# 海底堆積物中におけるグルコースの嫌気的無機化過程


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# Mineralization Process of Glucose and Low Molecular Fatty Acid Production in an Anoxic Marine Sediment Slurry

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The mineralization pathways of glucose and the kinetics of low molecular fatty acid production within the sediment slurry system were examined under anoxic conditions. Glucose was decomposed into some low molecular fatty acids such as acetate, formate, lactate, propionate, and *n*-butyrate. These intermediates were estimated to be directly produced from glucose. Among these fatty acids, the production rates of formate, acetate, and lactate from glucose conformed to Michaelis-Menten type saturation kinetics. The maximum production rate of formate was highest (0.303 mM/h), followed by acetate (0.138 mM/h) and lactate (0.090 mM/h).

Among the fatty acids produced from glucose, acetate and formate were completely mineralized in the anoxic condition. Most of the lactate was fermented to acetate, propionate, and formate. Propionate and *n*-butyrate were oxidized to acetate by sulfate reduction. Consequently, acetate was thought to be the most important intermediate in the mineralization process of organic matter.

Low molecular fatty acids are produced in anaerobic ecosystems and are commonly detected in aquatic sediments.[1-7] These fatty acids are known to be important intermediates in the terminal steps of the degradation process of organic matter in anoxic aquatic sediments. These are finally mineralized to carbon dioxide by various microbiological processes.[8]

Acetate, in general, is the major component of fatty acids in anoxic aquatic sediments[1-7] and is the major substrate for sulfate-reducing bacteria in anoxic marine sediments.[9,10] Most of the acetate is mineralized by sulfate reduction.[9-11] Other fatty acids also act as electron donors and/or the carbon source for sulfate-reducing bacteria. Sulfate reduction, therefore, plays an important role in the terminal oxidation of fatty acids to carbon dioxide. However, only few studies have been performed on the production of fatty acids in aquatic sediments.[12]

The present paper describes the kinetic analysis of the anaerobic decomposition process of organic matter in surface sediment from Uranouchi Inlet, Kochi, Japan, using the sediment slurry system. The potential activities were measured at the optimal condition for sulfate-reducing bacteria in the slurry. Glucose was selected as the precursor of fatty acids because the fatty acid content in the sediment of Uranouchi Inlet was controlled by carbohydrate contents as described in a previous paper,[2] and because glucose could be one of the major components of carbohydrates in aquatic sediments.[13] We also propose the pathway of anaerobic decomposition of glucose in the sediment slurry system as a model for organic matter decomposition in anoxic marine sediments.

## Materials and Methods

### *Sample Collection*

Anoxic marine sediments were collected from the innermost part of Uranouchi Inlet, Kochi, Japan. The surface sediment (0–5 cm layer) was taken aseptically with a K-K core sampler[14] and placed in a sterilized bottle. The bottle was sealed tightly, kept cool in an ice box, and then brought to the laboratory within a few hours.

### *Sediment Slurry Experiments*

Fifty grams of the anoxic surface sediment sample was placed in a 500-m*l* Erlenmeyer flask containing 450 m*l* of sterilized artificial seawater[15] and glucose or fatty acids. The concentration of glucose varied from 0.1 to 2.0 g/*l* of the sediment slurry, while that of acetate, formate, lactate, propionate, or *n*-butyrate used as the

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model fatty acid was 10 mM of the sediment slurry. To inhibit sulfate reduction, molybdate (final conc. 20 mM) was added to the sediment slurry. Oxygen in the slurry was replaced with nitrogen gas to make conditions anaerobic. The flasks were incubated at 28°C in the dark and were shaken twice a day to mix the sediment and seawater thoroughly. The decomposition process of added glucose and fatty acids was monitored by measuring the concentration of fatty acids, glucose, and sulfides in the seawater fraction of the slurry at appropriate intervals.

*Chemical Analyses*

The seawater fraction of the slurry was filtered through a 0.45 $\mu$m cellulose acetate filter (Advantec, Toyo) and low molecular fatty acids in the filtrates were analyzed by high performance liquid chromatography as described in the previous paper.[2] Added glucose was analyzed by the phenol-sulfuric acid method.[16] Total sulfides in the sediment slurry were separated by steam distillation under acidic conditions and trapped in a 10% zinc acetate solution.[17] Trapped sulfide was determined spectrophotometrically by the methylene blue method of Shinra.[18]

## Results

*Low Molecular Fatty Acid Production from Glucose*

The decomposition processes of glucose in the sediment slurry are shown in Fig. 1. No fatty acids were produced in the slurry without glucose addition. When 0.1 g of glucose was used, only acetate and formate were detected. By increasing the amount of glucose concentration added, the kinds of fatty acids became greater in number and their production rates increased. Acetate, formate, lactate, propionate, and *n*-butyrate were produced in the sediment slurry only when the added glucose was at the highest concentration tested (2.0 g/*l*). Of these fatty acids, acetate yielded the highest concentration in the slurry followed by formate, lactate, and propionate, except for the cases of low glucose concentration examined. These intermediate fatty acids produced during the decomposition of glucose in the slurry experiment were similar to the fatty acids found in the *in situ* sediment in Uranouchi Inlet. Futhermore, the composition of fatty acids produced at the early stage of incubation was approximately the same, even if the concentration of added glucose was different. Thus, these fatty acids are estimated to be produced directly from glucose in anaerobic coastal sediments.

Using the linear part of fatty acid production during the early stage of incubation, the production rates of fatty acids from glucose were found to conform to Michaelis-Menten type saturation kinetics (Figs. 2 and 3). In all glucose concentrations tested, formate production had the highest rate followed by acetate and lactate productions (Fig. 2). From the Lineweaver-Burk plots (Fig. 3), maximum production rates (Pmax) and Michaelis constants (Ks) were calculated, and these are listed in Table 1. The Pmax of formate was the highest at 0.303 mM/h, followed by acetate (0.138 mM/h) and lactate (0.090 mM/h). The Ks values were 0.23 g glucose/*l* for acetate, 0.47 g glucose/*l* for formate, and 0.72 g glucose/*l* for lactate. These kinetic parameters show that of all the fatty acids formate was produced most actively during the anaerobic decomposition process of glucose in the sediment slurry.

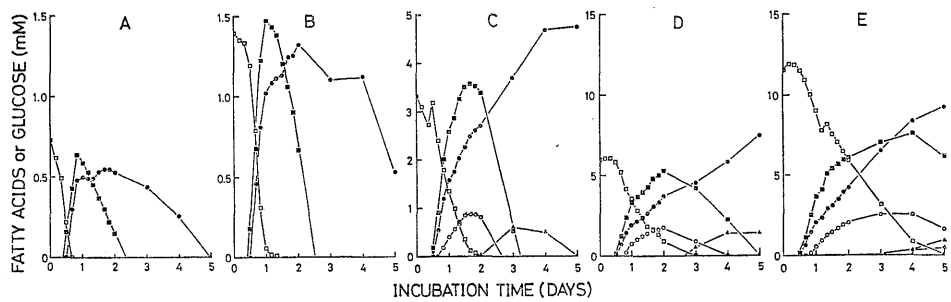


**Fig. 1.** Glucose decomposition (□) and fatty acid production (● acetate, ■ formate, ○ lactate, ▲ propionate, and △ *n*-butyrate) within anoxic marine sediment slurry systems.

Supplemented glucose concentrations were 0.1 (A), 0.2 (B), 0.5 (C), 1.0 (D), and 2.0 g/*l* of the slurry (E).

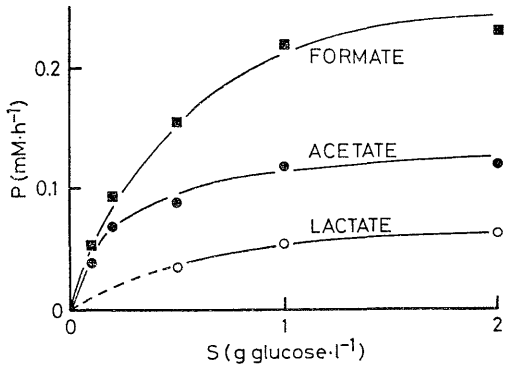


**Fig. 2.** Fatty acid production rates (P) versus glucose concentration (S) within anoxic marine sediment slurry systems.

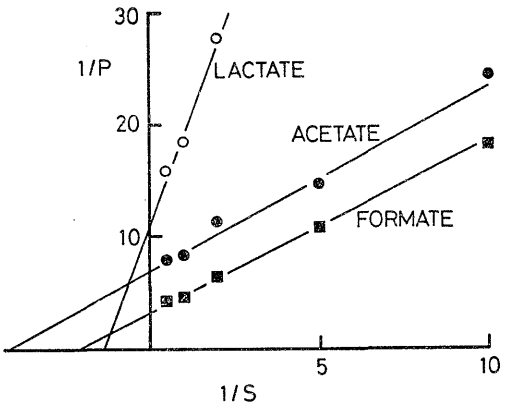


**Fig. 3.** Lineweaver-Burk plots of fatty acid production rates (P) from glucose (S) within anoxic marine sediment slurry systems.

**Table 1.** Calculated maximum production rates (Pmax) of fatty acids and Michaelis constants (Ks) within the anoxic marine sediment slurry system

| Fatty acid | Pmax (mM/h) | Ks (g glucose/*l*) |
|---|---|---|
| Formate | 0.303 | 0.47 |
| Acetate | 0.138 | 0.23 |
| Lactate | 0.090 | 0.72 |

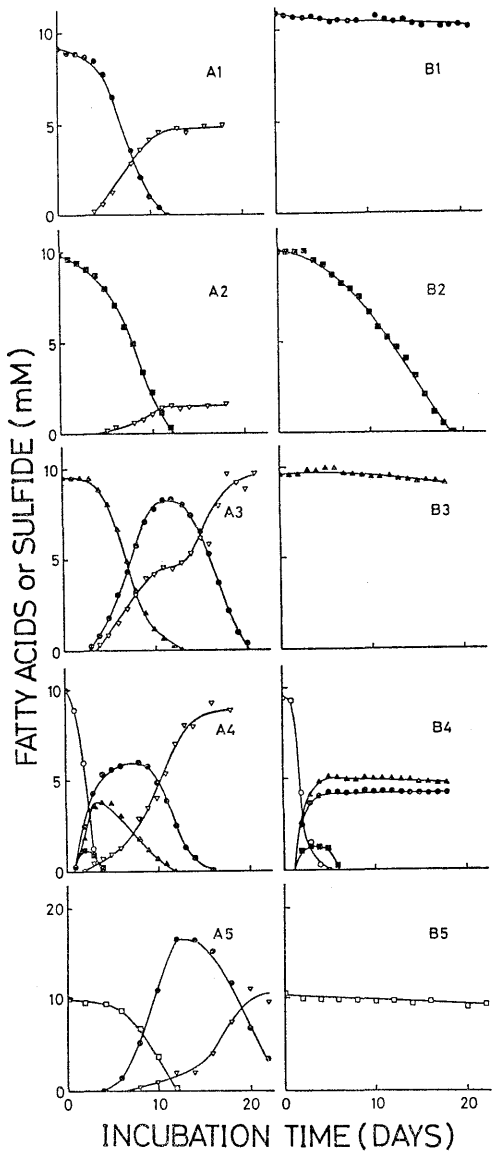


**Fig. 4.** Anaerobic decomposition of fatty acids in anoxic marine sediment slurries supplemented with 10 mM-acetate (A1, B1), 10 mM-formate (A2, B2), 10 mM-propionate (A3, B3), 10 mM-lactate (A4, B4), or 10 mM-*n*-butyrate (A5, B5); A1–A4, no inhibition; B1–B5, inhibition of sulfate reduction by 20 mM-molybdate. ● Acetate, ■ formate, ▲ propionate, ○ lactate, □ *n*-butyrate, or ▽ sulfide.

*Fatty Acid Decomposition*

Figure 4 shows the result of decomposition of fatty acids added to the sediment slurry. Sulfides in the slurry were used as an indicator of sulfate reduction. Since sulfide did not accumulate in the slurry without fatty acids, the accumulation of sulfide in the slurry was obviously due to the decomposition of added fatty acids. Acetate added to the sediment slurry was found to be completely decomposed with concomitant production of sulfides when the slurry was incubated without molybdate (Fig. 4, A1). However, in the slurry with molybdate, almost all of the added acetate remained during the incubation

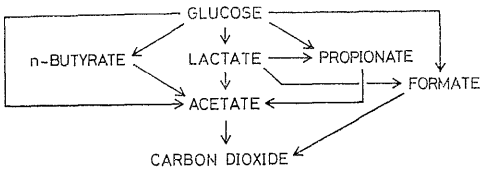


**Fig. 5.** Proposed pathways of anaerobic glucose decomposition in marine sediment.

period; this inhibitor completely inhibited the acetate decomposition.

Formate decomposition was not inhibited by molybdate but the concomitant production of sulfide was low, compared to the case of other fatty acid decomposition without the inhibitor (Fig. 4).

Propionate was decomposed to acetate and then acetate was completely decomposed with concomitant production of sulfide in the slurry without the inhibitor (Fig. 4). One molecule of propionate was found to produce almost the same molecule of acetate.

Lactate was rapidly consumed in both non- and molybdate-inhibited samples with consequent production of acetate, propionate, and formate (Fig. 4, A4 and B4); no lactate was detected in the slurry after four days' incubation.

*n*-Butyrate, as propionate, was decomposed to acetate (Fig. 4, A5). However, the molar ratio of acetate produced to *n*-butyrate decomposed was about 2. The acetate produced was then completely decomposed with concomitant production of sulfide in the slurry without molybdate. The decomposition of *n*-butyrate stopped in the presence of molybdate.

## Discussion

From the present results, glucose was decomposed to fatty acids in anoxic marine sediments, as intermediates during decomposition. Acetate, formate, lactate, propionate, and *n*-butyrate are estimated to be directly produced from glucose in anoxic sediments, and of these fatty acids, acetate yielded the highest concentration in the slurry. This result indicates that the main intermediate fatty acid derived from carbohydrates was acetate in the sediment of Uranouchi Inlet. Sansone[19] concluded that the main sources of acetate in marine sediments were carbohydrates or other complex organic compounds rather than volatile fatty acids or other metabolic intermediates. The present study also reveals that most of the acetate results from glucose decomposition but that some of the acetate can be produced from intermediates of glucose decomposition such as lactate, propionate, and *n*-butyrate.

The yield of formate was relatively high in the sediment slurry of Uranouchi Inlet. However, no production of formate in the sediment slurry system have been reported by other researchers.[20,21] In the present study, it should be noted that Pmax of formate from glucose was higher than that of acetate. Sorensen *et al.*[22] and Parkes *et al.*[23] determined that acetate accumulated in the molybdate-inhibited sediment at the highest rate, followed by propionate. Their results showed that the production of acetate was highest among low molecular fatty acids. In the present study, however, even if the rate of direct acetate production from glucose was lower than that of formate, the intermediates of glucose decomposition such as lactate, propionate, and *n*-butyrate may be the other precursors of acetate, resulting in the highest accumulation of acetate in most cases of glucose decomposition experiments (Fig. 1). Thus, the total production rate of acetate may be similar to or higher than that of formate in natural sediments.

Lactate is known to be an electron donor for most sulfate-reducing bacterial species represented by the genus *Desulfovibrio*. In the present study, however, lactate did not act as an electron donor for sulfate-reducing bacteria in the sediment slurry; lactate was mostly fermented to acetate, propionate, and formate. Analogous results have been recognized by Laanbroek and Pfennig[20] and Taylor and Parkes,[21] except for formate production.

Acetate seems to be decomposed by sulfate reduction as indicated by the accumulation of sulfides in the slurry. Similar observations were made by Laanbroek and Pfennig[20] and Taylor and Parkes.[21] Laanbroek and Pfennig[20] isolated acetate-oxidizing *Desulfobacter*-type organisms from the sediments used in their incubation experiments. On the other hand, Taylor and Parkes,[21] by using fatty acid biomarkers of specific organisms, concluded that *Desulfobacter* sp. dominated in their marine sediment slurries.

Sansone[19] indicated that there are two pathways of *n*-butyrate oxidation: (1) direct oxidation to one molecule of acetate and two molecules of $CO_2$ and (2) $\beta$-oxidation to form two molecules of acetate with subsequent oxidation of acetate

to $CO_2/CH_4$. He also indicated that both pathways are actually in operation simultaneously. However, in the present study, only $\beta$-oxidation of *n*-butyrate might have taken place in the slurry, because 2 molecules of acetate were produced from the decomposition of 1 molecule of *n*-butyrate, as mentioned previously.

Propionate was oxidized to acetate by sulfate reduction as indicated by the inhibition of propionate decomposition by molybdate. The molar ratio of propionate decomposed to acetate produced was approximately one. This reaction could be carried out by a *Desulfobulbus*-type bacterium.[24]

Based on the results of both experiments on the production and the decomposition of fatty acid, the pathway of anaerobic decomposition of glucose in anoxic sediments is as proposed in Fig. 5. Acetate is a key substrate for decomposition of complex organic compounds, as pointed out by many microbiologists.[11,19,25] However, not only acetate but also formate seem to be key substrates in the mineralization of organic matter, and most of formate oxidation may be independent of sulfate reduction.

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