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1 Curley, A., Sedlak, V. A., Girling, E. F., Hawk, R. E., Barthel, W. F., Pierce, P. E., and Likosky, W. H., *Science*, **172**, 65 (1971).
2 Lewald, G., *Habilitationschrift*, **31**, 4 (1857).
3 Reed, C. B., *Surg. Gynec. Obstet.*, **6**, 514 (1908).
4 Kiwimae, A., Swenson, A., Ulfvarson, V., and Westöö, G., *J. Agr. Food Chem.*, **17**, 1014 (1969).
5 Stoewsand, G. S., Anderson, J. L., Gutenmann, W. H., Bache, C. A., and Lisk, D. J., *Science*, **173**, 1030 (1971).
6 Berlin, M., and Ullberg, S., *Arch. Environ. Health*, **6**, 616 (1963).
7 Suzuki, T., Matsumoto, N., Miyama, T., and Katsunuma, H., *Industr. Health* (Kawasaki), **4**, 69 (1967).

Enhancement of Bacterial Methane Oxidation by Clay Minerals

METHANE is abundantly liberated by bacteria from the sediments of rivers and lakes[1]. If methane formation exceeds its solubility in water it is released into the atmosphere[2]. Several species of bacteria oxidize methane as an energy source, converting it into cellular materials or carbon dioxide gas[3]. Accumulation of carbon derived from methane is involved in the ageing of lakes because it causes some carbon to be recycled in the aquatic ecosystem rather than lost as gas. How much methane carbon is retained in the ecosystem and how much escapes into the atmosphere depends on conditions in the water. This report describes enhancement of bacterial methane utilization by clay mineral particles in a simulated aquatic ecosystem.

Bacterial inocula were taken from a mixed culture, isolated from Lake Erie, that was rich in methane oxidizing bacteria and had reproducible and predictable growth patterns. One ml. of this culture, chiefly Gram negative cocci and rods, was used as required to seed experimental vessels.

The common types of clay minerals tested and their respective cation exchange capacities (mequiv/100 g) were: kaolinite (9.7), illite (24.6), vermiculite (70.6) and bentonite (74.5). Organic material was removed from the clays by extraction with 0.5 N NaOH (ref. 4).

Experiments were performed in 74 ml. serum bottles fitted with rubber stoppers. Necessary additions were made to the bottles, then mineral salts medium was added to bring the volume to 25 ml. The bottles were filled with a methane : air mixture, and methane utilization and carbon dioxide production were monitored by gas chromatography.

When quantities of kaolinite (0.004% to 4.0%) were tested for their effect on methane uptake by bacteria, all concentrations enhanced methane oxidation. There was a decrease in the lag period before onset of methane oxidation, total methane uptake increased, and the rate of methane oxidation increased. The 4.0% kaolinite suspension produced maximum stimulation.

There were no significant differences in methane oxidation stimulation between the four clay minerals, which indicates that cation exchange capacity is not responsible for this enhancement. A surface phenomenon is implied because smaller clay

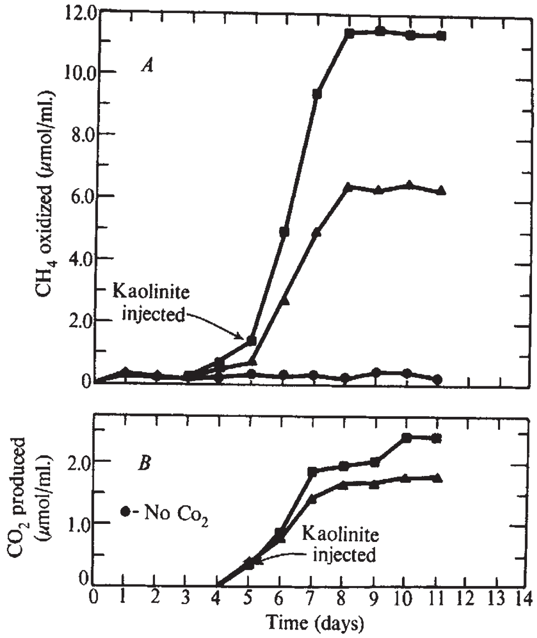

Fig. 1 Curves showing (*A*) amount of methane oxidized and (*B*) amount of CO_2 produced by a mixed bacterial population versus time of incubation when kaolinite (4%) was added to actively growing cells. ●, Control sterile medium. ▲, Control medium plus cells. ■, Control medium plus cells to which kaolinite was added on the fifth day.

particles (<2 µm) with a greater surface area/unit volume stimulated methane uptake more than did larger particles (>2 µm). The enhancement probably involves one of the following: (i) adsorption of a repressor or inhibitor, (ii) increased availability of nutrients, or (iii) provision of some unknown stimulating factor by the clays.

In nature it is probable that a continuously growing population of methane oxidizing bacteria is exposed to concentrations of clay particles which are varied by dispersal of bottom sediments into the water columns and surface runoff by wind and wave action. When kaolinite was injected into an actively growing culture of methane oxidizing bacteria the rate of utilization of methane was doubled and the total quantity taken up increased (Fig. 1).

We conclude that clay mineral particles enhance methane consumption by methane oxidizing bacteria. The increased utilization of methane in an aquatic ecosystem would increase methane carbon retention in water and thereby accelerate total carbon accumulation. The significance of this effect is emphasized by consistently high *in situ* measurements of methane production in Lake Erie sediments (1.71 cm^3 $CH_4/min/m^2$)[1]. Thus microbial fixation of methane can be an important contributor to the accumulation of organic material in lakes which may play a key role in lake eutrophication[5]. One might reduce methane carbon retention, and thereby eutrophication, by reducing runoff of clay minerals into rivers and lakes.

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1 Howard, D. L., Frea, J. I., and Pfister, R. M., *Proc. Fourteenth Conf. Great Lakes Res.*, 1971 (1971).
2 Swinnerton, J. W., Linnenbom, V. J., and Cheek, C. H., *Environ. Sci. Technol.*, **3**, 836 (1969).
3 Vary, P. S., and Johnson, M. J., *Appl. Microbiol.*, **15**, 1473 (1967).
4 Bremner, J. M., *J. Agric. Sci.*, **39**, 280 (1949).
5 Hutchinson, G. E., in *Eutrophication: Causes, Consequences, Correctives*, 17 (US National Acad. Sci., Washington, DC, 1969).