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Anaerobic dechlorination of pentachlorophenol in fixed-film and upflow anaerobic sludge blanket reactors using different inocula

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Abstract

Longterm performance and stability of two upflow anaerobic sludge blanket (UASB) reactors inoculated with granular sludge and treating a synthetic waste water containing pentachlorophenol (PCP) and phenol were studied. A similar system consisting of two fixed-film reactors inoculated with anaerobic digested sewage sludge were further studied. One reactor in each series received glucose in addition to the phenols. Dechlorination of PCP proceeded via two different dominating pathways in the respective reactor systems, suggesting that two distinct microbial populations were present, probably originating from the different inocula. Dechlorinating activity was maintained for more than 18 months in the UASB reactors and was generally higher than in the fixed-film reactors. In the fixed-film reactors, dechlorination of PCP suddenly decreased after 15.5 months of operation compared to earlier performance. Since no operational parameters had been changed, this indicated that the enriched culture was unstable on a longterm basis. Addition of yeast extract to the medium restored activity. General process stability in both reactor systems was clearly enhanced by the addition of glucose and was superior in the UASB/granular sludge system. The better performance and the higher stability in the UASB/granular sludge reactor highlights the importance of thorough screening of inocula prior to start-up of processes treating waste waters containing xenobiotic compounds.

Abbreviations: PCP – pentachlorophenol, TeCP – tetrachlorophenol, TCP – trichlorophenol, DCP – dichlorophenol, UASB – upflow anaerobic sludge blanket, HRT – hydraulic retention time

Introduction

Biodegradation of chlorinated aromatic compounds has been observed in various anaerobic environments, including sewage sludge (Suflita et al. 1982; Mikesell & Boyd 1985; Gibson & Suflita 1986; Larsen et al. 1991), freshwater sediments (Suflita et al. 1982; Gibson & Suflita 1986; Genthner et al. 1989; Zhang & Wiegel 1990; Larsen et al. 1991), aquifer material (Suflita & Miller 1985; Gibson & Suflita 1986), anaerobic soil (Ide et al. 1972) and marine sediments (Abrahamsson & Klick 1991). Studies on the dechlorination of PCP have demonstrated that the relative persistence as well as the specificity of dechlorination of this compound vary significantly between different environments. In some reports, PCP is totally resistant to microbial degradation (Battersby & Wilson 1989; Abrahams-

son & Klick 1991), while other studies have demonstrated extensive transformation (Abrahamsson & Klick 1991; Bryant et al. 1991; Madsen & Aamand 1991; Larsen et al. 1991) or complete mineralization (Mikesell & Boyd 1986). The distinctive dechlorination pathways of PCP found in the various studies indicate that removal of aryl chlorines is highly regiospecific.

Reductive dechlorination of chlorinated aliphatic compounds by methanogenic bacteria has been shown to represent a cometabolic process catalyzed by reduced cofactors typically present in these organisms, eg. corrinoids, coenzyme F_{430}, vitamine B_{12} (Fathepure & Boyd 1988; Krone et al. 1989; Gantzer & Wackett 1991). In contrast to this mechanism, dechlorination of 3-chlorobenzoate by *Desulfomonile tiedjei* was shown to be an energy-yielding process in which the chlorinated compound served as a final electron acceptor (Dolfing 1990; Mohn & Tiedje 1991). Usable electron donors for the process were hydrogen, formate, acetate, or pyruvate (DeWeerd et al. 1990; Mohn & Tiedje 1991; Dolfing & Tiedje 1991). This novel type of anaerobic respiration demonstrates the existence of bacteria which conserve energy for growth from reductive dechlorination. A similar mechanism was suggested for the dechlorination of PCE coupled to the degradation of benzoate (Scholz-Muramatsu et al. 1990).

Studies on biodegradation of chlorinated phenols in reactor systems are relatively scarce. Experiments have been made in continuous tank reactors (Guthrie et al. 1984), biofilm reactors (Hakulinen & Salkinoja-Salonen 1982), and UASB reactors (Woods et al. 1989; Krumme & Boyd 1988), all showing some dechlorinating activity. However, only in one study total mineralization was observed (Krumme & Boyd 1988).

We have previously reported on the initial performance of two lab-scale UASB reactors inoculated with granular sludge and treating a synthetic waste water containing PCP and phenol (Hendriksen et al. 1992). When glucose was supplied as an additional carbon source PCP removal reached 99%, whereas removal in the reactor without glucose typically ranged from 30 to 75%. Assuming that all chlorines were removed prior to ring cleavage, 94% of the PCP was fully dechlorinated in the glucose-amended reactor compared to 10 to 20% in the control. Experiments using fixed-film reactors inoculated with anaerobic digested sewage sludge also showed a potential for dechlorination of PCP (Hendriksen et al. 1991). PCP removal during steady-state, after 6 months of operation, in the glucose supplemented fixed-film reactor was 98%, compared to 60% in the control reactor. Estimates of the amount of PCP dechlorinated further than TCP during the same period were 70% and 25% for the glucose-amended and the control reactor, respectively.

In the present study we further investigated the longterm (1.5 to 2 years) performance and stability of the two reactor systems: The UASB/granular sludge reactors and the fixed-film/digested sludge reactors. The dechlorination pathway in the fixed-film reactors was determined and the maximum dechlorinating activity was localized in the reactor. The use of different inocula in the reactors prooved to be highly influential on the general performance and stability of the different reactor systems.

Materials and methods

Reactor design

UASB reactors

Experiments were made in two glass UASB reactors with a volume of approximately 2l. The inoculum used was a granular sludge previously grown on sugar-containing waste water. The hydraulic retention time (HRT) was initially 3 days but was eventually (day 75) reduced to 2 days. Part of the effluent was recirculated at a ratio of 4. This ratio was, however, increased at day 100 to 16 in an attempt to increase wash-out of fluffy sludge and free-living cells.

Fixed-film reactors

The fixed-film reactors had an empty reactor volume of 400ml and an active volume of 250ml. The reactors were made of glass and the inert carrier material was ceramic Raschig rings. The inoculum used was anaerobic digested sewage sludge ob-

tained from a municipal treatment plant receiving waste waters from both industrial and residential areas. The hydraulic retention time was initially 5 days but was later reduced stepwise to 4 days (day 41), 3 days (day 96) and finally to approximately 2 days (day 345). The recirculation ratio was 4.

Medium

All reactors were fed mineral medium (BA-medium without cysteine); (Angelidaki et al. 1990) with phenol and PCP added from ethanolic stock solutions. Concentrations of PCP and phenol in the medium varied between 2 to 4.5 and 10 to 22.5 mg/l, respectively, dependent upon reactor type and performance. The maximum concentration of ethanol added with the phenols was 3.5 mM for the fixed-film reactors and 1.1 mM for the UASB reactors. One of each of the reactors received glucose (0.9 g/l) as a supplemental carbon source, whereas the other served as an unsupplemented control. After 15.5 months of operation, the performance of the fixed-film reactors deteriorated drastically and addition of yeast extract (0.1 g/l) was initiated in an attempt to improve the dechlorination.

Samples from both reactor systems were taken once a week and analyzed for volatile fatty acids, chlorophenols, and phenol. Methane in the headspace was also measured regularly. A detailed description of the experimental set-up and start-up procedures of the four reactors has previously been reported (Hendriksen et al. 1991; Hendriksen et al. 1992). Experiments were run at 37° C.

Batch experiments

To evaluate the degradation pathway in the fixed-film reactors and in an attempt to localize the activity in the reactor, batch experiments using material from different zones in the glucose-supplemented reactor were made. The reactor was divided into three parts and the liquid and the Raschig rings from each zone were transferred separately to anaerobic serum vials. The Raschig rings were gently washed with the liquid from the same section to remove excess sludge that adhered to the ceramic rings but was not immobilized. 80 ml of reduced medium was poured into the 250 ml vials holding the Raschig rings. The liquid portions from the reactor (60 to 80 ml) were placed in 100 ml serum vials. The sludge and liquid from the recirculation reservoir (ca. 80 ml) were also checked for activity. PCP (2–3 mg/l), glucose (0.9 g/l), and yeast extract (0.1 mg/l) were added to all vials which were incubated at 37° C with gentle shaking. Samples were taken regularly and PCP transformation was followed. Samples from the vials with the Raschig rings, were centrifuged prior to acetylation and extraction. In vials containing the liquid fraction (sludge slurry) samples were not centrifuged allowing extraction of chlorophenols bound to the sludge.

Analytical methods

Chlorophenols were analyzed after acetylation on a HP-5890 series II gas chromatograph equipped with a megabore DB-5 capillary column (J&W Scientific) or a megabore HP-5 column (Hewlett Packard) and an electron capture detector (ECD). The acetylation procedure and the operation of the gas chromatograph were as previously reported (Hendriksen et al. 1992; Larsen et al. 1991). Phenol was analyzed using HPLC as previously described (Hendriksen et al. 1992). Volatile fatty acids and methane were determined by gas chromatography (Angelidaki et al. 1990).

Results and discussion

Fixed-film reactors

Start-up and initial operation of the two fixed-film reactors were presented in an earlier paper (Hendriksen et al. 1991). After 9 months of stable operation (98% PCP removal in the glucose reactor, 60% in the control), the medium concentration of PCP was increased from 2 to 3 mg/l and of phenol from 10 to 15 mg/l. Figure 1 illustrates the response of the fixed-film reactors towards this sudden in-

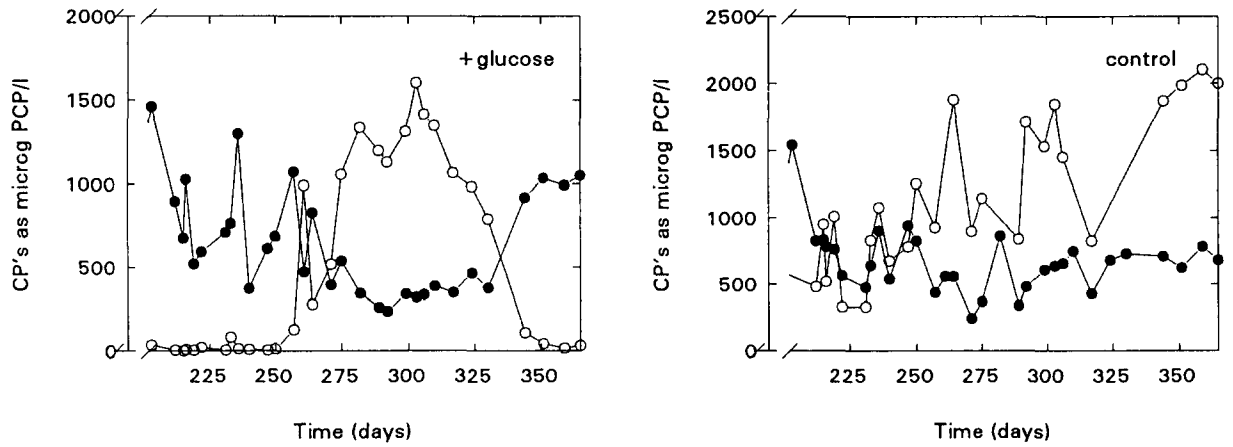

Fig. 1. Performance of the fixed-film reactors after an increase in the medium PCP concentration from 2 to approximately 3 mg/l, day 261. Symbols: ○ PCP, ● Sum of lesser chlorinated phenols calculated as µg PCP/l.

crease in the influent concentration of PCP. Transformation in the control reactor decreased to 21% and activity never really recovered from this perturbation. In the glucose reactor transformation was maintained at 98% after an acclimation period lasting 2 to 3 months. At this point (day 345), the HRT was reduced from 3 to 2 days without any significant change in performance in either of the reactors. Based on the total amount of chlorophenols in the effluent from the reactors, the estimated amount of PCP fully dechlorinated was 1% and 20 to 40% in the control and the glucose-amended reactor, respectively. Stable performance at this reduced efficiency continued for approximately 4 months.

After approximately 15.5 months of continuous operation (day 460), the dechlorination of PCP decreased drastically in the fixed-film reactors, especially in the glucose-amended reactor, accompanied by an increase in volatile fatty acids in the effluent from this reactor. Figure 2 shows the effluent concentrations of chlorinated phenols during this period of operation in both reactors.

No operational parameters were changed that

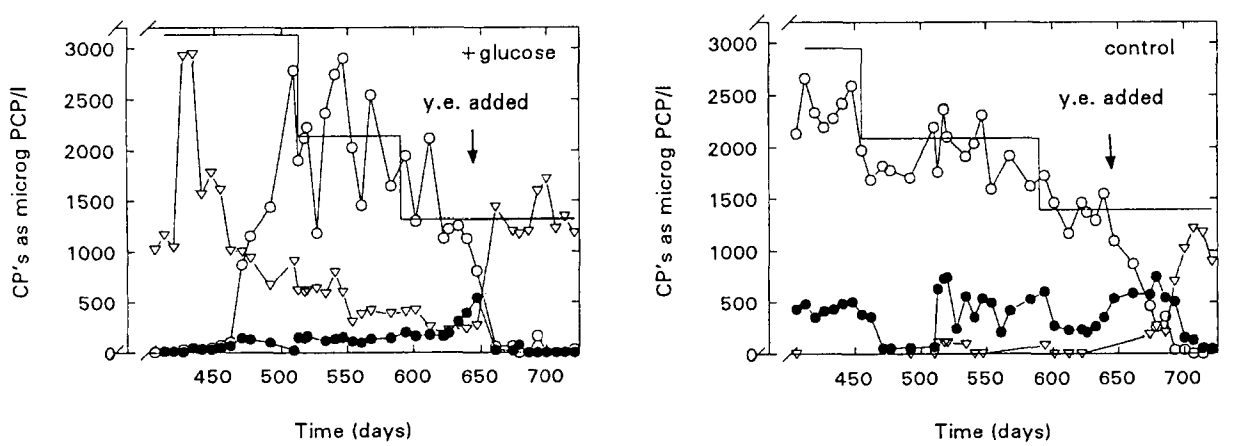

Fig. 2. Effluent concentrations of chlorinated phenols in the fixed-film reactors from day 400 to day 720. Symbols: — PCP concentration in the medium: Glucose reactor: day 408–512: 3.1 +/−0.5 mg/l, day 512–590: 2.1 +/−0.7 mg/l, day 590–720: 1.3 +/−0.2 mg/l. Control reactor: day 408–456: 3.0 +/− 0.3 mg/l, day 456–590: 2.1 +/−0.4 mg/l, day 590–720: 1.4 +/−0.4 mg/l. ◇ PCP, ● 2,3,4,5-TeCP, ▽ 3,4,5-TCP. Addition of yeast extract was initiated at day 644.

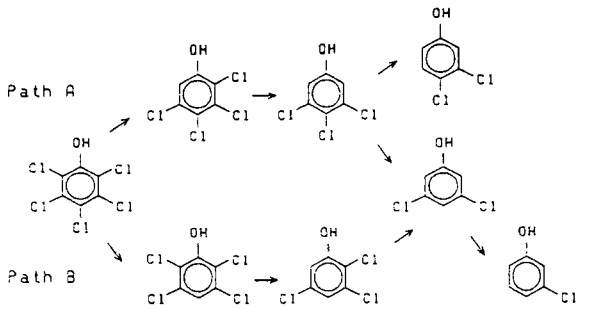

Fig. 3. Dechlorination pathways of PCP.

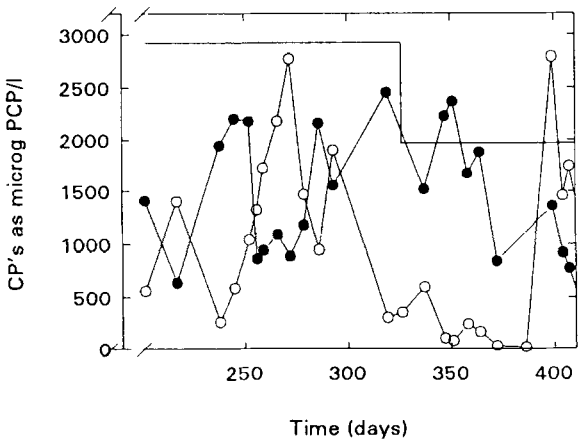

Fig. 4. Effluent concentrations of chlorinated phenols in the UASB control reactor from day 200 to day 410. Symbols: — PCP concentration in the medium, day 200–326: 2.9 +/–0.6 mg/l, day 326–410: 2.0 +/–1 mg/l. ◇ PCP, ● Sum of lesser chlorinated phenols calculated as µg PCP/l.

could explain this incident. The influent concentration of PCP was lowered from 3 to 2, and again to 1.5 mg/l in an attempt to increase transformation, but without any effect. Transformation was below 10% in both reactors. Consequently, yeast extract was added to the medium (day 644) and activity resumed immediately in the glucose-amended reactor (Fig. 2). A similar effect was observed in the control reactor but a longer period (1.5 months) was needed before stable operation was reached (Fig. 2). PCP removal in both reactors reached 98%. Addition of yeast extract thus increased PCP dechlorination in the control reactor to a level similar to the glucose-amended reactor, showing that yeast extract could replace glucose as a source of additional carbon and/or electrons.

Results from the batch experiments using material from the glucose-supplemented fixed-film reactor showed that initial dechlorination in the ortho position was favoured. This indicated a dechlorination pathway as illustrated in Fig. 3, path A, and fitted well with data from the routine operation of the reactors. The major product was 3,4,5-TCP and no dichlorophenols or monochlorophenols were detected. A gradual loss of 3,4,5-TCP in the vials indicated that dechlorination did occur but products were not observed, probably due to immediate transformation. In the effluent from the reactor 3,4-DCP could occasionally be detected, demonstrating some dechlorination of 3,4,5-TCP. Extraction and analysis of samples containing sludge revealed that especially 3,4,5-TCP had adsorbed to the biomass during the continuous operation of the reactor, suggesting that dechlorination of 3,4,5-TCP was rate limiting. PCP and 2,3,4,5-TeCP could also be extracted but in significantly smaller amounts than 3,4,5-TCP. The products of dechlorination detected in the control reactor indicated that a similar dechlorination pathway was functioning here.

UASB reactors

The stable performance and high removal of PCP obtained in the glucose-supplemented UASB reactor during initial operation (10 months; (Hendriksen et al. 1992)) continued for the entire experimental period (18 months). In contrast to this, the performance of the control reactor fluctuated as illustrated in Fig. 4. For a period of 1.5 months, PCP removal reached 95%, compared to 30 to 75% during initial operation, while total dechlorination could be estimated to 20%. Instability in the medium flow (day 390) did, however, cause an immediate rise in the effluent concentration of PCP and a general deterioration of the dechlorination process.

Figure 5 shows the performance of the two UASB reactors following a three-day stoppage of the medium flow. Resuming the medium flow (at day 390 in the control reactor and at day 410 in the glucose reactor) resulted in an immediate increase

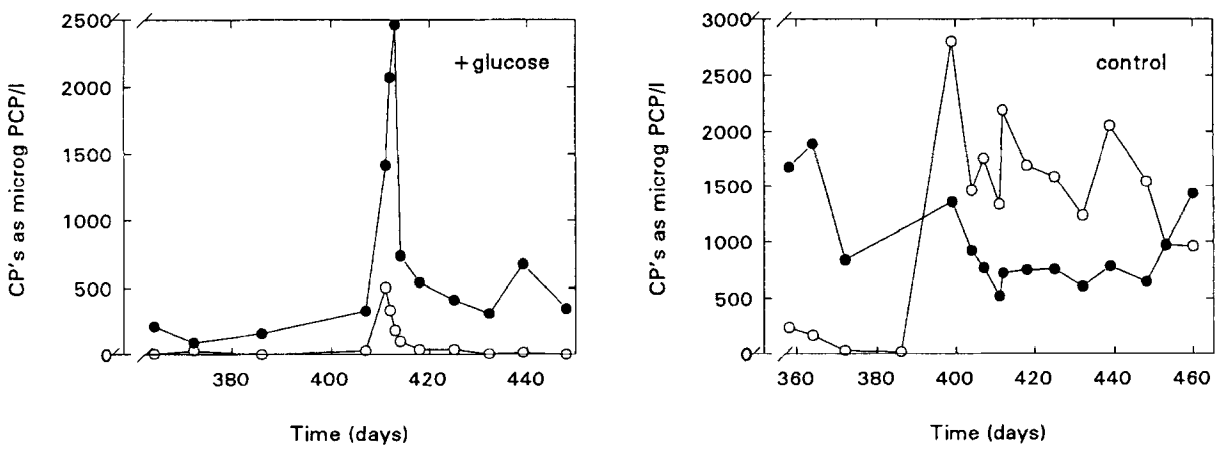

Fig. 5. Performance of the UASB reactors after a stoppage in the medium flow from day 407 to 410 in the glucose reactor and from day 386 to 390 in the control reactor. Symbols: ○ PCP, ● Sum of lesser chlorinated phenols calculated as μg PCP/l.

in effluent concentrations of chlorophenols. The glucose-supplemented reactor recovered from this perturbation in approximately one week and performance returned to normal. In contrast the control reactor took several months before a decrease in the concentration of PCP in the effluent could clearly be established (day 448). Parallely, the concentration of lesser chlorinated phenols increased.

Effect of extra carbon source

Addition of glucose clearly enhanced dechlorination in both reactor systems. For the UASB reactors this effect was previously shown to be caused partly by an increased amount of biomass in the glucose-amended reactor and partly by stimulation by glucose on the dechlorinating organisms (Hendriksen et al. 1992). Also the stability of both reactor systems towards any sudden change in operation was greatly enhanced by the addition of glucose, resulting in lower response times before performance returned to normal (Fig. 1, Fig. 5). This effect probably reflected the much higher concentration of biomass present in the glucose-amended reactors.

The sudden need for an extra complex carbon source in the fixed-film reactors could indicate that some essential growth factors had been exhausted from the system. Since glucose was still added as a possible electron donor in one reactor, the energy supply should not be the limiting factor. In the control reactor carbon supply was significantly lower but the amount of added phenol was still theoretically sufficient to account for the electrons needed for the full dechlorination of PCP. Other studies of dechlorinating cultures have shown a gradual loss of activity in original or primary enrichments (Genthner et al. 1989; Bryant et al. 1991), also demonstrating the existence of an essential compound that is eventually depleted. In addition, many highly enriched cultures using defined electron sources can only be grown in the presence of an extra complex carbon source (Dietrich & Winter 1990; Madson & Aamand 1991). In the reactorsystem used in the present study, however, any soluble component should have been rapidly washed-out. Alternatively, other microorganisms in the consortium could have produced essential cofactors or vitamins supplied to the dechlorinating organisms by cross feeding, suggesting that these organisms could live as scavengers. The observed drop in dechlorinating activity indicated a shift in the dominant microbial population affecting production of specific compounds or changing the interspecies competition for these compounds. The parrallel rise in volatile fatty acids (data not shown) probably reflects a toxic effect of the increased steady-state concentration of chlorophenols on either the methanogens or the acetogens. A

similar intermediate rise in VFA's was observed after the influent concentration of PCP was increased at day 261. Glucose degradation was never inhibited.

In a study by Allard et al. (1991) similar observations of longterm unstable enrichments were reported. In one enrichment culture the rate of dechlorination was substantially reduced during maintenance over several years. In another enrichment culture the specificity of dechlorination changed after some years of culturing, resulting in a novel dechlorination pathway. These results indicate that enrichment cultures containing several different microorganisms might not be metabolically stable for extended periods of time.

Dechlorination pathways

The dechlorination route observed in the fixed-film reactors, involving initial ortho cleavage, has been observed in a number of studies on PCP dechlorination using sewage sludge as inoculum (Madsen & Aamand 1991; Mikesell & Boyd 1986; Woods et al. 1989). However, in these experiments dechlorination typically proceeded further giving 3-CP (Madsen & Aamand 1991) or phenol (Mikesell & Boyd 1986) as the final product. Similar to the present study, Woods et al. (1989) observed only limited dechlorination of PCP and production of 3,4,5-TCP in an anaerobic reactor.

In the UASB reactors dechlorination of PCP proceeded via both path A (5 to 10%) and B (90 to 95%), Fig. 3, indicating that two populations, each using a different pathway, were probably present in these reactors (Hendriksen et al. 1992). The presence of several active dechlorinating populations was also demonstrated in a study of PCP degradation in a marine sediment (Abrahamsson & Klick 1991) where the degradation pathway changed during incubation. At first ortho cleavage was favoured but after a second addition of substrate a second, alternate pathway involving initial para cleavage developed. Furthermore, addition of a five-fold amount of PCP resulted in production of a trichlorophenol not previously observed. Dechlorination of hexachlorobenzene and polychlorinated biphenyls were similarly shown to proceed via two different pathways, which was explained as the presence of different microbial populations (Fathepure et al. 1988; Quensen et al. 1990). Dechlorination in our fixed-film reactors exclusively followed path A (Fig. 3), indicating that just one dechlorinating population was present in these reactors. Hence, the two different inocula used for the UASB and the fixed-film reactors could represent two distinct, dominant microbial populations or enrichment of distinct populations had occurred, based on the characteristic differences in the two reactor types. Alternatively, dechlorination via both pathways was catalyzed by a single organism showing different preferences in the respective reactors. The presence of two populations in the UASB reactors could perhaps explain the increased stability of this reactor system compared to the fixed-film reactors.

The estimated amount of PCP fully dechlorinated to phenol was much higher in the UASB/granular sludge system than in the fixed-film/digested sludge system. In the glucose-supplemented UASB reactor, dichlorophenols were the major product in the effluent, representing less than 10% of the influent PCP and indicating that 90–95% of the PCP was fully dechlorinated (Hendriksen et al. 1992). The binding of 3,4,5-TCP to the sludge in the fixed-film reactors made the estimation of the amount of PCP fully dechlorinated difficult. Furthermore, nothing was known about the reversibility and the capacity of this binding. Consequently, the actual amount fully dechlorinated was probably lower than first presumed, and the 20 to 40% found in the second period of stable performance could be a more realistic estimate. The production and accumulation of 3,4,5-TCP in the fixed-film reactors could explain the lower performance of these reactors compared to the UASB reactors, since this compound is rather toxic and very persistent compared to the other chlorophenols (Madsen & Aamand 1991; Ruckdeschel et al, 1987). Batch toxicity assays comparing the effect of 3,4,5-TCP and 2,4,6-TCP on acetate degradation showed that 3.3 mg/l 3,4,5-TCP caused 100% inhibition of methanogenesis, while 100 mg/l of 2,4,6-TCP could be applied before a similar response was seen (Woods 1985).

Immobilization

Visual examination of the Raschig rings from the fixed-film reactors showed no signs of immobilization or development of a biofilm. Some wall-growth was seen in the glass tubing associated with the glucose-supplemented reactor and in the recirculation reservoir of this reactor, but the major part of the biomass was located as a sludge slurry in the bottom of the reactor. A comparison of the activity in the batch experiment from the different parts of the reactor receiving glucose showed similar characteristics. The liquid fractions (sludge slurries) from the bottom and the middle part, had the highest absolute dechlorination rates, indicating that the major part of PCP was transformed here. Some dechlorination was observed in the vial with Raschig rings from the bottom and very little activity was found in the top liquid fraction and the Raschig rings from the top and middle parts. No dechlorinating activity was observed in samples from the recirculation reservoir. This distribution of activity indicated that plug flow was dominant in the fixed-film reactors, which was expected from the rather low flow rate and gas production rate.

Visual examination of samples of the granular sludge from the glucose-supplied UASB reactor showed that the granules originally present were able to maintain their structure. However, new biomass growth resulted in formation of a fluffy, dispersed sludge layer located in the upper part of the sludge bed. Some wall growth was also observed. The development of a more dispersed sludge would be expected from the very low COD loading rate applied in this reactor and any significant effect of the chlorophenols on the granules can therefore not be evaluated. In the control reactor the granules slowly disintegrated, probably due to carbon limitation.

The use of preformed granules in the UASB reactors was considered necessary since effective granulation would be difficult to obtain with the very low COD loading rate employed. Since no significant biofilm could be developed in the fixed-film reactors and consequently no effective immobilization, start-up of this reactor would probably have benefitted from the use of a preformed biofilm, as for the UASB reactors. Alternatively, the applied flow rate in the fixed-film reactors was too low to effectively select for organisms with the ability to initiate and form biofilms.

Comparing the two reactor systems employed in the present study, the use of granular sludge was clearly superior for transformation of PCP containing waste waters. Transformation of PCP was higher in the UASB/granular sludge reactor and the process was more stable when sudden changes in operation were introduced, as well as on a long-term basis. These differences can probably be explained by the distinct microbial populations dominating the two reactor systems and originating from the different inocula. An obvious difference between the inocula used was the physical structure. The large bacterial aggregates present in the granular sludge might protect sensitive microorganisms against PCP and, thereby, reduce toxicity. Also microniches characterized by especially favourably conditions for dechlorination could arise. The close physical contact between the different active organisms could increase the potential for dechlorination. This was true for the degradation of 3-chlorobenzoate in a dechlorinating consortium where separation of the dechlorinator, *Desulfomonile tiedjei,* and the benzoate degrader by a membrane resulted in reduced dechlorination rates (Tiedje & Stevens 1988). Finally, the diversity of the granular sludge could be greater than the dispersed sewage sludge since inactive species might lie dormant and immobilized in the granules, avoiding wash-out. The different recirculation ratios used in the two reactor systems could also have influenced the dechlorinating efficiency, since the PCP concentration in the bottom of the reactor would be somewhat higher in the fixed-film reactors than in the UASB reactors. The recirculation ratio in the UASB systems was, however, initially kept at 4, and changing this ratio to 16 had no effect on the dechlorinating performance of the reactors.

The observed differences in activity between the two reactor systems and the presence of different microbial populations emphasizes the importance of thorough screening of possible inocula prior to start-up of treatment processes involving xenobiotic compounds.

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