Degradation of Eighteen 1-Monohaloalkanes by *Arthrobacter* sp.

Strain HA1

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Four coryneform bacteria were isolated from enrichments with 1-chlorobutane, 1-chloropentane or 1-chlorohexane as sole source of carbon and energy. One organism, strain HA1, was identified as an *Arthrobacter* sp. It could utilize at least 18 1-chloro-, 1-bromo- and 1-iodoalkanes, but not the 1-fluoroalkane tested. Substrate utilization was quantitative and growth yields of about 5.5 g protein (mol C)⁻¹ were observed for hexanol and 1-chlorohexane with specific growth rates of 0.19 and 0.14 h⁻¹, respectively. The organohalogen atom was released quantitatively as the halide ion. Cells grown on 1-chlorobutane contained an inducible enzyme(s) that dehalogenated haloalkanes. The enzyme was soluble, required no cofactors, membrane components or oxygen for activity, and released halide and the corresponding n-alcohol from the 1-haloalkane. The halidohydrolase(s) in crude extract had a specific activity of about 0.7 mkat (kg protein)⁻¹ and dehalogenated a much wider spectrum of compounds (at least 29, including mid-chain and α,ω-dichlorosubstituted alkanes) than supported growth.

**INTRODUCTION**

Chlorinated alkanes were regarded as non-biodegradable until Omori & Alexander (1978a) and Brunner *et al.* (1980) demonstrated aerobic utilization of 1,9-dichlorononane and dichloromethane by pure cultures, and Bouwer & McCarty (1983a,b) and Vogel & McCarty (1985) confirmed anaerobic transformations of many haloalkanes. In contrast to work with chloroalkanoates, in which isolation of organisms seems to be simple (Motosugi & Soda, 1983), enrichments to degrade haloalkanes have succeeded only with dichloromethane, 1,2-dichloroethane (Stucki *et al.*, 1983; Jannsen *et al.*, 1985), vinyl chloride (Hartmans *et al.*, 1985), 1,9-dichlorononane, and, more recently, chloromethane (Hartmans *et al.*, 1986) and 1-chlorobutane (Yokota *et al.*, 1986); these isolates usually have a very narrow substrate specificity. However, some of these aerobic dechlorinations have relatively high specific activities in growing cells \([≥3 \text{ mkat (kg protein)}^{-1}]\), and the application of biodegradation to destroy waste dichloromethane has been demonstrated (Gälli & Leisinger, 1985).

The dechlorination of haloalkanes has been suggested to be NADH-linked (Omori & Alexander, 1978b), oxidative (Hartmans *et al.*, 1985, 1986; Yokota *et al.*, 1986) or hydrolytic (Kohler-Staub & Leisinger, 1985; Jannsen *et al.*, 1985; Yokota *et al.*, 1986). However, all models agree that dehalogenation is the first catabolic reaction. In the case of 1,2-dichloroethane, hydrolytic removal of the first chlorine atom is apparently followed by oxidation of the organic product to chloroethanoate from which the chlorine atom is removed by a haloalkanoate halidohydrolase (Jannsen *et al.*, 1985). Metabolism of haloalkanes independent of the halogen atom is also known (Murphy & Perry, 1983, 1984).

Monochloroalkanes have received little attention until now (Hartmans *et al.*, 1986; Yokota *et al.*, 1986). In biodegradation studies, these solvents and alkylating agents have the advantage of chemical and analytical simplicity. Further, some bromo- and iodoanalogues occur naturally.
(Gschwend et al., 1985) and we regard them as models from which biodegradation of more highly halogenated alkanes can be approached. This paper deals with the enrichment of biodegradative organisms, the mass balance of the degradation and the mechanism of the dehalogenation.

**METHODS**

**Materials.** The inoculum for enrichments was sludge (1% dry wt) from the settling tank of an industrial biological treatment plant which has treated chlorinated solvents for many years. The haloalkanes (99% purity) were purchased from Fluka, except for chloroethane (99%; Matheson) and 1-fluoropentane (Aldrich); purity (≥99%) was confirmed by GLC and the identities of 1-chlorohexane, 1-bromobutane and 1-iodoethane were confirmed by MS. Ethylene oxide, α-chlorinated alkanolic acids and other chemicals were purchased from Fluka and were of the highest purity available. Liquid cultures were grown in screw-cap Erlenmeyer flasks closed with Mininert valves (Hartmans et al., 1986).

**Apparatus and analyses.** GLC with flame ionization detection (Jutzi et al., 1982), GLC–MS (Cook et al., 1984), spectrophotometric analyses, OD measurements and anaerobic experiments were done with apparatus described previously (Thurnheer et al., 1986; Cook et al., 1984). Haloalkanes and alcohols were assayed by GLC after separation on a 1:8 m × 2 mm stainless steel column packed with Porapak P (cf. Stucki et al., 1981); for GLC–MS, a 10 m × 530 μm methylsilicone capillary column was used. Chloride ions were converted to 2-chloroethanol (Russel, 1970) which was then assayed by GLC on a 1:8 m × 2 mm Porapak Q column (at 170 °C) with a nitrogen flow rate of 30 ml min⁻¹. Chloride ions were also assayed with an ion-specific electrode (Cook & Hütter, 1984). Halide release was determined by the method of Bergmann & Sanik (1957): the standard curve always contained blank samples appropriate to the experiment because the colour development was inhibited in the presence of some ions. Growth was quantified as protein content in a modification of the method of Kennedy & Fewson (1968). Portions (5 ml) of cultures were brought to 0.5 M with respect to TCA and frozen. Each thawed sample was centrifuged (27000 g for 30 min) and the pellet suspended in 0.75 ml 1.32 M-NaOH before incubation at 80 °C for 20 min and then at 30 °C for 24 h. The sample was then diluted to 1.5 ml, centrifuged to remove turbidity (which did not contain protein), and the supernatant fluid was assayed. The Gram-reaction was determined directly by staining, indirectly by the Bactident L-aminopeptidase test (Merck), and was deduced from the surface characteristics of cells observed in the electron microscope (Hartmans et al., 1986).

**Growth medium and the isolation of organisms.** The growth medium was a mineral salts solution (Hartmans et al., 1986), containing trace elements (Cook & Hütter, 1981) and vitamins (Schlegel, 1981), to which a carbon source was added. The haloalkanes were found to be sterile and so were injected directly into the culture through the surface characteristics of cells observed in the electron microscope (Hartmans et al., 1986). Halide release was determined by the method of Bergmann & Sanik (1957): the standard curve always contained blank samples appropriate to the experiment because the colour development was inhibited in the presence of some ions. Growth was quantified as protein content in a modification of the method of Kennedy & Fewson (1968). Portions (5 ml) of cultures were brought to 0.5 M with respect to TCA and frozen. Each thawed sample was centrifuged (27000 g for 30 min) and the pellet suspended in 0.75 ml 1.32 M-NaOH before incubation at 80 °C for 20 min and then at 30 °C for 24 h. The sample was then diluted to 1.5 ml, centrifuged to remove turbidity (which did not contain protein), and the supernatant fluid was assayed. The Gram-reaction was determined directly by staining, indirectly by the Bactident L-aminopeptidase test (Merck), and was deduced from the surface characteristics of cells observed in the electron microscope (Hartmans et al., 1986).

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Biodegradation of 1-mono haloalkanes

Enrichments were prepared with 1-chloroethane, 1-chloropropane, 1-chlorobutane, 1-chloropentane and 1-chlorohexane, but only with the latter three substances was growth and substrate disappearance observed. Four strains, all requiring vitamins, were isolated: strain HA1 (from 1-chlorohexane), strain HA2 (from 1-chloropentane) and strains HA3 and HA4 (from 1-chlorobutane). All were coryneform bacteria with pleomorphic, Gram-positive rods or cocci (depending on the state of growth and substrate) and frequent V and Y forms. As strains HA1, HA2 and HA3 were apparently identical (in their spectrum of substrate utilization and morphology), only strains HA1 and HA4 were examined further. Strain HA1 gave an eight (of eight) character fit in Seiler's (1983) group A and was identified as an Arthrobacter sp. Strain HA4 gave at best a six (of eight) character fit in group C and was not further identified.

Growth physiology

Strains HA1 and HA4 were found to have wide substrate specificity for halogenated compounds. Strain HA1 could utilize at least one haloalkane more than strain HA4, as well as 3-chloropropionate (as opposed to the 4-chlorobutyrate utilized by strain HA4); as it gave a linear growth response to substrate concentration (as opposed to poor growth of strain HA4 at low substrate concentrations), we chose Arthrobacter sp. strain HA1 for detailed work.

Strain HA1 utilized at least 18 haloalkanes (Table 1), each of which was stable in sterile control experiments. This organism thus has the widest substrate spectrum of all organisms reported to degrade haloalkanes. These growth substrates were 1-monosubstituted alkanes; disubstituted (α,ω) compounds and those with mid-chain substituents were not utilized. In contrast to the chloro-, bromo- and iodosubstituents, neither the fluorosubstituted (1-fluoropentane tested) nor the unsubstituted hydrocarbon (hexane) was utilized. The fact that 1-chloroethane was not utilized by our new isolates (Table 1) or by other strains (Janssen et al., 1985; Hartmans et al., 1986) does not mean that it is not biodegradable. We found that Pseudomonas sp. strain P4 could degrade ethanol or 1-chloroethane with a yield of about 4 g protein (mol C)⁻¹.

Utilization of each substrate by strain HA1 was quantitative as measured by substrate disappearance and, where measurable, by release of halide (Table 1). In the case of the chlorohydrocarbons, the identity of the halide released was confirmed by using an ion-specific electrode and, after derivatization to 2-chloroethanol, by GLC. It was not possible accurately to quantify all data for halide release because substrate toxicity was frequently too great (Table 1). In general, the propyl or butyl derivative was the least toxic. The longer the chain and the larger the halogen atom, the more toxic was the substrate. Table 1 shows the non-toxic limit above which the growth rate was reduced; at substrate concentrations about 120% of this value, no growth occurred.

Strain HA1 quantitatively utilized n-alcohols with chain lengths from C₂ to C₈ with a growth yield of 5-5 g protein (mol C)⁻¹ (Table 1), but the yield decreased rapidly if the growth rate was
Table 1. Alkanes substituted in the 1-position as growth substrates for Arthrobacter sp. strain HA1

Growth yields are presented as g protein (mol C)-1. Specific growth rates and specific degradation rates [mkat (kg protein)-1] are given. Non-toxic limits for non-growth substrates were tested during growth with 1-chlorohexane. The solubility of C1-C4 1-haloalkanes is higher than the non-toxic limit; larger compounds are described in handbooks as 'insoluble'.

<table>
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<tr>
<th>Number of carbon atoms in substrate molecule</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
<th>C7</th>
<th>C8</th>
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<td></td>
<td></td>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>5.6</td>
<td>5.8</td>
<td>5.8</td>
<td>5.8</td>
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<tr>
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<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
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<td>Non-toxic limit (mM)</td>
<td>3</td>
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<td>14</td>
<td>14</td>
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<tr>
<td>Growth</td>
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<td>+</td>
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<td>+</td>
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<td>4.1</td>
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<td>2.8</td>
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<td>Bromide released (%)</td>
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<td>97</td>
<td>97</td>
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<td>9.3</td>
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<tr>
<td>Growth</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Growth yield</td>
<td>4.2</td>
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<td>Iodide released (%)</td>
<td>104</td>
<td>116</td>
<td>100</td>
<td>103</td>
<td>100</td>
<td>103</td>
<td>103</td>
<td>103</td>
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<tr>
<td>Non-toxic limit (mM)</td>
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<td>3.1</td>
<td>2.3</td>
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</table>

depresseb by the toxicity of the substrate. This is a typical value for complete utilization of hydrocarbons (Anthony, 1982; Cook et al., 1983; Thurnheer et al., 1986). Yields with more oxidized compounds (e.g., succinate and citrate) were about 3 g protein (mol C)-1.

Strain HA1 also utilized the chlorohydrocarbons with a yield of about 5.5 g protein (mol C)-1, where this could be tested (Table 1). The chlorinated compounds were thus utilized for growth as efficiently as were the n-alcohols: negligible amounts of dissolved organic carbon were found in growth media. We thus have mass balance of carbon from the (halo)alkane into cell material and carbon dioxide, and mass balance of the organochlorine as the chloride ion.

The growth yields from bromo- and iodoalcohohydrocarbons were lower (Table 1). These compounds, however, supported slower growth than the alcohols and chloroalkanes so we presume the difference in yield to be due to maintenance energy requirements (Tempest & Neijssel, 1984).

The growth rate of strain HA1 was examined with several substrates as sole source of carbon and energy for growth: data for 1-chlorohexane are shown in Fig. 1. Growth was concomitant with substrate utilization (release of halide ion), and the rate was about 75% of that with the homologous alcohol (Table 1). Specific degradation rates were calculated to lie between 1.2 and 2.9 mkat (kg protein)-1 for the different substrates (Table 1).

**Dechlorination**

Resting cells of strain HA1 degraded 1-chlorobutane with a specific rate of 800 µkat (kg protein)-1, whether cells were harvested during exponential growth or in the stationary phase.
Biodegradation of 1-monohaloalkanes

Fig. 1. Growth of Arthrobacter sp. strain HA1 with 1.7 mM-1-chlorohexane (●) or 3.2 mM-n-hexanol (■) as the source of carbon and energy. Inset are the utilization of 1-chlorohexane (as release of Cl⁻) and the residual concentration of n-hexanol during growth.

Table 2. Substrate specificity of the haloalkane halidohydrolase(s) in cell-free extracts of strain HA1 grown with 1-chlorobutane

Reaction rates are presented relative to the reaction with 1-chlorobutane [100%; 700 µkat (kg protein)⁻¹]. All tests were done under the standard conditions with 5 µmol substrate per 1 ml reaction mixture.

<table>
<thead>
<tr>
<th>Substituent</th>
<th>C₁</th>
<th>C₂</th>
<th>C₃</th>
<th>C₄</th>
<th>C₅</th>
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<th>C₈</th>
<th>C₉</th>
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<tbody>
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<td>10</td>
<td>23</td>
<td>100</td>
<td>90</td>
<td>67</td>
<td>34</td>
<td>24</td>
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</tr>
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<td>NA</td>
<td>159</td>
<td>163</td>
<td>78</td>
<td>40</td>
<td>56</td>
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<td>116</td>
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<tr>
<td>1-Iodo-</td>
<td>53</td>
<td>148</td>
<td>186</td>
<td>89</td>
<td>58</td>
<td>28</td>
<td>22</td>
<td>NA</td>
<td>NA</td>
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</tbody>
</table>

NA, Not assayed.

Whole cells could degrade at least one substance, 1,6-dichlorohexane, which did not support growth, and both chlorine atoms were released.

Crude extracts from strain HA1 dechlorinated 1-chlorobutane (Table 2). In the reaction, substrate was quantitatively converted to product: 5.0 ± 0.2 mM-Cl⁻ was released from 5 mM-1-chlorobutane and, in parallel assays by GLC, the substrate disappeared with concomitant release of putative n-butanol (5 mM). The organic product from 1-chlorohexane, 1-bromobutane or 1-iodoethane was identified as the corresponding n-alcohol by co-chromatography with authentic material and by GLC-MS. Both the organic product and the halide were released stoichiometrically [1 mol (mol substrate)⁻¹]. The substrate was stable in the absence of enzyme and the enzyme preparation released no halide in the absence of substrate. The rate was proportional to the amount of extract present to a maximum of 1 mg crude extract in a 2 ml assay, and to the incubation time for at least 2 h.

The specific rate of enzymic dechlorination was 700 µkat (kg protein)⁻¹; this is very similar to the rate observed in growing cultures, so presumably the activity accounted for the reaction in intact cells. The reaction was catalysed by a soluble enzyme(s) which did not require cofactors,
because removal of membrane proteins by ultracentrifugation (110000 g; 1.5 h) and removal of small molecules by chromatography on Sephadex G-25 did not significantly alter the dechlorination activity. The reaction was presumably hydrolytic, since it occurred as rapidly anaerobically as aerobically.

The substrate spectrum for dehalogenation was wider than the spectrum of growth substrates (Table 2). Thus, substrates with chain lengths longer and shorter than those of the growth substrates were dehalogenated. Further, 2-chlorobutane [120 µkat (kg protein)⁻¹] and 3-chloroheptane [250 µkat (kg protein)⁻¹] were substrates and α,ω-dichlorohydrocarbons (at least C₄-C₆) were dechlorinated rapidly to give 2 mol Cl⁻ although they did not support growth.

The dehalogenation was inducible. Cells grown with butanol had no dechlorinating activity; this was observed only in cells grown with haloalkanes. Extracts of cells grown with 3-chloropropionate as sole added source of carbon and energy could dechlorinate 3-chloropropionate [data were corrected for spontaneous hydrolysis of the substrate (4% d⁻¹)], but not 1-chlorobutane, and extracts of cells grown with 1-chlorobutane could not dechlorinate 3-chloropropionate. Degradation of the haloalkanoates was thus independent of the metabolism of haloalkanes (cf. Janssen et al., 1985).

**DISCUSSION**

We have obtained from a single inoculum at least two different coryneform bacteria (strains HA1 and HA4) able to degrade a very wide spectrum of haloalkanes. Similar successes were reported by Janssen et al. (1985) and Hartmans et al. (1985, 1986), in contrast to the work of Omori & Alexander (1978a), who obtained only three successful enrichments from over 500 soil types. This comparison emphasizes the advantage of inocula chosen for their likelihood to contain organisms which have evolved to utilize specific xenobiotics (Cook et al., 1983). 1-Bromopentane occurs naturally (Gschwend et al., 1985), so its degradation might be expected (Dagley, 1975), and evolution to utilize the chloro-analogue is not difficult to envisage. We used a simple mineral salts medium in which successful enrichments are easily scored (OD, GLC, halide release) and, fortunately, non-toxic substrate concentrations. The nature of the observed toxicity (or toxicities) is unclear but longer chains were more toxic, whereas with the alkanes the reverse is true (Britton, 1984). The toxicity of 1-iodoethane to bacteria may be due to its efficiency as an alkylating agent. However, the toxicity to growing cells is independent of the reaction rate of the halidohydrolase(s) with the different substrates (cf. Tables 1 and 2). The enrichment of Arthrobacter and related strains is not surprising, because they classically degrade aliphatic and aromatic hydrocarbons (e.g., Schlegel, 1981), and they are known to degrade chlorinated derivatives (Janssen et al., 1985; Stanlake & Finn, 1982). However, our strains do not utilize the unsubstituted alkanes.

The present work extends that of Janssen et al. (1985), Omori & Alexander (1978a,b) and Yokota et al. (1986), and, with data from Hartmans et al. (1986), shows that the monochloroalkanes from C₁ to C₈ are all biodegradable, though different organisms utilize substrates of different chain lengths. Longer chain compounds are probably degradable, but the only data available (Murphy & Perry, 1983, 1984) do not include mass balances. Ours is the first report of the degradation of all the C₂-C₈ monobromo- and C₂-C₇ monioiodohydrocarbons. The relatively high specific activities and the quantitative dehalogenation could allow the organisms to be used to destroy these chlorinated solvents in waste streams (cf. Galli & Leisinger, 1985).

The mechanism of dechlorination seems to be hydrolytic, corresponding to the well defined halidohydrolase of Janssen et al. (1985) (see also Kohler-Staub & Leisinger, 1985) and the system of Yokota et al. (1986), although it is not possible to make quantitative comparisons with the latter results. There is no evidence for the oxidative systems known or suspected for chloromethane, vinyl chloride (Hartmans et al., 1985, 1986) and 1,9-dichlorononane (Yokota et al., 1986) or the NADH-coupled system implied by Omori & Alexander (1978b). Much of the dehalogenation activity in crude extracts can be explained by a single halidohydrolase which is currently being purified (R. Scholtz, A. M. Cook & T. Leisinger, unpublished).

The enzymic dehalogenation is inducible. Some substrates for the enzyme (e.g. 1-
chloropropene) are apparently not inducers though the reaction product is a growth substrate (Table 1), so regulatory and catalytic proteins recognize different molecules. In addition, many substrates (e.g. with mid-chain substituents) of the halidohydrolase do not support growth, so, if degradative pathways for the dehalogenated products were present, yet more haloalkanes would be biodegradable.

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REFERENCES

ANTHONY, C. (1982). The Biochemistry of the Methylo- 
BERGMANN, J. G. & SANIK, J. (1957). Determination of 
trace amounts of chlorine in naphtha. Analytical 
Chemistry 29, 241-243.
tions of 1- and 2-carbon halogenated aliphatic 
organic compounds under methanogenic conditions. 
Applied and Environmental Microbiology 45, 1286- 
1294.
tions of halogenated organic compounds under 
denitrifying conditions. Applied and Environmental 
Microbiology 45, 1295-1299.
BRITTON, L. N. (1984). Microbial degradation of 
aliphatic hydrocarbons. In Microbial Degradation of 
Organic Compounds, pp. 89-129. Edited by D. T. 
Bacterial degradation of dichloromethane. Applied 
and Environmental Microbiology 40, 950-958.
nitrogen sources for bacteria. Journal of Agricultural 
and Food Chemistry 29, 1135-1143.
bacterial dechlorination, deamination, and complete 
degradation. Journal of Agricultural and Food Chem- 
istry 32, 581-585.
COOK, A. M., GROSSENBACHER, H. & HÜTTER, R. 
(1983). Isolation and cultivation of microbes with 
biodegradative potential. Experientia 39, 1191-1198.
COOK, A. M., GROSSENBACHER, H. & HÜTTER, R. 
(1984). Bacterial degradation of N-cyclopropylmel- 
amine: the steps to ring cleavage. Biochemical 
Journal 222, 315-320.
problems of environmental pollution. Essays in 
Biochemistry 11, 81-138.
strains for the removal of dichloromethane from 
industrial waste. Conservation and Recycling 8, 91- 
100.
GÄSSLER, P. M., MACFARLANE, J. K. & NEWMAN, 
compounds released to seawater from temperate marine 
HARTMANS, S., DE BONT, J. A. M., TRAMPER, J. 
HARTMANS, S., SCHMUCKLE, A., COOK, A. M. 
occurring toxicant and C-1 growth substrate. Journal 
of General Microbiology 132, 1139-1142.
JANSSEN, D. B., SCHEPER, A., DIJKHUIZEN, L. & 
halophoric aliphatic compounds by Xanthobacter autotrophicus 
GJ 10. Applied and Environmental Microbiology 49, 
673-677.
degradative pathway of the s-triazine melamine: the 
steps to ring cleavage. Biochemical Journal 208, 679- 
684.
the mandelate pathway in bacterium NCIB 8250. 
Biochemical Journal 107, 497-506.
KÖHLER-STAB, D. & LEISINGER, T. (1985). Dichloro- 
methane dehalogenase of Hyphomicrobium sp. strain 
In Bergey's Manual of Systematic Bacteriology, vol. 1, 
Baltimore: Williams & Wilkins.
MOTOSUGI, K. & SODA, K. (1983). Microbial degra- 
dation of synthetic organochlorine compounds. 
Experientia 39, 1212-1220.
chlorinated alkanes into fatty acids of hydrocarbon-
utilizing mycobacteria. Journal of Bacteriology 156, 
1158-1164.
chlorinated alkanes by hydrocarbon-utilizing fungi. 
OMORI, T. & ALEXANDER, M. (1978a). Bacterial and 
spontaneous dehalogenation of organic compounds. 
Applied and Environmental Microbiology 35, 512- 
516.
dehalogenation of halogenated alkanes and fatty 
acids. Applied and Environmental Microbiology 35, 
867-871.
gas chromatography. Angewandte Chemie, Interna- 
edn. Thieme, Stuttgart.
bacteria derived by numerical taxonomy studies. 
Journal of General Microbiology 129, 1433-1471.
characterization of a pentachlorophenol-degrading 
bacterium. Applied and Environmental Microbiology 
44, 1421-1427.
STUCKI, G., GÄLLI, R., EBERSOLD, H.-R. & LEISINGER, 
T. (1981). Dehalogenation of dichloromethane by 
cell extracts of Hyphomicrobium DM2. Archives of 
Microbiology 130, 366-371.


