# PARTICLE FORMATION FROM DISSOLVED MATERIAL BY THE USE OF A CONTINUOUS HARVESTING SYSTEM

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# PARTICLE FORMATION FROM DISSOLVED MATERIAL BY THE USE OF A CONTINUOUS HARVESTING SYSTEM

By

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## Introduction

Experimental evidence for *in situ* particle formation originally presented by Baylor et al. (1962) has been challenged by various workers. Riley (1963) and Riley et al. (1964, 1965) did further experimental work on the formation of particles by bubbling and developed a new hypothesis for possible spontaneous renewal of organic particles in the ocean. Later, Menzel (1966) repeated similar experiments with great care to minimize the organic contamination in the bubbling experiment and showed that no significant quantity of organic particles was produced from "particle-free" seawater. Barber (1966) reported that particles did not form in a bubbling experiment without bacteria present. He concluded that the interatcion between bubbles and bacteria was necessary in the formation of particles from dissolved matter. Batoosingh et al. (1969) and Sharp (1972) examined the phenomenon of particle formation by bubbling and evaluated the earlier works by Riley and co-workers cited above. They claimed that although there was some confusion in the earlier works because of the variety of experimental tecniques used, there is no doubt that particulate material is actually produced by some physical processes such as bubbling and agitation. They found that a high yield of particles was obtained by the use of the continuous harvesting system. Riley considers that the continuous harvesting system is a kind of mode simulating the relationship between grazing consumption and renewal of particulate matter occurring in natural oceans.

The author carried out a series of laboratory experiments on particle formation from dissolved material, natural or artificially added, by the use of a continuous harvesting system, and the results are described in this paper. To avoid the difficult problem of external contamination, a radioisotopic tracer technique was adopted. Although the technique necessarily introduced some artificiality in the experiments, the results were compared with a few experimental observations for natural seawater, and discussed with particular references to the possible part played by bacteria in the adsorptive accretion of particles.

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## Materials and Methods

Three liters of sample waters filtered through a 0.45 $\mu$ Millipore filter were put into a bubbling chamber (5 l capacity Erlenmeyer flask). The flask was placed on a magnetic stirrer, which introduced air bubbles in the sample water by vigorously rotating a teflon coated rotor. The sample water was circulated with a peristaltic pump via glass and tygon tubes. A filter trap was attached in the circulation system. The sample water was continuously circulated through a bubbling chamber and a filter trap, and the particle free water returned through the filter into the chamber. Flow rates of the water were variable (0–40 ml/min), and in the present study the flow rate of the sample water was in a range between 4 and 8 ml/min. The experimental system was a closed system as shown schematically in Fig. 1. The sample waters used were taken at Hakodate Bay and Onagawa Bay. Three different series of bubbling experiments were carried out as described below.

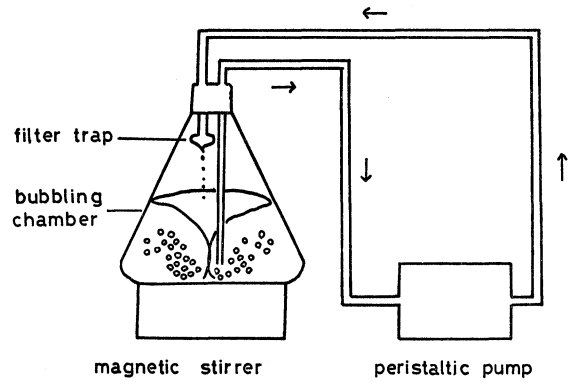


Fig. 1. Scheme of experimental system on the formation of particles by bubbling under continuous harvesting.

(1) Sample water obtained at Hakodate Bay was filtered through a 0.45 $\mu$ Millipore filter, and bubbling experiments were carried out in the laboratory by the method described above. A precombusted 25 mm glass fiber filter was used in the filter trap to retain particles produced by bubbling. The filter was removed and replaced by a new one every day during the period of the experiment. The removed filter was washed with a 3% NaCl solution and dried in a 60°C oven. After drying, particulate organic carbon and nitrogen were analyzed with a Hitachi 026 CHN Analyzer. Each experiment in this series was continued for 7 to 30 days.

(2) The sample water taken at Onagawa Bay was filtered through a 0.45 $\mu$ Millipore filter and the filtered water was put into a sterilized Erlenmeyer flask (5 l capacity). The sample water, glass tubes, tygon tubes, and filter trap used in this experiment were autoclaved for about 40 minutes in advance with normal pressure. Fifty $\mu$ Ci of uniformly labeled $^{14}$C-glucose was added to the sample water. Bubbling experiments were carried out in the same manner described earlier. Particles produced by bubbling were retained with a 25 mm 0.45 $\mu$ Millipore filter. The filter was generally replaced every day and dried in a 40°C oven. Each filter was placed into a scintilla-

tion vial containing 15 ml of toluene based fluor. The radioactivity of samples in the scintillation vials was counted with a Hitachi-Horiba liquid scintillation counter. Each sample was counted twice each for one minute. The counting efficiency of each sample was determined by the channel ratio method. Colony numbers of heterotrophic bacteria in the sample water were determined by the plate counting method. One milliliter of sample water was cultured in ZoBell's 2216E agar (ZoBell, 1941) at 20°C for 3 to 5 days. Bubbling experiments in this series were continued for 40 to 50 days. Occasionally, radioactivity in the sample water was counted.

(3) Further experiments with a tracer technique were carried out. An unialgal culture of *Skeletonema costatum* was grown in filtered seawater enriched with Matsudaira's medium (unpublished). Efforts were made to minimize bacterial contamination. The culture were illuminated with a fluorescent lamp at room temperature. The cells were cultured in a glass flask (2 l capacity) containing 1 l of seawater for a week. At the last day of the incubation, $^{14}C$-$NaHCO_3$ (200–1,000 $\mu$ Ci) was added to the flask. After exposure of the culture with radioactive carbon for six hours, the cells were filtered off using a 0.45 $\mu$ Millipore filter. Extracellular $^{14}C$ labeled organic products in the filtrate was obtained with the method described by Ignatiades and Fogg (1973). The filtrate was acidified with HCl to pH 2.8, and air was bubbled vigorously for an hour to remove all inorganic carbon. After bubbling, pH value was adjusted to 7.8 with a NaOH solution. About one liter of this filtrate was diluted to three liters with sterilized filtered seawater. Bubbling experiments were carried out in the same method as mentioned in (2). Each experiment in this series was continued for about two weeks.

## Results

There is a possibility that some of the suspended organic particles in the sea are not the direct disintegration product of organisms (detritus) but are synthesized "aggregates" produced by accretion of organic material dissolved in seawater. The possibility has been tested by a few investigators, and the results obtained so far are not so consistent as to produce a unified picture of the ecological significance of this phenomenon. The present author developed three series of bubbling experiments and the methodological design was described earlier, and tried to pave the way for understanding the controversial problems on a critical, experimental basis.

*Chemical analyses* (Series 1)

The results of daily yields of particulate organic nitrogen produced by bubbling under continuous harvesting through a glass filter are shown in Fig. 2. The sample water used was taken from the shore of Hakodate Bay and filtered through a Millipore filter HA type. The trap filter in the circulation system was a Whatman GF/C filter. The daily production of particles by bubbling was very high within a few days with a maximum of 27 $\mu$ gN/day occurring on the third day, and gradually decreased with time, converging to a negligible small daily yield after about 20 days. The total

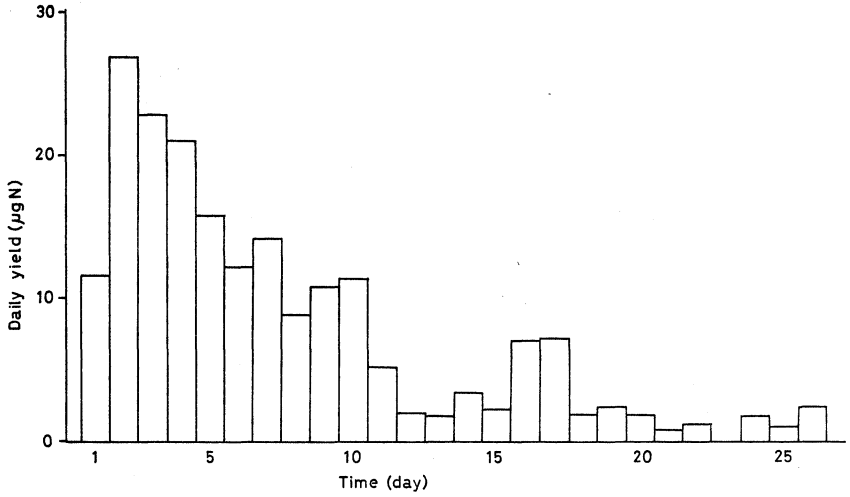


Fig. 2. Daily yield of particulate organic nitrogen. Water was continuously circulated through a bubbling chamber, through a filter, and back into the chamber (Exp. No. 1).

quantity of produced particulate nitrogen for 25 days was 194.5 $\mu$ gN. The total amount of particulate nitrogen in three liters of the original sample water retained on a glass fiber filter was 19.2 $\mu$ gN. Thus, the total yield of particulate nitrogen obtained by experimental bubbling was ten times the particulate nitrogen originally contained in the sample water. No measurements of dissolved organic nitrogen were made in this series of experiments.

Subsequent experiments in terms of organic carbon were carried out. Two controls were prepared; in the first one the sample water was bubbled with a magnetic

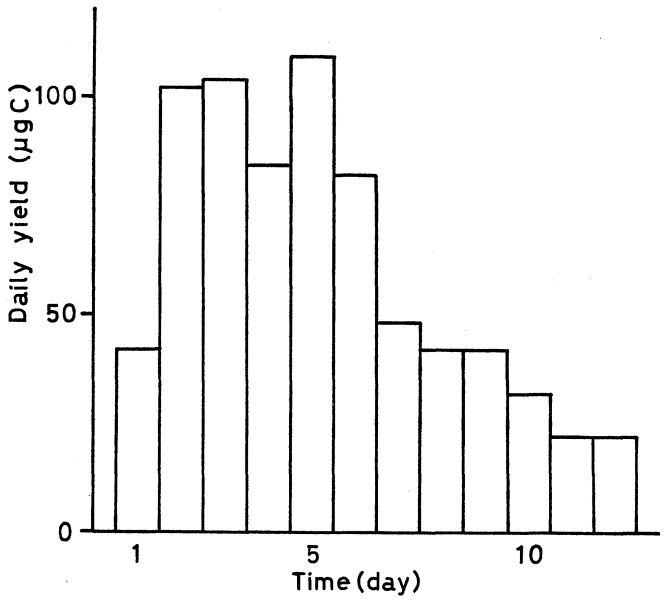


Fig. 3. Daily yield of particulate organic carbon. Sample was bubbled in a stoppered bubbling chamber with magnetic stirrer and filtered every day (Exp. No. 2).

stirrer in a tightly packed flask and harvested every day through a 47 mm glass fiber filter, and in the second the sample water was kept unbubbled in a stoppered flask. The daily yields of particulate organic carbon obtained in the intermittent filtering are shown in Fig. 3. The yields obtained from the continuous harvesting system are erroneously high above 100 $\mu$ gC per day and the total yield of carbon in the experimental period of 14 days was more than 3.5 mgC, suggesting serious external contamination occurring in this system. The production pattern in the intermittent filtration system, however, seemed essentially similar to that of particulate nitrogen under the continuous harvesting system described above; the carbon yield rapidly increased in the first several days with a maximum of 110 $\mu$ gC/day and decreased gradually, converging to about 20 $\mu$ gC/day. The total yield in 12 days attained to 0.7 mgC.

In this series of experiment measurements of dissolved organic carbon in the sample water were made. The initial concentration of dissolved organic carbon in the continuously circulated system was already higher (3.7 mgC/1) than that of the untreated control (2.2 mgC/1). This might be due to organic contaminants introduced from the air during a short initial test of operation of the experimental system. Menzel (1966) found that a few hours of bubbling with laboratory air increased the dissolved organic carbon by a factor of 2 to 4. The dissolved organic carbon was decreased slightly (3.4 mgC/1) on the 3rd day but increased again (4.0 mgC/1) on the 6th day of the experiment. The concentration did not show any decreasing tendency in the later period of the experiment (4.0 mgC/1). The concentration were significantly higher than that of the two controls throughout the entire experimental period. The initial concentration of dissolved organic carbon in the intermittent filtering sample was 3.7 mgC/1 and it gradually decreased with time, 2.2 mgC/1 on the 6th day. On the last day of the experiment, however, the dissolved carbon increased again to the same level as the initial value (3.7 mgC/1). On the other hand, there was no variation of dissolved carbon in the unbubbled control in the packed flask (2.2 mgC/1) in the entire period. These results indicate that the carbon contamination in the sample water was serious in spite of the great care taken to prevent it in all of the experimental materials. The author could not obtain satisfactory organic carbon data in this series of experiments.

*Tracer techniques* (Series 2 and 3)

In order to avoid the troublesome problems of external contamination, further experiments using radio isotope technique were prepared.

The results of an experiment in which 50 $\mu$ Ci of uniformly labeled $^{14}$C-glucose was added to the filtered water in the bubbling flask are shown in Fig. 4. The daily yield of labeled particles was 356,000 dpm/day on the first day. The yield rapidly decreased, and levled off at a value of 20,000 dpm/day after ten days. After this stable period, another rapid increase of particle production occurred with a maximum peak of 385,000 dpm/day on the 14th day. The yield, then, gradually decreased and converged again to 20,000 dpm/day in the remainder of the experimental period.

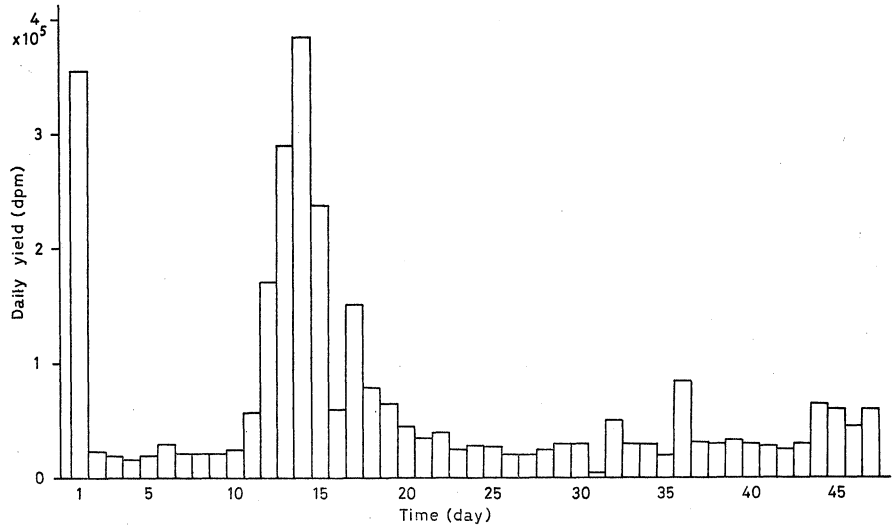


Fig. 4. Daily yield of particulate matter. Water was continuously circulated through a bubbling chamber, through a filter, and back into the chamber (Exp. No. 3).

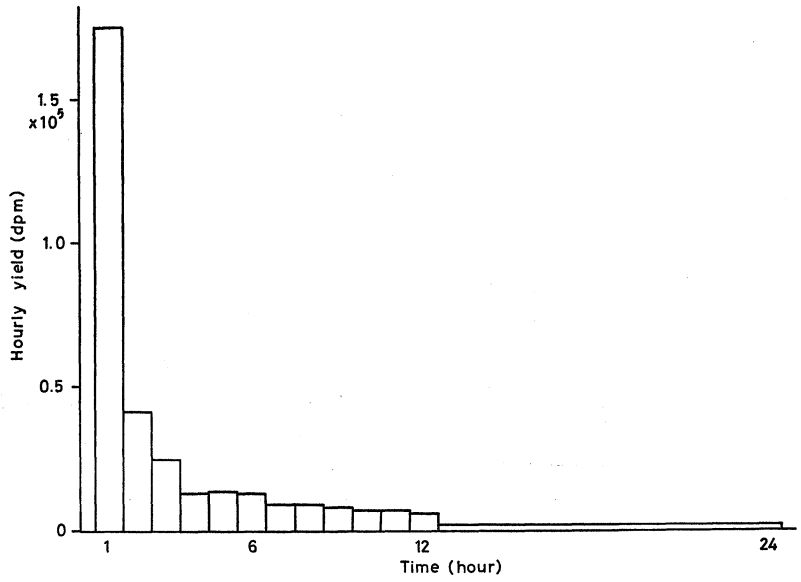


Fig. 5. Hourly yield of particulate matter in the first day of the experiment (Exp. No. 3).

Fig. 5 shows the hourly variation in production of labeled particles within the first day of this experiment. The yields obtained here reached a maximum of 175,000 dpm/hr in the first hour, rapidly decreased with time, and tended to converge to two orders of magnitude lower production rates (2,000 dpm/hr) after twelve hours.

Variation in colony number of heterotrophic bacteria in the chamber was estimated by the plate counting method (Fig. 6). In the first few days, there were several thousand bacterial cells per milliliter. The number of bacteria increased exponentially and reached, one week later, the order of several hundred thousand per milliliter. After that,

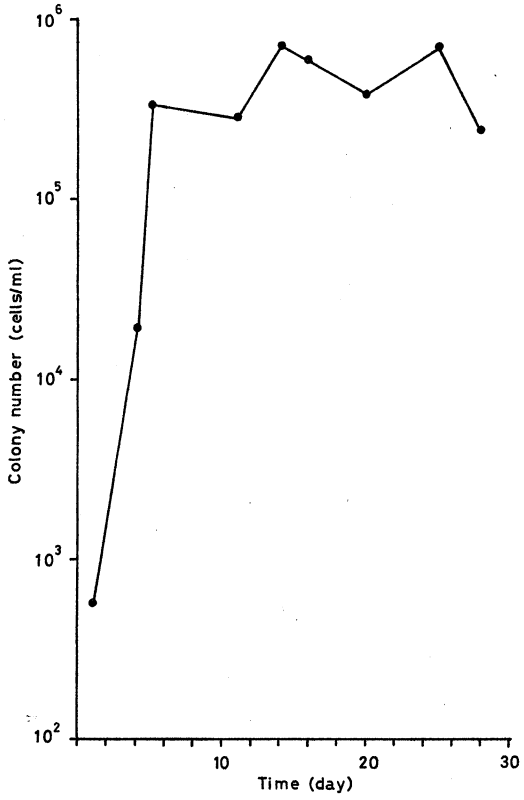


Fig. 6. Colony numbers of heterotrophic bacteria in the bubbling chamber (Exp. No. 3).

the bacterial population was relatively steady, keeping a level of several hundred thousand per milliliter. During the entire period of 33 days of bubbling, dissolved labeled carbon decreased from 38,682 dpm/ml to 24,143 dpm/ml. The total amount of radiocarbon retained on the filters during 33 days was 1.1 $\mu$ Ci. Thus, about 2% of the radiocarbon was converted to particulate matter in 33 days and most of the remainder of the decreased carbon (35%) would have been consumed by bacteria.

Fig. 7 shows the results of a similar experiment. In this experiment, the sample water was filtered "twice" through a 0.45 $\mu$ Millipore filter before the sample was drained into the bubbling flask. No peak of particle yield appeared in the early period of the experiment. About two weeks later, two successive cycles of particle production began to develop with maxima of 110,000 dpm on the 15th day and 330,000 dpm on the 34th day. The rate of particle production did not show any decreasing tendency in the last stage of the experiment, and the rate tended to keep a relatively high level over a period of more than 30 days.

No colony of bacteria was detected on the first few days. Four days later, bacterial growth showed a rapid increase with time, and reached a more or less steady state at several thousand per milliliter after about 20 days. The concentration of radiocarbon in the bubbling flask declined from 40,136 dpm/ml to 25,073 dpm/ml in the entire experimental period (40 days). Thus about 38% of labeled glucose disappeared from the sample water. The total quantity of particulate radiocarbon retained on the filter

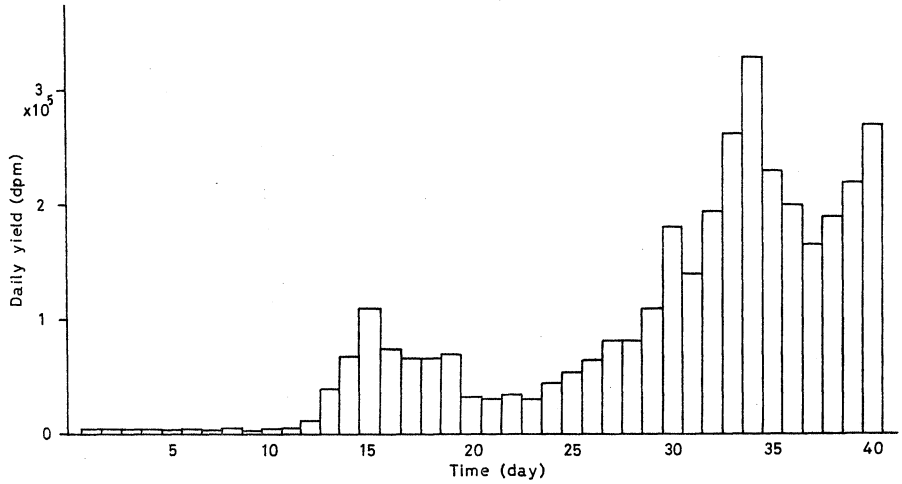


Fig. 7. Daily yield of particulate matter. Water was continuously circulated through a bubbling chamber, through a filter, and back into the chamber (Exp. No. 4).

was 1.58 $\mu$ Ci, which means that about 2.9% of the radiocarbon was converted to particulate matter. Most of the radiocarbon lost would have been utilized by bacterial metabolism.

As the last series of the experiment, the $^{14}C$-labeled extracellular product released from cultured *Skeletonema costatum*, which was incubated with $^{14}C$-$NaHCO_3$ (1,000 $\mu$ Ci) for six hours, was added to filtered seawater and aerated. After removing inorganic

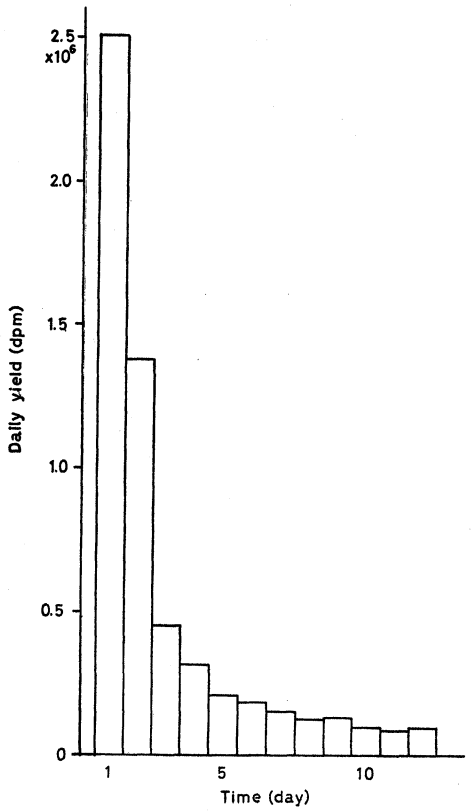


Fig. 8. Daily yield of particulate matter. Water was continuously circulated through a bubbling chamber, through a filter, and back into the chamber (Exp. No. 5).

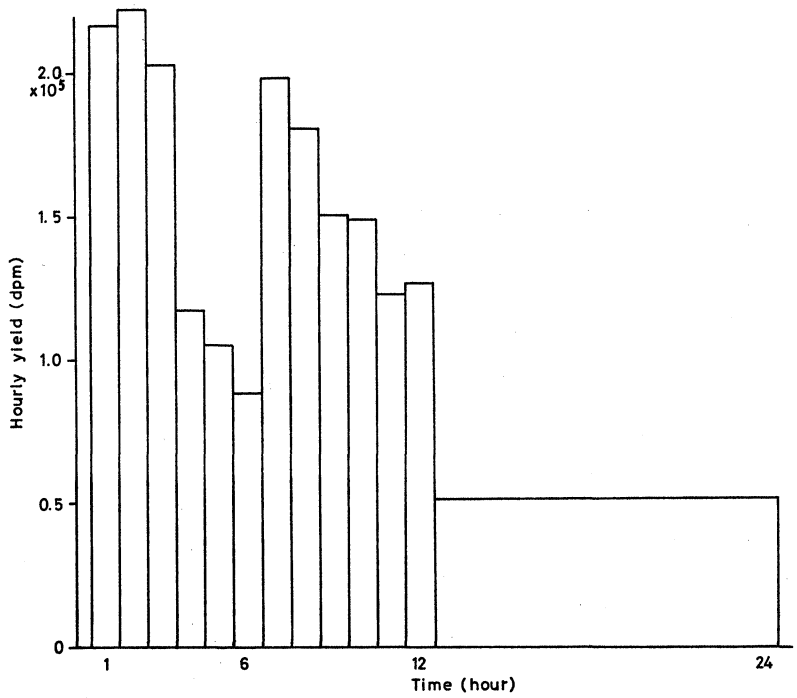


Fig. 9. Hourly yield of particulate matter in the first day of the experiment (Exp. No. 5).

radiocarbon, about 36 $\mu$ Ci of radioactive extracellular products were contained in the 3 l of sample water. The maximum yield (25,000,000 dpm/day) was obtained on the first day and the daily yield rapidly declined in the first few days, and continued to decrease gradually with time. About ten days later, the daily yield leveled off and converged to 100,000 dpm/day, and no secondary maximum was obtained during the entire experimental period of twelve days (Fig. 8). Fig. 9 shows the variation of hourly yield of particulate radiocarbon during the first day. The high yield in the first few hours decreased with time but had a second maximum seven hours later, and tended to decline to about 50,000 dpm/hr after another 12 hours. The concentrations of labeled carbon in the sample water declined from 27,484 dpm/ml to 4,403 dpm/ml on the last day of the experiment. About 83% of the radiocarbon in the bubbling flask disappeared and 7% of the lost radiocarbon was retained on the filter. No bacterial counts were done in this experiment.

A similar experiment was repeated using 26.1 $\mu$ Ci labeled extracellular products obtained from a *Skeletonema costatum* culture. The maximum yield (102,636 dpm/day) was obtained on the first day and the daily yield decreased gradually with time, leveling off eleven days later at about 20,000 dpm/day, and keeping the same level during the remainder of the experimental period (Fig. 10). The variation in hourly yield of labeled particles in the first day was quite similar to the glucose series experiment; the maximum hourly yield was obtained from the first harvesting and the yield dropped down by one half in the next hour. The yield decreased gradually with time, and

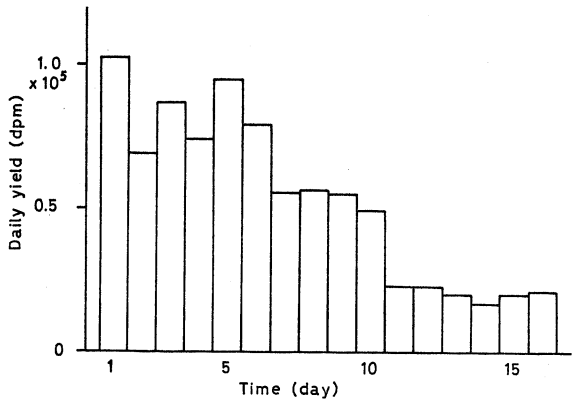


Fig. 10

Fig. 10. Daily yield of particulate matter. Water was continuously circulated through a bubbling chamber, through a filter, and back into the chamber (Exp. No. 6).

Fig. 11. Colony numbers of heterotrophic bacteria in the bubbling chamber (Exp. No. 6).

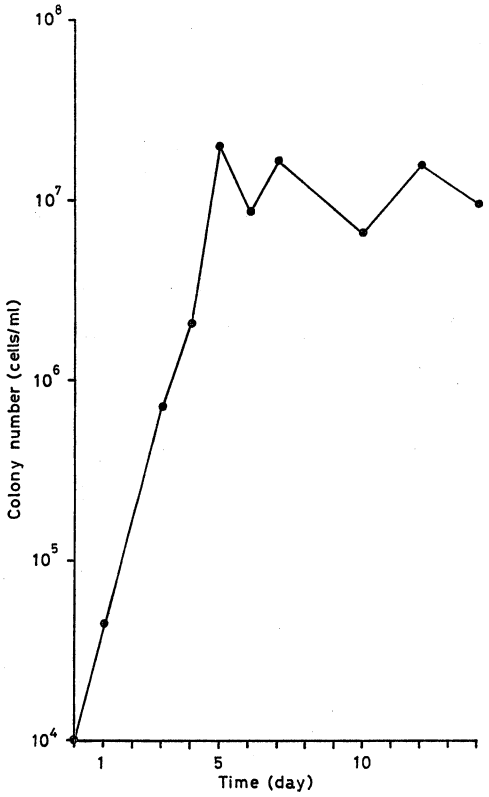


Fig. 11

the mean hourly yield declined to 2,000 dpm/hr twelve hours later and in the remainder of the sampling period. In this experiment the dissolved radioactive carbon was initially 19,281 dpm/ml. On the final day (16 th day) it declined to 780 dpm/ml. Thus, only 5% of the labeled extracellular materials remained in the flask on the final day. The total amount of radioactivity retained on the filter during the entire period was 0.8 $\mu$ Ci. Thus about 4% of the observed decrease was converted to particulate material. Fig. 11 shows the bacterial growth curve in the bubbling flask. Results showed that there were more than ten thousand bacterial cells per milliliter on the first day of the experiment. Subsequently, the bacterial population grew logarithmically until it stabilized at the cell concentration of ten million per milliliter during the remainder of the experimental period. The sample water in the flask became turbid within first few days and visual examination revealed the presence of large flakes and aggregates in the sample water.

## Discussion

It is well known that the formation of particulate material from filtered seawater with or without bubbling is a kind of equilibrium process, and the formation virtually stops when the concentration of produced particles arrives at a certain level that is primarily determined by the concentration of dissolved organic matter (Sheldon et al., 1967; Nakajima and Nishizawa, 1968; Riley, 1970). The continuous harvesting

system was originally designed by Batoosingh et al. (1969) to obtain a high yield of particles from a fixed amount of filtered seawater simply by continuously harvesting produced particles and thus constantly preventing the establishment of the equilibrium in the system.

Riley (1970) carried out bubbling experiments involving continuous harvesting for 120 hours with sample water from off the coast of Nova Scotia. The results showed that the rate of production of particles by bubbling was high in the initial period and decreased with time; the total yield was as high as 1.5 mgC/l. Unfortunately no measurement of dissolved organic carbon in the bubbling chamber was made. The present author could not succeed in preventing organic carbon contamination in the continuous harvesting system in spite of the repeated trials. Several hours of continuous harvesting increased the dissolved organic carbon by a factor of 2 and, even after a week, a slight increase of dissolved carbon in the bubbling chamber was observed. This error should not be ascribed to the air in the laboratory since the experimental system was a closed system. The silicone tubing under peristaltic motion of the pump system is a doubtful source of carbon contamination. However, a particle production mode essentially similar to the results of Riley (1970) was obtained under the continuous harvesting condition for particulate nitrogen for which no significant contamination from the pump tubing was anticipated (Fig. 2). Also, the results obtained by intermittent filtration (Fig. 3) gave another example of the same mode of production.

It is difficult to separate biological and physical parameters in experiments on particle formation by bubbling. Barber (1966) reported that bacterial activity was needed to get a significant yield of particulate matter by bubbling. Batoosingh et al. (1969), however, could not find a significant difference in the yield between a KCN treated sample and an untreated one in the bubbling experiments.

In the present study, using the tracer technique, high particle yields were obtained on the first day in each of the three series of experiments (Figs. 4, 8 and 10), and further, a maximum hourly yield on the first day was obtained in the first one hour. The bubbling chamber was sterilized immediately before the experiment and the number of bacteria colony in the chamber was negligible in the initial hour. At least the rapid occurrence of this aggregation process denies the possibility that the material yielded is merely the bacterial biomass produced in the initial hour. Sharp (1972) showed that a few hours of shaking of the flask gave a significant yield of particulate carbon, assumed to be produced mainly by physical processes.

In one case (Fig. 7), the first outburst of particle production was not observed. In this experiment the sample water was filtered twice through a Millipore filter (HA type) and several hours cold run test was taken before adding $^{14}C$-labeled glucose to the bubbling chamber. Riley (1970) has clearly shown that a severe harvesting of existing particles inhibits subsequent particle formation, suggesting that the process proceeds only when there are particles to serve as nuclei of aggregation.

After the rapid particle formation in the first day, the daily yield rapidly declined

to a level one order of magnitude lower, and kept this low level thereafter (Fig. 4). The subsequent second cycle of high yield might have to do with bacterial growth. However, it is noteworthy that the development of this second cycle of particle formation did not coincide in phase with the growth of bacteria. In Exp. 3 (Figs. 4 and 6), the observed logarithmic increase in bacterial number during the first 7 days was not reflected in the particle yield, the latter being nearly unchanged, and the maximum production of particles occurred one week later than the establishment of a steady maximal bacterial biomass. In Exp. 4 (Fig. 4), the second cycle of particle formation occurred during the period of logarithmic growth of bacteria, and when the bacteria attained the largest population the rate of particle formation declined to a lower level. Growth of bacteria would probably play an important role in the aggregation process (Riley, 1970), but the present results indicate that rapid particle formation could occur under a condition of near sterility. Further, the interrelation between bacterial development and particle formation is not linear even in the second cycle of particle formation, as well as in later stages. The exact nature of the interrelation is not known, but it may safely be said that the particles formed in the entire stage are not mere bacterial biomass, and include "something else" which is probably a non-living "detrital" substance (cf. Barber, 1966), and constitutes the predominant fraction of the total mass of produced particles including bacteria.

It is well known that marine phytoplankton in a laboratory culture, as well as in nature, excrete part of the organic matter that is assimilated by photosynthesis (Fogg. 1958; Fogg et al., 1965; Guillard and Wangersky, 1958; Watt, 1966; Anderson and Zeutschel, 1970; Thomas, 1971). The relative amount of extracellular products ranged from several percent to more than fifty percent of the total assimilated carbon depending on physical and biological conditions (Ignatiades, 1973; Ignatiades and Fogg, 1973). This phenomenon brought a serious problem to the conventional estimation of primary production by the traditional $^{14}C$ method (Riley, 1970). However, interests have been focused on the possible significance of extracellular production by phytoplankton. Riley et al. (1964) first indicated that extracellular products of phytoplankton could be easily converted to particulate matter by bubbling. Seven species of diatoms and one dinoflagellate were used in their experiments. Particulate carbon produced by bubbling was highly variable. The mean yield was 20% for the diatoms and 2% for the dinoflagellates of cellular carbon. In the present study, excreta from cultured *Skeletonema costatum* was also found to be rapidly converted to particulate form. No observation was made of assimilated labeled carbon during the incubation. Extracellualr $^{14}C$-labeled organic matter ranged from 4 to 12% of added inorganic radiocarbon. However, the results show that most of the extracellular products are lost from the bubbling chamber in two weeks. Although 92% of the lost carbon was considered to be metabolized by bacteria, the most rapid metabolization occurs only in the latter half of the first week of the experiments (Figs. 8 and 10). This seems to suggest that the particle formation developed at the initial stage of the experiment

supplies suitable matrices for bacteria to grow on, and the excreta are utilized effectively by bacterial colonies developed on these matrices. The utilization is straightforward and no second cycle of particle formation was observed.

An identification of extracellular products from *Skeletonema costatum* was made by Ignatiades and Fogg (1973). It was shown that high molecular as well as low molecular substances were released in the culture media; carbohydrates and glycollic acid were major components. Carbohydrates are common substances in the dissolved organic matter in the ocean. Gordon (1970a) reported that naturally occurring organic aggregates are stained strongly with acid-Schiff reagent. Sharp (1972) showed that particles produced by relatively short time bubbling were also stained with the same reagent.

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