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On the Mineralization of Organic Substances at the Intertidal Zone

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Although the intertidal zone is not well studied yet, it is evaluated to play an important role in the marine ecosystem. It is well known that the intertidal zone serves as the nursery ground for shrimps such as *Penaeus japonicus*.1) It is also supposed that such organisms as Periphyte, Nematoda, Polychaeta and benthic Copepoda dwell, and are eaten by the animals of higher trophic level. Therefore, the intertidal zone is essential for the biological production in coast.

On the other hand, a large number of bacteria exists in sand of the intertidal zone, they are concerned in the process of mineralization of organic substances, denitrofication, nitrification etc. For these reasons, the intertidal zones contribute to the purification of sea water. However, the role of the intertidal zone on the purification of marine environment is not clarified yet in detail.

Here, the authors determined the mineralization rate of organic solute (U-[^{14}C]-glucose) at the intertidal zone. Moreover, the characteristic of the mineralization rate is discussed in relation to the grain size composition of the sand at the intertidal zone in this article.

Materials and methods

Sampling

The sand was obtained with a spoon at the seven stations at the intertidal zone located at the south coast of Beppu Bay on March and August, 1979. The sand sample at the deeper layer of sand column was obtained by the following method. The pipe made of chlorinated vinyl (50mm in internal diameter, 1000mm in length) was driven into the sand and pulled out. The pipe was cut off at intervals of 10cm, the sand in the pipe was obtained.

The sand samples were cooled under ice and brought back to the laboratory.

Bacterial counts

One gram of sand was added to 9 ml of sterile sea water and stirred vigorously for 5 min. The supernatant was serially diluted by decimal intervals in sterile sea water. The samples of 1 ml of each dilution were inoculated to the liquid medum. The culture medium was similar to ZoBell 2216 E. Inoculated media were incubated at 25°C for two weeks. The most provable number was determined.

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Mineralization rate

The mineralization rate of glucose at the intertidal zone was determined by the kinetic approach described by M. J. HARRISON *et al.*[2] and R.T. WRIGHT.[3] The apparatus used in this research was described in the previous article.[4] It is a 50 ml amber glass Erlenmyer flask, in which the inner chamber (internal diameter, 10mm) is equipped. 0.5 μCi of U-[^{14}C]-glucose, adequate amount of the sand sample (weighed precisely) and 5 ml of artificial sea water (ASP-6 modified[5]) were put in the outer chamber. Unlabeled substrate (glucose) was also in the outer chamber at several concentrations (5.19, 10.38, 15.57 and 20.76 μg). One ml of 1 M methanol solution of hydroxide of Hyamine 10-X (p. (diisobutyl-cresoxyethoxyethyl) dimethylbenzyl ammonium hydroxide) and an accordion-folded piece of filter paper (3 × 4 cm) were introduced into the inner chamber. The Hyamine 10-X was used as a reagents for absorbing carbon dioxide evolved, and the filter paper was used to expand the surface area. Four pairs of flasks of the four kinds of concentration of unlabeled substrate (eight flasks) per one sand sample were prepared for the determination of the mineralization rate.

The incubation was carried out after sealing with a double rubber cap in a shaking incubator (TOYO monoshin II) for 15 minute at 27 °C. At the end of incubation time, the reaction was stopped and the medium was acidified with 1 ml of 6 N HCl. The acid was introduced to the outer chamber through the rubber cap by a syringe with a needle. The flask was shaked again for 1 hour after acidifying to liberate carbon dioxide derived from mineralization and to absorb it into Hyamine 10-X.

After finishing the incubation, the Hyamine solution and the filter paper were put into a glass vial and the inner chamber was rinsed with the liquid scintillation solution (5.005 g. PPO (2,5-diphenyloxazole) and 0.495 g. POPOP (1,4-bis-2-(4-methyl-5-phenyloxazole)-benzen) per 1 liter of toluene) and the rinsed toluene solution was added to the glass vial. The radioactivities based on absorbed [^{14}C]CO_2 were measured by a liquid scintillation spectrometer (Packard Instrument Company, Inc. Model 3255). Quenching was corrected with the external standard method, and data were corrected for blank activity and applied as in the following equation above t/f (turnover time), which was plotted agaist added substrate concentration.

$$t/f = (K+S)/V_{max} + A/V_{max}$$

where ; t ; incubation time (hr.)

f ; the fraction of available substrate mineralized

K ; a constant related to uptake

V_{max} ; the maximal velocity for minaralization

S ; the natural substrate concentration

A ; the concentration of added substrate

The maximal velocity of the mineralization was obtained from the inverse of the slope of the regression line.

Ignition loss (crude organic matter), and carbon and nitrogen contents

Ignition loss was determined by burning the dry mud at 750 °C until the weight becomes constant.

Carbon and nitrogen contents were determined by a CHN Corder (Yanagimoto Co Ltd., Model MT2).

Grain size composition

The sand was dryed in a oven at 110 °C, and mixed throughly with a glass rod. About 10 g of sand were sifted by a series of nine sieves, and the amount of sand on each sieve was weighed. Then, the grain size composition in percentage was caluculated.

Results

Kinetics of mineralization of organic solute

The kinetic plots of mineralization of glucose are shown in Fig. 1. The good correlations were obtained and the correlation coefficients(r) were over 0.8. It is possible from

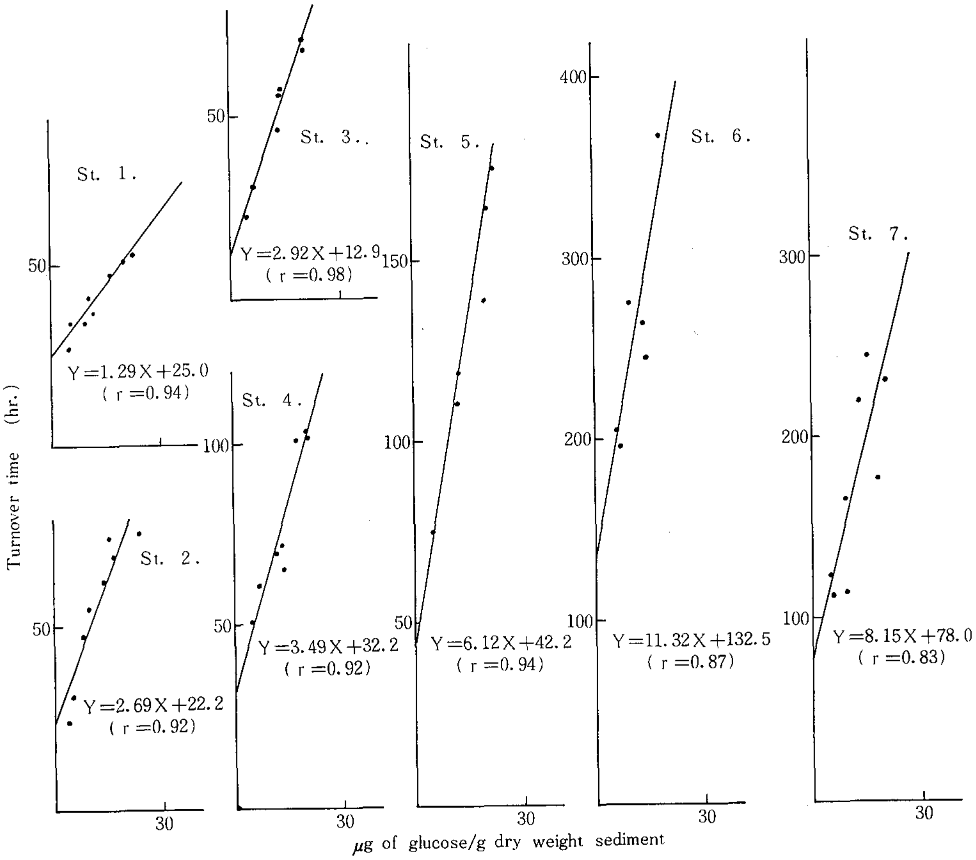

Fig. 1. Kinetics plot of mineralization of labeled glucose.

this figure that the maximal mineralization rates (V_{max}) are determined.

Number of aerobic heterotrophs and mineralization rate

These results are shown in Fig. 2 and Table 1. The mineralization rate and the number of aerobic heterotrophs were larger at the stations near the mouth of the Ohno River, where the ignition loss and the carbon and nitrogen contents were larger than those of the southeastern parts of this intertidal zone. It is apparent that the number of aerobic heterotrophs was larger in summer than in spring. The mineralization rate was also slightly higher in summer than in spring.

The mineralization rates fell between 0.049 and 0.488 μgC/g. dry sand/hr, and these values were lower than those of the bottom sediment.

Vertical distribution of mineralization rate, number aerobic heterotrophs, ignition loss, carbon and nitrogen contents and C/N ratio

From the data sumarized in Table 2, it is obvious that the mineralization rate and number of aerobic heterotrophs gradually decreased accoding to the sand column depths with becoming deeper. There were not found obvious characteristics in the ignition loss and C and N contents of the samples of sand column. The C/N ratios were

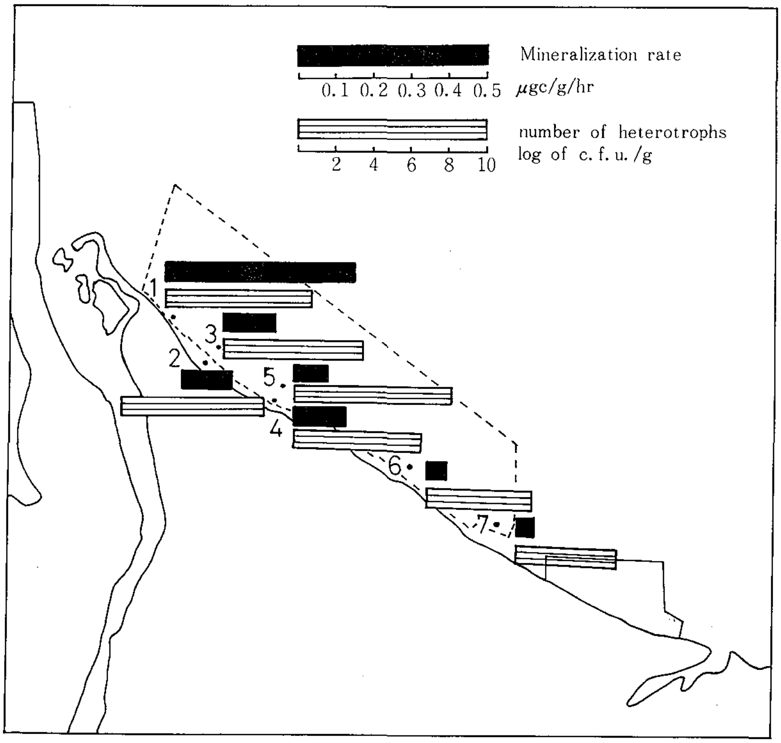

Fig. 2. Distribution of mineralization rate and heterotrophs at the intertidal zone of Beppu Bay.

Table 1. Seasonal differences of mineralization rate, heterotrophs, ignition loss, C and N contents, C/N ratio and sand temperature.

Date	St. No.	Mineralization Rate (μgC/g/hr)	No. of Heterotrophs (c.f.u./g)	IL (%)	C (mg/g)	N (mg/g)	C/N	Sand temp. (°C)
16th, Mar., 1979.	1	0. 310	1.0×10^{6}	1. 83	1. 59	0. 20	7. 95	15. 5
	2	0. 149	3.4×10^{6}	3. 59	7. 57	0. 67	11. 30	14. 2
	3	0. 137	4.4×10^{6}	2. 59	3. 26	0. 33	9. 88	16. 2
	4	0. 115	2.5×10^{6}	2. 54	2. 38	0. 29	8. 21	16. 5
	5	0. 065	1.5×10^{6}	1. 81	0. 88	0. 14	6. 29	14. 6
	6	0. 035	1.3×10^{5}	2. 37	1. 23	0. 14	8. 79	18. 0
	7	0. 049	7.3×10^{5}	2. 26	1. 09	0. 12	9. 08	16. 8
7th, Aug., 1979.	1	0. 488	6.5×10^{7}	4. 72	9. 83	0. 81	12. 14	31. 8
	2	0. 126	3.5×10^{7}	3. 28	6. 00	0. 59	10. 17	34. 5
	3	0. 135	2.8×10^{7}	3. 34	3. 38	0. 40	8. 45	31. 2
	4	0. 144	1.7×10^{7}	2. 52	2. 65	0. 27	9. 81	31. 8
	5	0. 091	1.8×10^{8}	2. 36	1. 96	0. 29	6. 76	33. 4
	6	0. 051	3.0×10^{5}	2. 54	1. 40	0. 17	8. 24	31. 0
	7	0. 051	2.0×10^{5}	2. 02	0. 86	0. 10	8. 60	33. 8

Table 2. Vertical differences of mineralization rate, heterotrophs, ignition loss, C and N contents and C/N ratio.

St. No.	Depth of Sand Column (cm)	Mineralization Rate (μgC/g/hr)	IL (%)	C (mg/g)	N (mg/g)	C/N	No. of Heterotrophs (c.f.u./g)
5	0— 0. 5	0. 091	2. 36	1. 96	0. 29	6. 76	1.8×10^{8}
	15. 0—18. 0	0. 042	2. 21	1. 33	0. 19	7. 00	1.5×10^{4}
	40. 0—42. 0	0. 012	2. 84	3. 23	0. 22	14. 68	3.2×10^{5}
7	0— 0. 5	0. 051	2. 02	0. 86	0. 10	8. 60	2.0×10^{5}
	20. 0—25. 0	0. 066	2. 15	1. 05	0. 10	10. 50	4.4×10^{4}
	60. 0—65. 0	0. 020	2. 23	1. 56	0. 11	14. 18	9.6×10^{3}

larger at the deeper layer than the surface layer of the sand column. The organic materials were more greatly decomposed at the deeper layer than at the surface.

Discussion

Near the river mouth of Ohno such as stations 1, 2, 3 and 4, the mineralization rate and number of heterotrophs were larger significantly as well as ignition loss and C and N contents. Concerning the grain size composition, the grains were distributed over wide ranges of the sieves at St. 1, 2, 3 and 4, on the other hand, grains between 250 μ and 500 μ were predominant at St. 5, and grains between 125 μ and 500 μ were predominant at St. 6 and 7. It is supposed that the smaller grain adsorbs large amount of organic materials. The organic debris is also accumulated to the region where the small grain (smaller than 63 μ) is found. Therefore the content of organic materials used by heterotrophs is greater at these stations (St. 1, 2, 3 and 4) than at other stations (St. 5. 6 and 7). As known that bacteria usually adhere to the surface of small particles,[6] it is also supposed that the number of heterotrophs is greater at the area where the small grain (smaller than 63 μ) is found. Hence, the number of heterotrophs is large at the area where the content of organic materials is large, and the mineralization rate is also high as the result of the large biomass of heterotrophs.

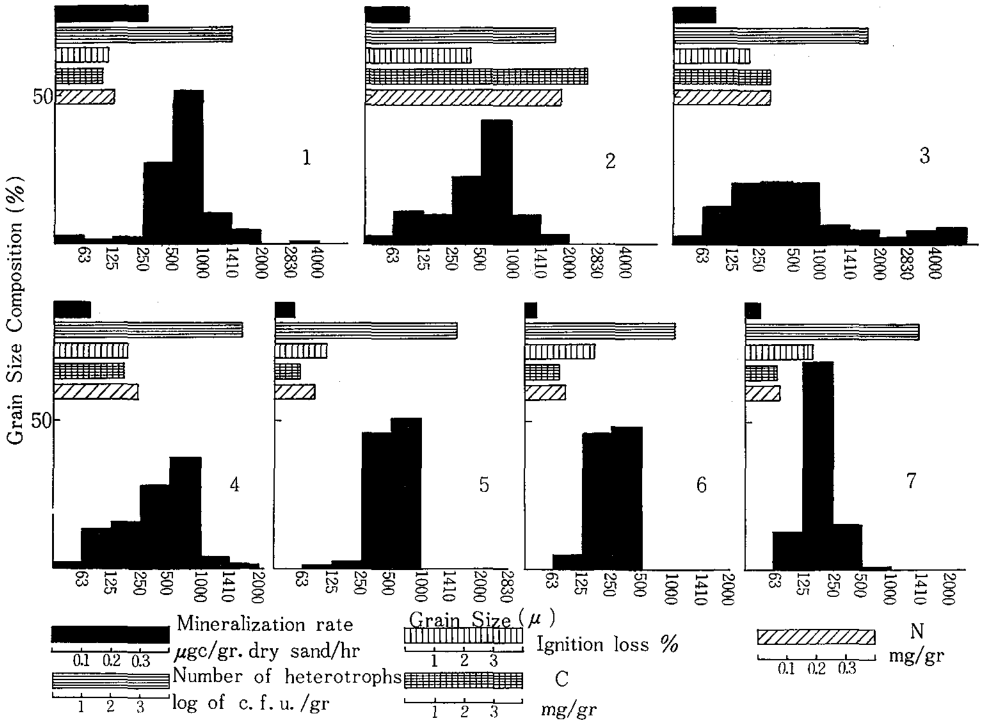

Fig. 3. The relation between grain size composition and mineralization rate, ignition loss, heterotrophs, C and N contents.

Judging from the vertical distribution of C/N ratio in the sand column, it is considered that the sand of this intertidal zone is not disturbed by the wave movement and sedimented chronologically. Because it is generally known that the mineralization of nitrogen compounds is superior to that of carbon compounds, and C/N ratios become gradually large according to the layers of sand column become deeper. According to K. HAYASHI,[7][8] it is recognized at a sandy beach that the number of aerobic heterotrophs and bacterial activities (activity of phosphatase, dehydrogenase and nitrification) are larger at the surface than at the bottom layer of the sand column. As the same results are obtained in the present study, it is considered that the surface layer of sand column is most important for the microbial processes.

The mineralization rate of organic solute at the foreshore was compared with that at sea water and the bottom sediments (Table 3). Although the bottom sediment was superior in the mineralization rate comparing to the other two areas, the foreshore mineralized about five hundred times as much organic solute as sea water did. Since the mineralization rate is remarkably decreased according to the sediment column depths with becoming deeper, that is, the mineralization rate at 10 cm deep is a eighteenth part at the surface of the sediment column,9) it is supposed that the mineralization of organic solute is almost carried out at the surface of the sediment. On the other hand, since the mineralization rate is gradually decreased according to the sand column depths with becoming deeper and the interstitial water flow easily in the sand than in the sediment, it is supposed that the deeper layers of the sand column contribute to the mineralization of organic solute. Although the mineralization rate is lower at the foreshore than at the bottom sediment, the layer concerning with the mineralization of organic solutes is thicker at the foreshore than at the bottom sediment. Therefore, it is reasonable to consider that the intertidal zone plays an important part in the metabolisms at coastal waters. Further studies related to the intertidal zone should be carried out in detail.

Table 3. Comparison of mineralization rate of sea water, bottom sediments and sand at the foreshore.

Location	Mineralization Rate	Ratio of Mineralization Rate
Sea Water*	5. 66^{+}	1
Bottom*	6651. 43^{++}	1175
Foreshore**	2952. 00^{++}	522

* estimated in Jan., 1979.
** estimated in Mar., 1979.
\+ μgC/l/day
\+\+ μgC/Kg/day

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Refferences

1) Norin Suisan Gizitsu Kaigi, 1979: Senkaiiki niokeru Zoyoshokugyozyo no Kaihatsu nikansuru Sogokenkyu (Studies on the developmentof coastal aquaculture grounds). (in Japanese)

2) Harrison. M.J., R.T. Wright, & R.Y. Morita, 1971: Method of measuring mineralization in lake sediments. Applied Microbiology, 21, 698-702.

3) Wright, R.T., 1973: Some difficulties in using ^{14}C-organic solute to measure heterotrophic bacterial activity. Estuarine Microbial Ecology (ed by H.L. Stevenson & R.R. Colwell), University of South Carolina Press, 199-217.

4) Yamada, H., A. Murakami, & M. Kayama, 1979: Mineralization of organic substances in sea water, Bull. Japan. Soc. Sci. Fish., 45, 1299-1305.

5) Tsukidate, J., 1977: Microbiological studies of porphyra plants-VI An investigation of bacteria-free culture of porphyra with a shaking apparatus, Bull. Nansei Reg. Fish. Res. Lab., 10, 113-121

6) Hattori, T., 1978: Biseibutsu Seitai Nyumon (Introduction to Microbial Ecology), University of Tokyo Press, xi + 137 (in Japanese)

7) Hayashi, K., 1979: On the purification at the sandy beach and the foreshore. Symposium on the some problems of the purification of marine environment held by Japanese Society of Scientific Fisheries.

8) Hayashi, K., 1978: On the hydrogenase activity of the sand at beach and bottom sediments. Abstract of Japanese Society of Scientific Fisheries held at Shimizu.

9) Yamada, H. & A. Murakami, 1979: On the mineralization rate of organic materials at the bottom sediments. Abstract of Japanese Society of Scientific Fisheries held at Hakodate.

干潟域における有機物の無機化について

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別府湾南岸の干潟で，グルコースをモデル基質として用い，M.J. Harrison 等の方法により無機化速度を測定し次の結果を得た。

1) 無機化速度は63μ 以下の微細砂粒子が存在し，IL 及びC，N量，従属栄養細菌数が大きい，大野川河口域で高い。

2) 無機化速度，従属栄養細菌数は干潟表面で大きく，深部になるに従って減少する。

3) 海水，干潟，底泥での無機化速度を比較すると，底泥で高く，ついで，干潟，海水の順である。干潟域での無機化速度は海水の500倍であり，沿岸域での物質循環にしめる干潟の役割は大きいものと推察された。

本研究は「埋立の漁業環境への影響に関する調査研究」の一環として行った。