Growth of bacteria at low oxygen concentrations
Gerritse, Jan

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1993

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Chapter 7

Mineralization of the herbicide 2,3,6-trichlorobenzoic acid by a co-culture of anaerobic and aerobic bacteria

Jan Gerritse and Jan C. Gottschal

Chapter 7

Mineralization of the herbicide 2,3,6-trichlorobenzoic acid by a co-culture of anaerobic and aerobic bacteria

Jan Gerritse and Jan C. Gottschal

Department of Microbiology, University of Groningen, Haren, Netherlands

Received 3 December 1991
Revision received 16 March 1992
Accepted 17 March 1992

Key words: Co-culture; Anaerobic plus aerobic bacteria; 2,3,6-Trichlorobenzoic acid; Reductive dechlorination; 2,5-Dichlorobenzoic acid; Microaerobic mineralization

1. SUMMARY

Bacteria from an anaerobic enrichment reductively removed chlorine from the ortho- position of 2,3,6-trichlorobenzoic acid (2,3,6-TBA) producing 2,5-dichlorobenzoate (2,5-DBA). The strictly aerobic bacterium Pseudomonas aeruginosa JB2 subsequently used 2,5-DBA as a growth substrate in the presence of oxygen. The anaerobic dechlorinating microbial population was grown with P. aeruginosa JB2 in continuous culture. Inside the liquid culture, a nylon netting, on a stainless-steel support, contained vermiculite particles to provide a strictly anaerobic environment within the aerated culture. Complete mineralization of 2,3,6-TBA depended on the extent of oxygen input into the reactor. Under strictly anaerobic conditions 2,5-DBA and Cl\(^{-}\) were produced stoichiometrically through the reductive dechlorination of 2,3,6-TBA. This process of reductive dechlorination was not inhibited by (moderate) aeration resulting in an O\(_2\)-concentration of 0.3–0.5 \(\mu\)M in the culture liquid.

2. INTRODUCTION

Halogenated aromatic compounds are serious pollutants of the environment world-wide. Many of these man-made chemicals, such as 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDE), polychlorinated biphenyls (PCB), chlorinated dibenzo-p-dioxins, chlorinated benzenes and chlorinated phenols, persist in nature. Some of these xenobiotics can be degraded by (micro) organisms. The metabolism of halogenated aromatic compounds has been studied extensively during recent years and information on their degradation has been reviewed by several authors [1–5]. Degradation depends on a variety of factors. The chemical structure of the compound, the presence of organisms with appropriate catabolic capacities and the prevailing environ-
Mental conditions are of critical importance. The presence of molecular oxygen is of remarkable influence on the degradation. Under aerobic conditions, the persistence of aromatic compounds generally increases with increasing halogen substituents [6]. Quite the opposite is true for reductive dehalogenation reactions, catalyzed by some (facultative) anaerobes. Increased halogen substitution may indeed enhance degradation [5,7]. Quite the opposite is true for reductive dehalogenation reactions, catalyzed by some (facultative) anaerobes. Increased halogen substitution may indeed enhance degradation [5,7].

3. MATERIAL AND METHODS

3.1. Source and growth of organisms

P. aeruginosa JB2 was kindly supplied by Dr. F.K. Higson and Dr. D.D. Focht. The organism grows on several mono-, di-, and trihalogenated benzoic acids [19]. We routinely grew P. aeruginosa JB2 at 30°C in a low-chloride mineral salts medium (LMM) containing the following components (per liter): MgSO₄·7H₂O (0.1 g), Ca(NO₃)₂·4H₂O (0.05 g), EDTA (1 mg), FeSO₄·7H₂O (0.2 mg), ZnSO₄·7H₂O (0.1 mg), MnCl₂·4H₂O (0.03 mg), H₃BO₃ (0.3 mg), CoCl₂·6H₂O (0.2 mg), CuCl₂·2H₂O (0.01 mg), NiCl₂·6H₂O (0.02 mg), Na₂MoO₄·2H₂O (0.026 mg), Na₂SeO₃·5H₂O (0.02 mg) and Na₂WO₄·2H₂O (0.033 mg). After autoclaving, the medium was completed by the addition of a K(NH₄)PO₄-buffer (25 mM, pH 7.0) and (chloro)benzoate (2.5 mM) from separately autoclaved stock solutions.

The anaerobic reductively dechlorinating enrichment used in this study was described earlier in a preliminary report [18]. Successful 2,3,6-TBA dehalogenating enrichments could be obtained repeatedly from 3 different sources. For further growth of these enrichments the following components were added to 1 l of LMM-medium: yeast extract (1 g), peptone (1 g), resazurin (0.1 mg), sodium benzoate (2 mmol) and a volatile fatty acid mixture (10 mM). The latter mixture contained acetate (120 mM), propionate (40 mM), butyrate (20 mM), 2-methylbutyrate (5 mM), isobutyrate (5 mM), valerate (5 mM) and isovalerate (5 mM). After autoclaving, vitamins (1 ml) [20], 1,4-naphtoquinone (0.2 mg) and 2,3,6-TBA (0.68 mmol) were added from filter-sterilized stock solutions. The medium was prepared under an N₂-atmosphere and reduced with Na₂S (35 mg). Anaerobic batch cultures were incubated statically at 30°C in the dark, and transferred (5-10% inocula) every 3-5 months in freshly prepared media.
A reactor was set up to grow mixed cultures of anaerobic and aerobic bacteria (Fig. 1). A chemostat similar to that described before was used [17]. It was equipped with probes allowing continuous monitoring and regulation of temperature, dissolved oxygen concentration, redox potential and pH of the culture liquid. The pH was maintained by automatic titration with H₃PO₄. A Servomex 1100 oxygen analyzer was used in combination with a gas flow meter to quantify the oxygen consumption by the bacteria in the reactor. The total volume of the liquid phase of the reactor was 900 ml. Particles of vermiculite, a clay mineral abundant in soils [21], enclosed in a porous nylon bag (pore size 1 mm) were present as a solid phase in the reactor liquid. The bag contained 100 g vermiculite and occupied a volume of 570 ml. The reactor-liquid was stirred by means of a magnetic stirrer (1000 rpm) immediately below the nylon bag. Oxygen was supplied by mixing air with N₂-gas supplied via a needle positioned just below the vermiculite bag. The reactor was operated at 30°C.

3.2. Analytical procedures

Chloride was measured colorimetrically according to the method of Bergman and Sanic [22] with NaCl as a standard. Methane concentrations in the head-space gas were measured gas chromatographically [17]. Chlorinated benzoates were analyzed, after methylation and extraction in CHCl₃, through capillary gas chromatography (Packard, GC model 438 A) using a flame ionization detector (275°C) and split injection (250°C, vent ratio 1:40). A Chrompack CP-sil-8-CB column (25 m × 0.25 mm) was used to separate methyl esters of chlorobenzoates with a linear temperature gradient (10°C per min from 70 up to 250°C). Standard solutions of commercially obtained chlorobenzoates were used for quantification. Using 4-chlorobenzoic acid as an internal standard, integration of the peak areas recorded with a Shimadzu C-3A integrator yielded a linear response over a concentration range from 10 μM to 10 mM. Coefficients of variation [standard deviation/mean × 100] of the concentrations ranged from 2 to 5%. Protein was measured according to Lowry et al. [23] with bovine serum albumin as the standard.

Substrate oxidation kinetics of P. aeruginosa JB2 were determined in a (Yellow Springs-type) biological oxygen monitor equipped with a polarographic oxygen electrode. Cells were washed and resuspended in an isotonic K(NH₄)PO₄ buffer with chloramphenicol (30 mg 1⁻¹) to block protein synthesis. Oxygen tracings were performed at 30°C, and kinetic parameters were obtained from direct linear plots.

For fluorescent microscopy a Carl Zeiss G42-110-e Axioscope was used. The microscope was equipped with an Osram high-pressure mercury lamp, and a Zeiss BP 400-44, FT 460, LP 470 filter-combination, and allowed detection of fluorescence of methanogenic bacteria.

3.3. Chemicals

All chemicals were of analytical grade, except 2,3,6-trichlorobenzoic acid (Pfaltz and Bauer, Flushing, NY), which was demonstrated (by capillary GC) to contain 71.5% 2,3,6-TBA, 4.6% 2,3,5-trichlorobenzoic acid, 3.5% 2,4,6-trichlorobenzoic acid, 0.2% 2,4-dichlorobenzoic acid, 0.4% 2,6-dichlorobenzoic acid, 1.1% 2,5-dichlorobenzoic acid, 0.2% 2,3-dichlorobenzoic acid, and 18.5% unidentified compounds.

4. RESULTS

4.1. Degradation of chlorobenzoates by P. aeruginosa JB2

As reported by Hickey and Focht [19], P. aeruginosa JB2 was capable of growth with benzoate or 2,5-DBA as the sole energy and carbon sources in mineral medium. No growth occurred in a batch culture supplied with 2,3,6-TBA (2.5 mM) within 100 days of incubation. In batch cultures containing a mixture of benzoate (2.5 mM) and 2,3,6-TBA (2.5 mM) within 100 days of incubation. In batch cultures containing a mixture of benzoate (2.5 mM) and 2,3,6-TBA (2.5 mM) growth occurred on benzoate while no 2,3,6-TBA was metabolized. Thus P. aeruginosa JB2 is not able to co-metabolize 2,3,6-TBA with benzoate as a primary substrate.

Oxygen consumption kinetics of P. aeruginosa JB2 were analyzed with cells grown on 2,5-DBA.
in a statically incubated (O₂-limited) and a well aerated (O₂-saturated) batch culture. With 2 mM 2,5-DBA as a substrate an apparent \( K_m \) for oxygen of 25 \( \mu \)M was obtained with cells grown under both conditions. The maximum specific oxygen consumption rate (\( Q_{\text{O}_2}^{\text{max}} \)) of the cells grown O₂-saturated was 7.2 \( \mu \)mol mg protein \(^{-1} \) h \(^{-1} \). The \( Q_{\text{O}_2}^{\text{max}} \) of the cells grown O₂-limited was somewhat higher, 12.0 \( \mu \)mol mg protein \(^{-1} \) h \(^{-1} \).

4.2. Reductive dechlorination of 2,3,6-TBA in an anaerobic reactor

An anaerobic reactor was set up to establish a population of anaerobic bacteria capable of reductive dechlorination of 2,3,6-TBA (Fig. 1). Preliminary results of enrichments from freshwater sediments, reductively dechlorinating 2,3,6-TBA and producing 2,5-DBA, have been published elsewhere [18]. Because initial experiments indicated a very low growth rate for such cultures (doubling time 25–50 days) a nylon bag filled with particles of the clay-mineral vermiculite was placed inside the reactor to prevent wash-out of the dechlorinating bacteria. The reactor was inoculated with an actively dechlorinating batch culture (150 ml) grown in the presence of vermiculite. Reductive dechlorination continued without a lag and the retention time of liquid phase of the reactor was set at 830 h. When 2,3,6-TBA was degraded to a concentration < 0.01 mM, the retention time of the culture liquid was decreased stepwise to 200 h, 100 h, and 50 h over a period of 30 days. Under these conditions the 2,3,6-TBA concentration remained below 0.2 mM. A further decrease in the retention time to 40 h resulted in accumulation of the substrate, 2,3,6-TBA, in the reactor liquid, and a concomitant decreasing concentration of the dechlorination products, 2,5-DBA and chloride.

An analysis of substrates consumed and products formed was made after the reactor was operated anaerobically for two months at a retention time of 50 h (Table 1). Under these growth conditions the redox potential measured in the liquid phase of the reactor remained between −200 and −215 mV. About 35% of the total carbon supplied to the reactor in the form of benzoate, yeast extract and peptone was recovered as methane. With fluorescent microscopy no fluorescent bacteria were detected in samples of 1–10 \( \mu \)l taken from the liquid phase, indicating the presence of a very low number of methanogens, probably < 10\(^3\)–10\(^5\) per ml. A thick biofilm on the vermiculite, and a relatively low protein concentration in the culture liquid (< 45 mg l \(^{-1} \)) indicated that most of the bacteria in the reactor were present on the vermiculite particles. Ben-
zoate was metabolized to a concentration < 1 \( \mu \)M. The residual concentration of 2,3,6-TBA in the reactor liquid (0.045 mM) showed that > 90% of the 2,3,6-TBA entering the reactor (0.68 mM) was consumed. In the reactor liquid 0.99 mol of chloride and 0.93 mol of 2,5-DBA were recovered per mol 2,3,6-TBA consumed. In addition to 2,5-DBA, minor concentrations (< 0.03 mM) of some other dichlorobenzoates (2,4-DBA and 3,5-DBA) were produced.

A control experiment was performed to investigate whether 2,3,6-TBA was dechlorinated in vermiculite containing non-sterile media not inoculated with the dechlorinating enrichment culture, but containing a sufficient number of non-dehalogenating bacteria to ensure reducing conditions over long periods of time. No reduction of the concentration of 2,3,6-TBA was observed within 200 days indicating that the reductive dechlorination was not an aspecific reaction catalized by vermiculite or many bacteria not selected for the ability to dechlorinate.

4.3. Mineralization of 2,3,6-TBA by the combined activity of the anaerobic reductively dechlorinating enrichment and P. aeruginosa JB2

When a sample of the anaerobic reactor liquid (100 ml) was incubated aerobically, no further degradation of 2,5-DBA occurred, which indicates that 2,5-DBA degrading aerobic bacteria were not present in the dechlorinating enrichment culture. However, when such a sample was inoculated with P. aeruginosa JB2, 2,5-DBA was metabolized immediately (Fig. 2). Chlorine release correlated well with 2,5-DBA consumption.

In an experiment designed to investigate whether reductive dechlorination of 2,3,6-TBA and oxidative degradation of 2,5-DBA could occur simultaneously, P. aeruginosa JB2, grown on 2,5-DBA in an O2-limited batch culture, was concentrated by centrifugation and inoculated into the dechlorinating reactor. This procedure resulted in an initial density of approximately 70 mg P. aeruginosa JB2 protein per liter reactor liquid. It was observed that individual cells of P. aeruginosa JB2 could be detected in the reactor microscopically, using the same filter combination to detect fluorescence of methanogenic bacteria (see MATERIALS AND METHODS). Immediately after inoculation the flow of N2-gas was replaced by air, which was bubbled through the reactor liquid at a

<table>
<thead>
<tr>
<th>Reactor conditions</th>
<th>( \text{O}_2 ) reading ((\mu \text{M}))</th>
<th>Redox reading ( (\text{mV}) )</th>
<th>Consumption ((\mu \text{mol h}^{-1}))</th>
<th>Production ((\mu \text{mol h}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>( \text{O}_2 )</td>
<td>( \text{BA} )</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>0</td>
<td>-214</td>
<td>0</td>
<td>38.8</td>
</tr>
<tr>
<td>Microaerobic</td>
<td>0.4</td>
<td>+299</td>
<td>3840</td>
<td>38.8</td>
</tr>
</tbody>
</table>

The reactor was operated under anaerobic and microaerobic conditions at a retention time of 50 h. Substrates in the reservoir medium: 2,3,6-TBA (0.68 mM), and BA (1.94 mM).
2.0

2.0

1.5

1.0

0.5

0.0

-20 -10 0 10 20 30 40 50 60

Time (days)

2.3,6-TBA
2.5-DBA
Cl-

0

1

2

3

4

5

Anoxic

Microoxic

Anoxic

O_2-consumption (mmol.h⁻¹)

Cl⁻-concentrations in an aerated reactor containing a mixed culture of anaerobic 2,3,6-TBA dechlorinating bacteria and the aerobic 2,5-DBA degrading P. aeruginosa JB2. The reactor was operated at a liquid retention time of 50 h. The reservoir medium contained 0.68 mM 2,3,6-TBA. The actual oxygen concentration in the liquid phase remained below 1 µM. □. 2,3,6-TBA; △, 2,5-DBA; ○. Cl⁻; and ---, rate of oxygen consumption.

Further increase of the air-flow resulted in concomitant increased O_2-consumption (Fig. 3; day 15) and a further increase of the redox reading from +134 to +299 mV. Although under these conditions the O_2-concentration in the culture liquid still remained < 1 µM, CH₄ could no longer be detected in the head-space gas. After 7 volume changes the concentration of 2,5-DBA in the reactor liquid had decreased to < 1 µM and that of 2,3,6-TBA to 17 µM (Fig. 3; day 34). Chlorine production (2.85 mol per mol 2,3,6-TBA consumed) proved that under these conditions 95% of 2,3,6-TBA had been dechlorinated completely (Table 1).

When, at day 34, air was replaced by N₂, anaerobic conditions were restored and the redox potential dropped to -210 mV within several hours. Gradually, 2,5-DBA and Cl⁻ returned to their initial anaerobic concentrations (Fig. 3).

5. DISCUSSION

The degradation of 2,3,6-TBA by the combined activity of anaerobic and aerobic bacteria was studied. To the best of our knowledge this is the first report in which the complete mineralization of this persistent herbicide is demonstrated.

Chlorinated benzoic acids have often been used as model compounds in biodegradation studies of halogenated aromatics. Although information of their occurrence in nature is scarce, it is certain they have been released into the environment due to extensive use as herbicides [24]. Furthermore, chlorobenzoates are known to be produced during the aerobic metabolism of PCBs and alkyl benzenes [1,3]. 2,3,6-Trichlorobenzoic acid itself has been used in large doses to control weeds, often as a component of mixtures with other herbicides. Although slow (partial) decomposition of 2,3,6-TBA in soil may occur [25], this compound was repeatedly found to be persistent and effective for many months (up to five years) after application [24,26,27].

The persistence of poly-chlorinated benzoic acids under aerobic conditions has been noticed by various investigators [26-28]. Especially chlorobenzoates containing two ortho-substituted chlorines, like 2,3,6-TBA or 2,6-dichlorobenzoic acid (2,6-DBA), resist aerobic degradation. It has
been suggested that an unsubstituted carbon on the aromatic ring next to the carboxyl group is important for the oxidation of chlorobenzoates by dioxygenases [19,29,30]. The only aerobic bacterium known to metabolize 2,3,6-TBA is a *Brevibacterium* sp. [31]. When grown on benzoate this organism co-oxidized 2,3,6-TBA. The product formed, 3,5-dichlorocatechol, was toxic for this bacterium and was not metabolized further.

P. *aeruginosa* JB2 can metabolize a wide range of halogenated benzoic acids [19]. The following chlorinated benzoic acids are used as the sole sources for carbon and energy by this bacterium: 2,3,5-TBA, 2,3- and 2,5-DBA, and 2- and 3-chlorobenzoate. In addition, when grown on benzoate *P. aeruginosa* JB2 co-metabolizes (chlorine release) 2,4-, 2,6-, 3,4-, and 3,5-dichlorobenzoate. In the present study it was substantiated that *P. aeruginosa* JB2 did neither grow on, nor co-metabolize 2,3,6-TBA, thus underlining the resistance of this compound to oxidative degradation.

Under anaerobic conditions many chlorinated benzoic acids can be dechlorinated via reductive mechanisms [5,7,32,33]. Anaerobic enrichments transforming 2,3,6-TBA into 2,6-DBA, by substitution of the *meta*-chlorine for a proton were described by Horowitz et al. [32]. No decrease of the concentration of 2,6-DBA was observed after a year of further anaerobic incubation of these enrichments. Recently, we obtained anaerobic enrichment cultures that reductively dechlorinate 2,3,6-TBA, and produce 2,5-DBA [18]. Although, 2,3,6-TBA degradation in these batch cultures was very slow, this enrichment was successfully grown in a reactor by using vermiculite for the attachment of the reductively dechlorinating organisms.

The production of low concentrations of dichlorobenzoates other than 2,5-DBA in the reactor was probably caused by reductive dehalogenation of (trichlorobenzoates present as impurities in the 2,3,6-TBA we used. Removal of the *meta*-substituted chlorine from 2,3,6-TBA did not occur since 2,6-DBA was not produced. Dichlorobenzoates formed in the reactor (e.g., 2,5-DBA, 2,4-DBA and 3,5-DBA) were not degraded under anaerobic conditions. Apparently, bacteria like *Desulfomonile tiedjei* DCC1, able to dechlorinate several dichlorobenzoates [7], were not selected in the enrichment used in the present study.

Removal of an *ortho*-substituted chlorine from 2,3,6-TBA is crucial for further mineralization of this compound since aerobic chlorobenzoate catabolizers like *P. aeruginosa* JB2 are capable of growth on 2,5-DBA, but not on 2,3,6-TBA or 2,6-DBA [19,29,30]. Indeed, this bacterium was shown to metabolize 2,5-DBA aerobically, which was formed anaerobically by reductive dechlorination of 2,3,6-TBA in the enrichment culture. The degradation of other chlorinated compounds such as PCBs [1,8] and the pesticide Methoxychlor [1,1bis (p-methoxyphenyl)-2,2,2-trichloroethane] [34] can also be enhanced by a period of anaerobiosis preceding a period of aerobicosis. In addition, reductive dechlorination of tri- and tetrachloroethylenes was shown to depend on a transition from aerobic to anaerobic conditions [35]. These observations suggest that alternating anaerobic and aerobic conditions may improve degradation of polychlorinated compounds in general. In the environment such conditions frequently occur in soils [36] and sediments [37], and it is tempting to hypothesize that habitats dominated by fluctuating oxygen concentrations are best suited for the development of microbial communities capable of mineralizing such xenobiotics.

A complicating factor occurring when employing alternating anaerobic and aerobic conditions to degrade substantial amounts of chlorinated compounds is that (toxic) intermediates may accumulate during the anaerobic or aerobic phase. In addition, the anaerobic bacteria involved may not survive the period of aerobicosis. The fact that reductive dechlorination of 2,3,6-TBA and oxidative mineralization of the product 2,5-DBA occurred simultaneously in the aerated reactor and resulted in almost complete mineralization of 2,3,6-TBA shows that anaerobic and aerobic bacteria can successfully be combined at low oxygen tensions (Fig. 4).

Because the apparent *K*m for *O*₂ of *P. aeruginosa* JB2 with 2,5-DBA as the substrate was 25 μM, and the oxygen concentration in the reactor remained < 1 μM, the rate of 2,5-DBA oxidation...
Fig. 4. Proposed scheme of chlorobenzene degradation in the mixed culture reactor under aerated conditions. 2,3,6-TBA enters the nylon bag with vermiculite where reductive dechlorination takes place. The anaerobic dechlorination product 2,5-DBA is degraded further by P. aeruginosa JB2 once it has reached the microaerobic liquid phase.

by P. aeruginosa JB2 must have been limited by O2. However, due to the availability of additional organic substrates such as benzoate, yeast extract, peptone and fermentation products of the anaerobic population P. aeruginosa could probably maintain a sufficiently high cell-density to oxidize all 2,5-DBA in spite of the low specific oxidation rate as a result of the low oxygen concentration. Half-saturation constants for O2 of aerobic (halo)aromatic catabolizing bacteria are usually relatively high [6,38]. For example, for dioxygenases, which are essential enzymes in the metabolism of these bacteria, Km values for O2 ranging from 9 μM to 33 000 μM have been reported. This would imply that relatively high oxygen concentrations are necessary for an efficient degradation of chlorinated aromatics by aerobic bacteria. This obviously complicates growth of co-cultures composed of such organisms and anaerobes.

Aeration of the reactor reduced the activity of the strictly anaerobic methanogenic bacteria present in the mixed culture. However, reductive dechlorination was not significantly inhibited under these conditions. It is known that strict anaerobes can be protected from O2 by the respiratory activity of (facultative) aerobes maintaining the dissolved O2-concentration at a very low level (< 0.5 μM) [17]. Oxygen, present at such low concentrations, surely does not always block reductive dechlorination [39–41]. Moreover, in this particular reactor, O2 concentrations (and redox potential) in the nylon bag with vermiculite were probably much lower than in the liquid phase, thus ensuring suitable growth conditions for strict anaerobes.

The experiments described in this paper demonstrate that mixed cultures of strictly anaerobic and aerobic bacteria may be applied successfully to the degradation of poly-halogenated aromatic compounds. Use of biological purification filters with habitats for both anaerobic and aerobic bacteria could have several advantages over systems which are only (or sequentially) aerobic or anaerobic for the following reasons: (1) both aerobic and anaerobic species with different catabolic capabilities can be active in close cooperation. This may reduce the problem of accumulation of (toxic) intermediates or dead-end products; (2) inactivation or death of aerobes or anaerobes due to the periodic absence or presence of O2, respectively, is prevented; (3) only one filter-system is needed, which can be operated continuously under O2-limitation.

ACKNOWLEDGEMENTS

This work was supported by the Netherlands Integrated Soil Research Programme. The authors thank Dr. D.D. Focht, and Dr. F.K. Higson for their kind gift of Pseudomonas aeruginosa JB2.
REFERENCES


