Co-metabolism of *m*-Chlorobenzoate by Natural Microbial Populations Grown Under Co-substrate Enrichment Conditions

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The degradation of chlorinated benzoates by natural microbial populations has been reported to occur in the absence of additional carbon and/or energy sources by a co-metabolic process (HORVATH 1973; HORVATH 1972a; HORVATH 1972b; HORVATH 1971). However, use of the co-substrate enrichment technique (HORVATH 1973) clearly resulted in an increased rate of mineralization by the microorganisms present.

In view of the complete degradation of m-chlorobenzoate by microorganisms present in co-substrate enrichment systems, it seemed logical to determine the pathway by which this compound was degraded and the effect that this pathway had on the total cell crop obtained in this system. The results of this investigation establish the role of co-metabolism in the initial attack on m-chlorobenzoate and the degradation of this compound to inorganic chloride and organic intermediates which eventually can serve as carbon and energy sources for microbial growth.

Materials and Methods

The medium employed in this study was the same as that previously reported (HORVATH and ALEXANDER 1970). The co-substrate enrichment flask received m-chlorobenzoate at a concentration of 100 mg per liter in addition to 500 mg glucose per liter while the control flask contained no m-chlorobenzoate. All systems contained 500 ml of medium per 1-liter Erlenmeyer flask and were incubated at 25°C.

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

The concentration of m-chlorobenzoate was measured by the spectrophotometric procedure of WHITESIDE and ALEXANDER (1960). The ARNOW (1937) procedure was used to quantitate 3-chlorocatechol. Inorganic chloride was determined by the method of BERGMANN and SANIK (1957) and 2-hydroxy-3-chloro-muconic semialdehyde was quantitated by the procedure of HORVATH (1970). The presence of a free aldehyde moiety in the latter compound was confirmed by the ability of the compound to reduce Tollen's reagent (ENGLISH 1961).

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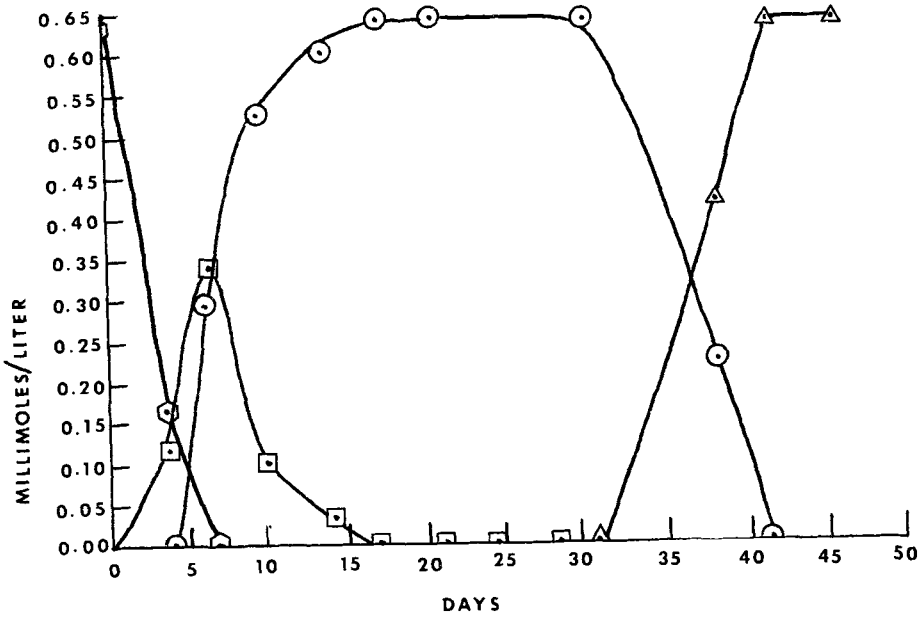

Figure 1. Co-metabolic degradation of m-chlorobenzoate (⬡) resulting in the formation of 3-chlorocatechol (⊡), 2-hydroxy-3-chloro-muconic semialdehyde (⊙) and inorganic chloride (△).

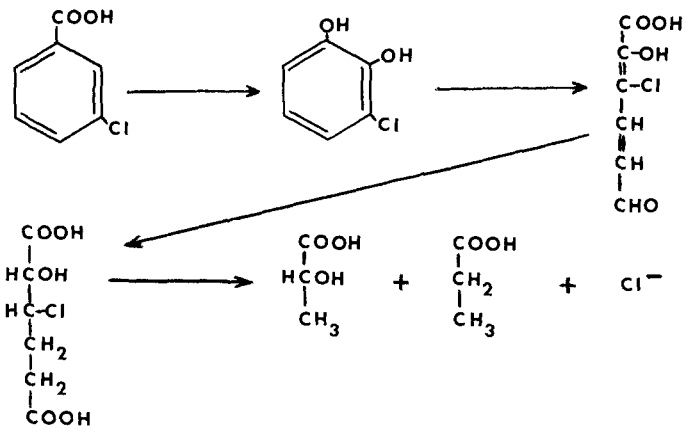

Figure 2. Co-metabolic pathway for the microbial degradation of m-chlorobenzoate.

Identification of products was accomplished by u.v. spectral analysis performed with a Varian Techtron model 635 spectrophotometer. Cell numbers were determined by standard plate count techniques using the same medium as found in the experimental flask plus 15 g ion agar per liter of medium.

Results and Discussion

In the presence of the co-substrate, glucose, m-chlorobenzoate was subject to immediate microbial degradation which was complete on day 7 (Fig. 1). This co-metabolic attack resulted in an accumulation of a chlorocatechol which reached a maximum concentration on day 7. The u.v. spectra of this compound showed a peak at 275 nm in neutral solution and two peaks at 270 nm and 317 nm at a pH of 10.0. These results were identical to those reported by REINER and HEGEMAN (1971) for 3-chlorocatechol and on this basis, this identity was assigned to the accumulated catechol.

From day 7 through day 17, 3-chlorocatechol showed a decrease in concentration which was accompanied by a corresponding increase in a compound which exhibited u.v. spectra in acidic and basic solutions that indicated the occurrence of keto-enol tautomerism. This compound reduced Tollen's reagent, establishing the presence of a free aldehyde structure. This material exhibited peaks at 281 nm at a pH of 2.0, 274 nm at a neutral pH and 307 nm at a pH of 10.0 and was identified as 2-hydroxy-3-chloro-muconic semialdehyde on this basis (BAYLY and DAGLEY 1969).

This chlorinated muconic semialdehyde, resulting from a meta-cleavage of 3-chlorocatechol, reached a concentration of 0.64 mmole/liter on day 17. At this time, all of the initially supplied m-chlorobenzoate had been converted into 2-hydroxy-3-chloro-muconic semialdehyde. This concentration remained constant until day 31 at which time a rapid destruction of the semialdehyde occurred, accompanied by a stoichiometric increase in inorganic chloride concentration (Fig. 1).

The co-metabolic degradation of m-chlorobenzoate appeared to follow the pathway shown in Figure 2. The fact that this series of reactions failed to support microbial growth is clearly shown in Figure 3 and thus establishes the mode of degradation as co-metabolism. Note that the cell crop obtained in basal salts plus glucose medium is identical to that obtained in basal salts plus glucose plus m-chlorobenzoate medium up to day 27. At this point, a decrease in cell numbers appeared to result due to a possible toxic environment caused by the muconic semialdehyde in the co-substrate enrichment system.

However, on day 34 when the degradation of the chloro-muconic semialdehyde was about 50% complete, a second log growth phase began in the co-substrate enrichment flask. This indicated that the products resulting from the co-metabolic break-down of the semialdehyde, lactic acid and/or propionic acid, were capable of

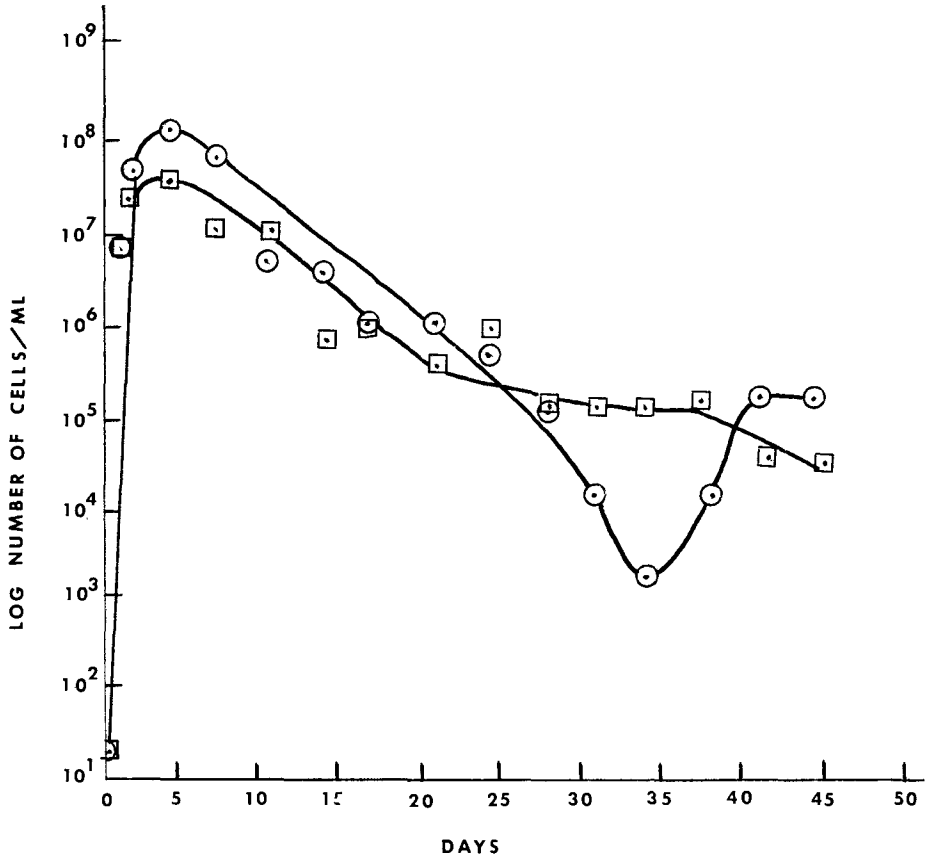

Figure 3. Growth of the natural microbial community in glucose-basal salts media in the presence (⊙) and absence (⊡) of m-chlorobenzoate.

serving as a metabolizable carbon and energy source. Thus, co-metabolism in the co-substrate enrichment system, appeared to allow the formation of products which could eventually support microbial growth. A similar phenomenon had been reported by HORVATH and KOFT (1972) for the microbial degradation of alkyl benzene sulfonate under co-substrate enrichment conditions.

It is not presently known whether one species of microorganism is responsible for all co-metabolic steps shown in Figure 2 or if population changes are induced by formation of the intermediate products resulting from degradation of m-chlorobenzoate. The growth curve shown in Figure 3 indicates the participation of at least two microbial populations in the total degradative pathway but proof of this is lacking. The species of bacteria involved in this degradative system, and their role in the system, are presently under investigation and will be reported at a later date.

Acknowledgement

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