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Degradation of Chlorophenols by a Defined Mixed Microbial Community

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Synthetic sewage containing phenol, acetone, and alkanols plus 4-chlorophenol or a mixture of isomeric chlorophenols is completely degraded by a defined mixed culture with *Pseudomonas* sp. strain B13 as a chlorocatechol-dissimilating member of the community. Total degradation of the organic carbon was indicated by release of stoichiometric amounts of chloride and low content of dissolved organic carbon in the cell-free effluents. During adaptation to high loads of chlorophenols the initial meta-cleavage activity was completely replaced by ortho-cleavage activity of type I and II. In the fully acclimated culture, hybrid strains such as *Alcaligenes* sp. strain A7-2 were detected, which are more competitive than *Pseudomonas* sp. strain B13 with respect to chlorophenol degradation.

High loads of haloaromatics are not satisfactorily removed by the traditional activated sludge. Because of incomplete degradation of the halosubstituted aromatic ring the effluents of the settling tank are deeply colored and contain high concentrations of dissolved organic carbon (DOC). In addition the haloaromatics' inherent toxicity or the toxicity generated through accumulation of metabolites such as chlorocatechols or chlorosubstituted ring fission products severely inhibit growth and may also suppress the development of haloaromatic-utilizing microorganisms that may preexist as marginal members of the community.

Principally halogenated aromatics can be acted on microbiologically. Since members of several bacterial genera have been isolated from very different places of the world (2, 5, 13), it must be assumed that catabolic enzymes or even complete degradative sequences for haloaromatics preexist in nature. Information is needed on whether and how such activities can become established under practical conditions of activated sludge.

The present investigation was designed to examine the degradation of monochlorophenols by a defined mixed community as a model ecosystem. Furthermore, the study was initiated to demonstrate that instabilities of the population due to the presence of chlorophenols can be counteracted by the addition of a chloroarene-utilizing bacterium.

Since ring cleavage of chlorocatechols has been identified as a bottleneck of chloroaromatics breakdown (3, 4), the introduction of genes encoding halocatechol assimilation and the establishment of the latter activity within the population is expected to be of crucial importance for the stability of the microbial community and for complete degradation of synthetic waste containing chlorophenols.

MATERIALS AND METHODS

Organisms. *Pseudomonas* sp. strain B13 has been described by Dorn et al. (3), and *Alcaligenes* sp. strain A7 has been described by Schwien and Schmidt (11). *Pseudomonas extorquens* was characterized as a methanol-degrading strain (12).

Media and culture conditions. For growth of *Pseudomonas* sp. strain B13 and *Alcaligenes* sp. strain A7, we used the culture conditions of Schwien and Schmidt (11). For cultivation of *P. extorquens* the mineral medium was supplemented with 5 mM methanol.

The continuous cultures were run at 30°C in a 2-liter fermentor (Biolafitte, Poissy, France) containing 1 liter of medium. Fresh medium was added continuously (1 liter per day) by means of a peristaltic pump (LKB, Bromma, Sweden). As a synthetic sewage the mineral medium (11) was supplemented with 15 mM methanol, 3 mM ethanol, 3 mM acetone, 3 mM isopropanol, and 5 mM phenol. 4-Chlorophenol or a mixture of the isomeric chlorophenols as critical components of the synthetic sewage were supplied as a linear gradient (0 to 6 mM) generated in the reservoir. Polypropylene glycol (P2000) at a final concentration of 0.003% (vol/vol) was added to the medium as an antifoaming agent.

Preparation of cell-free extracts. Cell suspensions in 50 mM Tris-hydrochloride buffer (pH 8.0) were disrupted with a French press (Aminco, Silver Spring, Md.). Cell debris and particles were removed by sedimentation at 100,000 × *g* for 60 min.

Assays for pyrocatechase. Catechol 2,3-dioxygenase

(EC 1.13.11.2) was assayed by the method of Nozaki (9). Catechol 1,2-dioxygenase (EC 1.13.11.1) activity was measured by the procedure of Dorn and Knackmuss (3). Extinction coefficients for the ring fission products were those reported by Dorn and Knackmuss (4). When high levels of catechol 2,3-dioxygenase were present, this activity was eliminated by the addition of H_2O_2 at a final concentration of 0.1% (vol/vol).

Analytical methods. The concentrations of phenol and chlorophenols were determined by high-pressure liquid chromatography with a reverse-phase RP8 column (Merck, Darmstadt, Federal Republic of Germany). Vacuum-degassed 10 mM phosphoric acid containing 0.5% (vol/vol) propanol and 70% (vol/vol) methanol was used as the eluent. Peaks were detected in a variable-wavelength model 770 spectrometric detector (Schoeffel Instrument Corp., Westwood, N.J.) and were identified with authentic compounds by their retention times.

The concentrations of the alkanols and acetone were measured by gas chromotography with a 3-m glass column filled with Porapak S (80-100 mesh). Peaks were detected by use of a flame ionization detector (3920; Perkin Elmer, Überlingen, Federal Republic of Germany).

For quantification of DOC, 50 μl of medium was injected in a total organic carbon analyzer (915 B; Beckman Instruments, Inc., Fullerton, Calif.) after removal of cells and particles by sedimentation for 3 min in a centrifuge (model 5412; Eppendorf, Hamburg, Federal Republic of Germany).

Chloride ion concentrations were measured with an ion selective combination electrode (model 96/17; Orion Research Inc., Cambridge, Mass.) which was calibrated with KCl (0.1 mM up to 10 mM) in mineral medium before each measurement.

RESULTS

Model ecosystem. To investigate degradation of chlorophenols by a mixed culture under conditions similar to those in industrial sewage, a synthetic waste water was used that contained representative components such as methanol, ethanol, acetone, and isopropanol. Phenol was added as a readily degradable aromatic component which on one hand might serve as a potential inducer of chlorophenol-cooxidizing activity and on the other hand might interfere with the total degradation of chlorophenols.

Baumgarten (1) has shown that members of the genus *Pseudomonas* and *Alcaligenes* are dominating organisms of activated sludge populations, which digest effluents from chemical industry similar to the synthetic sewage described above. Therefore *Alcaligenes* sp. strain A7 was chosen as a model bacterium which could utilize ethanol, isopropanol, and acetone. This organism was isolated through its ability to utilize phenol and its ability to form colonies on solid media containing high concentrations (≤2 mM) of this chemical (11). *P. extorquens* (12) was added to the model culture for its ability to utilize methanol.

To evaluate the possibility of establishing a chlorophenol-degrading organism in a mixed community stressed by chlorophenol, *Pseudomonas* sp. strain B13 was selected. Its ability to utilize 4-chlorophenol is based on a phenol hydroxylase which normally functions in the utilization of phenol and a chlorocatechol-degrading system obtained by selection with 3-chlorobenzoate (3, 7, 10).

Continuous growth in the presence of 4-chlorophenol. A mixed culture of *Alcaligenes* sp. strain A7 and *P. extorquens* was grown in a 1-liter chemostat. The fresh medium containing synthetic sewage was pumped into the fermentor at a rate of approximately 1 liter per day. A stable population was obtained after 4 days and was subsequently stressed with a linear gradient of 4-chlorophenol (0 to 6 mM within 30 days).

Although 4-chlorophenol could not be detected in the culture fluid throughout the course of stable growth (35 days), the amount of chloride released from 4-chlorophenol oxidation was always far below equimolarity (Fig. 1). This, together with a steady increase in DOC of the cell-free effluent, indicated incomplete degradation of the organic carbon. The deep yellow brownish color (λ_{max}, 379 nm) of the cell free culture fluid indicated the accumulation of 5-chloro-2-hydroxymuconic semialdehyde (6). At that time the DOC value reached a plateau of about 360 ppm, corresponding to 30% of total organic carbon in the fresh medium. After 35 days the culture became unstable and the DOC increased

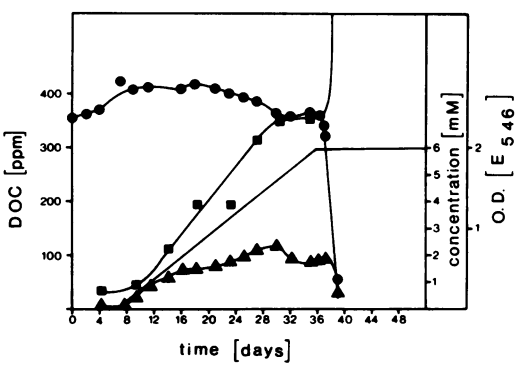

FIG. 1. Degradation of 4-chlorophenol by a two-membered community involving *Alcaligenes* sp. strain A7 and *P. extorquens*. The organisms were grown continuously in a synthetic waste water containing phenol, acetone, and alkanols (DOC, 828 ppm). Increasing amounts of 4-chlorophenol were added to the fresh medium (the concentration in the reservoir was 0 to 6 mM, corresponding to 0 to 432 ppm). The line without points exhibits the gradient applied. The OD at 546 nm (●), chloride micromolar concentration (▲), and content of DOC (■) in the cell-free effluent were measured at intervals.

beyond 1,000 ppm. At the same time 4-chlorophenol accumulation and rapid washout of the population was observed.

A parallel culture augmented with a 3-chlorobenzoate-grown inoculum of *Pseudomonas* sp. strain B13 behaved completely differently when treated under the same conditions. Under the selective pressure of the 4-chlorophenol load *Pseudomonas* sp. strain B13 became a stable member of the community.

Despite a stronger fluctuation in the optical density (OD) during the initial phase of chlorophenol loading, chloride elimination clearly paralleled chlorophenol concentration in the fresh medium. This, together with the low DOC values in the later phase of adaptation, clearly demonstrated total degradation and utilization of the organic compounds in the synthetic sewage. The culture fluid was almost colorless, which indicates that the haloaromatic ring was not channelled into the unproductive meta-cleavage pathway.

Exposure to isomeric chlorophenols. The model culture with *Alcaligenes* sp. strain A7 and *P. extorquens* was much more sensitive to a mixture of 2-, 3-, and 4-chlorophenol than to 4-chlorophenol alone. This was also the case in the presence of *Pseudomonas* sp. strain B13 with its 4-chlorophenol-degrading capacity. As indicated by Fig. 3 and Fig. 4, OD measurements and population analyses were severely disturbed because the cells formed larger aggregates during the early stage of chlorophenol exposure.

The reference culture without the auxiliary strain B13 became unstable and was washed out rapidly as soon as total concentrations of the chlorophenols in the reservoir exceeded 1 mM. In contrast, a stable culture was obtained when *Pseudomonas* sp. strain B13 was present in the

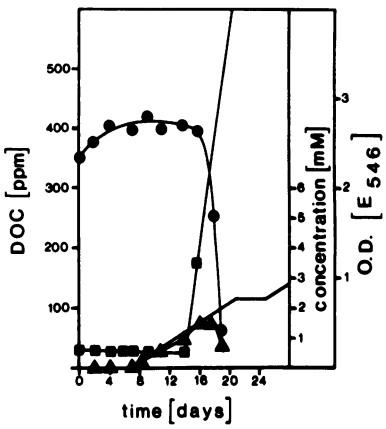

FIG. 3. Continuous culture of *Alcaligenes* sp. strain A7 and *P. extorquens* exposed to a mixture of monochlorophenols. The mixed culture was grown continuously in a synthetic waste water and stressed with increasing concentrations of the three monochlorophenols (0 to 2 mM each in the reservoir). The OD at 546 nm (●), chloride concentration (▲), and content of DOC (■) in the cell-free effluent were measured at intervals.

population. Elimination of equimolar amounts of chloride and low DOC contents in the cell-free culture fluid throughout the entire loading experiment indicate total degradation of the organic carbon of the synthetic sewage.

Key activities of chlorophenol degradation. Phenol hydroxylase activities were measured by O_2 consumption assay and were followed during the entire course of the loading experiments. This activity, however, did not respond significantly to an increase of the concentration of chlorophenols in the culture fluid and could therefore not be used as an indicator for the adaptation status of the culture. Instead of that, the activities of ortho-ring cleavage with cate-

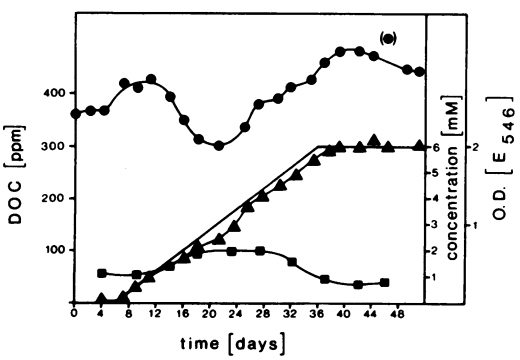

FIG. 2. Degradation of 4-chlorophenol by a two-membered community augmented with *Pseudomonas* sp. strain B13. The mixed culture growing continuously on a synthetic waste water was exposed to increasing concentration of 4-chlorophenol (culture conditions and symbols as in Fig. 1).

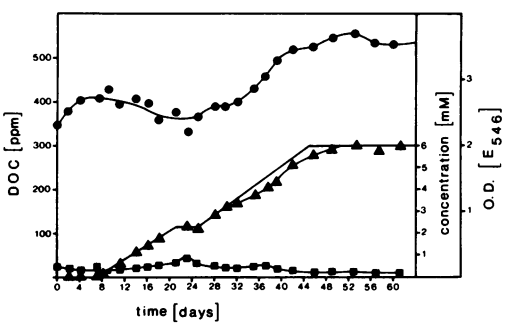

FIG. 4. A two-membered community augmented with *Pseudomonas* sp. strain B13 exposed to a mixture of monochlorophenols. The mixed culture was stressed with increasing concentrations of the three monochlorophenols (culture conditions and symbols as in Fig. 3).

chol and 3-chlorocatechol as well as of meta cleavage with catechol proved to be a useful aid for the description of the chlorophenol acclimation of the population.

As described earlier, *Alcaligenes* sp. strain A7 degraded phenol via meta-cleavage so that this was the only measurable ring cleavage activity during growth with synthetic sewage in the absence of chlorophenols. As a consequence, exposure to 4-chlorophenol instantaneously generated a deep yellow color in the culture fluid. In the continuous culture without *Pseudomonas* sp. strain B13 the catechol 2,3-dioxygenase activity was present until the culture had been destroyed by a 4-chlorophenol overload (Fig. 5). In contrast, when *Pseudomonas* sp. strain B13 was present in the population, high ortho-cleavage activity appeared, whereas the meta-cleavage activity gradually decreased and finally disappeared in the fully adapted status. It is noteworthy that ortho-cleavage activity during the early stage of 4-chlorophenol loading (up to 30 days, ≤1 mM 4-chlorophenol in the reservoir) was measurable only with catechol, but not with 3-chlorocatechol as the substrate. The heavily loaded culture (≥1 mM 4-chlorophenol in the reservoir), however, also exhibited ortho-cleavage activity with 3-chlorocatechol as the substrate (pyrocatechase type II).

A rapid decrease and a complete disappearance of the apparent meta-cleavage activity was observed during loading with the chlorophenol mixture (Fig. 6). In the absence of the auxiliary strain B13, no ortho-pyrocatechase activity was induced, whereas this activity was high in the

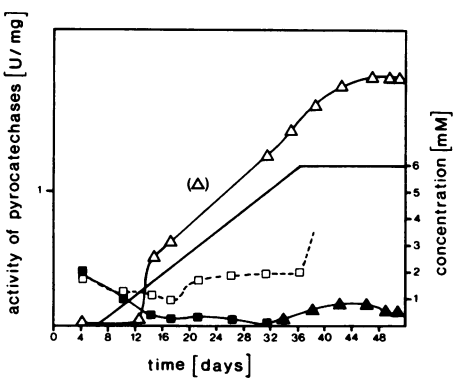

FIG. 5. Ring cleavage activities of a defined mixed culture degrading synthetic sewage plus 4-chlorophenol (Fig. 1 and 2). The specific activities of pyrocatechases were measured in crude extracts. The *Pseudomonas* sp. strain B13-augmented, mixed culture exhibited meta-cleavage activity (■) as well as ortho-cleavage activity of type I (△) and of type II (▲). In the culture without *Pseudomonas* sp. strain B13, only meta-cleavage activity (□) was found.

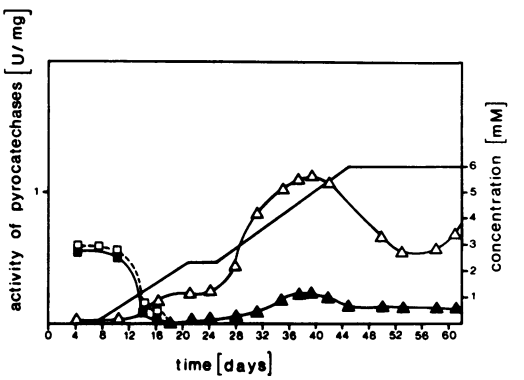

FIG. 6. Ring cleavage activities of a defined mixed culture degrading synthetic sewage plus a mixture of the monochlorophenols (Fig. 3 and 4). The *Pseudomonas* sp. strain B13-augmented, mixed culture exhibited meta-cleavage activity (■) as well as ortho-cleavage activity of type I (△) and of type II (▲). In the culture without *Pseudomonas* sp. strain B13, only meta-cleavage activity (□) was found.

Pseudomonas sp. strain B13-augmented population. In addition, type II ortho-pyrocatechase activity (3-chlorocatechol as substrate) was induced at a relatively early stage of loading with the mixture of isomeric chlorophenols.

Analysis of the population. To follow any changes in the population during continuous exposure to chlorophenols, samples of the population were plated on mineral agar with selective carbon sources. Growth with nicotinate was typical for *Alcaligenes* sp. strain A7. *P. extorquens* was recognized through its ability to grow with methanol, and *Pseudomonas* sp. strain B13 was identified by its capability to form colonies on 3-chlorobenzoate agar. The latter substrate was used instead of 4-chlorophenol because the inherent toxicities of the chlorophenols did not allow us to count cell numbers reproducibly on solid medium. For total viable cell counts nutrient broth agar was used.

The results of the population analysis of the 4-chlorophenol loading experiment corresponded to those of the OD measurements (Fig. 1). In the absence of *Pseudomonas* sp. strain B13 as an auxiliary strain the culture was destroyed after 37 days as indicated by a rapid fall in turbidity. Before the wash out of the culture the initial cell density of *Alcaligenes* sp. strain A7 had already decreased from 2×10^9 (day 12) to 3×10^8 (day 35) cells per ml, and that of *P. extorquens* had decreased from approximately 6×10^8 (day 12) to 2×10^8 (day 35) cells per ml.

The establishment of the chloroarene-degrading capacity of *Pseudomonas* sp. strain B13 (Fig. 2) had a pronounced stabilizing influence on the population of *Alcaligenes* sp. strain A7 (2

$\times 10^9$ cells per ml initially and 1×10^9 cells per ml after 45 days) and of *P. extorquens* (4×10^8 cells per ml initially and 5×10^8 cells per ml after 45 days). The number of cells that were able to form colonies on 3-chlorobenzoate agar rose continually to about 10^9 cells per ml of synthetic sewage with increasing loads of 4-chlorophenol.

Similar results were observed in the cultures loaded with the mixture of isomeric chlorophenols. Before the rapid fall in bacterial cell density, the culture without *Pseudomonas* sp. strain B13 contained 3×10^9 cells of *Alcaligenes* sp. strain A7 per ml and about 6×10^8 cells of *P. extorquens* per ml. The *Pseudomonas* sp. strain B13-amended culture again distinguished itself by a pronounced stability against high loads of chlorophenols. The initial cell density of *Alcaligenes* sp. strain A7 was about 3×10^9 cells per ml and decreased slightly to about 10^9 cells per ml, whereas that of *P. extorquens* increased from 5×10^8 to 6×10^8 cells per ml. Corresponding to the 4-chlorophenol loaded culture the 3-chlorobenzoate-utilizing cells increased from 10^7 cells per ml initially to about 10^9 cells per ml in the fully acclimated stage.

Replica plating on nicotinate and 3-chlorobenzoate agar revealed that the chlorophenol-acclimated populations contained cells which could utilize both substrates, indicating that *Alcaligenes* sp. strain A7 had acquired the ability to utilize 3-chlorobenzoate and thus to participate in chlorophenol dissimilation. In the 4-chlorophenol-acclimated culture (Fig. 2) after 50 days of chlorophenol exposure only 10% of the population was able to utilize both nicotinate and 3-chlorobenzoate. In contrast loading with the mixture of isomeric chlorophenols favoured the rapid development of hybrides such as *Alcaligenes* sp. strain A7-2. This mutant has also been obtained by conjugation on solid medium (11). Thus, after 50 days of exposure already 50% of cells were able to utilize both substrates, nicotinate and 3-chlorobenzoate.

The remaining nicotinate-positive cells, which were unable to grow with 3-chlorobenzoate, exhibited the same physiological properties as *Alcaligenes* sp. strain A7, except that phenol is utilized via the ortho-cleavage pathway. From this population a strain A7-1 could readily be isolated. The cell-free extracts of phenol-grown cells of this isolate contained no meta-cleavage activities, but high ortho-cleavage activities for catechol.

Maximum loading rates. As mentioned above, the culture without *Pseudomonas* sp. strain B13 was destroyed after 20 days when loaded with the isomeric chlorophenols. Immediately before the rapid fall in OD (after 15 days; Fig. 3) neither solvents nor any of the phenols could be detected in the cell-free culture fluid. A sample taken 40 h later (day 16 of the experiment), however, contained phenol ($\leq$2.4 mM), chlorophenols ($\leq$0.3 mM each), and some solvents (acetone, $\leq$0.1 mM; isopropanol, $\leq$0.4 mM). Although methanol and ethanol were still completely degraded at that time, washing out of the population could not be averted by halting or reducing the chlorophenol load.

The fully acclimated culture with *Pseudomonas* sp. strain B13 as a stable partner of the community digested 1 liter of synthetic sewage containing 6 mM chlorophenol per day. The loading rate could be increased by 200 ml per day without affecting total degradation of the chlorophenol-loaded sewage (high-pressure liquid chromatography and DOC analysis) until the dilution rate exceeded 3.8 liters per day. Beyond this maximum loading rate, first of all methanol and ethanol, but no phenols, appeared in the cell-free culture fluid. Obviously under these conditions the growth rate of *P. extorquens* and turnover of alcohols was the limiting factor of this model system.

DISCUSSION

The present investigation shows that a synthetic sewage containing alkanols, acetone, and phenol (DOC, 828 ppm) can readily be degraded by the stable coexistence of two organisms, the methanol-degrading bacterium *P. extorquens* and a recently isolated *Alcaligenes* sp. strain A7 that can degrade phenol at high concentrations in addition to ethanol, isopropanol, and acetone (11). This phenol- and solvent-degrading community (OD at 546 nm, 2.5 to 3.0) tolerated a relatively high load of 4-chlorophenol as a critical component of the synthetic sewage. However, chlorophenol degradation was incomplete, as indicated by high DOC in the cell-free effluents and unstoichiometric chloride release. One of the main dead end products seemed to be 5-chloro-2-hydroxymuconic semialdehyde. It is noteworthy that dechlorination was almost quantitative as long as the relative amounts of 4-chlorophenol and phenol were $\leq$1 mM and 5 mM, respectively, which has also been discussed by Janke and Fritsche (6). When the culture was exposed to higher amounts of 4-chlorphenol, the accumulation of 5-chloro-2-hydroxymuconic semialdehyde increased significantly, resulting in a deep yellow brownish color of the medium.

Qualitatively the same observations with incomplete degradation of the organic carbon were made in a corresponding culture being stressed with a mixture of isomeric chlorophenols. Compared with the corresponding experiment with 4-chlorophenol, the culture proved to be more sensitive against the mixture of chlorphenols, so that the threshold overall concentra-

tion of chlorophenols in the reservoir was ≤0.5 mM.

In contrast to the loading experiment with 4-chlorophenol the culture remained almost colorless when exposed to increasing concentrations of isomeric chlorophenols.

When a mixture of isomeric chlorophenols is degraded, hydroxylation of 2- and 3-chlorophenol must generate 3-chlorocatechol as an additional critical metabolite. This compound has been recognized as an inhibitor for catechol 2,3-dioxygenases (7). Consequently, meta-cleavage activity rapidly decreased when increasing concentrations of isomeric chlorophenols were present in the fresh medium.

For the treatment of an industrial sewage containing 4-chlorophenols or a mixture of isomeric chlorophenols the function of *Pseudomonas* sp. strain B13 as a chlorophenol-degrading and thus stabilizing member of the community could clearly be demonstrated. In both experiments unproductive meta-cleavage activity decreased even more rapidly in the three-species community compared with the control culture without *Pseudomonas* sp. strain B13. Instead of meta-cleavage activity catechol 1,2-dioxygenase activities of type I and type II were induced with increasing loads of chlorophenols. A significant observation was that the stability of the *Pseudomonas* sp. strain B13-augmented community was clearly indicated by the appearance of pyrocatechase II activity, which was induced during exposure to high loads of chlorophenols. High ortho-cleavage activities in cell free extracts always paralleled complete dehalogenation of the chlorophenols. In the fully acclimated status the low DOC content accompanied by quantitative chloride release indicated complete degradation of the organic carbon. After prolonged exposure of the continuous culture to high loads of chlorophenols the *Pseudomonas* sp. strain B13-augmented population contained increasing numbers of cells of *Alcaligenes* sp. strain A7 which have acquired the capacity to metabolize chlorophenols and thus to form colonies on 3-chlorobenzoate mineral agar. From this population a strain *Alcaligenes* sp. strain A7-2 was isolated, which was also accessible by classical conjugation experiments (11). These studies supported the idea that the selection of this kind of organism with greatly elevated levels of a phenol hydroxylase and higher tolerance against increasing concentrations of chlorophenols are the basis for the progressive replacement of *Pseumonas* sp. strain B13 by *Alcaligenes* sp. strain A7-2 as the chlorophenol-degrading member of the community. *Alcaligenes* sp. strain A7-2 has completely and irreversibly lost the ability of the parent strain A7 to induce meta-cleavage activity. Since degradation of non-halogenated aromatics such as phenol via meta cleavage appears to be incompatible with the productive break down of haloaromatics, at high loads of chlorophenols a strong counterselection exists against organisms that induce catechol 2,3-dioxygenases.

This readily explains the selective advantage of mutants such as *Alcaligenes* sp. strain A7-1, which cannot yet grow with chlorophenols or 3-chlorobenzoate, but, in contrast to the wild-type strain A7, utilizes phenol via ortho-cleavage. Apparently, as long as moderate amounts of chlorophenols are present, strain A7-1 induces high levels of ortho-pyrocatechase type I, which to a certain extent may avoid accumulation of chlorocatechols. *Alcaligens* sp. strain A7-1, however, is not competitive at high loads of chlorophenols, because, unlike strain A7-2, it cannot induce enzymes of chlorocatechol assimilation such as ortho-pyrocatechase type II.

In conclusion the present results indicate that chlorophenols as critical components of industrial sewage can be decomposed completely by a stable consortium after *Pseudomonas* sp. B13 has been established as a chlorocatechol-degrading member of the community during an adaptation period. Significantly meta-cleavage activity is completely replaced by ortho-cleavage activity of type I and II in the fully acclimated culture.

Furthermore, population analysis revealed that it is the establishment of the chlorocatechol-degrading capability more than the number of viable cells of *Pseudomonas* sp. strain B13 that describes the status of chlorophenol acclimation. Under the conditions of the present model culture hybrid organisms such as *Alcaligenes* sp. strain A7-2, which acquired the halocatechol-degrading genes from *Pseudomonas* sp. strain B13, are much more competitive with respect to phenol and chlorophenol utilization (11). To deal with chlorophenols as problem pollutants of industrial sewage, microbial populations of waste treatment systems must develop a new breakdown potential. The present experiments indicate that this process of adaptation comprises the following three major events: (i) the prevention of the meta pathway as an unproductive route for chlorophenol degradation, (ii) the establishment of a chlorocatechol-assimilating sequence through the acquisition of appropriate genetic information from a separately evolved laboratory strain, and (iii) the induction of high levels of phenol hydroxylase activities to function in chlorophenol degradation and detoxification (11). The model system clearly shows that all three conditions must be fulfilled for productive breakdown. However, more sophisticated population analyses have to be designed to determine the sequence of events during the course of adaptation.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Baumgarten, J.** 1980. Bakterienpopulationen von Belebtschlamm und ihr Einfluß auf die Klärleistung für industrielle Abwässer. Forum Mikrobiol. **5**:284–291.
2. **Dorn, E., M. Hellwig, W. Reineke, and H.-J. Knackmuss.** 1974. Isolation and characterization of a 3-chlorobenzoate degrading pseudomonad. Arch. Microbiol. **99**:61–70.
3. **Dorn, E., and H.-J. Knackmuss.** 1978. Chemical structure and biodegradability of halogenated aromatic compounds: two catechol 1,2-dioxygenases from a 3-chlorobenzoate-grown pseudomonad. Biochem. J. **174**:73–84.
4. **Dorn, E., and H.-J. Knackmuss.** 1978. Chemical structure and biodegradability of halogenated aromatic compounds: substituent effects on 1,2-dioxygenation of catechol. Biochem. J. **174**:85–94.
5. **Evans, W. C., B. S. W. Smith, P. Moss, and H. N. Fernley.** 1971. Bacterial metabolism of 4-chlorophenoxyacetate. Biochem. J. **122**:509–517.
6. **Janke, D., and W. Fritsche.** 1979. Dechlorierung von 4-Chlorphenol nach extradioler Ringspaltung durch *Pseudomonas* putida. Z. Allg. Mikrobiol. **19**:139–141.
7. **Klečka, G. M., and D. T. Gibson.** 1981. Inhibition of catechol 2,3-dioxygenase from *Pseudomonas putida* by 3-chlorocatechol. Appl. Environ. Microbiol. **41**:1159–1165.
8. **Knackmuss, H.-J., and M. Hellwig.** 1978. Utilization and cooxidation of chlorinated phenols by *Pseudomonas* sp. B13. Arch. Microbiol. **177**:1–7.
9. **Nozaki, M.** 1970. Metapyrocatechase (Pseudomonas). Methods Enzymol. **17A**:522–525.
10. **Schmidt, E., and H.-J. Knackmuss.** 1980. Chemical structure and biodegradability of halogenated aromatic compounds: conversion of chlorinated muconic acids into maleolyacetic acid. Biochem. J. **192**:339–347.
11. **Schwien, U., and E. Schmidt.** 1982. Improved degradation of monochlorophenols by a constructed strain. Appl. Environ. Microbiol. **44**:33–39.
12. **Stocks, P. K., and C. S. McClesky.** 1964. Identity of the pink-pigmented methanol-oxidizing bacteria as *Vibrio* extorquens. J. Bacteriol. **4**:1065–1070.
13. **Vandenbergh, P. A., R. H. Olsen, and J. F. Colaruotolo.** 1981. Isolation and genetic characterisation of bacteria that degrade chloroaromatic compounds. Appl. Environ. Microbiol. **42**:737–739.