THE CATALYTIC ACTIVITY OF SEA-WATER III. THE EFFECT OF THE CATALYTIC ACTIVITY UPON THE GROWTH OF DIATOM

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By

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From the result reported in a preceding paper (Matsudaira, 1951)[1] in which negative catalysts with especially strong inhibitory power for activity is quite in accord with the substances known as promotors of diatom growth, the effect of catalytic activity upon the growth of diatom is expected. The present paper deals with culture experiments of diatom under different grades of the catalytic activity.

Materials and Methods

1. *Natural Sea-water:*

The surface sea-water collected from Fukuoka Bay is used. The sample is filtered through the absorbent cotton of good quality washed with boiled distilled water, in order to remove the plankton and detritus. It is stored in water-bottles with a capacity of 20 L in a dark place until use.

2. *Artificial Sea-water:*

The preparation was reported in the preceding paper.[3]

3. *Culture Method and Culture Apparatus:*

The actively growing cells of *Skeletonema costatum* (Grev.), 1866. subcultured in the modificated Miquel's sea-water are used for the experiments. The culture method and the culture apparatus were already described in the preceding paper.[3]

4. *Determination of Diatom Population:*

In preceding experiments,[3][4] the diatom population in culture had been determined by a counting method. As the dimension of the diatom cell differs by culture conditions, it is hard by the counting method to give the exact result for the population cultured under the various environmental conditions such as those in present experments; consequently, the present method is based on wet combustions with potassium permanganate.

The culture bottles are shaken to obtain a homogeneous diatom population and 10 c.c of diatom suspension is measured into a centrifuge tube. The diatom is separated from the fluid by a centrifuger. The supernatant fluid in the centrifuge tube is abandoned and 10 c.c of distilled water is added. The content of the centrifuge tube is mixed thoroughly and decanted into a 100 c.c Erlenmyer's flask. In order to combust the diatom, 2.5 c.c of 1.1 *n* NaOH solution and 5 c.c of 0.015 *n* $KMnO_4$ solution are added. The flask is heated in a steam-bath at 100°C for forty minutes.

The quantity of $KMnO_4$ consumed by the combustion of the diatom is measured after water analysis by iodometric method which has been proposed by T. Tomiyama.[5] One c.c of 10 per cent potassium iodide is added to the flask in the steam-bath. The flask is taken out from the steam-bath and allowed to stand until it cools to room temperature. And then, one c.c of sulphuric acid (1 : 3) is added. The iodine evolved is titrated with 0.01 *n* thiosulphate solution with starch indicator. In the same manner, a blank test is carried out in 10 c.c of culture medium. Cubic centimeters of 0.01 *n* $KMnO_4$ solution consumed by wet combustion per 100 c.c diatom suspension are calculated as follows:

$$P = (Bl. - T) \times \frac{1}{R} \times \frac{100}{S}$$

P: Cubic centimeters of 0.01 *n* $KMnO_4$ consumed by wet combustion per 100 c.c diatom suspension.

Bl: Reading of the titration in the blank test.

T: Reading of the titration in the experiment.

R: The factor of 0.01 *n* thiosulphate solution for standard 0.01 *n* iodine solution.

S: Cubic centimeters of the sample (10 c.c).

According to Tomiyama, the concentration of the organic matter in the water is linear to the quantity of potassium permanganate consumed by combustion unless more than a third of that added is consumed. Also in the present experiment, the same attention should be paid. When the consumption of potassium permanganate is above a third of that which was the sample is diluted to a half with the culture medium.

In this method, there is uncertainity as to the completeness of the destruction of the organic compounds. Some organic materials may be completely converted into carbon dioxide, water and so on, by such a procedure; other compounds are only partially decomposed and still others are not attacked at all. Therefore, as the chemical constituents of the diatom are not measured, it is difficult to evaluate the accuracy of determination made by this method. However, as the present experiment has been made in a species

only, the relative value of the diatom population gives acculate result at least. The result shown in Table I and Fig. 1 permits the use of this method for the present experiments.

Table I. Diatom Population in Numbers of Potassium Permanganate Units.

Concentration of diatom in per cent %	Numbers of cells per liter $\times 10^5$	Diatom population measured by $KMnO_4$ units (c.c 0.01 n $KMnO_4$/100 c.c sample)
10	255	1.9
20	510	5.1
30	764	7.9
40	1019	11.0
50	1274	13.5
60	1529	16.4
70	1784	19.6
80	2038	22.0
90	2293	24.5
100	2548	27.8

The dimension of *Skeletonema* used in this experiment is 14.5 μ long and 6.6μ wide on the average of 10 cells taken from different chains. As the relation between the consumption of potassium permanganate and the dilution of diatom suspension is linear, the diatom population can be measured by cubic centimeters of 0.01 n $KMnO_4$ solution consumed by the diatom. When one cubic centimeter of 0.01 n $KMnO_4$ solution per 100 c.c of the sample is consumed by the combustion, we call it one potassium permanganate unit of diatom. In the present experiment, one potassium permanganate unit is equivalent to the sample containing about 9165×10^3 cells of diatom per liter.

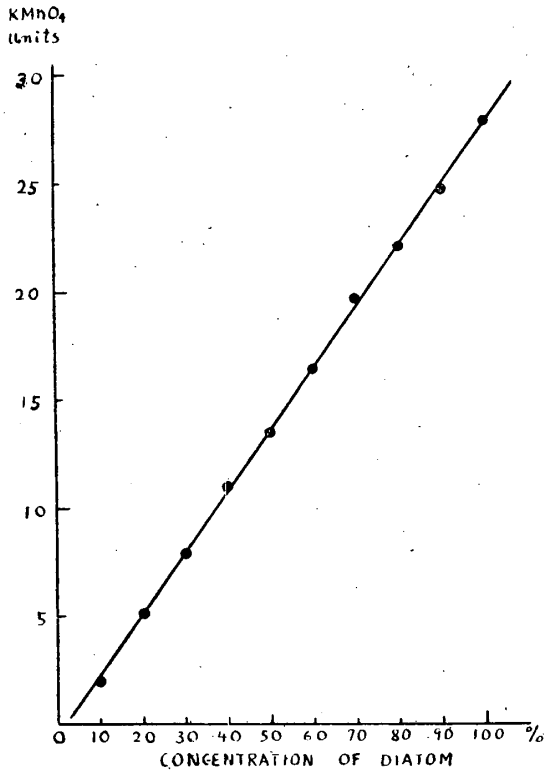

Fig. 1. Relation between the Concentration of Diatom and the Population in Numbers of $KMnO_4$ Units.

Experimental

As already reported (1939)[4], the culture media prepared with water samples taken at different depths and on different dates show different grades of effect upon the growth of diatom. As the sea-water moves in the sea, the water at a certain place changes its property occasionally and it becomes sometimes coastal and others oceanic in its nature. Culture media prepared with coastal water gives better growth than those with oceanic water. The so-called "miquelization" which is reaction causing the precipitation of ferric phosphate and ferric hydroxide in the preparation of Miquel's sea-water enhances the growth of diatom in the sea-water samples (Allen and Nelson, 1910, Matsudaira, 1939)[4]. On the other hand, some growth promoting substances are known. The culture of diatom in the artificial sea-water is difficult without the addition of growth promoting substances such as organic or inorganic sulphides and so on. These point to the following conclusion. The sea-water may possess unknown physico-chemical characteristics controlling the growth of diatom. Its characteristics may differ in different sea-water such as oceanic, coastal and artificial sea-water and by the treatment of the sea-water, for example 'miquelization' and the addition of growth promoting substances. According to the preceding result on catalytic activity[1)2)5)], the catalytic activity of sea-water differs in various sea-waters and by the treatment of the sea-water. Therefore, it is expected that the characteristics of the sea-water controlling the growth of diatom may be characterized by the catalytic activity of sea-water. The present experiment is carried out to obtain the relation between the catalytic activity of sea-water and the growth of diatom. The experiment consists of the following four experiments in which the method of the regulation of the activity differs from each other.

Experiment 1.

The sea-water (Cl 16.5‰) collected from Fukuoka Bay is filtered and its chlorinity is adjusted to Cl 16‰ by adding distilled water. And then, in order to enrich the sea-water with nutritive salts, it is miquelized and treated as described in the preceding paper[3]. The activity of the sample drops from K_{30} 0.060 to 0.034. On the other hand, when the artificial sea-water (Cl 17‰) is miquelized in the same manner as the natural sea-water, its activity drops from K_{30} 0.368 to 0.174. Next, the two samples thus obtained are mixed in different ratio as described in Exp. 1 of Table II. The activity in each mixture is determined before the culture. 0.5 c.c of the subculture of *Skeletonema* ($KMnO_4$ unit ca. 20) is added in 100 c.c of the samples and they are placed into the cuture apparatus for six days. The population of *Skeltonema* is determined by the already described method. The growth of *Skeletonema*

under the different grades of activity is obtained with the observations of cells as shown in Exp. 1 of Table II.

Experiment 2.

In experiment 2, the natural sea-water in the experiment 1 is replaced by the artificial sea-water prepared from the well-water. The well-water has been collected from the well near the Kyushu University in Fukuoka. The culture media with different grades of the activity are prepared as same as Exp. 1. The result of the culture experiment is shown in Exp. 2 of Table II.

Experiment 3.

The natural sea-water in the experiment 1 is replaced by the artificial sea-water treated with ferric chloride. The treatment with ferric chloride is carried out as follows. One c.c of ferric chloride solution ($FeCl_3 \cdot 6\ H_2O$ 1 gm. per 100 c.c) is added to 1 liter of the artificial sea-water. It is heated at 100°C for a half hour in the steam-bath, cooled at room temperature and allowed to stand for a day. The ferric hydroxide precipitates at the bottom of the beaker. Using a siphon, supernatant fluid is separated from the precipitates. The supernatant fluid thus obtained is miquelized and mixed with non-treated artificial sea-water as in Exp. 1. The result of the culture is shown in Exp. 3 in Table II.

Experiment 4.

In this experiment, the natural sea-water is replaced by the artificial sea-water treated with active carbon. One gm. of active carbon is added to 1 liter of the artificial sea-water in a beaker. The beaker is placed on an electric heater. The fluid is agitated with a glass rod until it boils for a minute. The beaker is taken off from the heater and cooled at room temperature. The fluid is filtered to exclude the active carbon by a filter paper. The filtrate is miquelized and mixed with non-treated Miquel's artificial sea-water. And then, the same experiment as described above is carried out. The result of the culture is shown in Exp.4 in Table II.

The relations between the growth of *Skeletonema* and the catalytic activity which have been obtained in above four experiments are plotted into Fig. 2. As shown in the figure, a definite relationship is found between the growth of *Skeletonema* and the catalytic activity. When the activity of the culture media is in the range of K_{30} $0.050 \sim 0.090$, there is no effect of the activity upon the growth and cell form although a difference in the growth has been observed among four experiments, perhaps resulting from the difference of the culture conditions. In the range of K_{30} $0.090 \sim 0.120$, the growth decreases sharply to the side of higher activity. The form of the cell changes from slightly abnormal to abnormal and the pigment is discoloured. When

Table II. The Growth of Diatom in Different Grades of the Catalytic Activity.

Experiment 1. The catalytic activity is regulated by adding the natural sea-water to the artificial sea-water.

Addition of natural sea-water in per cent %	Catalytic activity of culture media K_{30}	Growth of diatom after six days in numbers of $KMnO_4$ units	Form of cells after calture
0	0.115	3.5	abnormal
20	0.103	13.5	slightly abnormal
40	0.088	16.0	normal
60	0.064	17.5	normal
80	0.047	18.5	normal
100	0.034	21.5	normal

Remarks: Temp. 23.0°C pH 8.2 Cl 16.0‰

Experiment 2. The catalytic activity is regulated by adding the artificial sea-water contained well-water to the artificial sea-water

Addition of artifcial sea-water contained well-water in per cent %	Catalytic activity of culture media K_{30}	Growth of diatom after six days in numbers of $KMnO_4$ units	Form of cells after culture
0	0.115	3.5	abnormal
20	0.117	8.8	abnormal
40	0.111	10.0	slightly abnormal
60	0.091	11.0	normal
80	0.086	10.5	normal
100	0.074	10.0	normal

Remarks: Temp. 23.0°C pH 8.2 Cl 17.0‰

Experiment 3. The catalytic activity is regulated by adding the artificial sea-water treated with ferric chloride to the artificial sea-water.

Addition of artificial sea-water treated with ferric chloride in per cent %	Catalytic activity of culture media K_{30}	Growth of diatom after six days in numbers of $KMnO_4$ units	Form of cells arter culture
0	0.135	2.5	abnormal
20	0.121	4.5	abnormal
40	0.115	5.5	abnormal
60	0.108	10.5	slightly abnormal
80	0.098	12.5	normal
100	0.096	12.5	normal

Remarks: Temp. 22.2°C pH 8.2 Cl 17.0‰

Experiment 4. The catalytic activity is regulated by adding the artificial sea-water treated with active carbon to the artifificial sea-water.

Addition of artificial sea-water treated with active carbon in per cent %	Catalytic activity of culture media K_{30}	Growth of diatom after six days in numbers of $KMnO_4$ units	Form of cells after culture
0	0.174	1.5	abnormal
20	0.140	4.7	abnormal
40	0.125	5.0	abnormal
60	0.113	10.5	slightly abnormal
80	0.096	19.7	normal
100	0.064	20.4	normal

Remarks: Temp. 22.3°C pH 8.2 Cl 17.0‰

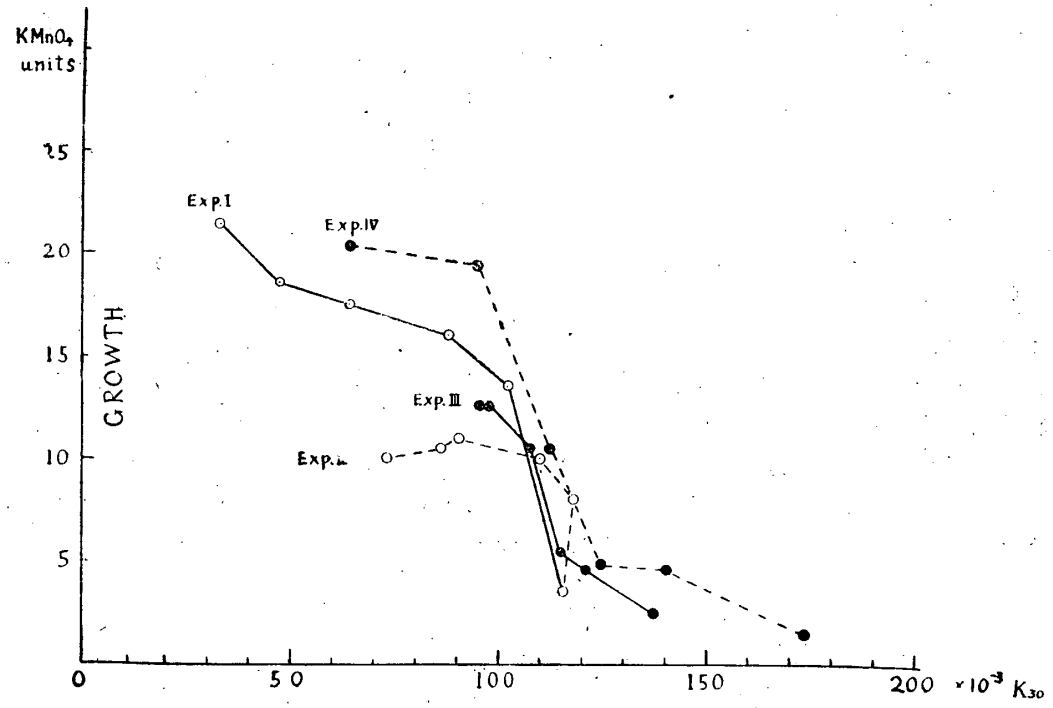

Fig. 2. Relation between the Growth of *Skeletonema* and the Catalytic activity.

the activity is above K_{30} 0.120, the growth is almost inhibited or stopped. All cells are abnormal and dead cells are observed.

Discussions

From the present experiment, the following conclusions are obtained.

1). The growth of *Skeletonema* is sharply inhibited by the activity above K_{30} 0.090 and when the activity is higher than K_{30} 0.120, normal growth does not take place.

2). The inhibition of the growth in the present experiment is not related to the nutritive salts limiting the growth because a definite relation between mixing percentage of two sea-water samples and the diatom growth is not found from the result shown in Table II.

3). The culture of *Skeletonema* in the artificial sea-water is possible without addition of organic matter by lowering the activity below K_{30} 0.090.

4). In the present experiments, the growth at activity below K_{30} 0.050 has not been observed. Therefore, whether optimum activity for the growth of this diatom exists or not is not yet clear.

From the above conclusions, a few interesting problems which must be investigated in future are drawn. The one is the physiological mechanism of the activity effecting physiological processes in diatom cells such as assimilation, respiration and etc.. However, prior to dealing with this problem, it is

necessary to clarify positive catalysts for the catalytic activity of sea-water. From the result that the normal growth of diatom occurs in culture media treated with active carbon and ferric chloride, the presence of some heavy metals in sea-water and their effect for the activity are expected. They may act as oligodynamic action to diatom and work also as a catalyst for the catalytic activity. Sometimes, they may lose their poison being adsorbed on or combined with negative catalysts such as active carbon and ferric hydroxide. Details of catalysts associated with heavy metals will be reported in next paper.

On the other hand, there is a problem of the adaptation for the catalytic activity. If the range of the catalytic activity which accompanies normal physiological processes differs among diatoms or other marine organisms, the catalytic activity should take an important rôle as an environmental factor for marine organisms. This is important to be investigated in future.

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