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Quinones as terminal electron acceptors for anaerobic microbial oxidation of phenolic compounds

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Abstract

The capacity of anaerobic granular sludge for oxidizing phenol and *p*-cresol under anaerobic conditions was studied. Phenol and *p*-cresol were completely converted to methane when bicarbonate was the only terminal electron acceptor available. When the humic model compound, anthraquinone-2,6-disulfonate, was included as an alternative electron acceptor in the cultures, the oxidation of the phenolic compounds was coupled to the reduction of the model humic compound to its corresponding hydroquinone, anthrahydroquinone-2,6-disulfonate. These results demonstrate for the first time that the anaerobic degradation of phenolic compounds can be coupled to the reduction of quinones as terminal electron acceptor.

Introduction

Phenols are common constituents of industrial aqueous effluents from processes such as polymeric resin production, oil refining and coking plants. Phenol is both toxic and lethal to fish at relatively low concentrations (e.g., 5–25 mg l^{-1}) and imparts objectionable tastes to drinking water at much lower concentrations (Hill & Robinson 1975). Due to their widespread use, phenolic compounds are common contaminants of water bodies, which receive untreated streams containing these compounds. It has been shown that phenol can be degraded by microorganisms participating in methanogenic consortia (Wang et al., 1986). Additionally, phenol can be degraded anaerobically by pure cultures using alternative electron acceptors such as sulfate (Bak & Widdel 1986), nitrate (Tschech & Fuchs 1987) and ferric iron (Lovley & Lonergan 1990).

In this study humus is considered as a terminal electron acceptor for phenolic compounds degradation. Humus is the stable organic matter accumulating in sediments and soils. It has been recently reported to play an active role in the anaerobic oxidation of various organic compounds, such as functioning as a terminal electron acceptor for the microbial oxidation of acetate (Lovley et al. 1996). A microbial humus-respiring consortium obtained from an organic rich streambed sediment was also shown to mineralize vinyl chloride and dichloroethene under anaerobic conditions (Bradley et al. 1998). The fact that humus was serving as the electron acceptor for the anaerobic oxidation of these contaminants was demonstrated by stimulating the oxidation through the addition of humic acids or the humic model compound, anthraquinone-2,6-disulfonate (AQDS).

Quinone moieties are the most likely candidates for the redox reactions observed in humus. This is supported by recent experiments, which correlated the humic substance quinone content with their electron accepting capacity (Scott et al. 1998). Therefore, quinone model compounds should be able to replace the function of humus as terminal electron acceptor. Most known humus-reducing microorganisms are capable of transferring electrons to AQDS, reducing it to anthrahydroquinone-2,6-disulfonate (AH_2QDS) (Lovley et al. 1996; Coates et al. 1998; Francis et al. 2000). Furthermore, isolation of AQDS-reducing mi-

croorganisms from a variety of sediments consistently resulted in the recovery of microorganisms that could also reduce humic acids (Coates et al. 1998).

The fact that there is a wide variety of organic compounds which can be utilized by a humus-respiring consortia (Field et al. 2000) leads to the question of whether humus or humic model compounds can also achieve the oxidation of phenolic compounds by acting as terminal electron acceptors. In this study, the capacity of two different anaerobic granular sludges for oxidizing phenolic compounds with AQDS as a terminal electron acceptor was explored.

Materials and methods

Inocula and basal medium

Methanogenic granular sludge from a full-scale upflow anaerobic sludge blanket (UASB) reactor treating effluent from an alcohol distillery of Nedalco (Bergen op Zoom, The Netherlands) and from a full-scale UASB reactor treating wet oxidized industrial effluent of Shell Nederland Chemie (Moerdijk, the Netherlands) were used for the present study. These consortia were chosen based on their capacity for both degrading phenolic compounds under methanogenic conditions and for reducing AQDS with readily biodegradable substrates such as hydrogen and acetate. Both granular sludge sources were washed and sieved to remove the fine particles before use in the batch tests. Both biomass sources were stored at 4 °C before use. The basal medium used in all batch experiments contained (g l^{-1}): $NaHCO_3$, (5); NH_4Cl, (0.03); K_2HPO_4, (0.02); $MgCl_2{\cdot}6H_2O$, (0.012); $CaCl_2{\cdot}2H_2O$, (0.005); Na_2S, (0.013); and 1 ml l^{-1} of both trace elements and vitamins solutions. The trace elements solution contained (mg l^{-1}): $FeCl_2{\cdot}4H_2O$, (2000); H_3BO_3, (50); $ZnCl_2$, (50); $CuCl_2{\cdot}2H_2O$, (38); $MnCl_2{\cdot}4H_2O$ (500); $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, (50); $AlCl_3{\cdot}6H_2O$, (90); $CoCl_2{\cdot}6H_2O$, (2000); $NiCl_2{\cdot}6H_2O$, (92); $Na_2SeO{\cdot}5H_2O$, (162); EDTA, (1000); and 1 ml l^{-1} of HCl (36%). The vitamins solution contained (mg l^{-1}): biotin, (20); *p*-aminobenzoate, (50); pantothenate, (50); folic acid dihydrate, (20); lipoic acid, (50); pyridoxine, (100); Nicotinamide, (50). Thiamine, riboflavin and cyanocobalamine were prepared separately in individual flasks and were added to the basal medium at the final concentrations of 100, 50 and 10 μg l^{-1}, respectively.

The basal medium was flushed with N_2/CO_2 (80/30) by passing this gas mixture through the liquid bulk for 10 minutes and was used without sterilization in the experiments.

Bioassays for the biodegradation of phenolic compounds under anaerobic conditions

The assays were conducted in batch mode by triplicate cultures in 117-ml glass serum bottles with a liquid volume of 50 ml (67 ml as headspace). Two sets of assays were run. In the first set, basal medium was transferred directly to the vials and then, inoculation took place by adding 1 g of volatile suspended solids (VSS) per liter in the cultures. The vials were sealed with butyl rubber stoppers and aluminum crimps and then flushed with N_2/CO_2 (80/30) for 10 minutes. Finally, either phenol or *p*-cresol was added as substrate at the final concentration of 300 mg of theoretic chemical oxygen demand (COD) per liter; namely, 1.35 mM for phenol and 1.1 mM for *p*-cresol. Another set was amended with AQDS (25 mM) and run under the same experimental conditions. Furthermore, two more sets of assays were conducted (one with and the other without AQDS) in the presence of the methanogenic inhibitor, 2-bromoethanesulphonic acid (BES) at a final concentration of 50 mM. Controls without phenolic compounds to correct for endogenous methane production and AQDS reduction were also conducted. Sterilized controls were also included to discard chemical transformations. The pH under these conditions was monitored and remained at 7.3 $\pm$ 0.1 in all the assays.

Analyses

Analysis of AH_2QDS was carried out on anaerobically collected samples in an anaerobic chamber under N_2/H_2 (96:4) atmosphere. The anaerobic chamber was a Type B Coy chamber (Coy Laboratory Products Inc.) made of pressed polished clear vinyl with a manual airlock installed. Samples (0.5 ml) were collected by using 1 ml disposable syringes and centrifuged (10,000 g, 5 min) under these conditions and then diluted in 1 cm disposable plastic cuvettes containing anaerobic bicarbonate buffer (60 mM, pH 6.7 $\pm$ 0.1). Concentrations of AH_2QDS were determined by monitoring absorbance at 450 nm and using an extinction coefficient of 2.25 absorbance units per mM obtained from a calibration curve of AQDS chemically reduced by dithionite.

Phenol and *p*-cresol were analyzed on previously centrifuged samples (10,000 g, 5 min) by gas chromatography using a Hewlett Packard 5890 gas chroma-

tograph equipped with 2 m × 6 mm × 2 mm glass column packed with Supelcoport (100–120 mesh) coated with 10% Fluorad FC 431. The temperatures of the column, the injector port and the flame ionization detector were 130, 200 and 280 °C, respectively. The carrier gas was nitrogen saturated with formic acid (40 ml/min). The retention times were 9.3 and 13.7 min for phenol and *p*-cresol, respectively. The sample injection volume was 10 μl.

Using a flame ionization gas chromatograph model 438/S (Packard-Becker, Delft, The Netherlands), methane production was determined. The gas chromatograph was equipped with a steel column (2 m × 2 mm) packed with Porapak Q (80/100 mesh, Millipore Corp., Bedford, MA). The temperatures of the column, injector port, and the flame ionization detector were 60, 200 and 220 °C, respectively. Nitrogen was used, as carrier, at a flow rate of 20 ml/min and the sample injection volume was 100 μl. Volatile fatty acids (VFA) were analyzed as previously described (Kortekaas et al. 1998).

The intermediates benzoate and *p*-hydroxybenzoate were analyzed with high performance liquid chromatography (HPLC) at the end of the experiments. Samples from the batch experiments were centrifuged (10,000 g, 5 min) and diluted in demineralized water, and 10-μl samples were injected with a Marathon autosampler (Separations, Hendrik Ido Ambacht, The Netherlands). These compounds were detected spectrophotometrically with a Spectroflow 783 UV detector (Kratos Analytical, Hendrik Ido Ambacht, The Netherlands) at their maximum absorbance (218 nm). Methanol with 2% demineralized water (A) and triethylamine (5 mM) in acetate buffer (10 mM) (B) were used as liquid phase and were pumped (Separations High Precision Pump Model 104, Separations, Hendrik Ido Ambacht, The Netherlands) at a flow rate of 500 μl min^{-1} first through a Separations GT-103 degaser (Hendrik Ido Ambacht, The Netherlands) and afterwards through a reverse-phase C18 column (Chromosphere C18, Chrompack, Bergen op Zoom, The Netherlands). The liquid phase composition was 15% of solution A and 85% of solution B; namely, the final composition was 4.25 mM of triethylamine and 3.7 mM of methanol in acetate buffer (8.5 mM). The retention times were 2.45 min and 4.38 min for p-hydroxybenzoate and benzoate, respectively.

The VSS content of the granular sludges was determined by subtracting the ash content from the dry weight after the sludge was incubated overnight at 105 °C. The ash content was determined after the dry sludge was heated at 550 °C for 2 hours. The sample size used in the analyses was 10 g of wet sludge.

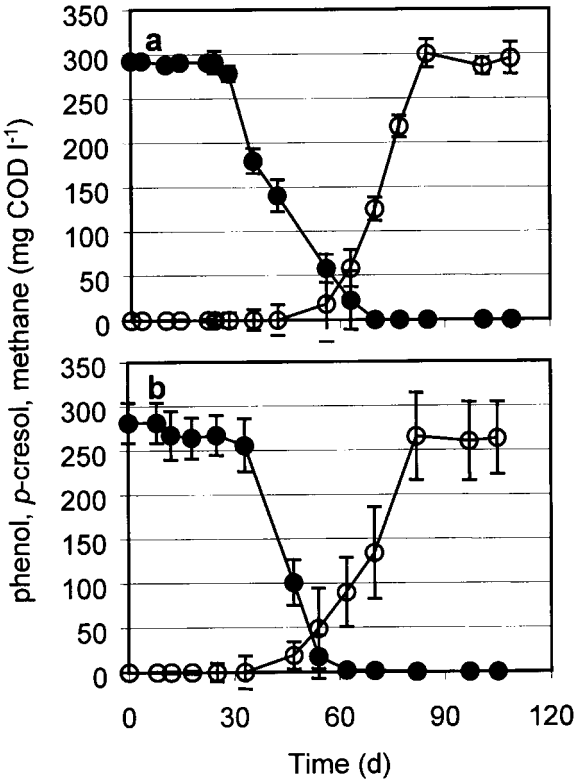

Figure 1. Conversion of phenolic compounds (●) to methane (○) by "Nedalco" granular sludge. (a) Phenol. (b) *p*-cresol. Methane production reported as mg COD per liter of culture fluid and corrected for endogenous methanogenesis.

Results

Complete conversion of both phenol and *p*-cresol to methane was observed when batch experiments were conducted in the absence of AQDS and BES by the anaerobic granular sludge obtained from a full-scale UASB reactor treating effluent from an alcohol distillery of Nedalco (Figure 1). There was a lag phase of about 30 days before this granular sludge started to consume the phenolic compounds, but after this period, degradation took place in both bioassays. Evidence of complete degradation is based on the elimination of the phenolic compounds and recovery of the stoichiometric amounts of methane in excess of that produced in the endogenous substrate control cultures (Tables 1 and 2).

Table 1. COD balance (in mg COD l^{-1}) in different cultures for phenol and *p*-cresol biodegradation by "Nedalco" sludge after 15 weeks of incubation.

Culture (added substrate)	Endogenous[a]	Phenol	*p*-Cresol	Benzoate	CH_4[c]	AH_2QDS[d]	Recovery[e] (%)
Phenol (293)	406 ± 18	ND[b]	ND	ND	701 ± 57	–	100.7
Phenol-BES (293)	12 ± 2	ND	ND	312 ± 4	15 ± 6	–	107.5
Phenol-AQDS (292)	161 ± 14	205 ± 7	ND	ND	ND	262 ± 54	104.8
Phenol-AQDS-BES (283)	157 ± 12	202 ± 7	ND	159 ± 0.7	ND	137 ± 19	120.5
p-cresol (281)	214 ± 41	ND	ND	ND	477 ± 29	–	93.6
p-cresol-BES (299)	10 ± 4	ND	132 ± 35	8 ± 3	10 ± 6	–	46.8
p-cresol-AQDS (265)	117 ± 7	ND	84 ± 45	ND	ND	297 ± 101	99.6
p-cresol-AQDS-BES (263)	113 ± 10	ND	149 ± 4	ND	ND	224 ± 21	98.9

[a] Endogenous production of methane (AQDS not present) or AH_2QDS (AQDS present) in sludge controls.
[b] ND: Not detected.
[c] Total production of methane not corrected for endogenous methane production. Methane concentration expressed as mg COD per liter of culture fluid.
[d] COD calculated only refers to the hydrogen linked to the structure, total concentration of AH_2QDS measured not corrected for endogenous AH_2QDS production.
[e] Recovery = (identified products – endogenous COD)/(initial COD), no VFA were detected in all the samples at the end of the experiment.

Phenol was completely consumed by "Nedalco" granular sludge also in the presence of BES in which negligible methane production was observed (Figure 2A). Further analyses revealed that phenol was completely converted to benzoate under these conditions (Table 1). On the other hand, *p*-cresol was partially converted after 105 days of incubation by "Nedalco" sludge, only 56% of *p*-cresol was consumed (Figure 2B) and benzoate was only detected at low levels under these conditions (Table 1). The very low recovery observed in the balance for the *p*-cresol-BES culture (Table 1) suggests that this phenolic compound was converted to another unidentified intermediate by this consortium. The missing COD could not be attributed to either VFA or to *p*-hydroxybenzoate, which were only present at trace levels at the end of the experiment (data not shown).

Phenol degradation could also be coupled to AQDS reduction by "Nedalco" granular sludge (Figure 3A), but at a 3-fold lower extent of degradation as compared to methanogenic conditions (Table 2). The coupling between phenol degradation and AQDS reduction is evidenced by the amount of phenol degraded, which fits with the COD recovered as hydrogen in AH_2QDS corrected for the endogenous AQDS reduction (Table 2). No benzoate was detected at the end of the experiment under these conditions. When BES was included in the phenol-AQDS culture, the extent of phenol degradation was slightly lower (Table 2) and it was partially converted to benzoate (Table 1). In

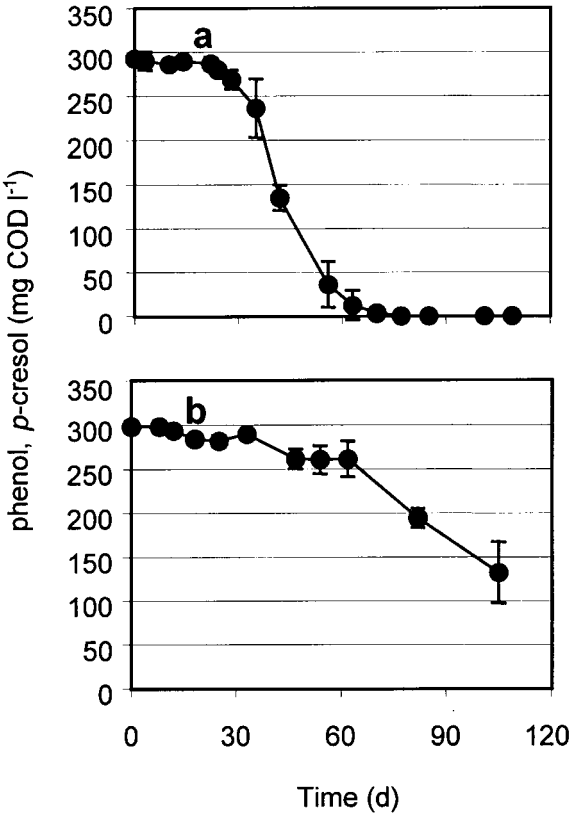

Figure 2. Phenol (a) and *p*-cresol (b) degradation by "Nedalco" granular sludge in the presence of BES (50 mM).

Table 2. Extent of degradation and ratio reduced products: substrate consumed (RRPSC) for phenol and *p*-cresol by "Nedalco" and "Shell" sludge under different anaerobic conditions after 15 weeks of incubation.[a]

Culture conditions	Extent of degradation (%)		RRPSC (%)[b]	
	"Nedalco" sludge	"Shell" sludge	"Nedalco" sludge	"Shell" sludge
Phenol	100 ± 0	100 ± 0	101 ± 8	113 ± 5
Phenol-BES	100 ± 0	100 ± 0	Not applicable[c]	Not applicable
Phenol-AQDS	30 ± 3	38 ± 12	116 ± 21	47 ± 27[d]
Phenol-AQDS-BES	28 ± 3	41 ± 3	Not applicable	Not applicable
p-cresol	100 ± 0	100 ± 0	94 ± 6	102 ± 3
p-cresol-BES	56 ± 12	70 ± 5	Not applicable	Not applicable
p-cresol-AQDS	69 ± 16	96 ± 1	99 ± 34	79 ±14
p-cresol-AQDS-BES	44 ± 2	100 ± 0	98 ± 8	106 ± 8

[a] Data from experiments with "Shell" sludge after 20 weeks of incubation.
[b] RRPSC = (total CH_4-endogenous CH_4)/(phenol or *p*-cresol consumed) for methanogenic culture, RRPSC = (total AH_2QDS-endogenous AH_2QDS)/(phenol or *p*-cresol consumed) for the AQDS containing cultures. Ratio based on mg COD per liter of culture fluid. COD calculated as AH_2QDS only refers to the hydrogen linked to the structure.
[c] Not applicable refers to the lack of coupling between degradation and methanogenesis or AQDS reduction.
[d] Total recovery 75% including 28 mg COD l^{-1} as benzoate.

the presence of BES no significant AQDS reduction was observed as compared to the endogenous AQDS reduction (Figure 3B). Further experiments confirmed that benzoate oxidation can be coupled to AQDS reduction by this granular sludge (about 100 mg COD l^{-1} of benzoate recovered as AH_2QDS, corrected for endogenous AQDS reduction, after 5 months of incubation). Neither phenol conversion nor AQDS reduction was observed in sterilized controls with autoclaved "Nedalco" sludge. Moreover, there was no methanogenic activity detected in the presence of AQDS.

Oxidation of *p*-cresol could also support AQDS reduction by "Nedalco" granular sludge both in the presence and in the absence of BES (Figure 4). Evidence is based on decrease in p-cresol and concomitant increase in AH_2QDS production beyond the level observed in the endogenous substrate control. Furthermore, the COD recovered as hydrogen in AH_2QDS (corrected for endogenous AH_2QDS production) agrees with the amount of *p*-cresol consumed in both experiments (Table 2). There was no benzoate nor VFA detected at the end of the experiment under these conditions (Table 1). There was no methanogenic activity in the presence of AQDS in the experiments for *p*-cresol degradation. In sterilized controls neither *p*-cresol conversion nor AQDS reduction was observed. The extent of degradation of *p*-cresol observed in the presence of AQDS was about the same level compared to that observed in the p-cresol-BES culture, but about 1.5–2.0-fold lower than under methanogenic conditions (Table 2).

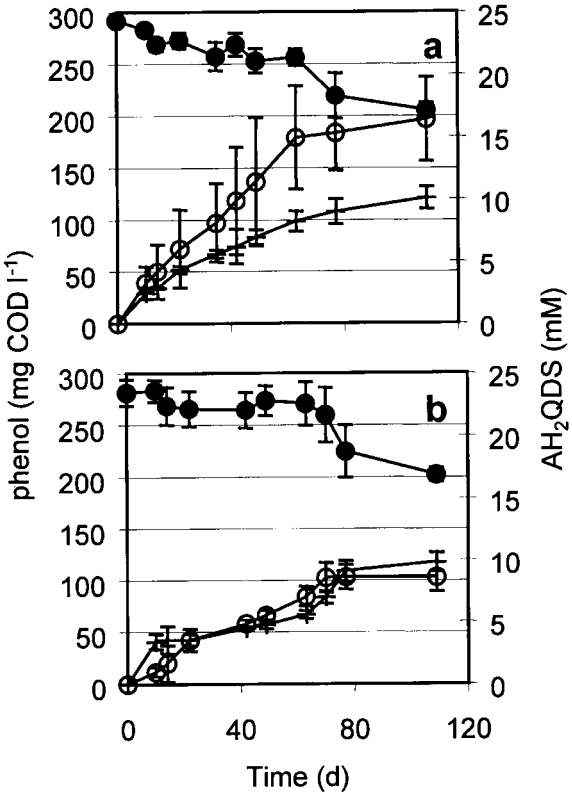

Figure 3. Degradation of phenol (●) by "Nedalco" granular sludge. (a) In the presence of AQDS (25 mM). (b) In the presence of AQDS (25 mM) and BES (50 mM). (○), AH_2QDS; (+), endogenous AH_2QDS production.

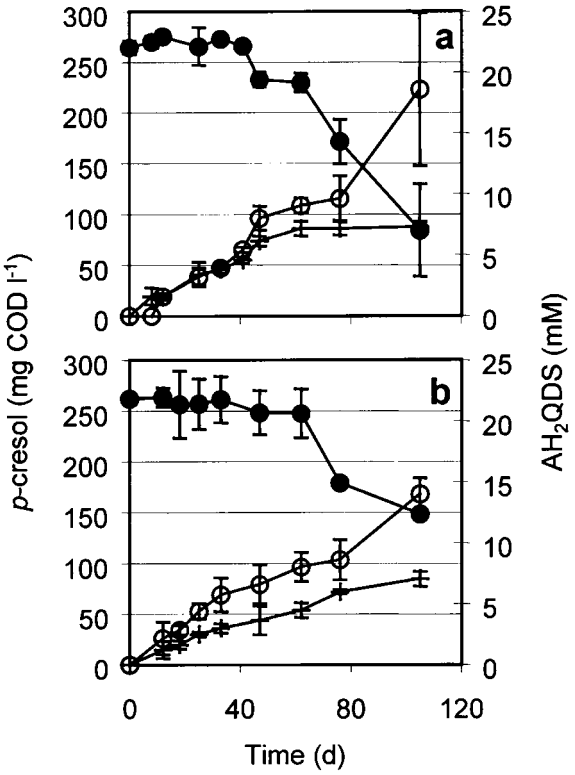

Figure 4. Degradation of *p*-cresol (●) by "Nedalco" granular sludge. (a) In the presence of AQDS (25 mM). (b) In the presence of AQDS (25 mM) and BES (50 mM). (○), AH_2QDS; (+),endogenous AH_2QDS production.

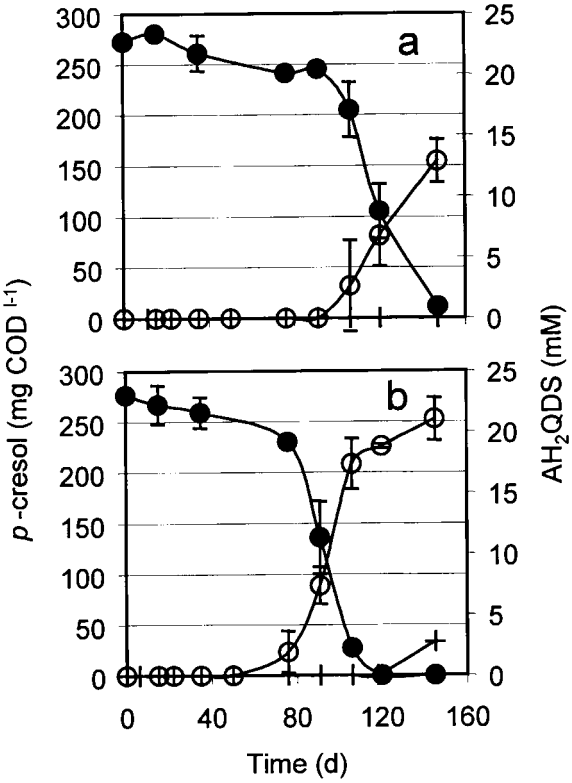

Figure 5. Degradation of *p*-cresol (●) by "Shell" granular sludge. (a) In the presence of AQDS (25 mM). (b) In the presence of AQDS (25 mM) and BES (50 mM). (○), AH_2QDS; (+),endogenous AH_2QDS production.

Additional experiments were carried out with anaerobic granular sludge obtained from a full-scale UASB reactor treating wet oxidized industrial effluent of Shell Nederland Chemie. This sludge referred to as "Shell" sludge was able to completely degrade *p*-cresol coupled to AQDS reduction both in the absence and in the presence of BES (Figure 5). This is evidenced by the consumption of this phenolic compound, which fits with the COD recovered as hydrogen in AH_2QDS corrected for the endogenous AQDS reduction (Table 2). However, there was only minor phenol degradation linked to AQDS respiration by "Shell" sludge after 5 months of incubation. The COD recovered as AH_2QDS accounted only for 47% (Table 2) of the phenol degraded (about 100 mg COD-phenol l^{-1}) and benzoate was detected as an intermediate (28% of the phenol degraded). When BES was included in the phenol-AQDS culture, no coupling between phenol degradation and AQDS reduction was observed by "Shell" sludge. There was no methane production by "Shell" sludge in the AQDS amended media. Moreover, neither conversion of the phenolic compounds nor reduction of AQDS was observed in the sterilized controls with autoclaved "Shell" sludge.

Both phenolic compounds were completely converted to methane when bicarbonate was the only electron acceptor available by "Shell" granular sludge after 3 months of incubation (data not shown). The COD recovered as methane (corrected for the endogenous methane production) agreed with the amount of phenol and p-cresol consumed (Table 2). Phenol was completely converted to benzoate (98% of recovery) when the methanogenic inhibitor, BES, was included in the culture; whereas about 70% of *p*-cresol was consumed under the same conditions. p-cresol was mainly converted to VFA, but this COD only accounted for about 60% of the consumed *p*-cresol indicating that this phenolic compound was transformed to another unidentified intermediate also by "Shell" sludge when BES was included in the medium.

Table 3. Reactions involved in the degradation of phenolic compounds under anaerobic conditions (all $\Delta G^{0\prime}$ values are calculated from data of Thauer et al. 1977 or of Hollinger et al. 1988).[a]

Reaction	$\Delta G'$ (kJ reaction^{-1})
Phenol	
$C_6H_6O + 6.5H_2O \rightarrow 3.5CH_4 + 2.5HCO_3^- + 2.5H^+$	−155.3
$C_6H_6O + 17H_2O + 14AQDS \rightarrow 14AH_2QDS + 6HCO_3^- + 6H^+$	−302.0
p-Cresol	
$C_7H_8O + 7.5H_2O \rightarrow 4.25CH_4 + 2.75HCO_3^- + 2.75H^+$	−187.5
$C_7H_8O + 20H_2O + 17AQDS \rightarrow 17AH_2QDS + 7HCO_3^- + 7H^+$	−365.7
Benzoate	
$C_7H_5O_2^- + 7H_2O \rightarrow 3CH_3COO^- + HCO_3^- + 3H^+ + 3H_2$	+70.4
$C_7H_5O_2^- + 7.75H_2O \rightarrow 3.75CH_4 + 3.25HCO_3^- + 2.25\ H^+$	−124.1
$C_7H_5O_2^- + 19H_2O + 15AQDS \rightarrow 15AH_2QDS + 7HCO_3^- + 6H^+$	−281.5

[a] $\Delta G^{0\prime}$ for reactions with AQDS include reduction of AQDS by hydrogen according to Nernst equation with data from Sober HA (1970). $\Delta G^{0\prime} = -44.4$ kJ mol^{-1}.

Discussion

The observation that phenol and *p*-cresol degradation occurred under methanogenic conditions is consistent with numerous, previous reports, which indicate that these pollutants can be utilized by methanogenic consortia (Boyd et al. 1983; Dwyer et al. 1986; Wang et al. 1989; Tawfiki et al. 2000). This also agrees with thermodynamics, which indicates that conversion of these phenolic compounds is favorable under methanogenic conditions (see Table 3). The lag phase observed during these experiments was due to the inocula used, which were not previously exposed to the phenolic contaminants. Time was required for the growth of the responsible degrading bacteria and the development of the enzymatic systems involved in the degradation pathway.

The complete conversion of phenol to benzoate in the presence of the methanogenic inhibitor, BES, agrees with previous reports, which showed the same pattern by inhibiting the culture. Field & Lettinga (1989) observed complete conversion of phenol to benzoate in methanogenic cultures that were inhibited by an excess concentration of phenol. Knoll & Winter (1989) used an atmosphere of 80% H_2 and 20% CO_2 to stimulate feedback inhibition and this also led to the accumulation of benzoate during degradation of phenol under methanogenic conditions. Conversion of phenol to benzoate is feasible according to thermodynamics if hydrogen is available from endogenous substrates ($\Delta G' = -64.9$ kJ mol^{-1}). Considering the proposed pathway of phenol degradation, which proceeds through benzoyl-CoA (Heider & Fuchs 1997), it seems that equilibrium is reached between benzoyl-CoA and benzoate when inhibitory conditions predominate in the culture and no further transformation occurs towards saturating the aromatic ring, which are the next steps in the pathway (Figure 6). This may be explained by thermodynamics which indicates that conversion of benzoate to acetate is an unfavorable reaction, whereas the global conversion of benzoate to methane is thermodynamically favorable (Table 3), but this last reaction did not occur under these conditions due to the presence of BES.

The limited conversion of *p*-cresol achieved when BES was included in the medium suggests that this methanogenic inhibitor has a stronger effect on the degradation of *p*-cresol compared to the effect observed during phenol degradation by both inocula tested. Since there was no major accumulation of benzoate or *p*-hydroxybenzoate during these experiments, other intermediates such as *p*-hydroxybenzyl alcohol and *p*-hydroxybenzaldehyde, which are formed during the conversion of *p*-cresol to *p*-hydroxybenzoate (Cunane et al. 2000), might have accumulated.

In this study we observed that quinones can be used as alternative electron acceptors for supporting the anaerobic oxidation of phenols. Upon addition of the model compound, AQDS, the flow of electrons was diverted away from methanogenesis and was directed towards quinone reduction. The coupling of phenol and *p*-cresol degradation to quinone reduction was supported by the stoichiometric recovery of electrons in the reduced quinone, AH_2QDS, as compared to the amount of phenols degraded (see Table 2).

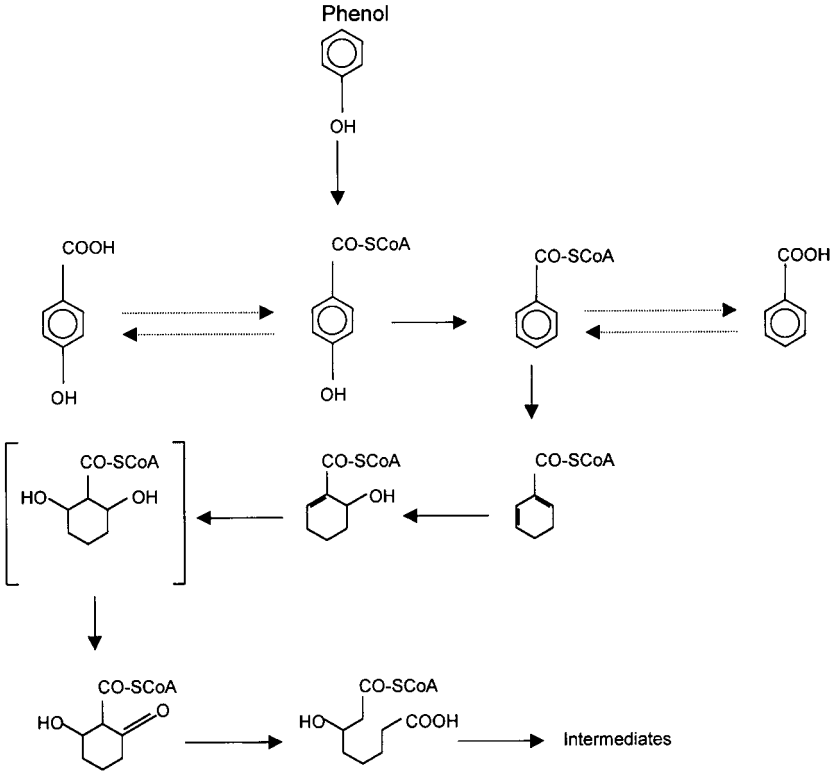

Figure 6. Proposed pathway involved in the anaerobic degradation of phenol (Field et al. 1995; Heider & Fuchs 1997).

AQDS reduction was most likely related to the oxidation of intermediates (e.g., benzoate) and not to the direct oxidation of phenol by "Nedalco" sludge. This is suggested by the negligible AQDS reduction observed (compared to the endogenous control) when BES was included in the phenol-AQDS culture in which partial conversion of phenol to benzoate was observed by "Nedalco" sludge. This theory is also supported by the fact that benzoate degradation led to AQDS reduction by this anaerobic granular sludge. On the other hand, the coupling between *p*-cresol degradation and AQDS reduction was observed both in the presence and in the absence of BES, without any accumulation of intermediates by both consortia evaluated. This may indicate that *p*-cresol degradation was carried out directly through quinone respiration by both sources of anaerobic granular sludge.

Addition of AQDS to the consortia prevented methanogenesis. This may be due to the fact that AQDS was inhibitory to methanogens or that AQDS was the preferred electron acceptor over bicarbonate. AQDS increased the redox potential of the culture fluid (data not shown). This high redox potential probably interferes with biochemical processes required for methanogenesis.

Thermodynamically, AQDS reduction is more favorable than methanogenesis (Table 3) and therefore, it can be expected that AQDS reduction would proceed instead of methanogenesis according to this point of view. However, this does not agree with the slower degradation for both phenolic compounds observed with AQDS as alternative electron acceptor compared to that obtained under methanogenic conditions by both sources of granular sludge. The slower degradation rates may be attributed to the type of inocula used in these experiments, which are characterized by a high methanogenic activity and thus, may only contain few quinone respiring microorganisms. This may also explain the longer lag phase observed during biodegradation of p-cresol via AQDS reduction by "Shell" sludge (Figure 5) compared to that observed under methanogenic conditions (only one month as lag phase).

To our knowledge, this investigation is the first report of quinones serving as a terminal electron acceptor to support the oxidation of phenolic compounds under anaerobic conditions. Thus, the results have im-

portant implications for bioremediation of anaerobic sites contaminated with phenolic compounds. In fact, the results suggest that humus, which is very abundant in many anaerobic sites and rich in quinone moieties, may contribute to the bioremediation capacity of sites contaminated with aromatic compounds by serving as a terminal electron acceptor. These results also suggest that quinones may play an active role in the biodegradation of plant material, which contains a variety of simple and complex phenolic substances (Harborne 1980). Therefore, quinones in humus may contribute to important carbon cycling process in the biosphere.

Conclusions

The results presented in this study indicate that quinones can contribute in the oxidation of phenolic compounds by serving as terminal electron acceptors. The results also suggest that humus may be a potential electron acceptor for the biodegradation of aromatic compounds in anaerobic sites. This information needs to be considered in future studies of electron and carbon flow in soils and sediments as it may have important implications for the biotransformation of organic matter.

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