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THE GROWTH OF TWO MARINE-MICROALGAE (Chaetoceros gracilis and Tetraselmis chuii) IN SEMI-CONTINUOUS CULTURE METHOD

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ABSTRACT

The growth performance of two marine-microalgal species, i.e. a centric diatom-Chaetoceros gracilis and a green algae-Tetraselmis chui , were investigated. Semi continuous-200 litre plastic bags facilitated with a 0.2 µm hydrophobic filter membrane for each were required to culture each species of the microalgae. Aseptical method was employed during algal culture, harvesting, and refilling. The algal density in the bag culture was monitored every 2 days. Results indicated that the average values of mean generation time for C. gracilis and T. chui were 18.83 and 25.14 hours, respectively. The average values of bacterial counts taken from inoculated C. gracilis and T. chui culture samples were 9.9 x 10E6 and 7.8 x 10E4 bacteria/ml media. The importance of bacterial monitoring in relation to recommended culture method is discussed briefly.

Key words: growth, marine-microalgae, culture

I. INTRODUCTION

Semi-continuous culture differs from batch culture in that as the alga grows fresh medium is added while an equal volume of culture (i.e. medium plus organisms) is removed. Although culture of limited volume relatively simple to set up and a large number of culture may be be grown simultaneously, both the environment and the algae in a batch culture change continuously. As the organisms grow they take up nutrients, evolve oxygen, generate extracellular products, and, as the culture becomes dense, the amount of light each cell receives decreases. In poorly buffered media, large change in pH may also occur.

The basic requirements for growth (e.g. water, light, and certain inorganic solutes) of the algae which constitute the phytoplankton are much the same as those for other photo-synthetic plants. However, light is only available near the water surface whereas, because growth of phytoplankton converts them into particulate from subject to sedimentation, some essential elements are often only available in deep water.

In this investigation, two species of marine-microalgae, a centric diatom-*Chaetoceros gracilis* and a green algae-*Tetraselmis chuii*, were each cultured in a semi-continuous-200 litre plastic bag. A 0.2 μm hydrophobic filter membrane was employed. It enables to control bacterial level in algal culture (Brown, 1989). Lewis et al. (1988) found that the use of 0.2 μm filter resulted in significantly lower bacterial counts comparing to pasteurization.

Another advantage of using 0.2 μm filter is able to get rid of all of *Vibrio* sp. from sea water and air. Even though the number of bacteria that pass through the filter are still relatively high, Lewis et al. (1988) didn't recommend the use of 0.1 μm filter. It is probably to avoid a predominant of certain bacteria in the algal culture. However, the number of bacteria allowed in the cultural media shouldn't be higher than 2.4×10^6 cells/ml (Lewis et al., 1986).

The aim of the investigation is to study algal growth performance, including the growth pattern before and after harvesting, and the values of algal-mean generation time.

II. MATERIALS AND METHODS

2.1. Preparing a Bag Culture

The following procedure was conducted aseptically; and all of the fittings used have been autoclaved. First of all, a plastic bag was put in laminar hood after it was checked for flaws, holes or other damages. Hands, scissors and one corner of the plastic bag were sprayed with metho. The corner then was cut and a prepared-sterilized air-outlet filter was inserted in quickly. Soon, it was fitted and fixed tidily and tightly with electrical tape. The same steps were employed for multipurpose inlet to another corner.

Before the bag was thoroughly put into wire-mesh frame, the frame was cleaned and checked for any debris or sharp edge. The bag was blown with 0.2 μm-filtered air through the multipurpose inlet and then partially was filled with filtered sea water to shape it. Sea water was filtered through a series of filters of 5 μm (nominal), 1μm (nominal), 1μm (absolute), 0.45 μm (nominal) and 0.2 μm (absolute). The shape of bag culture was maintained with no flaws or folds to avoid a death area. Finally, after a spike was stabbed into the bag aseptically at about the middle, a tap was inserted into this hole.

2.2. Inoculation of Starter Culture and Nutrients

Each bag was inoculated with an axenic starter culture from 3 litter flask using a sterilized silicon tubing transfer

line through multipurpose inlet. The same operation was done for transferring f2 medium as nutrients. In *Chaetoceros gracilis* -bag culture, sodium metasilicate was added aseptically into the f2 medium. It was done in laminar hood. The important feature in this procedure is that each step was done quickly; and metho was used liberally to ensure the aseptic technique.

2.3. Harvest and Refill

Harvesting as much as 60 litres each was done in exponential phase just before a phase of declining relative growth rate. First harvest was in day-8 while the second was in day-12 for both cultures. One end of chlorinated silicon tubing was connected to tap and the other end was placed into a container. Before and after harvesting, the tap was sprayed with metho. Moreover, the tap should be turned back at the same position with the former position.

After harvesting, the bag was refilled with 0.2 µm-filtered sea water and was then added with 50 % volume of nutrients of f2 medium through multi-purpose inlet. This operation was done aseptically as well.

The average value of mean generation time can be calculated using the following formula:

$$G = (\ln 2 \times t) / (\text{Ln } Nt - \text{Ln } No)$$

(Fogg and Thake, 1987);

In which G is a mean generation time; t is time in hours; Nt is number of cells at time-t; No is number of cells at time-0 (initial).

2.4. Culture Conditions

Algal culture media was conditioned as shown in the Table 1.

Table 1.

Algal culture condition during the experiment

Condition	Level	Unit
Light intensity	350	$\mu Em^{-2}s^{-1}$
Photoperiod	16/8	L/D
Temperature	20	^0C
Salinity	35	ppt
pH	8.0 - 8.4	-
Aeration rate	?	l/min
CO_2	0.5	%

III. RESULTS AND DISCUSSION

3.1. Results

Cell densities over 2 weeks of the two species were plotted in each curve as depicted in Figure 1. The exponential growth phase can be predicted from these curves and therefore, the average value of mean generation time can be calculated.

The results of mean generation time calculations are represented in Table 2.

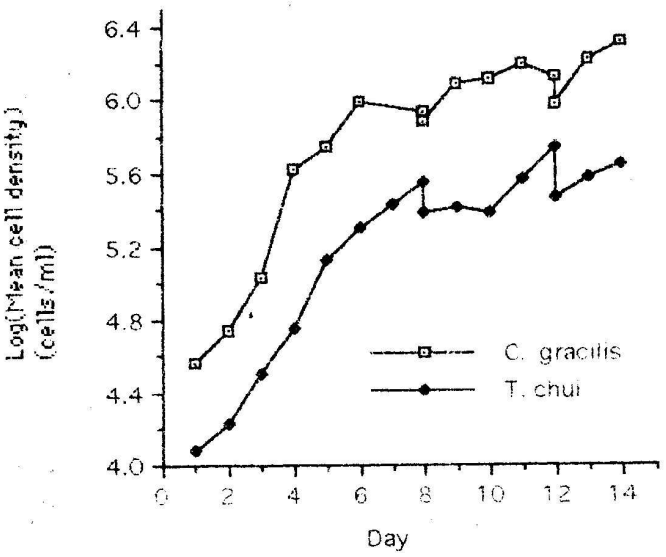

Figure 1

Growth curves of *Chaetoceros gracilis* and *Tetraselmis chui* in 200 litre semi continuous-bag culture

Table 2.

The calculations of mean generation time during the exponential phase

Count	Cell number of each species (cells/ml)	
	Chaetoceros gracilis	*Tetraselmis chui*
$Ln(N_0)$	$Ln(5.5 \times 10^4) = 10.92$	$Ln(1.72 \times 10^4) = 9.75$
$Ln(N_1)$	$Ln(1.06 \times 10^5) = 11.57$	$Ln(3.22 \times 10^4) = 10.38$
$Ln(N_2)$	$Ln(4.22 \times 10^5) = 12.95$	$Ln(5.67 \times 10^4) = 10.95$
$Ln(N_3)$	-	$Ln(1.32 \times 10^5) = 11.79$
G1*	25.60	26.41
G2*	12.06	29.19
G3*	-	19.81
G-average	(G1 + G2)/2 = 18.83	(G1 + G2 + G3)/3 = 25.14

*) t = 24 hours

Samples required to estimate bacterial levels in each culture were collected at day-12 coinciding with second harvesting. The total number of marine bacteria was predicted by semi-quantitative method using SWAV plates readings. While the presence of Vibrio sp. was detected using TCBS plates readings. Both *Chaetoceros gracilis* and *Tetraselmis chui* cultures were free from *Vibrio* sp.

3.2. Discussion

3.2.1. Algal growth phases

Growth patterns of *Tetraselmis chui* and *Chaetoceros gracilis* cultures are represented in Figure 1. Lag phase does not appear obviously as an ideal growth curve. It may be due to the starter cultures used which were exponentially growing populations or due to the sampling which was done daily instead of every hour. High level of ions Ca^{+}, Mg^{+}, or phosphorus is able to extend lag phase as well (Hoff and Snell, 1989).

Exponential phase is considered occurring from day-1 to day-4 in *Chaetoceros gracilis* -curve or from day-2 to day-5 in *Tetraselmis chui* -curve as in that time cell density increases rapidly according to a logarithmic function and the curve then tends to decline. This assumption is used to calculate G-values.

If the cell densities at harvest time are compared, it seems that *Chaetoceros gracilis* should be harvested at day-6 as the density reaches nearly 1.9×10^6 cell/ml. It is the reason for the drastic decrease of this curve which occurs before first harvest. While first harvest for *Tetraselmis chui* occurs in a correct time as recommended by Brown et al. (1991), that is done when the density is nearly 0.4×10^6 cell/ml. Harvesting time during exponential phase is important in terms of high level of biochemical composition content of the algae (Brown et al., 1989; Fogg and Thake, 1987).

The phase of declining relative growth rate occurs when media lacks nutrients. While in stationary phase, biochemical composition in algal cells tends to be minimum (Fogg and Thake, 1987). Finally, death phase occurs when nutrients in the media are extremely inadequate. Bacteria then grow rapidly in the algal culture.

3.2.2. Mean generation time (G)

Mean generation time value of *Chaetoceros gracilis* is 18.83 hours, whereas that of *Tetraselmis chuii* is 25.14 hours (see Table 2). The higher G-value of *Tetraselmis chuii* is due to the larger volume of the cell. Therefore, it requires more time in cell division. Fogg and Thake (1987) and Watson (1979) reveal that mean generation time is related to the cell size and light intensity.

3.2.3. The importance of bacterial monitoring

Bacteria are present in algal culture through 2 sources, i.e., from non-axenic starter inoculum of microalgae and from sea water used in the growth medium (Lewis et al., 1988). Furthermore, they found that the number of bacteria in sea water always exceed 30.2 cells/ml.

The level of bacteria is examined using SWAV and TCBS plates reading. When the level ranges from 10^5 to 10^7 bacteria/ml, the culture then is ready for harvest (Lewis et al., 1986). The level exceeding 2 x 10^5 bacteria/ml or the presence of *Vibrio* sp. harms the oysters larval rearing (Lewis et al., 1988). Therefore, filtering sea water through 0.2 µm membrane and harvesting cells in the exponential phase of growth enable to keep bacterial numbers down (Brown et al., 1989).

3.2.4. Bacterial level in the algal culture

Total numbers of marine bacteria present in *Chaetoceros gracilis* and *Tetraselmis chuii* culture were calculated. Compared to the allowed bacteria level in the media of algal culture, the total bacteria level in *Chaetoceros gracilis* culture is significantly high. It may be due to the starter culture of this algae which has been contaminated with bacteria (non-axenic). But Lewis et al., (1988) found that the use of axenic and non-axenic starter culture didn't result in the difference of bacterial counts.

Another mistake may occur during diluting the sample into 7 McCartney bottles. Lack of shaking may cause the bacteria in the liquid do not distribute homogeneously. Therefore, the data gained didn't represent the correct number of bacteria in the media culture.

In contrast, the bacterial level in the *Tetraselmis chuii* culture is much lower than those of allowed bacterial level. It may be due to this culture which was inoculated from an axenic starter culture. Another reason is that the cell size of *Tetraselmis chuii* is much larger than that of *Chaetoceros gracilis* (Brown, 1991). Therefore, it has a relatively smaller surface area, and so, is less potential to the bacterial growth.

Nevertheless, no bacteria were found on TCBS plates of the two cultures. It indicates that no external contamination occurred (Lewis et al., 1986) and the use of 0.2 µm filter was effective to get rid of *Vibrio* sp. from the culture.

3.2.5. Bacterial level allowed and recommended in the culture

Bacterial levels allowed in the algal medium culture before fed the medium to oysters larval rearing are $6.17 \times 10^5 \pm 4.17$ cells/ml and $7.4 \times 10^5 \pm 2.09$ cells/ml, while the level of $2.40 \times 10^6 \pm 5.89$ cells/ml may cause diseases for the larvae (Lewis et al., 1986).

IV. CONCLUSION

To provide algal live food continuously, semi-continuous culture is a recommended method to be applied. This method will also maintain the cultured algal grow leading to the exponential phase. Mean generation time of each algal species differs depending on many factors, such as cell size and media condition. Bacterial number in the algal culture can be reduced by employing 0.2 µm hydrophobic filter membranes. However, thorough bacterial monitoring

is required and therefore the bacterial growth and number could be limited as its high level will cause the cultured algae to grow slowly or even die. The bacterial count in the cultural media must be lower than 2.4x10E6 cells/ml.

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