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Effects of Chemical Composition and Temperature for the Production of Volatile Fatty Acids During Anaerobic Decomposition Process of Marine Sinking Particles

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Anaerobic decomposition experiments were performed to know the effect of chemical composition and temperature for the production of volatile fatty acids (VFAs) from marine sinking particles. Sinking particles were obtained with sediment traps set in Aburatsubo Inlet, Kanagawa Prefecture, Japan, in February, May and August. Sinking particles collected in May were composed of higher fraction of chl. a than the other two months. February and May samples were used to perform the decomposition experiments. VFAs production rates were higher in May sample than February. The production rates increased with increase of incubation temperature, and order of production rates of four VFAs were acetate>*n*-butyrate>propionate>*iso*-butyrate at 10℃ and 20℃. At 28℃, the production rate of propionate was higher than *n*-butyrate. Based on these results, it is considered that production of VFAs from sinking particles during anaerobic decomposition depends on the chemical composition and temperature.

Key words : particulate organic carbon, sinking particles, volatile fattay acids

Introduction

Much of the organic material produced in estuarine and coastal marine areas is decomposed by aerobic and anaerobic microbial processes in the marine sediments (Smith, 1974). Though it depends on the geological area in some extent, the dissolved and particulate organic materials in the coastal water are originated from decomposition of phytoplankton, macrophytes and other organisms, and from detrital material brought in from land at these area (Nedwell, 1984). These organic materials are sunk to the sediment. These are oxidized aerobically and are followed by anaerobic decomposition in the sediment.

During anaerobic decomposition, volatile fatty acids (VFAs) produced by fermentative bacterial groups are utilized mainly as substrates of sulfate-reducing bacteria and methane producing bacteria. The production of VFAs is affected by pH (Ben-Yaakov, 1973), Eh (Thorstenson, 1970), temperature (Kondo et al., 1993) and age of the organic material (Westrich and Berner, 1984) in the sediment.

In this study, production rates of VFAs from sinking particles during decomposition process affected by two factors, chemical composition and temperature, were studied.

Materials and Methods

Sampling

The samples of sediment, surface seawater and sinking particles were collected on February 28, May 24, and August 26, 1994, at Aburatsubo Inlet, Kanagawa Prefecture, Japan. Surface seawater and sediment samples were obtained with 1 liter sterile glass bottle and Phleger corer, respectively. The location of surveyed area is shown in Fig. 1. The water depth of st. A was about 7 m. The sediment trap which was used to sample sinking particles was set at depth of 3 m for 24hrs. This trap consisted of six transparent acrylic cylinders, about 30 cm long and 8 cm in diameter (Hamasaki et al., 1994). The samples obtained were stored in an ice box at 4℃ until the pretreatment was carried out within 5hrs after collection.

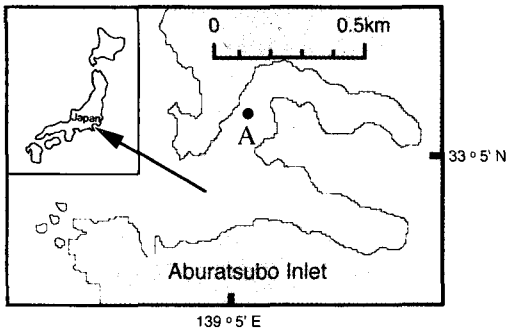

Fig. 1. Location of sampling station.

Preparation for decomposition experiments

The experiments for production rate of VFAs during anaerobic decomposition process of sinking particles were carried out with 50 ml Erlenmeyer flasks as follows; the samples obtained in February and May were dried at 60℃ for 24hrs and were analyzed for POC. The same amount of samples in February (dry weight 28.6 mg) and May (dry weight 12.0 mg) as carbon weight were taken, autoclaved and transferred to each sterile 50 ml flask, then 1 ml of sediment (taken on August 26) was added as source of microorganisms. The flasks were filled with sterile seawater which had been deoxygenated with nitrogen gas, capped with rubber stoppers and incubated at 20℃ in the dark. The concentration of sinking particles were 3 mg C/100 ml.

The other experiments for temperature effect on the production rates of VFAs were carried out. Low concentration of polypepton was added as artificial organic material, since both nitrogen source and trace elements are supplied. One gram of polypepton (final conc. 0.05%) and 1 g of sediments (taken on August 26) were added to 50 ml Erlenmeyer flasks as substrates and source of microorganisms, and then deoxygenated seawater was filled to the top. The flasks were capped with rubber stoppers and incubated at 10, 20 and 28℃ in the dark. The subsamples were taken with 1 ml syringe at appropriate intervals in the anaerobic chamber (Hirasawa Works, ANX-1, Japan).

Analysis of VFAs

Determination of acetate, *iso*-butyrate, *n*-butyrate and propionate were performed with HPLC-LC12A (Simadzu, Japan) using a column (ODS, 5 μm; 4.6×250 mm; Senshu Pak, Japan) with a precolumn (ODS, 5 μm; 4.6×20 mm; Senshu Pak), and SPD-10A UV detector (Simadzu), operated at 400 nm

The sample (20 μl) was eluted at a rate of 1.2 μl/min with acetonitrile in deionized water (27% v/v). The column temperature was 24 ± 2℃. The derivation procedure of VFAs was carried out according to the method of Mueller-Harvey and Parkes (1987). The reagents were made up as follows; 0.02 M 2NPH · HCl (2-nitrophenylhydrazine hydrochloride) was prepared by dissolving the reagent in distilled water. Pyridine solution (3% v/v) in ethanol and 0.25 M EDC · HCl [1-ethyl-3- (3-dimethylaminopropyl) carbodiimide hydrochloride] in ethanol were prepared, then a working EDC · HCl solution was prepared by mixing equal volumes of EDC · HCl and pyridine solutions. A KOH solution (15% w/v) in methanol-water (80 : 20 v/v) was prepared.

Two ml volume of screw-capped vials used for the derivation were heated at 450℃ for 4 hrs before use. EDC · HCl/pyridine reagent (0.4 ml) was added to a sample (0.1 ml) in the vials. The 2NPH · HCl reagent (0.2 ml) was then added immediately. The reaction mixture was incubated at 25℃ for 60 min, after which KOH solution (0.1 ml) was added followed by heating at 60℃ for 15 min. The vials were cooled on ice for 30 sec, before adding HCl (3 M, 0.1 ml) in order to adjust the pH to approximately 7.0. The solution was mixed for 60 sec and 20 μl of sample were injected into HPLC.

Chemical analyses

Sediment trap samples were collected on Whatman GF/F glass fiber filters (diameter 24 mm), which had been baked at 400℃ for at least 2 hrs. These filters were used for analyzing particulate organic carbon (POC) and chlorophyll (chl. a). Analysis of POC was done by CHN Corder (Yanagimoto, MT-2, Japan). Chl.

Table 1. Chemical analyses of sinking particles obtained at Aburatsubo Inlet

	February	May	August
Water temperature (℃)	12.3	21.5	27.3
POC (mg/dry g)	52.3	124.0	40.0
Chlorophyll a (μg/dry g)	34.5	491.3	83.7
(Chl. a/POC)×1000	0.7	4.0	2.1

Table 2. Production rates of VFAs from sinking particles obtained in February and May, and production rate of VFAs from polypepton at different temperatures

(μM/day)

	acetate	*iso*-butyrate	*n*-butyrate	propionate
February	13.2	0.0	6.8	5.2
May	32.5	9.4	19.2	12.2
10 (℃)	182.5	10.1	52.0	48.0
20	345.0	48.7	206.8	85.7
28	578.0	138.4	227.3	276.9

a was extracted with N,N-dimethylformamide and fluorometrically analyzed by the method of Suzuki and Ishimaru (1990). Spectrofluorophotometer (Simadzu, RF-1500) was used for the measurement.

Results and Discussion

Chemical analysis of sinking particles

Table 1 shows the results of POC and chl. a analyses in the sinking particles obtained during three sampling times at st. A. The maximum values of POC was 124.0 mg/dry g and chl. a was 491.3 μg/dry g in May, which are thought to be related to phytoplankton blooming. Gergis (1990) surveyed this area and reported that the maximum phytoplankton standing stock was observed in May during two years observation in 1987 and 1988. Chl. a/POC ratio was the highest in May compared with other two months. This probably indicates that sinking particles in May were composed of higher fraction of phytoplankton than other two months.

The production VFAs affected by sources of sinking particles and temperature

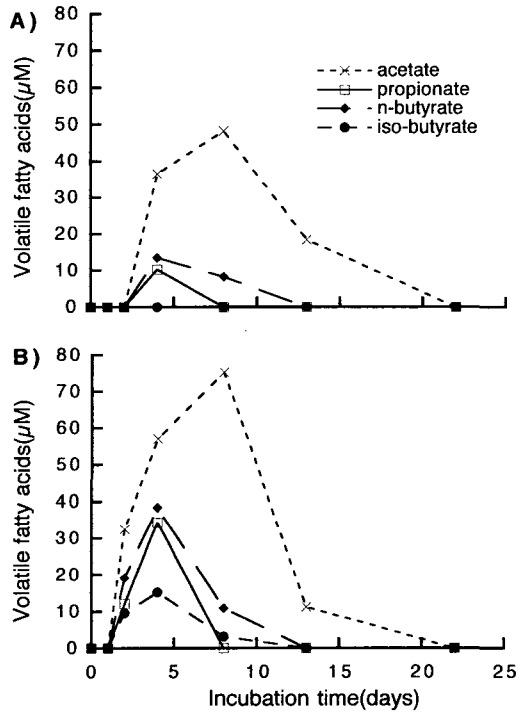

Fig. 2. Production of VFAs during the decomposition of sinking particles.
A) February, B) May

During the decomposition process, polymers such as polysaccharides, proteins, lipids and nucleic acids

are depolymerized to monomers and followed by fermentation in the anaerobic condition (Capone and Kiene, 1988). This process was thought to be affected by bacterial communities and environmental conditions of the sediment.

Fig. 2 and Table 2 show production patterns and rates of VFAs from sinking particle samples with different seasons. The production rates were calculated from linear increase of VFAs during the early stages of incubation. Fig. 2 shows the amount of VFAs produced during the decomposition process of sinking particles obtained in February and May. The production of VFAs started 1 day earlier in the sample of May than February. Wolfe and Higgins (1979) stated that anaerobic microbial communities have been differentiated into a number of functionally different groups of bacteria, each of which contributes to a different stage of anaerobic carbon mineralization. It is considered that the beginning of VFAs production depended on the characteristics of organic materials in the early stage of anaerobic decomposition, since the composition of microflora might be the same in this experiment. Table 2 shows that all the production rates of VFAs were higher in May sample than February. The production rate of acetate was the highest, followed by *n*-butyrate, propionate and *iso*-butyrate at each sample. The *iso*-butyrate was not produced at the decomposition process of samples of February. These results show that the organic material in sinking particles of May was more labile than that of February.

Fig. 3 and Table 2 show the effect of temperature on the VFAs production. The production of VFAs at different temperature, 10, 20, and 28℃ during the decomposition of process is shown in Fig. 3. The beginning of VFAs production was affected by these temperatures. The production rates increased with increases of incubation temperature, and order of production rates were, acetate> *n*-butyrate> propionate> *iso*-butyrate at 10 and 20℃. At 28℃, the production rate of propionate was higher than that of *n*-butyrate.

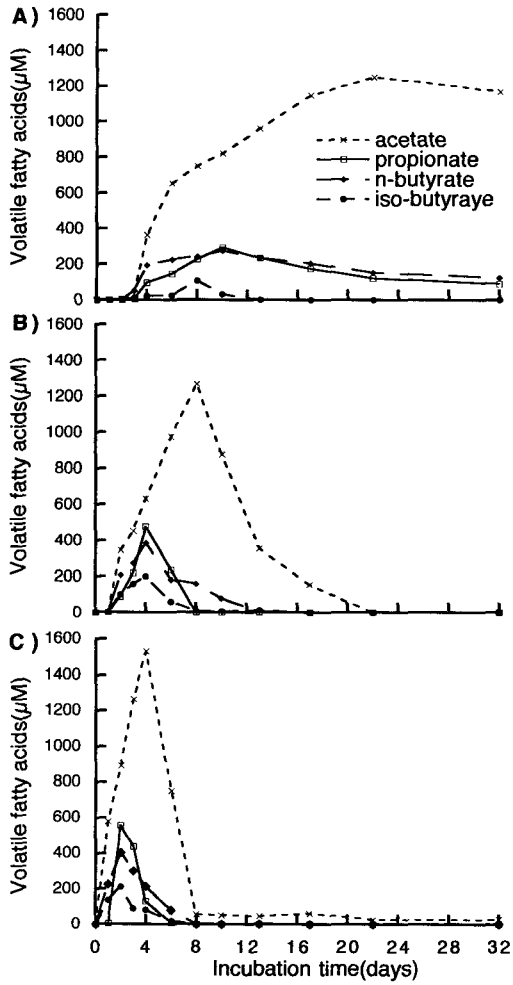

Fig. 3. Production of VFAs during the decomposition of polypepton at different temperatures.
A) 10℃, B) 20℃, C) 28℃

It was well agreed to the results of Kondo *et al.* (1993) that the production rates increased with increases of incubation temperature. However, propionate, *iso*-butyrate and *n*-butyrate were not produced at 10℃ in their experiment. It was considered that each of the fermentative bacterial population was affected by the different temperature during anaerobic decomposition process.

In conclusion, the production of VFAs from sinking particles during anaerobic decomposition is considered to depend on the chemical composition and environmental temperature in the sediment.

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