Enhancement of Co-Metabolism of Chlorobenzoates by the Co-Substrate Enrichment Technique

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Chlorinated benzoates were degraded by bacteria contained in an activated sludge inoculum by a co-metabolic mechanism. This decomposition began after an initial lag period of 4 days and accounted for 63 to 69% degradation in 28 days. The co-substrate enrichment technique, using glucose as co-substrate, increased both the rate of microbial decomposition of the benzoates and the total amount of substituted aromatic compounds degraded.

Chlorinated aromatic compounds which do not sustain microbial growth have been clearly shown to be susceptible to microbial decomposition by a co-metabolic mechanism (4-12, 17, 18; R. S. Horvath, J. M. Duxbury, and M. Alexander, Bacteriol. Proc., p. 9, 1930). Co-metabolism of halogenated-benzene derivatives by pure bacterial cultures generally results in oxidation of the materials to phenol- and catechol-like compounds (7, 8, 11) which, in turn, appear to be susceptible to further co-metabolism by other bacterial genera (6, 10; Horvath et al., Bacteriol. Proc.).

Recent investigations (9, 10) have indicated that co-metabolism could account for complete mineralization of these substances by mixed bacterial cultures if a carbon and energy source were supplied to the microorganisms in the form of a biodegradable analogue of the halogenated compound. Data obtained in this investigation established the ability of a structurally non-related substance, glucose, to serve as an enrichment agent for the degradation of m-chlorobenzoate and p-chlorobenzoate.

MATERIALS AND METHODS

The medium employed in this study was the same as that previously reported (11). The chlorinated aromatic substances were added to this medium at a concentration of 25 mg per liter. One flask of each aromatic salts medium received 500 mg of glucose per liter and served as the co-substrate enrichment system, whereas a second flask received no glucose. Uninoculated controls were included for each aromatic compound to determine any photodecomposition of the substance during the course of experimentation. All systems contained 500 ml of medium per 1-liter Erlenmeyer flask and were incubated at 25 C on a gyrotoery shaker.

The inoculum was obtained from a drainage ditch which received the effluent from the sewage treatment plant of Bowling Green, Ohio. A 2% inoculum (vol/vol) was used in all studies.

Halogenated benzoates were measured by the spectrophotometric procedure of Whiteside and Alexander (15). Chlorinated catechols were measured by the Arrow procedure (2).

RESULTS AND DISCUSSION

Degradation of m-chlorobenzoate occurred in the absence of the co-substrate, glucose (Fig. 1A). This decomposition reached a maximum of 50% in 14 days and occurred only after an initial lag period of 4 days. The decrease in concentration of the halogenated benzoate was accompanied by a corresponding increase in concentration of a chlorocatechol up to day 10. Active metabolism of the catechol appeared to occur after this time, resulting in complete degradation of this compound by day 14.

Similar results were obtained during the microbial degradation of p-chlorobenzoate in the absence of co-substrate (Fig. 1B). Decomposition began after an initial lag period of 4 days and was initially accompanied by an accumulation of a chlorocatechol. Further degradation of p-chlorobenzoate did not occur until the chlorocatechol had been completely metabolized. By day 28, 69% of the initial benzoate had been degraded.

In neither case was the degradation of substituted benzoates accompanied by an increase in cell numbers as determined turbidimetrically (λ = 545 nm) or by colony counts on
The degradation of the chlorobenzoates was followed by the disappearance of the co-substrate. The length of the lag period was reduced to 1 day in the presence of glucose, and 97% of the initial chlorobenzoate was degraded by day 21, compared to 31% degradation in the same time period when glucose was not supplied as the co-substrate. During this degradation process, a chlorocatechol did accumulate in stoichiometric amounts up to day 7 but was completely degraded by day 10.

The lack of microbial growth at the expense of the chlorobenzoates was indicative of the degradation of these substances by a co-metabolic process. The accumulation of chlorocatechols, in all cases, clearly showed an incomplete oxidation of the substrate and implicated the phenomenon of co-metabolism, at least in the initial oxidation of the chlorobenzoates.

The fact that both m-chlorobenzoate and p-chlorobenzoate were degraded in the absence of a co-substrate (Fig. 1A, B) established the ability of these substances to induce the requisite microbial enzymes for degradation, although neither compound could support growth of the microorganisms present. The ability of 2,3,6-trichlorobenzoate to induce enzymes necessary for its degradation by co-metabolism in the absence of an additional carbon and energy source had also been reported (9).

Addition of a co-substrate appears to serve primarily to increase the numbers of microorganisms capable of effecting an oxidation of the halogenated material and does not necessarily induce a specific microbial population. This latter function seems to be performed by the chloro-aromatic substances present in the environment. Glucose has been reported to be an effective co-substrate for decomposition of vanillin by soil microorganisms (14), alkyl benzenesulfonate by Pseudomonas sp. (3, 13), diazinon by Arthrobacter sp. (16), and o-anisidine by soil microorganisms (1). The wide range of compounds for which glucose serves as co-substrate supports the function of the co-substrate proposed above.

The results obtained in this study in the absence of co-substrate (Fig. 1A, B) were similar to those reported earlier by Alexander and
Lustigman (1), who noted that m-chlorobenzolate persisted for 32 days and p-chlorobenzolate persisted for 64 days when acted upon by soil microorganisms. The co-substrate enrichment technique employed in this study considerably reduced the time required for complete degradation of both substances.

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