Mem. Fac. Fish., Kagoshima Univ.
Vol. 31 pp. 153~160 (1982)

Ecological Succession of *Chlorella saccharophila*, *Brachionus plicatilis*, and Autogenous Bacteria in Culture Water

Hachiro HIRATA*[1], Masakazu USHIRO*[2], and Ikuo HIRATA*[3]

Abstract

The ecologiacl succession of *Chlorella saccharophila*, *Brachionus plicatilis*, and autogenous bacteria in culture water was observed for the purpose of analyzing their microcosms and to determine the dietary efficiency of bacteria for the culture of *B. plicatilis*.

B. plicatilis were fed *C. saccharophila* by using 3 *l* flasks at the beginning of the experiments. Several species of bacteria were observed to grow autogenously in the cultured water. Population densities of *C. saccharophila, B. plicatilis*, and autogenous bacteria were monitored daily. The behaviour of autogenous bacteria was closely observed by the introduction of BGA (bacterial growth ability). BGA was determined by measuring turbidity of population growth of autogenous bacteria which were sampled from the water and cultured in flasks.

Ecological succession in the flasks were clearly found in each observation. *B. plicatilis* preferably consumed *C. saccharophila* during the first few days. Population density of autogenous bacteria gradually decreased after *C. saccharophila* were consumed. Moreover bacterial density increased again when the population density of *B. plicatilis* finally decreased.

Introduction

Chlorella, rotifer, and bacteria in culture water play the vital roles of producers, consumers, and decomposers, respectively. The study of the ecological succession of these three organisms would not only serve as a critical key to the understanding of their microcosms[1)] but also play a very significant role in the mass culture of rotifers[2,3)].

Considerable research on the growth of rotifer by supplying marine *Chlorella* and baker's yeast was initiated by HIRATA and MORI[4)] in 1967. FURUKAWA and HIDAKA[5)] assessed the dietary efficiency of marine yeast for mass culture of rotifers. YASUDA and TAGA[6)] reported on the growth of rotifers by feeding certain species of cultured bacteria in batch incubation. Ecological succession of *Chlorella*, rotifer, and autogenous bacteria, however, has not been reported elsewhere.

The growth of autogenous bacteria by the introduction of BGA (bacterial growth ability)

*1) Faculty of Fisheries, Kagoshima University, Shimoarata 4, Kagoshima, 890, Japan
*2) 8-4, Niitaka 3-chome, Yodogawa-ku, Osaka, 532, Japan
*3) Kumamoto Prefectural Station for Protection of Minamata Pollution, 1-5-41, Shiomi-cho, Minamata, 867, Japan

was closely observed and given special emphasis in this research. Furthermore, the dietary efficiency of bacteria for mass culture of rotifer is discussed in this paper.

Acknowledgements: We wish to thank Mr. Shigehisa YAMASAKI for technical assistance, and to Miss Socoro C. CASTRO for looking over the English manuscript.

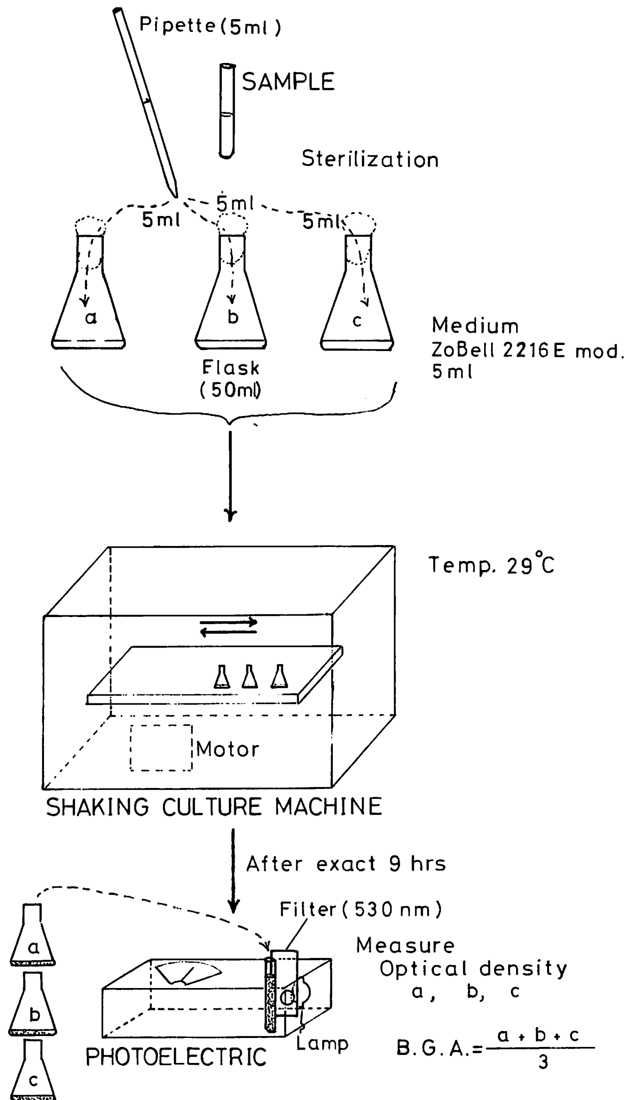

Fig. 1. Schematic view of shaking culture method for BGA (bacterial growth ability).

Materials and Methods

Culture Experiments

Chlorella saccharophila, *Brachionus plicatilis*, and several species of autogenous bacteria were utilized as producers, consumers, and decomposers in the culture water, respectively.

Five three-liter flasks (A~E), each placed with 1.5 *l* of culture water were used in the experiment. Temperature and salinity in the water were kept at 22℃ and 25 ‰, respectively. Likewise, photoperiod was controlled at L: D = 15: 9. The flasks were kept in an incubator (Sanyo MIR 250).

The experiments were done on two separate occasions, namely: Exp. I and Exp. II. *C. saccharophila* used in each experiment were cultured using bioconversion medium, basically composed of fermented trash fish[7,8]. In Exp. I, *C. saccharophila* were directly introduced into the flasks without filtration, whereas, in Exp. II, *C. saccharophila* were filtered through a paper with a mesh size of 5 μm. Prior to the start of each experiment, *B. plicatilis* were pre-cultured in the incubator for a period of about two weeks in order to acclimate the organisms to the experimental conditions mentioned above.

At the beginning of each experiment, the population density of *C. saccharophila* was maintained at 8~10 x 10^6 cells/ml in each flask, except Flask 2C which was prepared for '*Chlorella* control' (*Chlorella* absent). The densities of *B. plicatilis* were varied at 0, 1, 2, 5, and 10 indiv/ml in Flasks 1A, 1B, 1C, 1D, and 1E, respectively. Flasks 1A and 2A were employed for '*Brachionus* control' (*Brachionus* absent). The Flasks 2C, 2D, and 2E were all used for 10 indiv/ml.

Quantitative Observation of Organisms

The population densities of *C. saccharophila* and *B. plicatilis* were monitored daily by slide counting methods with the aid of microscopes. The density of autogenous bacteria was measured by three methods: 1) DCM; direct counting method, using an epi-flourescent microscope[9,10]: 2)MPN; most probable number, using three test tubes: and 3) BGA; bacterial growth ability, using optical density as an indication of bacterial density in the culture water.

In order to reduce tedious handling for observation of bacterial density and to assure more precise experimental data, simpler and modified BGA methods were introduced in this research. Prior to observe the BGA, *B. plicatilis* and other organic aggregates in the culture water were eliminated through a filter paper having a 5 μm mesh size. This filtering procedure was taken in DCM and MPN. The method is described as follow: 1) five ml of the filtered water was sampled and placed in a 50-ml flask containing 5 ml of modified ZoBell 2216E medium[11] : 2) the sample was then shaken in an agitator (Takasaki TOR-1) for nine hours at 29℃: and 3) the optical density of bacteria in the flask was determined using a photoelectric colorimeter with 530 nm filter (Erma AE-11). Readings of the optical density were then considered as BGA (Fig. 1). Average of three duplications in each sample was calculated.

In the case of DCM observation, microbacteria of less than 0.4 μm in diameter were excluded[9,10]. Total number of bacteria in the observation slides for the microscope was maintained at 200~400 cells per sample by diluting the culture water.

MPN method was applied only in Flask 1C containing *B. plicatilis* at a density of 5 indiv/ml as this method requires too many test tubes for daily observation.

Results and Disussion

Results obtained in Flasks 1A, 1B, 1C & 1D, Flasks 1E, 2A, 2C, & 2D, and Flask 2E are illustrated in Figs. 2, 3, and 4, respectively. Generally, *C. saccharophila* were preferably consumed by *B. plicatilis* during the first few days in each experiment. Such a trend is clearly shown in Flasks 1C, 1D, 1E, 2D and 2E. *C. saccharophila* grown without *B. plicatilis* (*Chlorella* control) showed steady increase in population during the culture period (Flasks 1A and 2A in Figs. 2 and 3). On the other hand, population density of *C. saccharophila* inoculated with *B. plicatilis* obviously decreased. Moreover, population density of *B. plicatilis* did not increase when *C. saccharophila* were absent in the Flask 2C (Fig. 3). From these apperent results, it may be considered that *C. saccharophila* are essential diets for growth of *B. plicatilis*. These findings are in complete agreement with the report published by the Seto Inland Sea Farming Fisheries Association[12] in 1964.

It is interesting to note that population density of autogenous bacteria in the culture water graually decreased after *C. saccharophila* were consumed by *B. plicatilis*. These results were observed in most culture flasks (1B, 1C, 1D, 1E, 2D, & 2E) which were inoculated together with *C. saccharophila* and *B. plicatilis*. Population density of *B. plicatilis* tended to increase even after *C. saccharophila* were completely absent in the water. Moreover, the peaks in population density of *B. plicatilis* coincided with decrease in density of bacterial population (Flasks 1C, 1D, 1E, 2D & 2E). Hence, it could be suggested that *B. plicatilis* also consumed autogenous bacteria in the culture water. Furthermore, the population density of *B. plicatilis* gradually decreased during the latter half of the experimental period (Flasks 1C, 1D, 1E. 2D, & 2E). This might be caused by lack of essential nutritive diets in the culture water. The population density of *B. plicatilis* were almost absent at the end of the experiments. Further observation showed that a decline in population of *B. plicatilis* simultaneously coincided with an increase and of regulation in density repopulation of autogenous bacteria. Such result may be attributed to the phenomenon of energy recycling in the culture water.

A few exceptions, however, were observed regarding repopulation of autogenous bacteria depending upon the presence of protozoans in water. When protozoans were encountered in the culture water, bacterial density was observed to be either stabilized or decreasing (Flasks 1C, 1D, & 2C). The trend in population succession of the organisms studied was complicated more by the presence rather than the absence of protozoans.

In this research, a technique of BGA (bacterial growth ability) was introduced to indicate

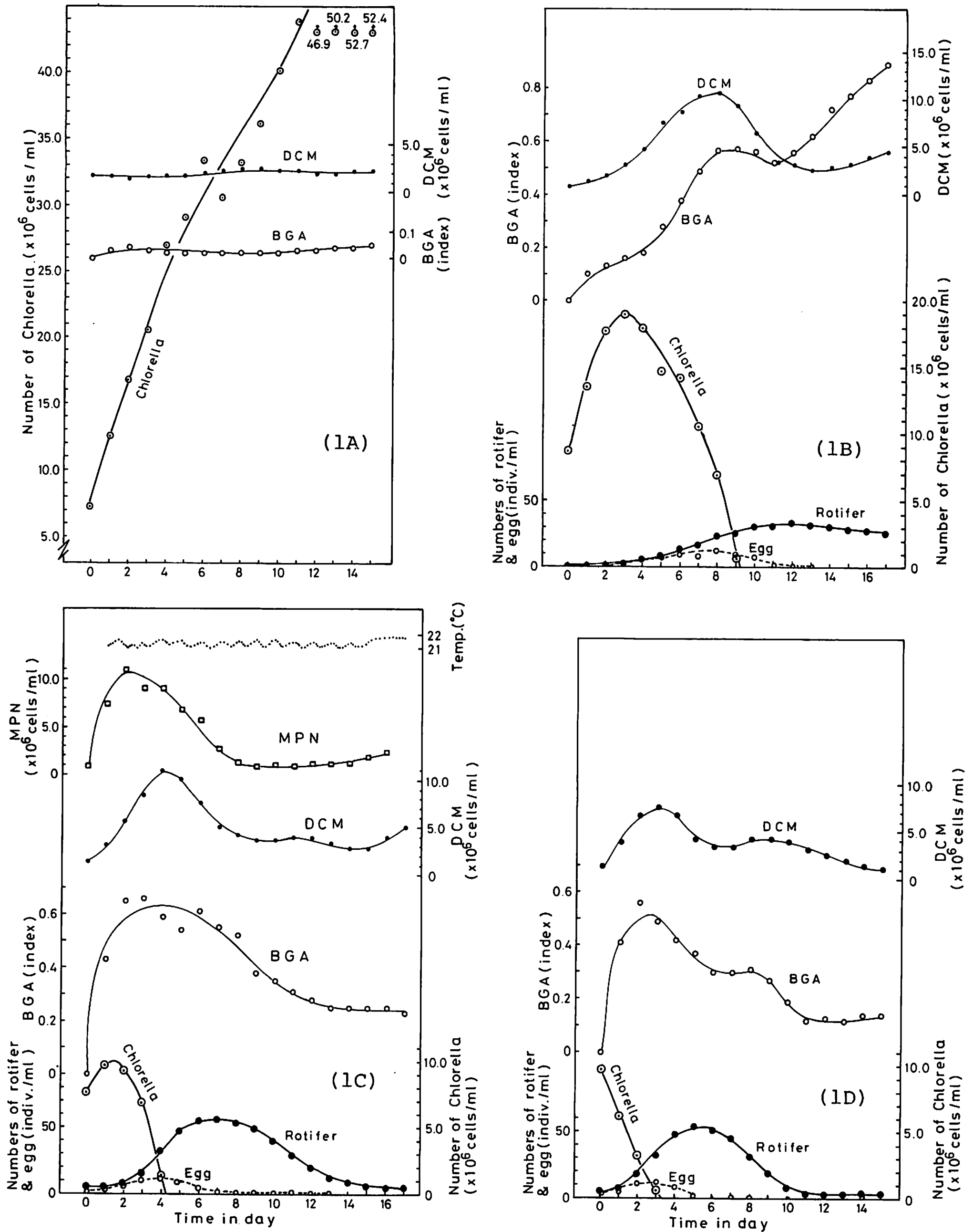

Fig. 2. Succession of rotifer, *Chlorella*, and autogenous bacteria in Flasks 1A, 1B, 1C, and 1D

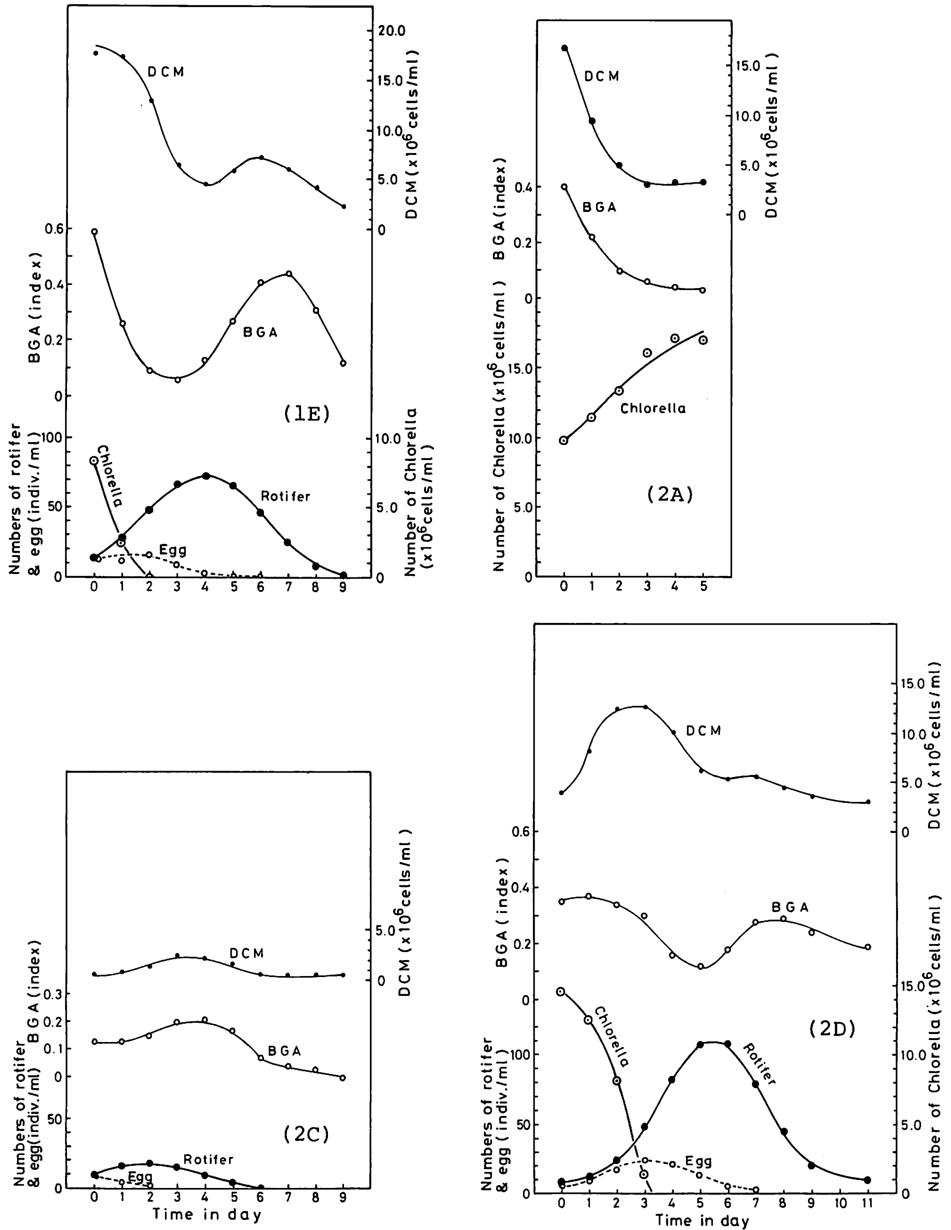

Fig. 3. Succession of rotifer, *Chlorella* and autogenous bacteria in Flasks 1E, 2A, 2C, and 2D.

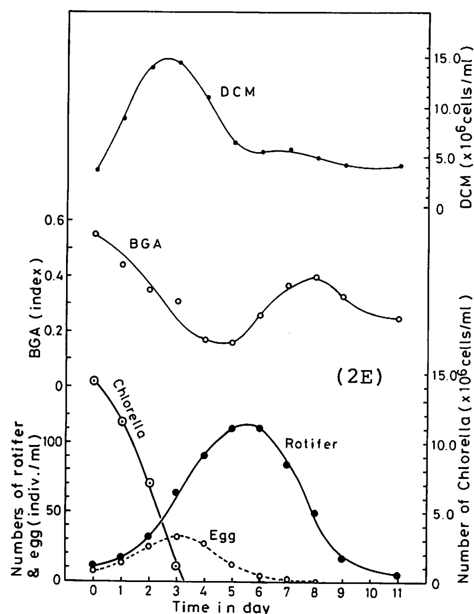

Fig. 4. Succession of rotifer, *Chlorella*, and autogenous bacteria in Flask 2E.

population density of autogenous bacteria in the culture water. As shown in the figures, the variation in BGA was closely correlated to the results using the MPN and DCM methods.

To test the practical applicability of the new technique introduced, a comparison with the conventional, more tedious agar plate counting method was made. The BGA method was significantly correlated with the agar plate counting method (r = 0.79). Based on the results obtained, the BGA method could well be applied for ecological succession studies of bacteria in culture water.

References

1) SUZUKI, T. and Y. KURIHARA (1981): The role of bacterial flcoks in the population dynamics of bacteria and protozoa in continuous culture. *Japan. J. Ecol.*, **31**, 23-29.

2) FUKUSHO, K., O. HARA and J. YOSHIO (1976): Mass culture of rotifer *Brachionus plicatilis* fed mixture diets of marine *Chlorella* and baker's yeast in large tank. *Aquiculture*, **24**, 96-101. (In Japanese)

3) HIRAYAMA, K. and K. NAKAMURA (1976): Fundamental studies on physiology of rotifers in mass culture-V. Dry *Chlorella* powder as a food for rotifers. *Aquaculture*, **8**, 301-307.

4) FURUKAWA, I. and K. HIDAKA (1973): Technological problems on the mass culture of rotifer *Brachionus plicatilis*. *Japan. Planktol. Rept.*, **20**, 61-71. (In Japanese)

5) HIRATA, H. and Y. MORI (1967): Cultivation of rotifer *Brachionus plicatilis* fed mixture diets of marine *Chlorella* and baker's yeast. *Saibai-Gyogyo*, **5**(2), 36-40. (In Japanese)

6) YASUDA, K. and N. TAGA (1980): Cultivation of rotifer, *Brachionus plicatilis*, fed cultured bacteria. *Japan. Soc. Sci. Fish.*, **46**, 933-939. (In Japanese)

7) MATSUDA, M. (1981): Studies on cultivation physiology of bioconversion systems by trash fish. *M.S.*

Thesis, Kagoshima Univ., pp 100. (In Japanese)

8) HUSAIN, A.M. (1982): Studies on the rearing of prawn larvae *Penaeus japonicus* BATE under bioconversion system. *M.S. Thesis, Kagoshima Univ.*, pp 192.

9) HOBBIE, J.E., R.J. DALEY and S. JASPER (1977):Use of nucleopore for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.*, **33**, 1225-1228.

10) KOGURE, K., U. SIMIDU, and N. TAGA (1979): A tentative direct microscopic method for counting living marine bacteria. *Can. J. Microbiol.*, **25**, 415-420.

11) ZOBELL, C.E. (1940): The effect of oxygen tension on the rate of oxidation of organic matter in sea water by bacteria. *J. Mar. Res.*, **3**, 211-223.

12) SETO INLAND SEA FARMING FISHEREIS ASSOCIATION (1965): Cultivation of live food organisms in Yashima Station-II.. *Saibai-Gyogyo News*, **4**, 4.