FERTILITY OF MATSUSHIMA BAY III. ACCUMULATION OF ORGANIC ACIDS IN THE COURSE OF FERMENTATION OF THE ORGANIC MATTER IN MARINE MUDS

FERTILITY OF MATSUSHIMA BAY
III. ACCUMULATION OF ORGANIC ACIDS IN THE COURSE OF FERMENTATION OF THE ORGANIC MATTER IN MARINE MUDS

By

Akiyoshi KAMATANI and Chikayoshi MATSUDAIRA

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

(Recieved May 25, 1965)

Introduction

In the previous report (I), attention has been drawn to the mineralization processes of organic matters such as glucose, starch, cellulose and eelgrass in the marine mud under aerobic and anaerobic laboratory conditions. According to the experimental results, less carbon dioxide was accumulated by the fermentation of these carbonhydrates under anaerobic than aerobic conditions. As organic carbons are incompletely fermentated under anaerobic condition, it is presumed that organic acids accumulate at the same time as the formations of carbon dioxide and hydrogen. The present study, therefore, is focused on the identification and determination of volatile fatty acids which have been derived from the anaerobic decomposition of carbohydrates added in marine muds.

Experimental methods

As the incubation method was described in the previous report (I), five grams of air-dried mud and 15 ml of sea water were took into a injector, and the mouth of the injector was tightly closed with a rubber-stopper. A series of these injectors was incubated at 30°C for a week. After the incubation, each of glucose, starch and cellulose of 30 mg as carbon was added into the each injectors. On the other hand, eelgrass was added in the amount of 160 and 320 mg, respectively. These injectors were kept again in the incubator. At sutiable intervals, the injector was taken out of the incubator and the content was transferred into a 250 ml flask. The organic acids in the sample were extracted according to the method as described by Miyoshi, et al.(2).

The amount of vloatile fatty acids in the extract was determined by the method of Westerhold (3) as modified by the authors. That is, an aliquote por-

tions of the extract which was alkalized by 0.5 N sodium hydroxide took into a evaporating dish and evaporated nearly to dryness on a steam bath. One drop of cresol red indicator was added in the residue. The residue was neutralized with 0.5 N sulfuric acid and then one or two drops of 5 N sulfuric acid were added. The acidified sample was mixed thoroughly with 5g of silica gel which was washed with distilled water and dried at approximately 125°C for 48 hours. The mixture was transferred by a spoon into the percolate as shown in Fig. 1. The organic acids absorbed on the silica gel were eluted with 50 ml of the solvent. The solvent used

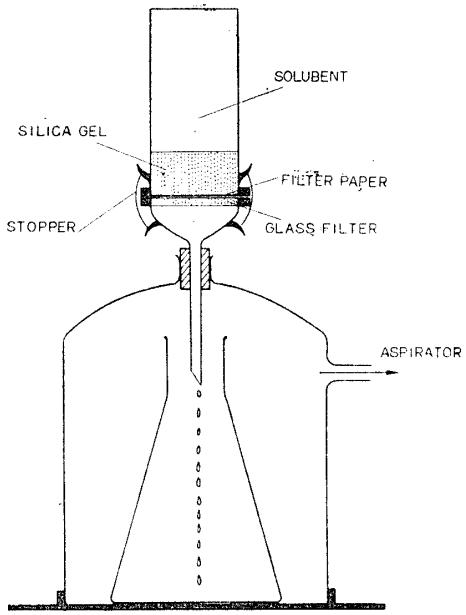

Fig. 1 Aparatus of chromatogram for the determination of total fatty acids

here consisted of 20 per cent n-butyl alcohol in benzen, which was equilibrated with 0.5 N sulfuric acid. The time used for the elution was about two minutes. A few drops of cresol red indicator were added in the eluate, and it was titrated with 0.01 N potassium hydroxide prepared by methyl alcohol under the condition free from the carbon dioxide.

A blank vlaue was determined in the same manner using distilled water instead of the sample, and the blank titration value was substrated from that of the sample.

In the present exmaination, the method of Belasco (4) as modified by Minato, et al. (5) was applied for the quantitative identification of volatile fatty acids.

Results and discussions

The analytical results of the amount of volatile fatty acids are shown in Table I. As can be seen in the table, there is not an appreciable accumulation of organic

acids in the control sample after the 7th day of incubation. On the other hand, much organic acid is derived from the fermentation of glucose and galactose such as mono-saccharide. On the addition of starch, there is no difference in the amount of organic acids in the comparison with the control sample for the 15th day of incubation, but after the time it can be recognzied that the considerable amount of organic acids is derived from the substrate. The start of the accumulation of organic acids is prolonged at the fermentation of cellulose than that of starch.

Table 1. Total amount of organic acids derived from the fermentation of carbohydrates. (mg as acetic acid)

Substrates	Incubation time (days)				
	7	12	15	25	35
Control	11.7	—	14.9	13.6	12.1
Glucose	—	76.5	85.0	88.1	92.1
Starch	—	—	17.4	47.2	60.5
Cellulose	—	—	14.5	15.0	23.3
Galactose	—	81.5	73.1	82.1	83.4

Table 2. Total amount of organic acids derived from the fermentation of eelgrass. (mg as acetic acid)

Substrates	Incubation time (days)				
	7	12	15	25	35
Control	11.7	—	14.9	13.6	12.1
Eelgrass					
160 mg	—	31.8	34.6	21.6	17.9
320 mg	—	72.3	50.6	38.8	23.2

Note : Eelgrass contained of 7.35 mg as acetic acid per 100mg according to the extraction method.

Taking into the consideration of results of the previous report (1), the start of the accumulation of organic acids was nearly corresponded with the start of the accumulation of carbon dioxide and the absorption of ammonium nitrogen.

As can be seen in Table 2, the pattern of the accumulation of organic acids derived from the fermentation of eelgrass is clearly opposite with that of the above-mentioned substrates. That is, the amount of organic acids gradually decreased in the course of fermentation of eelgrass. The phenomenon will be discussed in later.

Tables 3 and 4 show the content of organic acids in the sea waters and in the sediments collected at different five stations in Matsushima Bay. According to the analytical results, it could be concluded that the content of organic acids in the sea waters and also in the sediments is more abundant in summer than in winter season.

Table 3. Content of organic acids in sea waters (mg as acetic acid/L)

Dates	Sampling stations				
	1	2	3	4	5
July.-24-64	28.37	29.37	19.68	—	—
Oct.-29-64	15.46	10.48	17.86	16.81	19.27
Dec.-09-64	7.05	8.91	—	7.54	2.45
Jan.-13-65	2.32	0.97	2.06	1.68	1.41

Table 4. Content of organic acids in sediments (mg as acetic acid/10g of dried mud)

Dates	Sampling stations				
	1	2	3	4	5
Sept.-17-64	22.23	20.21	15.93	20.53	17.91
Dec.-09-64	7.74	7.45	7.05	7.52	8.12

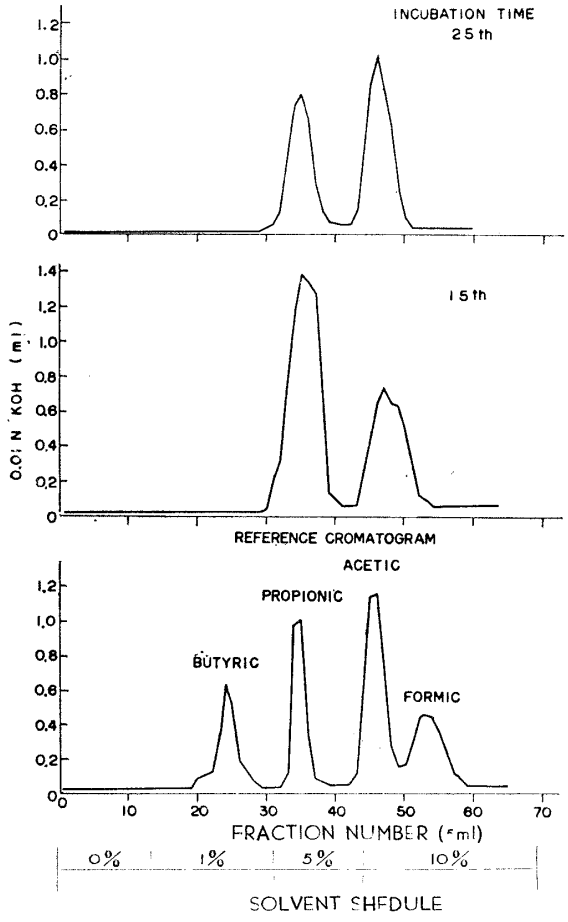

Fig. 2 Chromatograms of volatile fatty acids derived from lactate fermentation

On the next place, the organic acids were separated by the silica gel chromatography, and the typical figures are shown in Figs. 2, 3 and 4. As can be seen in Fig. 3, formic and acetic acids are derived quickly in nearly same mole from glucose at the early stage of the fermentation, but the formic acid was gradually disappeared with the passage of the incubation. Taking account of the results of previous report (1), the period forming much formic acid corresponded with the hydrogen gas phase, and the absorption of hydrogen was followed by the disappearance of formic acid. In the course of the fermentation of xylose and galactose, formic acid was not so much accumulated as observed in the fermentation of glucose, but hydrogen was remarkably accumulated in the early stage of the fermentation. In these fermentation processes, only acetic acid was a dominant organic acid. Also in the courses of the fermentation of starch, cellulose and eelgrass, acetic acid was dominant, and only a little amount of formic, butyric and propionic acids was detected at the same time.

The volatile fatty acids in the sea waters and the sediments are composed of acetic, formic, propionic and butyric acids (Fig. 4), which were arranged in the

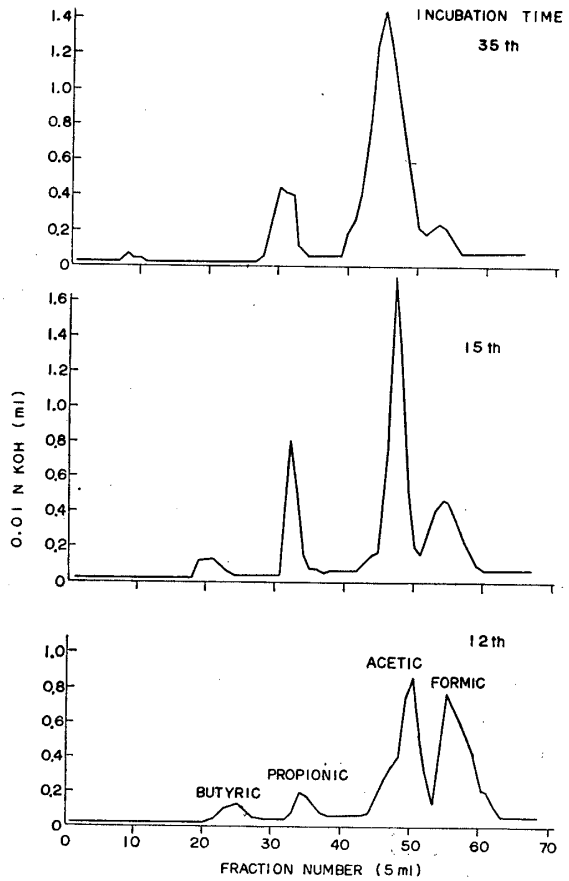

Fig. 3 Chromatograms of volatile fatty acids derived from glucose fermentation

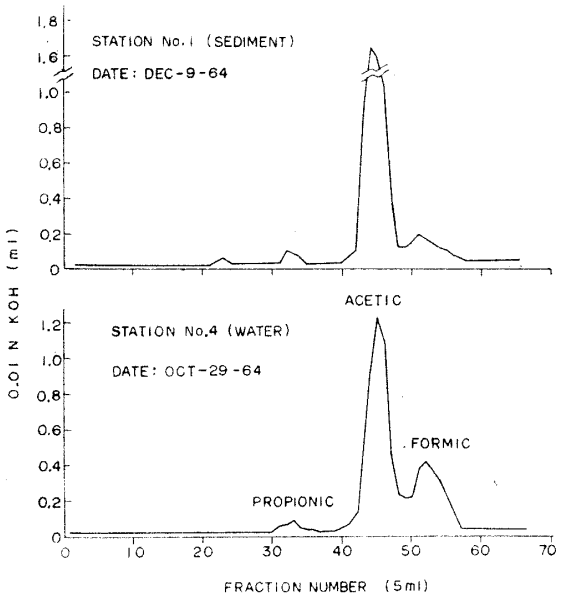

Fig. 4 Chromatograms of volatile fatty acids in seawater and sediment

order of their abundance.

When glucose was a substrate the acid appeared in the early stage of fermentation was formic and acetic acids, but the most of the formic acid disappeared after the 15th day of incubation. The vicissitudes of the formic acid were corresponded with that of the molecular hydrogen reported in the previous paper (1). From this fact, it was assumed that the formic acid was converted to carbon dioxide and hydrogen by formic hydrogenlyase. The mechanism was already demonstrated by Pakes and Jollyman (6). These workers showed that the production of carbon dioxide and hydrogen by a few organisms (mostly, *Enterobacteriaceae*) was chiefly due to the decomposition of formate produced from hexose as an intermediate product.

The acetic acid seems to be produced from the carbon dioxide and hydrogen as a sole product of the fermentation at the same stage as the production of hydrogen and carbon dioxide from formic acid, because the accumulation of acetic acid is followed to the disappearance of formic acid as can be seen in Fig. 3. The path way had been already confirmed by Wieringa (7) in a *Clostridium* isolated from canal and also recognzied in several other clostridia and anaerobes (8).

From this reason, 30 mg of sodium formate was used as a substrate. However, the hydrogen was not appeared in the course of fermentation. This path way could not yet explain clearly. On the other hand, when a solution of 40 mg of sodium pyruvate was a substrate, acetic acid was a sole product throughout the fermentation, and when 105 mg of sodium lactate was a substrate, acetic and prropionic acids were accumulated dominantly in the course of fermentation as shown in Fig. 2. The above-mentioned experimental results could be summarized briefly as in Fig. 5.

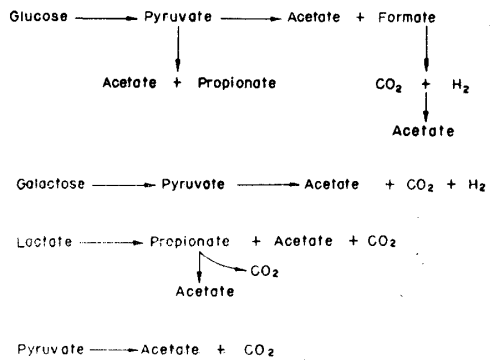

Fig. 5 Metabolic path ways

Miyoshi, et al. (2) pointed out that the organic acids in marine and lake sediments were absorbed or adhered tightly to the sedimental particles, therefore, it was more difficult to extract the organic acids from these sediments than from paddy soil. Then the extractant of 0.5 N sulfuric acid solution prepared with 80 per cent alcohol was adopted by these authors for the extraction of organic acids in sediments. The sediment was treated repeatedly four times with 48-hour extraction with the solvent to extract organic acids. The appreciable portions of organic acids extracted by the treatment seem to be derived from the break down products of organic matters in the sediment.

Eelgrass must contain many kind of organic compounds, which may be considered to convert easily by the acid treatment to the volatile fatty acids. The organic acids in the early stage of fermentation, therefore, may be composed both of the acids that is mechanically produced from the decomposable organic matters during the extraction and that have been already fermented by microorganism. However, the decrease of organic acids content in the later stage of fermentation would mostly due to decrease of the organic acids derived from the easily decomposable portions of eelgrass by the extraction and also by microorganioms. It will, therefore, be important to study moreover the extraction method of organic acids in marine sediments.

Summary

On the results of exmainations, formic and acetic acids were typically derived from the glucose at the early stage of fermentation, but most of the formic acid disappeared with the passage of the incubation. Taking into consideration the results of the previous report, it is presumed that the formic acid should be decomposed to hydrogen and carbon dioxide by hydrogenlyase and then that these gasses would be refermentated to acetic acid.

On the fermentations of xylose, starch, cellulose, pyruvate and eelgrass, acetic acid was a dominant organic acid. When lactate was a substrate, it was fermentated to acetic and propionic adids.

The following organic acids were detected in sea waters and marine sediments: acetic, formic, propionic and butyric acids. Among these acids, acetic acid was generally most abundant. Though it is presumed that there is an annual cycle or seasonal variation of organic acids in natural conditions, it is as yet dangerous to conclude the existence of the cycle or variation from only these data. A more detailed report and discussion of the anual cycle of organic acids in Matsushima Bay will be expected in the near future.

References

1) Kamatani, A. and C. Matsudaira (1965). Tohoku J. Agr. Res., **15**, 281.
2) Miyoshi, H., T. Shirai and H. Kadota (1962). Bull. Jap. Soc. Sci. Fish., **28**, 534.
3) Westerhold, A.H. (1963). J. Water Polution Cont. Fed., **35**, 1431.
4) Belasoc, I.J. (1954). J. Animal Sci., **13**, 748.
5) Minato, H., T. Sai, A. Endo, T. Murakami and T. Uemura (1963). J. Agr. Chem. Soc., Japan, **37**, 379.
6) Pakes, W.W.C. and W.H. Jollyman (1901). J. Chem. Soc., **79**, 386.
7) Wieringa, K.T. (1936). Ant. van Leeuwenkock, **3**, 263.
8) Gunsalus, I.C. and R.Y. Stanier (1961). The Bacteria vol. 2: Metabolism, p. 572, Academic Pre., N.Y.