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Utilization of Dichloromethane by Suspended and Fixed-Film Bacteria

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Dichloromethane (methylene chloride) was biodegraded by and supported growth of suspended and fixed-film bacteria enriched from sewage.

Dichloromethane (CH_2Cl_2), which is also called methylene chloride, is commonly used as a solvent for cleaning, extraction, refrigeration, and fumigation in many industrial and analytical applications (8). Being widely used and relatively water soluble (20 g/liter at 20°C [8]), CH_2Cl_2 is ubiquitous in aqueous environments. For example, treated sewage effluents have been found to contain up to 24 μg of CH_2Cl_2 per liter (3, 6a).

Despite the prevalence of CH_2Cl_2, we have found no previously reported information on its biodegradability. This paper demonstrates that CH_2Cl_2 is biodegraded and supports bacterial growth.

Dichloromethane was measured by a modification of the extraction techniques of Mieure (5) and Richard and Junk (7). To a 1.84-ml (0.5-dram) vial was added 0.1 ml of octane which had been refluxed to remove volatile organic compounds. A 1.0-ml aqueous sample was added to the vial, and the CH_2Cl_2 was extracted by vigorous shaking for 1 min. Five microliters of the octane phase was injected into a Tracor MT-220 gas chromatograph with electron capture detection and an ECL linearizer. The column packing was 10% squalene on Chromosorb W (80/100 mesh), the 95% argon-5% methane carrier gas flow rate was 60 ml/min, and the isothermal oven temperature was 70°C. Extraction recoveries with this technique are constant, give linear responses, and range from 78 to 97% for various tested compounds (5, 7). Dichloromethane had a linear response over the concentration range of 0.1 to 100 mg/liter.

Bacterial cultures were enriched from a seed of primary sewage effluent (Water Quality Control Plant, Palo Alto, Calif.) over a 12-month period. Mineral medium was made of the following (in milligrams per liter): KH_2PO_4, 8.5; K_2HPO_4, 28.5; Na_2HPO_4, 33.4; $FeCl_3 \cdot 6H_2O$, 0.25; $NaHCO_3$, 20.0; NH_4Cl, 1.7; $MgSO_4$, 22.5; and $CaCl_2$, 27.5. The pH was 7.1. The sewage seed was inoculated into the mineral medium in a series of 60- and 120-ml serum bottles. The enrichment involved feeding the bottles at various concentrations every 2 or 3 days. Before feeding, the contents were aerated with filtered air to ensure aerobic conditions. Then CH_2Cl_2 was added at a sufficiently low concentration (1 to 25 mg/liter) so that dissolved oxygen would not become limiting, a rubber serum cap was put in place to prevent losses of CH_2Cl_2 by volatilization, and the bottles were incubated in the dark at 20°C without agitation. Because a variety of feeding schedules were used, several enrichment cultures, having different bacterial densities, were developed. It was observed that culture turbidity changes and CH_2Cl_2 utilization were not significant if bicarbonate was omitted from the mineral medium.

Microscopic examination showed that the enriched bacteria were predominately gram-negative, motile rods; no further species identification was performed.

Batch, suspended-growth experiments were performed to demonstrate CH_2Cl_2 utilization. The batch runs were initiated by aerating the cultures and then inoculating the serum cap-sealed bottles with CH_2Cl_2. One-milliliter aqueous samples were removed with a syringe initially and periodically over 1 to 5 days. Figure 1 shows that control flasks containing only sterile mineral medium and CH_2Cl_2 had no loss of CH_2Cl_2, which demonstrated that the serum caps prevented volatilization losses. The CH_2Cl_2 utilization rates by cultures gradually increased during the enrichment period. Figure 1 shows the removal rates of CH_2Cl_2 for three different cultures near the end of the enrichment period. Bacterial counts were measured on tryptone-glucose extract agar with a 24-h incubation time at 35°C (1). This medium is not selective for CH_2Cl_2-utilizing ogranisms, and because the medium is not specific and the plate count technique is notoriously imprecise, the counts cannot be used for determination of kinetic constants. Nevertheless, Fig. 1 clearly demonstrates the

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biological utilization of CH_2Cl_2 by suspended bacteria.

Bacteria from the suspended-growth enrichment cultures were inoculated into glass columns (12 cm long by 2.5-cm diameter) filled with 3-mm glass beads. Inoculation involved adding the suspended-culture liquid to the column and letting the liquid remain in the column overnight. When continuous feeding was initiated the next day, most of the bacteria were washed from the column, but some cells remained to initiate biofilm growth. A CH_2Cl_2 feed solution, mineral medium containing nominally 25 mg of CH_2Cl_2 per liter and 8 to 9 mg of dissolved oxygen per liter, was fed continuously through the column at a flow rate of 2.0 liter/day, which is equal to a superficial flow velocity of 400 cm/day. Over about 4 weeks, a visible, brown biofilm formed in the first 8 cm of a seeded column fed the CH_2Cl_2-containing solution, but not in one fed mineral medium without CH_2Cl_2. The concentration of CH_2Cl_2 was measured in liquid samples removed with a 1-ml syringe from sampling ports sealed with serum caps to prevent volatilization losses along the length of the column. Figure 2 shows that the CH_2Cl_2 concentration was reduced to approximately 1 mg/liter by the biofilm. This removal was essentially steady state and was repeatedly measured over a 5-week period after the biofilm growth appeared.

The suspended-growth and biofilm experiments demonstrate that dichloromethane was utilized by bacteria and supported bacterial growth. Since neither CO_2 evolution nor intermediates were measured, it is not possible to state whether CH_2Cl_2 was completely mineralized or by what pathway it was metabolized. Nonetheless, bacterial growth with CH_2Cl_2 as the only exogenous energy source indicates that CH_2Cl_2 can be used as an energy source without serving as a cometabolite (4).

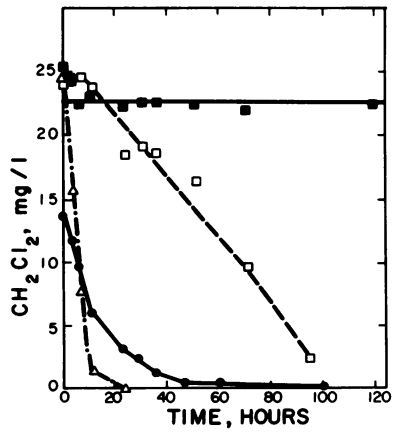

FIG. 1. *Biodegradation of CH_2Cl_2 by enrichment cultures. The incubation was at 20°C on a shake table. Standard plate counts (1) at the beginning of each run were as follows (per milliliter): □, 6.5×10^5; △, 1.6×10^6; ●, 7.2×10^6; and ■, sterilized control.*

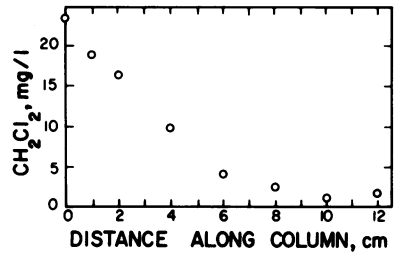

FIG. 2. *Steady-state utilization of CH_2Cl_2 by biofilm culture grown with CH_2Cl_2 as the only exogenous energy source. The flow velocity was 400 cm/day; the flow rate was 2.0 liters/day; the influent concentration was 23.8 mg/liter.*

Since CH_2Cl_2 is a C_1 compound, its biochemical pathway for synthesis is likely to be similar to that of other C_1 compounds, such as formaldehyde (2, 6). During C_1 metabolism, bacteria use the heterotrophic ribulose monophosphate or serine pathways, or they use the autotrophic Calvin cycle. Although not conclusive, the observation that suspended growth was reduced when bicarbonate was omitted from the mineral medium suggests that the autotrophic pathway was used.

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