ON THE FERTILITY OF MATSUSHIMA BAY II. THE MINERALIZATION OF ORGANIC MATTER IN MARINE MUD

著者	KAMATANI Akiyoshi, MATSUDAIRA Chikayoshi
journal or publication title	Tohoku journal of agricultural research
volume	15
number	3
page range	279-294
year	1965-03-25
URL	http://hdl.handle.net/10097/29453

ON THE FERTILITY OF MATSUSHIMA BAY
II. THE MINERALIZATION OF ORGANIC MATTER IN MARINE MUD

By

Akiyoshi KAMATANI and Chikayoshi MATSUDAIRA

Department of Fisheries, Faculty of Agriculture,
Tohoku University, Sendai, Japan

(Received December 1, 1964)

Introduction

In the preceding paper, we dealt with the organic carbon, total nitrogen and α-amino acid nitrogen contents in the marine deposits of Matsushima Bay (Kamatani and Matsudaira 1). The organic matter in these sediments was considered to have originated mainly from sea weeds, planktons and their remains. Since the mechanism of microbiological decomposition and mineralization of the native organic matter in the deposits are presumed to be quite complex, the present work was undertaken to find experimentally the mineralizing processes of the carbohydrates in the deposit. We attempted to do this under conditions less complex than those found in nature. For this purpose, glucose, starch, cellulose and eelgrass were added to the marine mud collected from Matsushima Bay and their decomposition was studied under aerobic and anaerobic conditions by measuring the gases evolved by the action of the microorganisms in the mud.

Methods

The mud used in this experiment was collected with an Ekman's sampler from Matsushima Bay. It was passed through a sieve (0.5mm in diameter), air dried and then pulverized sufficiently to fine powder. To this air dried mud, various amounts of the substrate such as glucose, starch, cellulose and eelgrass were added respectively. They were kept in an incubater maintained at 30°C under aerodic and anaerobic conditions using the apparatus shown in Fig. 1. At suitable intervals, the amounts of gasses and ammonium nitrogen derived from the mineralization of the organic matters were determined. At the same time pH and Eh were observed by the following methods.

During this experiment it was necessary to correct the oxidation-reduction potentials at different pH values to pH 7 by subtracting 0.06 volt for every unit on the acid side of neutrality, or adding 0.06 volt for every unit on the alkaline side of neutrality. A potential corrected in this way to pH 7 is written Eh_7.

(1) The anaerobic experiments

Five grams of the air dried mud and 15 ml of sea water were added to the injector (Fig. 1). They were mixed well for a while, left to stand for about 10 minutes until the occluded air bubbles escaped completely, and then the mouth of the injector was closed with a rubber stopper leaving no air bubbles between the stopper and the water. A number of such injectors were prepared for each sample and kept at 30°C in an incubator. The gases evolved in the injector were determined by the method reported by Koyama (2) and Yamane (3, 4).

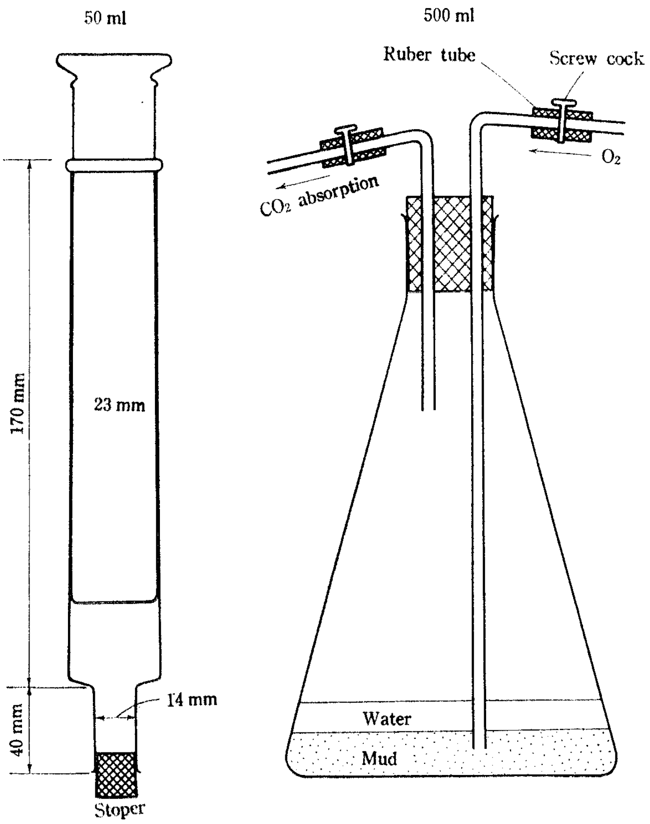

Fig. 1. Injector and flask.

For the measurements of Eh, pH and ammonium nitrogen, the tube used is as shown in Fig. 2. A series of glass tubes for the measurement of Eh were filled with 20g of the air dried mud and with the sea water. They were mixed thoroughly with a glass rod to exclude the air bubbles and closed with a rubber stopper equipped with both a platinum electrode and Bunzen's bulb. Then they were set in an incubator in parallel with the injectors for the measurement of gases.

For the measurement of pH and ammonium nitrogen, another series of glass tubes were filled with 20 g of the air dried mud and sea water in the same

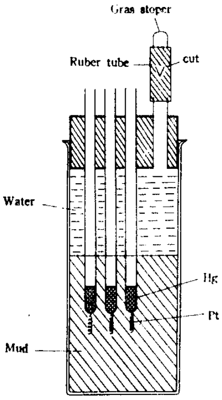

Fig. 2. Vessel for determination of Eh and pH.

manner as the Eh measurement and closed with the rubber stopper equipped only with a Bunzen's bulb. After a suitable intervals the samples were taken out of the incubator, the contents were mixed quickly with a glass rod and then the pH was measured. After the determination of pH, the content was transfered to a 300 ml flask. It was filled up to 100 ml with proper amounts of KCl and distilled water in order that the final concentration of KCl would be 10 percent. The mixtures of mud and KCl solution were shaken for 30 minutes and filtered with a filter paper. The ammonium nitrogen in the filtrate was determined by distillating it with MgO.

(2) The aerobic experiments

The aerobic experiment was done by using the apparatus shown in Fig. 1. Twenty grams of air dried mud and 120 ml of sea water were added to the flask (500 ml) and the air in the apparatus was replaced by oxygen. The flask was kept in the incubator and shaken several times every day.

The carbon dioxide liberated in the process of decomposition was driven off by passing oxygen bubbles at the rate of 200 ml per minutes through the culture medium for about 50 minutes. Then the gas was passed through the tubes containing a standard $Ba(OH)_2$ solution. The carbon dioxide was thoroughly absorbed.

Experimental Results

(1) The evolution of gases

In the aerobic decomposition of organic matter in the mud, the main gas evolved is carbon dioxide. While, under the anaerobic condition, the organic carbon is incompletely metabolized, the intermediary substances such as organic acids are accumulated, and a great amount of hydrogen and methane are produced.

Firstly, the analytical results of the gases evolved from glucose under an anaerobic condition are illustrated in comparison with pH and Eh_7 in Figs. 3 and 4. The peculiarity of this figure is that within one day after the addition

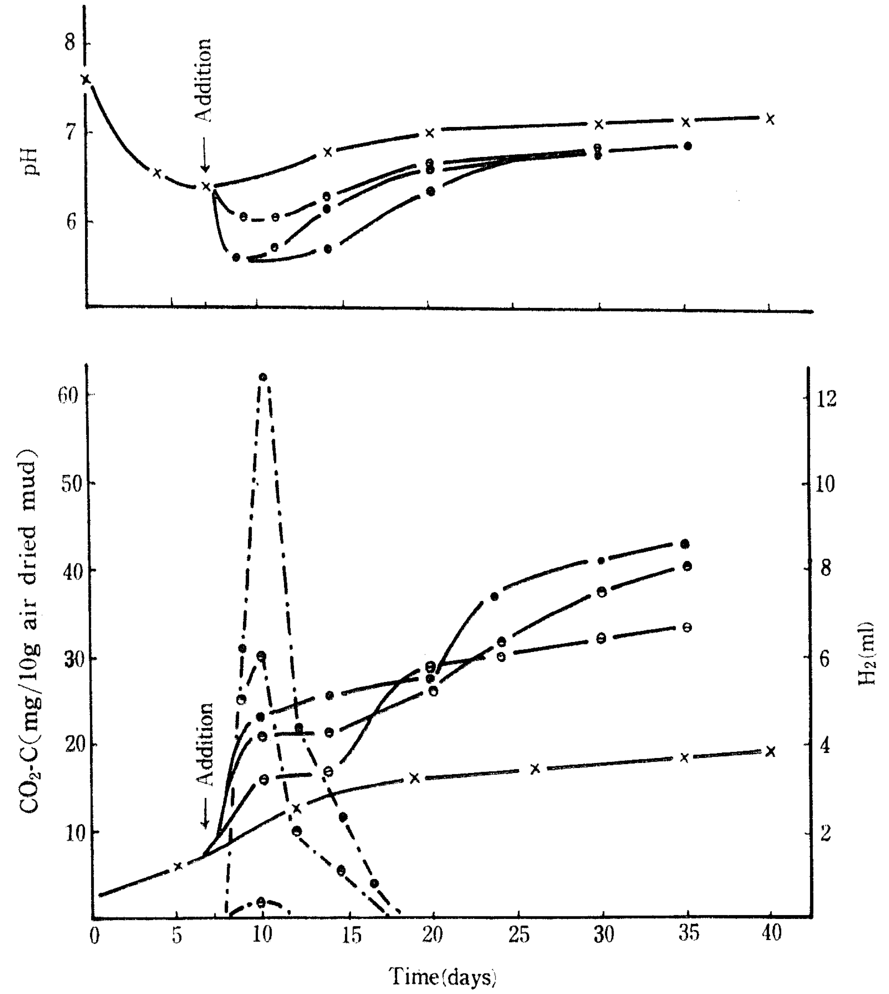

Fig. 3. Correlation of the change-over time of gases and pH in the marine mud under anaerobic condition.
-⊖-, -◓- and -●- indicate 20, 40 and 60 mg of carbon as glucose, respectively. -×- corresponds to the control.

of glucose, the production of hydrogen increases remarkably. Then after several days, the hydrogen is utilized by the microorganisms. On the other hand, it is found also that the more the glucose is added to the mud, the more the hydrogen evolves and also the longer is the period required for its disappeareance. The carbon dioxide does not increase during the evolution and disappearance of the hydrogen but increases after the disappearance of the hydrogen.

Fig. 4 illustrates the analytical results of the gases evolved from starch and cellulose in the process of anaerobic decomposition. According to these results, the hydrogen is not evolved during the processes of decomposition of these substrates added to the mud.

The vicissitudes of the gases in the processes of anaerobic decomposition of eelgrass are shown in Fig. 5 in comparison with pH and Eh$_7$. The process of accumulation of carbon dioxide has a pattern analogous with the decomposition of glucose. There was no accumulation of carbon dioxide during the gas phase,

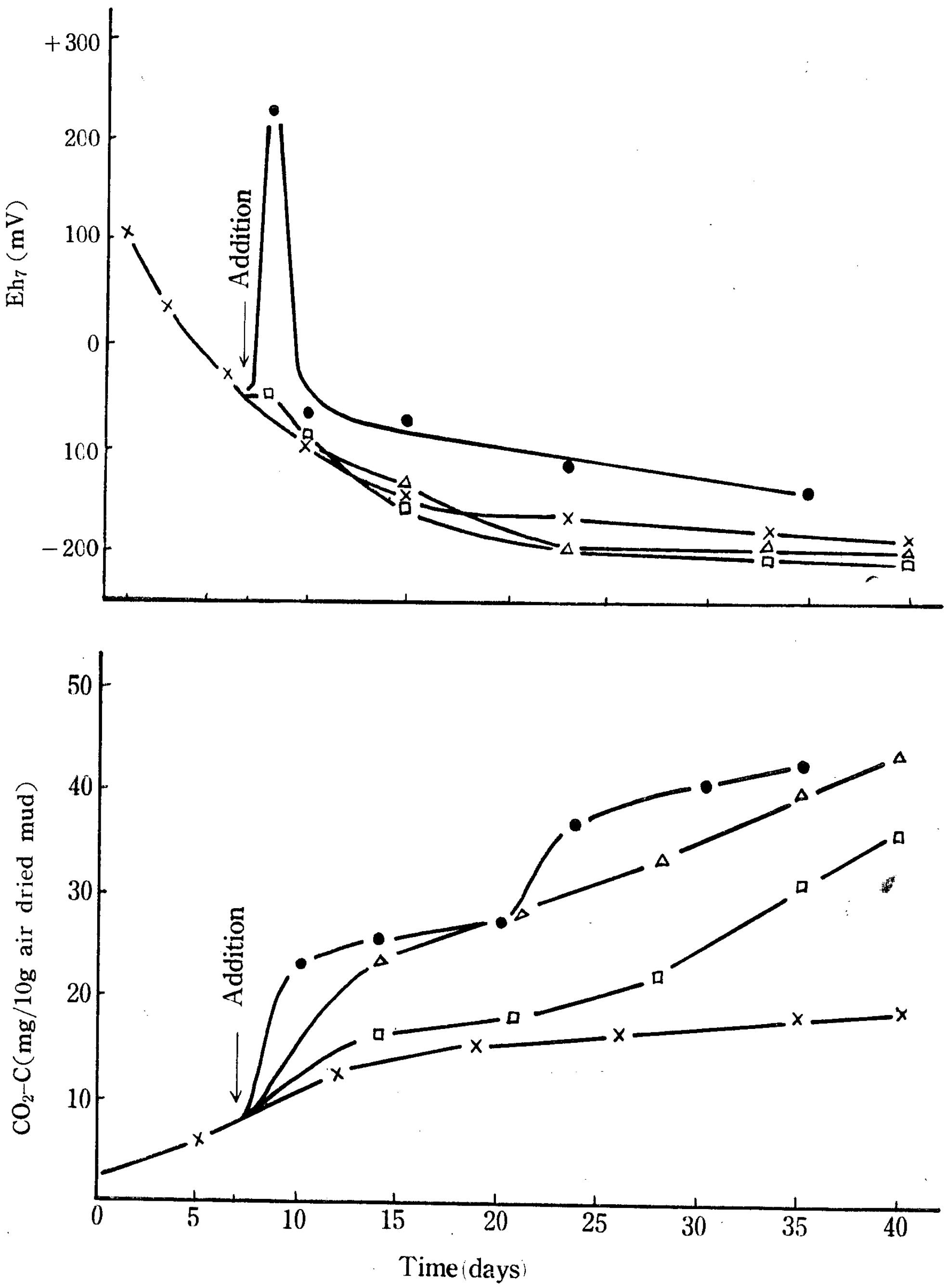

Fig. 4. Correlation of the change-over time of gases and Eh_7. The marks are the same as those employed in Fig. 2 except for -△- and -□- which correspond to starch and cellulose.

but after the gas phase carbon dioxide began to accumulate again. The components of the gases were not investigated, but it is presumed that they consist mainly of hydrogen. The gasses disappeared after five or six days. If they had consisted of carbon dioxide, they would not have such a low solubility. Yamane and Sato (5) affirmed also that gas was formed by the evolution of hydrogen, nitrogen and methane of low solubility. The formation and disappearance of hydrogen and methane were observable. In the anaerobic experiments, it seemed

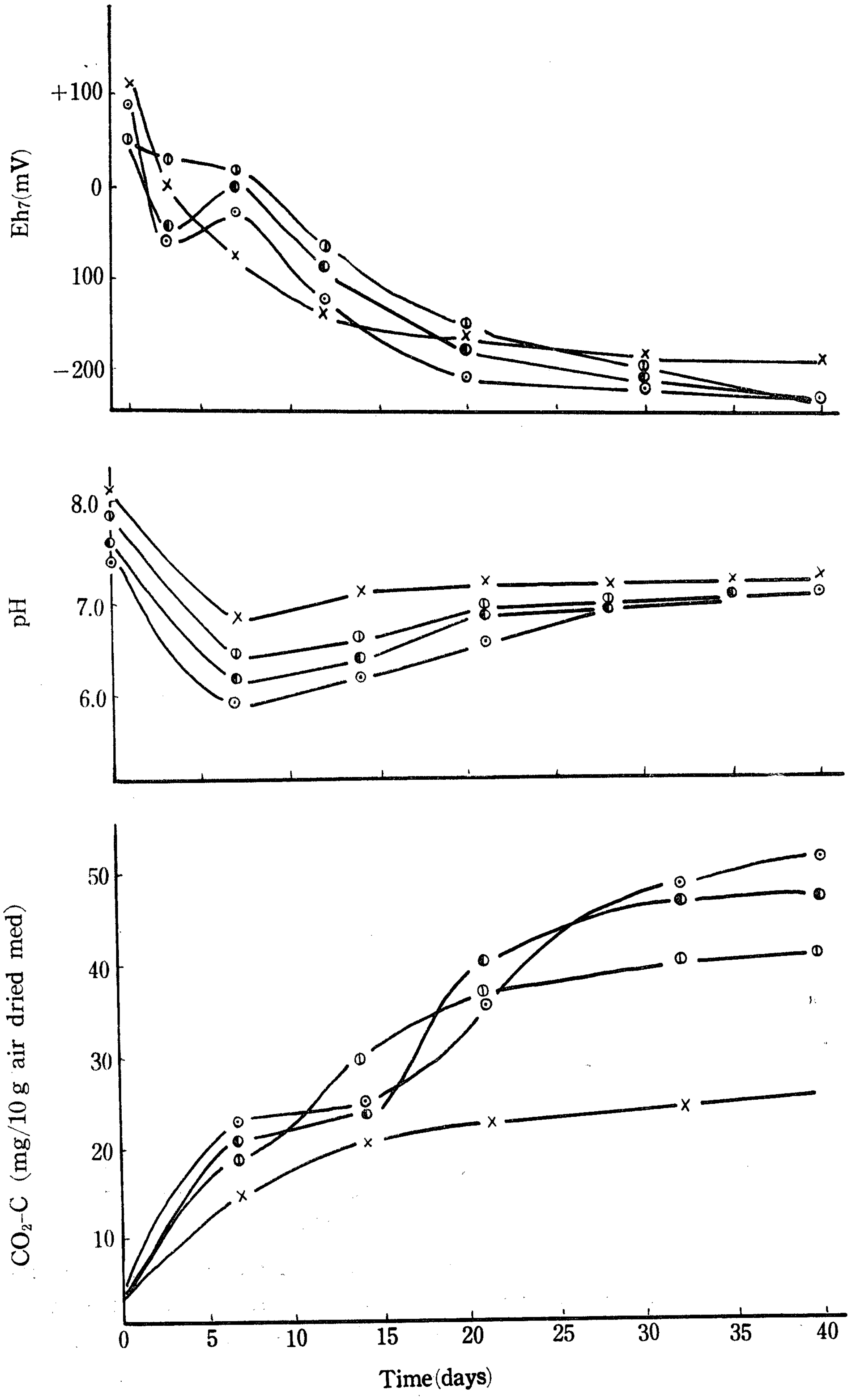

Fig. 5. Correlation of the change-over time of gases, pH and Eh$_7$ in the period of mineralization of eelgrass under anaerobic condition.
-⦶-, -◐- and -○- corespond to 160, 320 and 480 mg of eelgrass, respectively. The used eelgrass contained 1.22 per cent of nitrogen and 32.60 per cent of carbon.

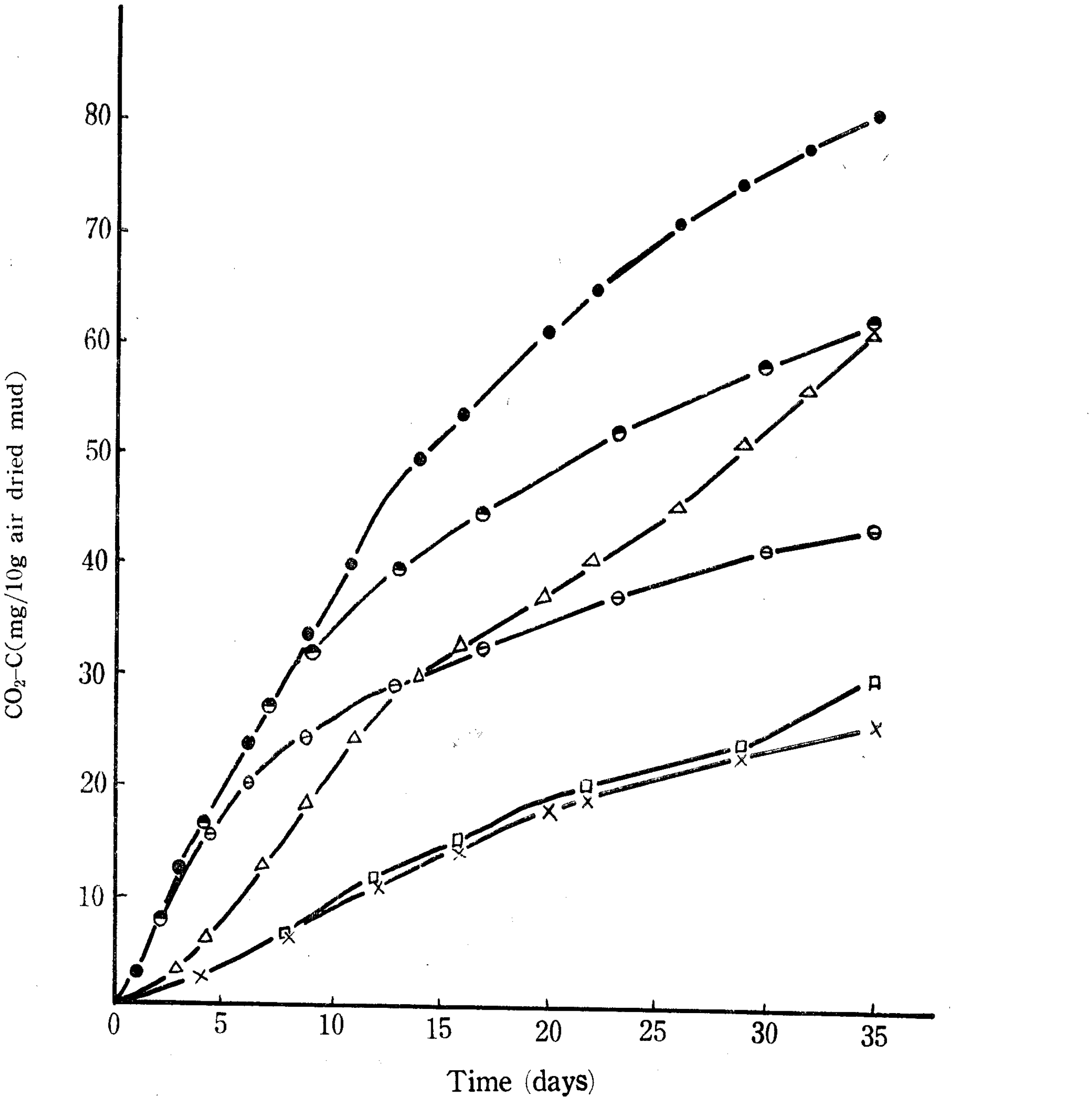

Fig. 6. Accumulation of CO_2-C in the period of mineralization of carbohydrates under aerobic condition.
The marks are the same as those employed in Figs. 3 and 4.

to be important that the methane was not detected during the mineralization process.

Figs. 6 and 7 show the accumulation of carbon dioxide in the processes of aerobic decomposition of glucose, starch, cellulose and eelgrass. In comparison with Fig. 5, the glucose and starch are much more mineralized than in the anaerobic condition during the experiment. On the contrary, there is evidence that the cellulose is more resistant to the microorganisms which are under the aerobic condition.

The rate of mineralization of the organic matter is decreased with the increase of the substrates such as glucose and eelgrass during the anaerobic decomposition. The rate of evolution of carbon dioxide, however, is in proportion to the increase

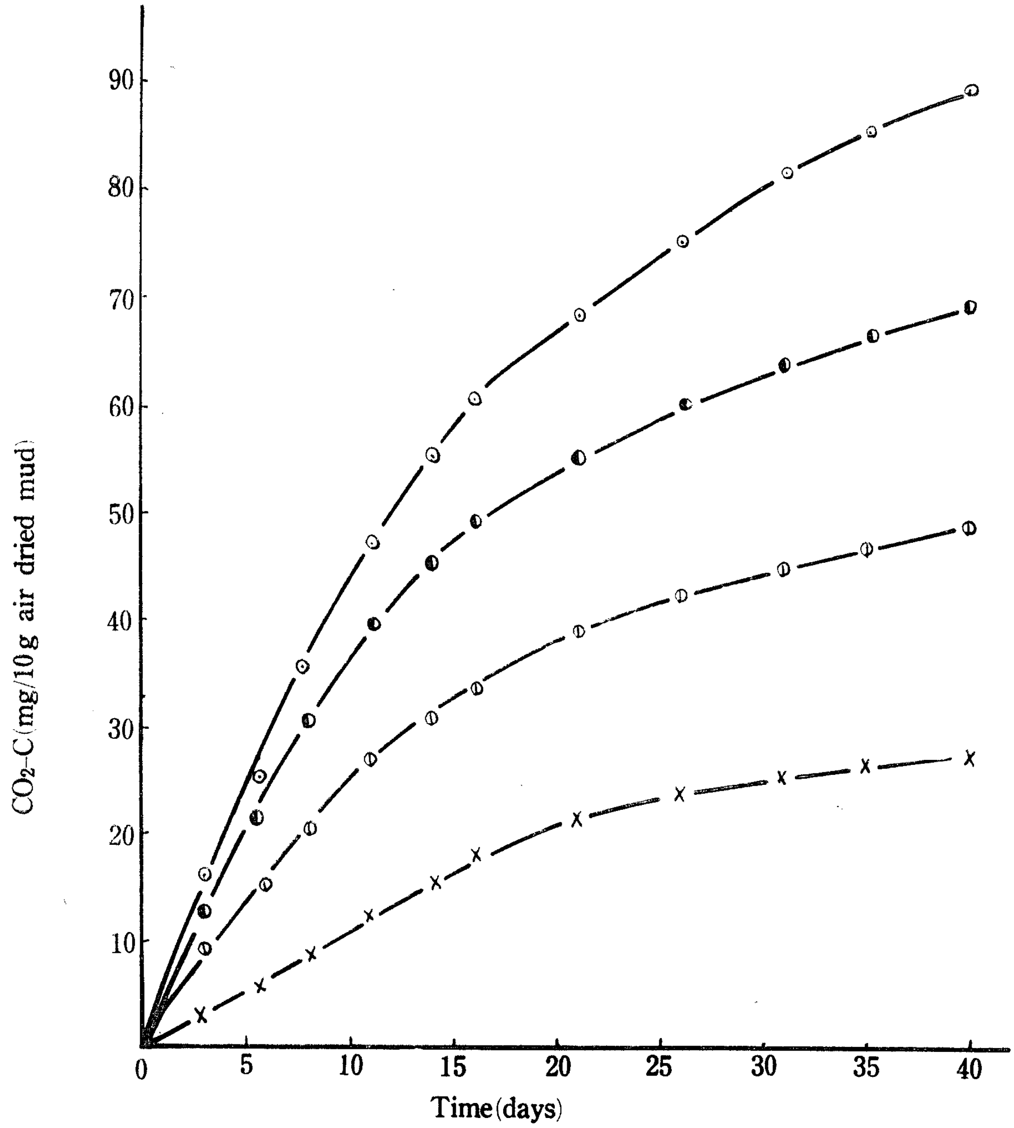

Fig. 7. Accumulation of CO_2-C in the period of mineralization of eelgrass under aerobic condition.
The marks are the same as those employed in Fig. 5.

of the substrate during the aerobic mineralization.

(2) The formation of ammonium nitrogen

The amount of ammonium nitrogen derived from the organic nitrogen contained in the mud under the anaerobic condition was determined on the samples which contained glucose, starch, cellulose and eelgrass. However, the experiment on the sample containing eelgrass was carried out under both the anaerobic and the aerobic conditions.

Each process of accumulation of ammonium nitrogen with the addition of glucose, starch and cellulose is illustrated in Fig. 8. As this figure shows, the total amount of ammonium nitrogen in the control sample gradually increased with the mineralization of the organic matter contained in the mud. On the other hand, when the substrate is added to the mud, a part of the ammonium nitrogen produced from the organic nitrogen is consumed. The consumption pattern of

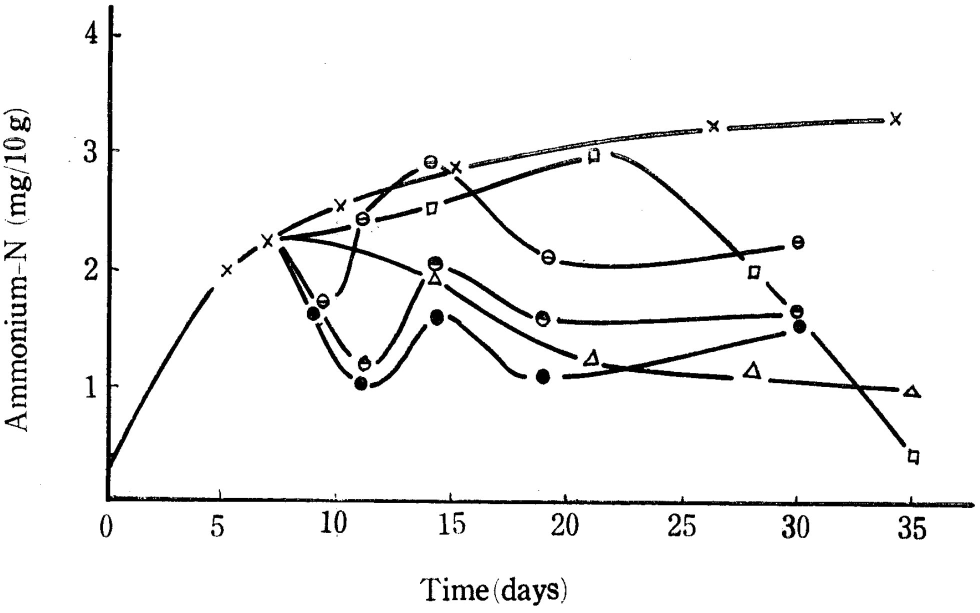

Fig. 8. Change of ammonium-N on the addition of carbohydrates. The marks are the same as those in Figs. 3 and 4.

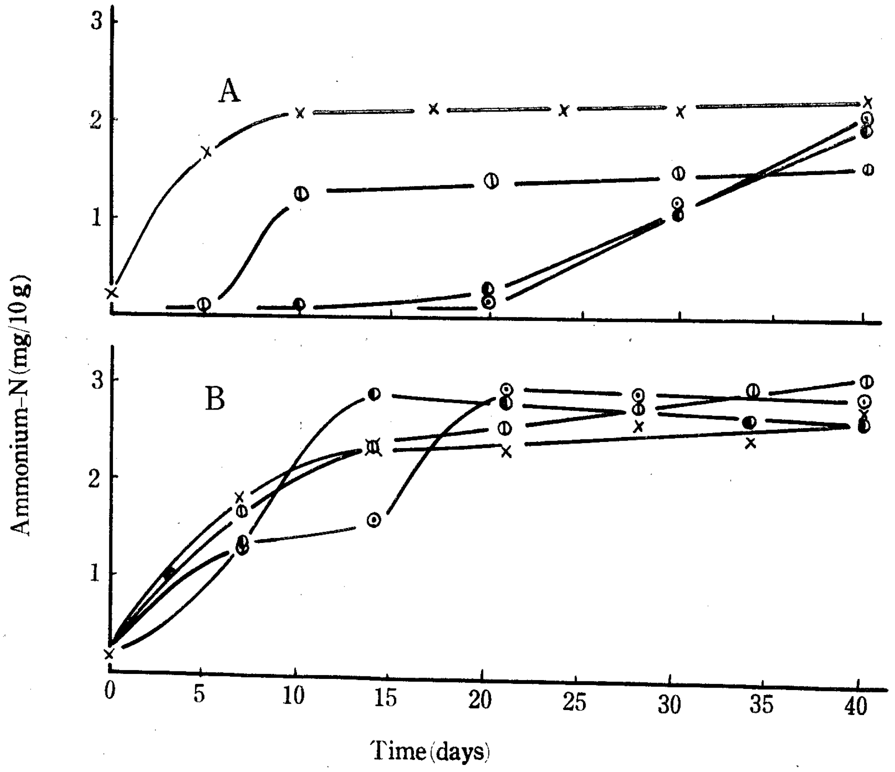

Fig. 9. Change of ammonium-N in the period of decomposition of eelgrass.
The marks are the same as those employed in Fig. 5. A : aerobic condition, B : anaerobic condition.

ammonium nitrogen may be dependent on the character of the added substrate. The consumption of ammonium nitrogen is rapid with the addition of glucose and moderate with starch. In the case of cellulose, however, the decrease of ammonium nitrogen begins two weeks after the addition and is remarkable.

The amount of ammonium nitrogen which is mineralized in the process of aerobic and anaerobic decomposition of the eelgrass is shown in Fig. 9. According to this figure, the formation of ammonium nitrogen appears in an earlier stage for anaerobic than for aerobic decomposition. Under the aerobic condition, the more the eelgrass is added to the mud the later is the liberation of ammonium nitrogen in the beginning.

(3) The changes of Eh_7 and pH

The change of Eh_7 and pH were determined only in the anaerobic condition as shown in Figs. 3, 4 and 5 respectively. The pH of the control sample generally decreased for a week after the start of incubation and then it is increased gradually. By the addition of glucose, the pH dropped remarkably. The addition of starch or cellulose, however, does not show such a distinct fall of pH, but maintained a pattern similar to the control sample.

The Eh_7 of the control sample dropped distinctly within the first few days of incubation and after this period the decline is not so remarkable. Except for the slightly increase in Eh_7 during the evolution of hydrogen, there was no remarkable difference with the control sample.

Discussions

(1) The vicissitudes of gases

In the anaerobic experiments, hydrogen distinctly evolved from the glucose added to the mud in the early stages of the decomposition, but in the case of starch or cellulose addition, hydrogen was not detected.

After a thorough investigation, it has been stated that in lake sediment and paddy soil the formation of methane was subsequent to the appearance of hydrogen during the anaerobic mineralization of carbohydrates contained in the mud (Koyama 6, 7. Yamane 3, 5, 8). In our experiments, however, the evolution of hydrogen was remarkable only in the early stages of the anaerobic mineralization of glucose. The methane formation was not subsequent to the appearance of hydrogen. According to ZoBell (9), the moleculer hydrogen is utilized under an anaerobic condition by several physiological types of bacteria, among which the sulfate reducers appear to be the most important in marine sediments. Sisler and ZoBell (10) concluded that the moleculer hydrogen was utilized by the sulfate reducing bacteria under an anaerobic condition. Therefore, it must be considered that most of the hydrogen would be consumed by this type of bacteria in our experiments.

Yamane (5) reported that the methane fermentation was very active after the disappearance of hydrogen in the paddy soil which had glucose added. However, Koyama (7) had found that the high concentration of sea salts in the soil water hindered methane fermentation considerably. In our experiments, the methane fermentation did not occur after the disappearance of hydrogen, consequently it seemed to be reasonable to assume that the high concentration of salts inhibited the methane fermentation.

As already mentioned, the pattern of evolution of carbon dioxide from the substrate under the aerobic condition differed according to the substrate. The process of accumulation of the carbon dioxide derived from the decomposition of glucose took a smooth curve but that of starch and cellulose showed a sigmoid curve. The process of accumulation of carbon dioxide in the mixture of glucose-starch and glucose-cellulose, different from starch or cellulose alone, take a smooth curve as shown in Fig. 10. This is similar to eelgrass which consisted of many kinds of carbohydrates.

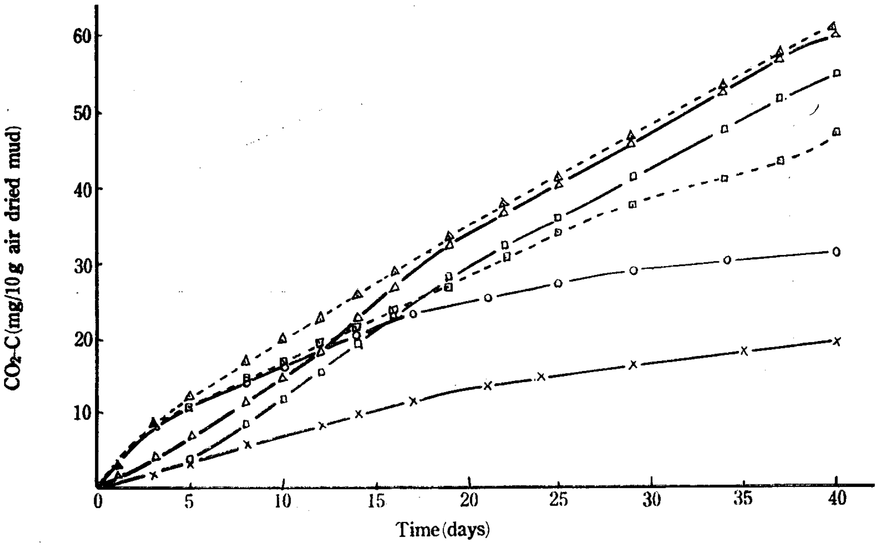

Fig. 10. Accumulation of CO_2-C in the processes of the decomposition of carbohydrates mixture.
-△- and -□- correspond to 50 mg of carbon as starch and cellulose, respectively. -○- is 10mg of carbon as glucose. -⚠- and -▣- are glucose-starch and glucose-cellulose. These sample are mixed in the same order as shown above.

(2) The decomposing velocity of carbohydrates

As carbon dioxide is one of the end products derived from the organic matter under an aerobic condition, the mineralization velocity of organic matter is computed from the amount of carbon dioxide and expressed as carbon per unit time (dCO_2-C/dt). According to this result, the velocity of mineralization in the decomposition of glucose or eelgrass reaches a peak at two to three days after the incubation and then decreased gradually. On the decomposition of starch or cellulose, the rate of mineralization is low and a conspicuous peak does not appear as in the case of glucose or eelgrass. The mineralizing processes seem to be attended to the pseudo first order reaction (Figs. 11 and 12), since the relation between the time and log ($C_t/C_0 \times 100$) is linear, where C_0 and C_t are the amount of organic carbon in the mud at the start and at an arbitrary time (t), respectively. As shown in Figs. 11 and 12, each mineralizing process consists of

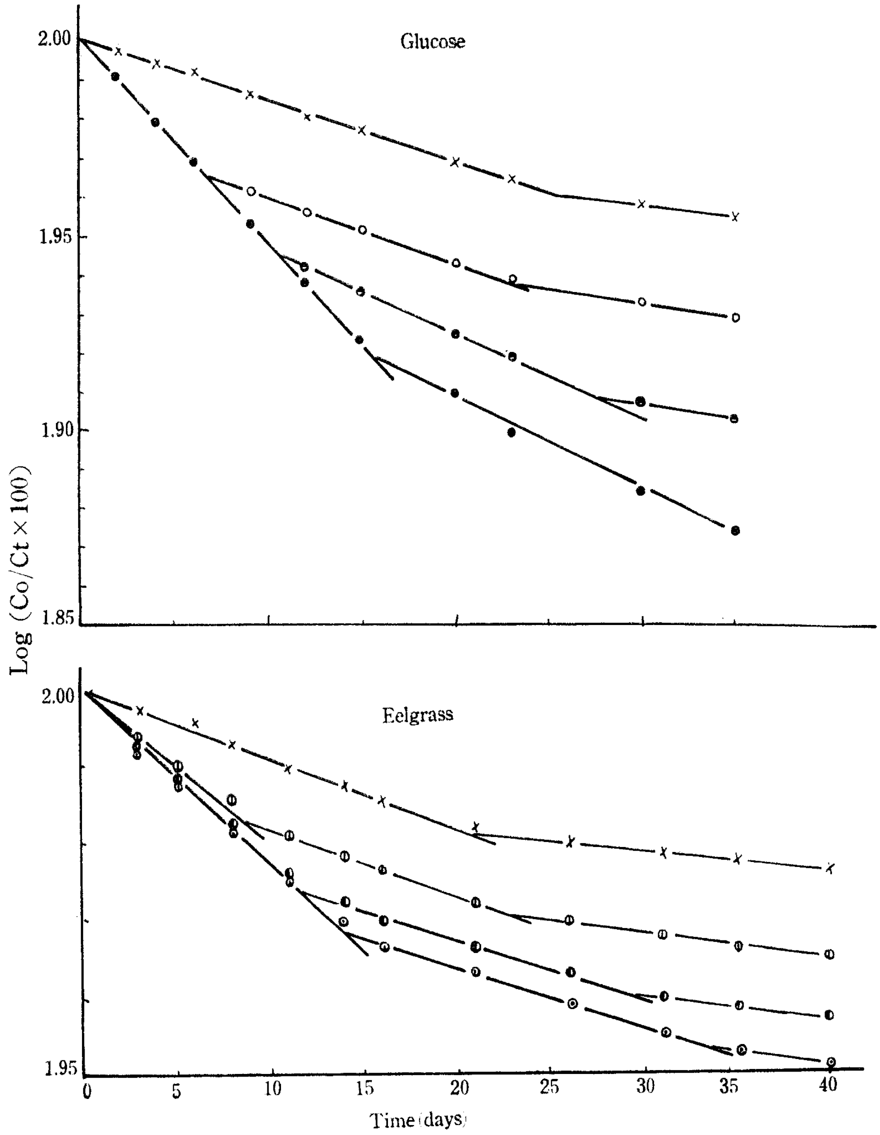

Fig. 11. Concentration-time diagram for glucose and eelgrass. The marks are the same as those employed in Figs. 3 and 5.

two or three straight lines. Therefore, the processes of mineralization can be divided into two or three stages. The rate constant in each stage calculated from the figure are shown in Table 1. In the glucose and the eelgrass, the rate constant of the first stage is conspicuous, and that of the second and third stages almost corresponded with that of the control sample. However, the starch and cellulose were highest in the second stage. When a bit of glucose is added, the rate constant was the largest in the first stage.

The production of carbon dioxide, namely the mineralization velocity is attributed to the easily available organic matter rather than to the total organic matter. On the investigation of the rate of oxidation of organic matter in sea culture, Waksman and Hotchkis (11) found that 1 mg. of organic matter in the

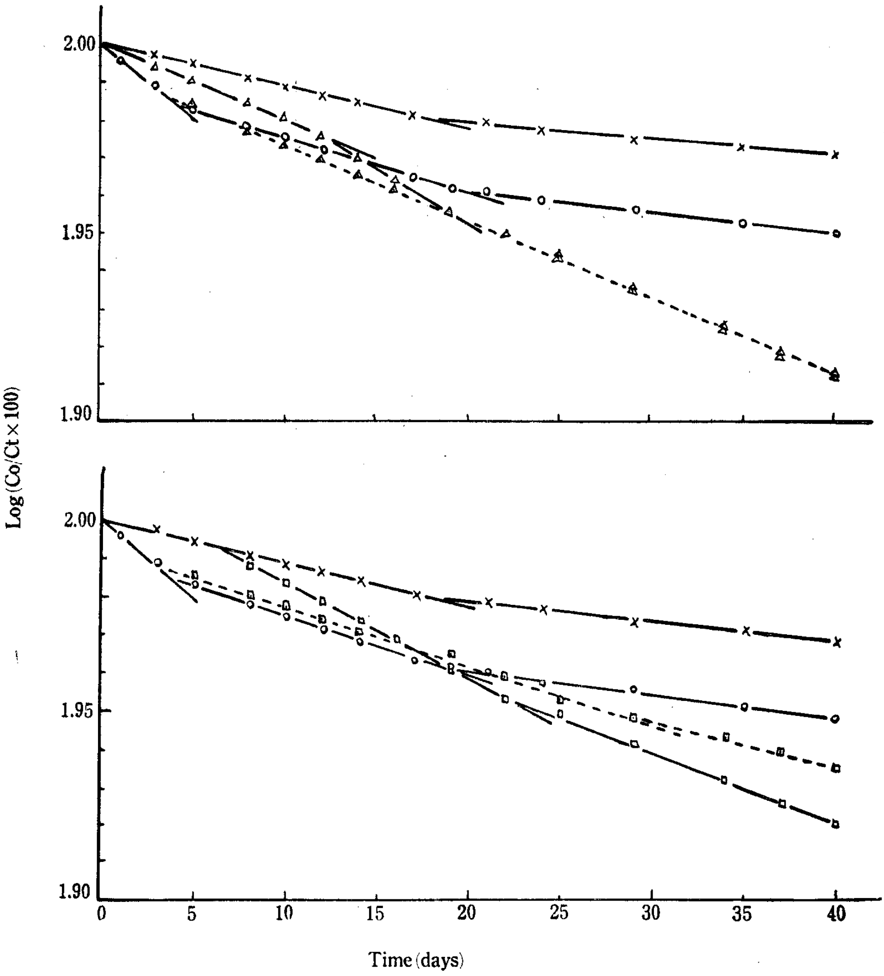

Fig. 12. Concentration-time diagram for carbohydrates mixture. The marks are the same as those employed in Fig. 10.

Table 1. Rate constants.

Substrates (mg/10g mud)		Rate constants of each stage $(\times 10^{-3})$*		
		K_1	K_2	K_3
Glucose	0	3.736	1.428	—
	20	11.97	3.892	1.753
	40	11.97	4.928	1.884
	60	11.97	5.302	—
Eelgrass	0	2.593	0.553	—
	160	4.487	1.957	0.777
	320	5.181	1.770	0.672
	480	5.181	1.815	0.672
Mixture	Control	2.703	1.152	—
	Glucose-10(A)	9.201	3.653	1.373
	Starch-50(B)	4.698	6.402	4.698
	Cellulose-50(C)	2.564	6.149	2.913
	(A)+(B)	9.201	4.733	—
	(A)+(C)	9.201	3.730	2.718

* The rate constants are arranged respectively K_1, K_2 and K_3 from left to right in Figs. 11 and 12.

bottom close to land consumed about 0.1 cc. of oxygen, under the most favorable conditions. Also the organic matter contained in the sea bottom was much more resistant to biological decomposition or oxidation than was that in sea water or that found is plankton organisms. On the basis of these results, it seems to be reasonable to assume that at the first stage, the easily available organic matter such as glucose in quite mineralized and in the second and third stages, the starch, cellulose and their related materials begin to decompose gradually in the sediments.

(3) The change of the ratio of carbon to nitrogen

Waksman and Carey (12) pointed out that if carbohydrates or oxidizable non-nitrogenous carbon compounds are present in excess, the nitrogen in decomposable nitrogenouse material may be utilized directly into bacterial cell substances and thus is not liberated immediatly as free ammonium nitrogen. In this experiments, the liberated ammonium nitrogen is consumed by microorganisms in

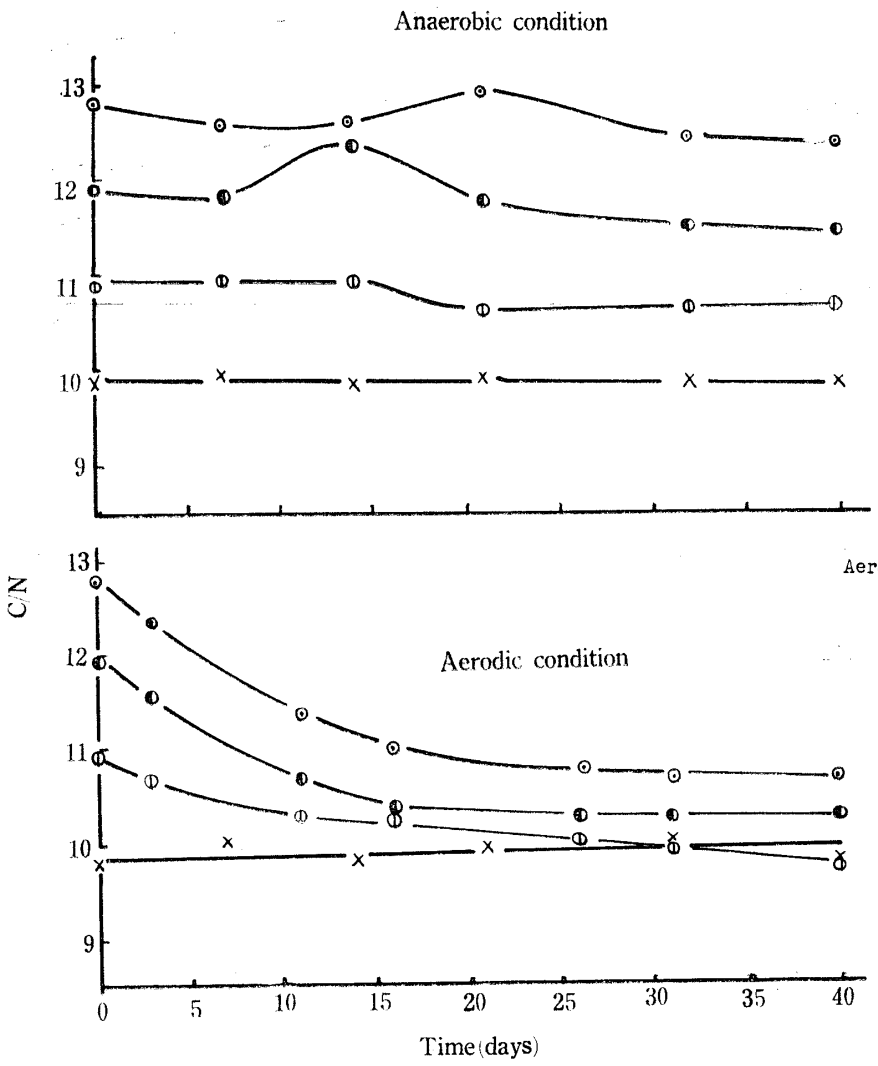

Fig. 13. Change of the ratio of carbon to nitrogen (C/N) remaining in the mud.
The marks are the same as those employed in Fig. 5.

addition of glucose, starch or cellulose (Fig. 8). By the addition of the substrate which have a high ratio of carbon to nitrogen such as eelgrass, the ammonium nitrogen was not liberated in the earlier stage in the process of aerobic decomposition, but it was liberated after the mineralization of the relatively available organic carbon for microorganism. On the other hand, under an anaerobic condition the ammonium nitrogen was liberated in the early stage. The ratio of carbon to nitrogen, therefore, is decreased remarkably at the early stage under an aerobic condition, but it is not observed under the anaerobic condition as shown in Fig. 13.

Summary

Under an anaerobic condition, the evolution of hydrogen was very remarkable within one day after the addition of glucose. After several days the hydrogen was utilized by the microorganisms. Carbon dioxide did not increase in the period of the vicissitudes of hydrogen. On the addition of starch or cellulose, hydrogen was not detected. Only carbon dioxide accumulated gradually.

The vicissitudes of gases in the processes of anaerobic decomposition of eelgrass showed pattern analogous with that of glucose.

The mineralization rate and the rate constant of the samples were computated according to the volume of carbon dioxide accumulated under the aerobic condition. The rate of mineralization on the decomposition of glucose or eelgrass reached the maximum at two or three days after the start of the experiment. On the decomposition of starch or cellulose, no conspicuous peak could be observed.

The processes of mineralization could be divided into two or three stages for each sample. The pH was remarkably decreased for a week after the start of incubation and after that period, it increased gradually. By the addition of glucose, the pH dropped remarkably. The addition of starch or cellulose, however, did not show such a remarkable fall of pH.

Except for the case of glucose addition in which Eh_7 was changed markedly by the evolution of hydrogen, there were no remarkable differences in Eh_7. That is, the Eh_7 dropped distinctly within the first few days of incubation and after that the decline was not so remarkable.

By the addition of carbohydrates, the liberated ammonium nitrogen was consumed by the microorganisms. The consumption pattern appeared to be dependent on the character of the added substrate. By the addition of eelgrass, the formation of ammonium nitrogen appeared in an earlier stage in the anaerobic condition than in the aerobic condition. The ratio of the carbon to nitrogen remainning in the mud was decreased remarkably in the early stage under an aerobic condition.

Acknowledgments

We wish to thank Dr. I. Yamane, Institute for Agricultural Research, Tohoku University, for his advice and criticism in the present study.

References

1) Kamatani, A. and C. Matsudaira (1963). Tohoku J. Agr. Res., **14**, 83.
2) Koyama, T. (1953). J. Earth Sci. Nagoya Univ., **1**, 101.
3) Yamane, I. (1958). Soil and Plant Food, **4**, 1.
4) Yamane, I. (1961). Soil Sci. and Plant Nutrition, **7**, 71.
5) Yamane, I. and K. Sato (1964). Soil Sci. and Plant Nutrition, **10**, 34.
6) Koyama, T. (1955). J. Earth Sci. Nagoya Univ., **6**, 1.
7) Koyama, T. (1964). Advances in organic geochemistry. p. 303, Pergamon Press. Ed. by U. Colombo and G. D. Hobson.
8) Yamane, I. and K. Sato (1963). Soil Sci. and Plant Nutrition, **9**, 28.
9) ZoBell, C. E. (1947). Bull. Am. Assoc. Petroleum Geol., **31**, 1709.
10) Sisler, F. D. and C. E. ZoBell (1950). J. Bact., **60**, 747.
11) Waksman, S. A. and M. Hotchkiss (1937). J. Mar. Res., **1**, 101.
12) Waksman, S. A. and C. L. Carey (1935). J. Bact., **29**, 531.