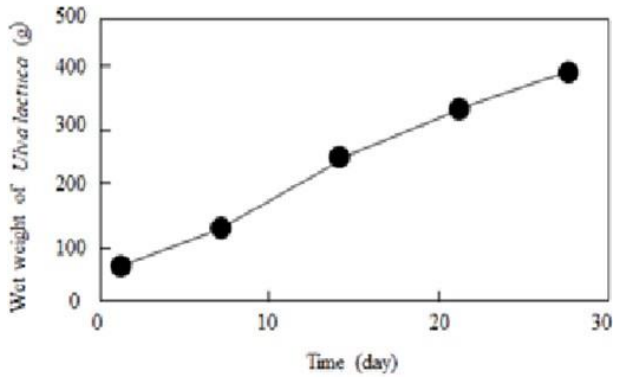

Figure 2: Changes in the growth rates of *Ulva* spp. on a wet weight basis during culture under conditions simulating the sea.

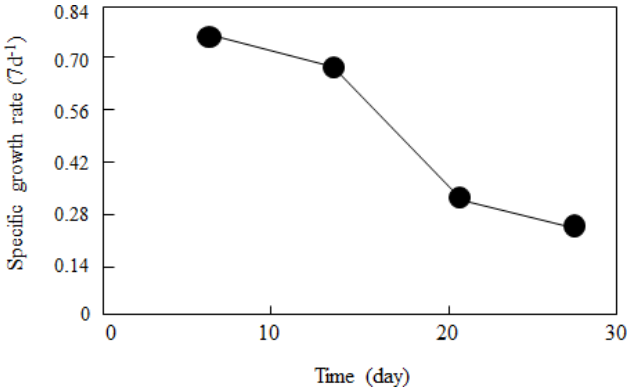

Figure 3: Productivity of *Ulva* spp. in model reactors based on the specific growth rate and Leaf Area Index (LAI).

As a result, as an index for grasping the degree of light used in the culture tank, we focused on the PPFD at the bottom of the tank. We then measured the transition to clarify the relationship with the growth rate. In other words, if the light condition that maintains a high specific growth rate can be found, it is thought to be possible to obtain more real-time information on the growth of *U. lactuca*. The PPFD at the bottom of the culture tank was therefore measured, and the results aggregated every week (which is the growth evaluation period for *U. lactuca*). Although it was measured three times a day, the average PPFD reached a maximum of 20.6 μmol m^{-2} s^{-1} after seven days (one week). It then decreased significantly after seven days, and seven after 28 days. In other words, it was found that the PPFD decreases to 4.0 μmol m^{-2} s^{-1} after 14 days, to 3.7 μmol m^{-2} s^{-1} after 21 days, to 1.2 μmol m^{-2} s^{-1} after 28 days, and to 1/17 after seven days. In this study, the PPFD was measured three times a day, and the relationship with the growth rate verified every seven days. In order to improve the degree of accuracy, it was necessary to increase the measurement frequency of the PPFD and the specific growth rate of *U. lactuca*.

Figure 4 shows the relationship between the LAI and the average value of the PPFD on the bottom of the culture tank. With the increase in LAI, the following became clear: The PPFD at the bottom of the culture tank decreased, and after seven days of culture, the PPFD at the bottom of the culture tank became 20.6 μmol m^{-2} s^{-1} under the condition of LAI 13.3. Up until this point, we have determined the specific growth rate using LAI as an index, and have shown that the specific growth rate peaks on the seventh day of culture in some *Ulva* spp. [14]. The LAI can be measured by 1) Harvesting *U. lactuca* from the culture tank, 2) Measuring and confirming the number of *U. lactuca*, 3) Measuring the area of *U. lactuca*, and 4) Calculating the total area of the measured *U. lactuca*. This involves a significant number of steps consisting of calculating the sum. Furthermore, in order to suppress the effects on *U. lactuca*, it was necessary to complete these operations in a short time and return the *U. lactuca* to the culture tank. Accordingly, LAI is an index that is difficult to use as an index for the efficient production of sterile *U. lactuca*. It was also found to be difficult to apply this index when the large-scale production of *U. lactuca* is assumed.

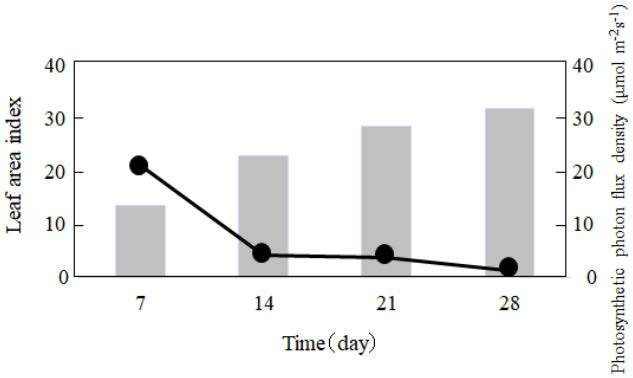

Figure 4: Relation between leaf area index and photosynthetic photon flux density in model reactors. ▒ leaf area index; ●Photosynthetic photon flux density

In this respect, the measurement of the PPFD at the bottom of the culture tank is simple. This method of finding the conditions for maintaining a high specific growth rate, based on the PPFD, is easy to apply to large-scale culture tanks. It is therefore highly likely that the technology will be directly linked to industrial applications. Nevertheless, the water temperature of the culture tank during the daytime when it is exposed to sunlight. After 20 days of culturing, the days when the temperature fell below 15°C became prominent even during the day. It was thought that the effect of the decrease in specific growth rate due to temperature might be added.

Evaluation of the growth rate of *Ulva lactuca*

Based on the above culture results, 28 days after culturing, *U. lactuca* was washed with water, the dry weight measured and the growth rate (g-dry·m^{-2}·d^{-1}) determined. As a result, it was as high as 12.6 g-dry·m^{-2}·d^{-1}. The water in the culture tank contained 0.8 mg/L (ppm) NO_3-N and 0.04 mg/L (ppm) PO_4-P, which are the normal concentrations recorded in Tokyo Bay. Therefore, it was shown that efficient biomass production is possible even under relatively low temperature conditions with this level of NO_3-N concentration, temperature, and air (CO_2) supply.

Potential for culture production of *Ulva lactuca*

During the 28 days of *U. lactuca* culture, no contamination of other seaweeds or adhesion of diatoms in the culture vessel were observed, and the biomass yield of the edible portion was 8.4 times that of rice cultivation. From this, it is considered that the *Ulva* spp. culture type shows a high productivity of edible parts.

Accordingly, an evolved version of the continuous production system of *U. lactuca* [16], has been proposed, using LAI as an index. Figure 5 shows the *U. lactuca* culture production system, using the PPFD at the bottom of the *U. lactuca* culture tank as an index. The production system for *U. lactuca* includes the following six steps: 1) Select a sterile *Ulva* sp., 2) Cultivate in a culture tank equipped with an aeration device under NO_3-N concentration control, using the PPFD as a growth index, 3) Collect and effectively use the plants approximately every seven days, 4) Adjust the collected part of *U. lactuca* that is cut out, 5) Transfer the cut out *U. lactuca* to the culture tank as a seed, and 6) Effectively use *U. lactuca* residue after drilling. This system enables the continuous production of *U. lactuca* under culture conditions while maintaining a high growth rate (Figure 5).

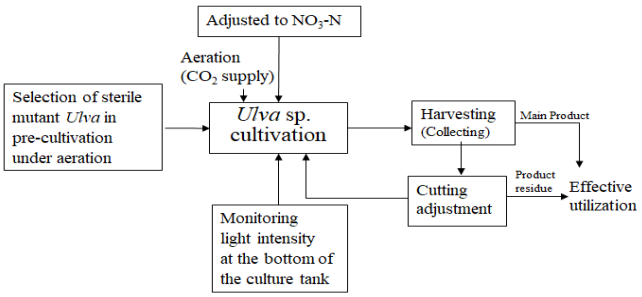

Figure 5: System for the efficient, continuous production of sterile, mutant *Ulva* spp. using enriched seawater.

Sterile *U. lactuca* can be used as a feed and functional food for farmed fish and poultry [15,16]. Therefore, this system has the potential to contribute to food and feed production. Furthermore, the installation of this production system in countries with warm seas would allow food self-sufficiency to be achieved, which is currently an issue for Japan. In particular, this system, which does not use LAI as an index, has the potential to become a labor-saving production system with less complicated operations for production control.

The development of a continuous culture production method for *U. lactuca*, which allows production and harvesting to occur in eutrophic areas, is becoming increasingly feasible. Furthermore, *U. lactuca* collected from the coast contain a useful sulfur-containing amino acid (D-cysteinolic acid) and have also been reported to have singlet oxygen and neutral fat suppression effects [16]. In addition, anti-Methicillin Resistant *Staphylococcus Aureus* (MRSA) suppression effect has also been reported [17-19]. The next step will therefore be to determine the content of useful substances in *U. lactuca* grown under culture conditions, with the ultimate goal of accumulating these substances in cultured plants, adding further to their value.

Conclusion

In this study, it was found that sterile *U. lactuca* can be effectively produced under enriched culture conditions, controlled by an index as the PPFD at the bottom of the culture tank. The measurement of the

PPFD at the bottom of the tank is simple, and this method of finding the conditions for maintaining a high specific growth rate based on the PPFD is easy to apply to large-scale culture tanks. As a result, it is highly likely that the technology will be directly linked to industrial applications. In other words, this Japanese production system namely Saga model applied to eutrophic inner bays in warmer regions of the world. Furthermore, since this production system is capable of high biomass production, it has potential as a food production system using enriched seawater.

Acknowledgment

We gratefully acknowledge the work of past and present members of Institute of ocean energy, Saga University.

Conflicts of Interest

No conflict of interests declared.

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