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ORIGINAL PAPER

Contribution of quinone-reducing microorganisms to the anaerobic biodegradation of organic compounds under different redox conditions

Francisco J. Cervantes · Claudia H. Gutiérrez · Kitzia Y. López · María Isabel Estrada-Alvarado · Edna R. Meza-Escalante · Anne-Claire Texier · Flor Cuervo · Jorge Gómez

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Abstract The capacity of two anaerobic consortia to oxidize different organic compounds, including acetate, propionate, lactate, phenol and *p*-cresol, in the presence of nitrate, sulfate and the humic model compound, anthraquinone-2,6-disulfonate (AQDS) as terminal electron acceptors, was evaluated. Denitrification showed the highest respiratory rates in both consortia studied and occurred exclusively during the first hours of incubation for most organic substrates degraded. Reduction of AQDS and sulfate generally started after complete denitrification, or even occurred at the same time during the biodegradation of *p*-cresol, in anaerobic sludge incubations; whereas methanogenesis did not significantly occur during the reduction of nitrate, sulfate, and AQDS. AQDS reduction was the preferred respiratory pathway over sulfate reduction and methanogenesis during the anaerobic oxidation of most organic substrates by the anaerobic sludge studied. In contrast, sulfate reduction out-competed AQDS reduction during incubations performed with anaerobic wetland sediment, which did not achieve any methanogenic activity. Propionate was a poor electron donor to achieve AQDS reduction; however, denitrifying and sulfate-reducing activities carried out by both consortia promoted the reduction of AQDS via acetate accumulated from propionate oxidation. Our results suggest that microbial reduction of humic substances (HS) may play an important role during the anaerobic oxidation of organic pollutants in anaerobic environments despite the presence of alternative electron acceptors, such as sulfate and nitrate. Methane inhibition, imposed by the inclusion of AQDS as terminal electron acceptor, suggests that microbial reduction of HS may also have important implications on the global climate preservation, considering the green-house effects of methane.

Keywords Competition · Denitrification · Humus reduction · Methanogenesis · Sulfate reduction

F. J. Cervantes · C. H. Gutiérrez · K. Y. López
Departamento de Ciencias del Agua y del Medio Ambiente, Instituto Tecnológico de Sonora, Av. 5 de Febrero 818 Sur, Cd. Obregón, SON 85000, Mexico

M. I. Estrada-Alvarado
Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora, Av. 5 de Febrero 818 Sur, Cd. Obregón, SON 85000, Mexico

E. R. Meza-Escalante · A.-C. Texier · F. Cuervo · J. Gómez
Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa, Av. San Rafael Atlixco 186, Col. Vicentina, Iztapalapa, DF 09340, Mexico

Present Address:
F. J. Cervantes (✉)
División de Ciencias Ambientales, Instituto Potosino de Investigación Científica y Tecnológica (IPICyT), Camino a la Presa San José 2055, Col. Lomas 4ª. Sección, C. P. 78216, San Luis Potosí, SLP, Mexico
e-mail: fjcervantes@ipicyt.edu.mx

Introduction

Reduction of humic substances (HS) has recently been recognized as a microbial respiratory process supporting growth of several distinct microorganisms (Lovley et al. 1996; Coates et al. 1998; Cervantes et al. 2002; Field and Cervantes 2005). Quinone moieties, which are very abundant in the humic acid fraction of humus (Stevenson 1994), are the main functional groups conferring electron-accepting capacity to HS (Scott et al. 1998). The capacity to reduce HS has been reported in anaerobic consortia from a wide diversity of environments, including fresh water and marine sediments, soils rich in organic matter, as well as sludge samples from wastewater treatment facilities (Coates et al. 1998; Cervantes et al. 2000a; Kappler et al. 2004). The HS-reducing capacity observed in many different habitats agrees with the wide variety of microorganisms, which are capable of using HS as terminal electron acceptors or as redox mediators. The diversity of HS-reducing microorganisms includes iron-reducing bacteria and archaea, sulfate-reducing, fermentative and halorespiring bacteria, and methanogenic archaea (Lovley et al. 1998; Benz et al. 1998; Cervantes et al. 2002).

Microbial reduction of HS supports the anaerobic oxidation of a large selection of ecologically important organic compounds, such as acetate, lactate, and propionate (Field et al. 2000). Furthermore, the anaerobic microbial oxidation of recalcitrant pollutants has also been linked to the reduction of HS or to the humic model compound, anthraquinone-2,6-disulfonate (AQDS). The first priority pollutants reported to be degraded by HS-reducing consortia were the carcinogenic solvents, vinyl chloride (VC) and dichloroethene (DCE). Bradley et al. (1998) reported the anaerobic microbial oxidation of VC and DCE was coupled to the reduction of humic acids or AQDS. Phenolic compounds were also shown to be degraded through the microbial reduction of AQDS (Cervantes et al. 2000b). Moreover, the anaerobic mineralization of toluene, by anaerobic sediments from different environments, was supported by the reduction of humic acids and AQDS (Cervantes et al. 2001).

Application of HS to restore contaminated aquifers has been proposed as a suitable strategy owing to a number of benefits (Field et al. 2000). First, HS are very abundant in many natural environments or can be obtained through composting of organic residues. HS do not necessarily have to be supplied abundantly in many contaminated sites as there are several recycling mechanisms, which allow bioremediation even at sub-stoichiometric levels. The main recycling mechanism explored is the chemical reaction of reduced HS with metal oxides, such as goethite or vernadite, which are very abundant in many anaerobic environments (Lovley et al. 1996; Kostka and Nealson 1998). Moreover, the use of HS in anaerobic contaminated sites does not promote the formation of undesirable by-products, such as those occurring during bioremediation supported by either denitrification (nitrite, N_2O) or sulfate reduction (sulfide) (Anderson and Lovley 2000; Hutchins et al. 1991).

Despite the potential to apply HS to clean up polluted aquifers, the literature provides little knowledge on the role of HS in the biodegradation of organic contaminants under different redox conditions. The aim of this study was to evaluate the role of quinone-reducing microorganisms to the biodegradation of organic compounds in the presence of nitrate and sulfate. Phenol and *p*-cresol were considered as model pollutants in the presence study as they are common contaminants of water bodies, which receive untreated streams containing these compounds due to their widespread industrial use (Cervantes et al. 2000b). Key anaerobic metabolites (e.g., acetate, lactate, and propionate) were also included as electron donors in the present study.

Materials and methods

Inocula and basal culture medium

Anaerobic sludge containing 12.9% of natural organic matter (NOM) was obtained from a stable full-scale upflow anaerobic sludge blanket (UASB) reactor treating effluents from a paper mill factory, Eerbeek, The Netherlands. This consortium showed quinone-reducing activity with glucose as electron donor during previous studies (dos Santos et al. 2004). Moreover, due to the wastewater composition (Oude-Elferink et al. 1998), the sludge was commonly exposed to several electron acceptors (sulfate, sulfite, thiosulfate, and HS), which allow the proper niche for a wide microbial diversity. The microbial

population of this consortium has been characterized showing phylogenetic stability during several years of scrutiny (Oude-Elferink et al. 1998; Roest et al. 2005). Anaerobic wetland sediment was collected from the ''Tóbari'' bay located in the Cortéz Sea (UTM Coordinates 60°24′09″ East, 29°97′23″ north, SON, Mexico), which also proved quinone-reducing activity during an earlier screening. The ''Tóbari'' bay is commonly exposed to different contaminants since it receives waste discharges from different municipalities and industrial activities. Sediment cores (8 cm diameter and 30–45 cm long) were collected and cooled immediately after sampling until performing the microbial incubations. High temperatures arise in the region resulting in a diurnal water temperature between 27 and 32°C for a large period of time (between May and September). The wetland sediment contained 2.2% of NOM. Both consortia were handled under strict anaerobic conditions during the inoculation procedure.

The composition of the basal culture medium used in all incubations was as follows (g/L): $NaHCO_3$ (1.68); NH_4Cl (0.3); KH_2PO_4 (0.2); $MgCl_2$ $6H_2O$ (0.03); $CaCl_2$ (0.1); Na_2S (0.1), and 1 mL/L of trace elements solution with the composition previously described (Cervantes et al. 2000b). The pH of the medium was controlled at 6.7 ± 0.2 by the bicarbonate added and a headspace composed of N_2/CO_2 (80%/20%).

Incubation procedure

The assays were conducted in batch mode in 117-mL glass serum bottles. Basal culture medium (50 mL) with the composition described above was dispensed in the serum bottles and then, inoculation took place by adding 10 g dried weight/L for each inoculum. The culture bottles were sealed with rubber stoppers and aluminum crimps. Anaerobic conditions were established by flushing the headspace (67 mL) with a mixture of N_2/CO_2 (80%/20% v/v) for 5 min. For incubations including AQDS, the basal medium was prepared with the initial concentration needed of this electron acceptor. Nitrate and sulfate were provided from stock solutions. All electron donors were supplied in excess to allow every respiratory process to occur. Therefore, acetate (16.6 mM), propionate (9 mM), lactate (10.4 mM), phenol (1.5 mM), and *p*-cresol (1.5 mM) were supplied as energy sources from stock solutions. For the experiments with phenolic compounds, the initial concentrations of nitrate, sulfate, and AQDS were 1, 0.625, and 2.5 mM, respectively, to set up similar electron accepting capacity for all electron acceptors. For the remaining experiments, the concentrations of nitrate, sulfate, and AQDS were modified to 3, 2, and 5 mM, respectively. AQDS was supplied at a lower electron accepting capacity for these experiments, compared to nitrate and sulfate, as previous studies indicate inhibitory effects of AQDS on methanogenesis at concentrations above 5 mM (Cervantes et al. 2000a). The batch cultures were incubated once and after complete reduction of all electron acceptors available (including endogenous), the bioassays were refilled under anaerobic conditions to proceed with the experimental determinations. Controls without external electron donor supplied were included in the experimental design in order to subtract the endogenous reduction of the electron acceptors. All experimental treatments were prepared in triplicate cultures and incubated at 30°C in the dark. The consumption of all substrates provided was quantified as well as the reduction of nitrate, sulfate, and AQDS. The headspace of the cultures was also monitored to quantify the production of methane and the denitrification intermediate, N_2O.

Respiratory rates were determined on the maximum slope observed on linear regressions considering at least three sampling points. The coefficient of determination (R^2) was higher than 0.9 for most respiratory rates calculated. Denitrification rates were based on nitrate, nitrite and N_2O concentrations and without considering dissimilatory nitrate reduction to ammonium as preliminary incubations did not show ammonium production by the consortia evaluated during denitrification with phenolic compounds.

Analyses

The concentration of phenolic compounds and volatile fatty acids was determined by chromatographic methods previously described (Cervantes et al. 2000a, b). The production of methane and N_2O was quantified using a gas chromatograph model 3800 (Varian, Mexico city, DF, Mexico) equipped with a thermal conductivity detector (TCD) and with a steel column ($2.4 \times 1.18 \times 2.0$ mm^3) packed with Porapak Q (80/100 mesh, Varian, Mexico). The temperatures

of the column, the injection port and the TCD were 35, 140, and 190°C, respectively. The TCD filament was kept at 240°C. Helium was used as a carrier gas at a flow rate of 20 mL/min. AQDS reduction was quantified by a spectrophotometric method previously reported (Cervantes et al. 2000a).

The concentrations of sulfate, nitrite and nitrate were measured in a high performance liquid chromatograph (HPLC, Waters 2690) in previously centrifuged samples (10,000*g*, 5 min). The HPLC was equipped with a high resolution anion column (IC-Pak A HR Waters 4.6×75 mm^2), which was kept at 35°C, and a conductivity detector (Waters 432). The eluent was supplied at a flow rate of 1 mL/min with the following composition (mL/L): concentrate borate–gluconate (20), *n*-butanol (20), and acetonitrile (120). The composition of the borate–gluconate concentrate was (g/L): boric acid (18), sodium gluconate (16), and sodium tetraborate decahydrate (25). The injection volume was 20 μL and deionized water was used during eluent preparation and sample dilutions.

Results

Respiratory activities of anaerobic sludge from a paper mill wastewater treatment plant under different redox conditions

All microbial processes monitored (denitrification, sulfate reduction, AQDS reduction, and methanogenesis) occurred in this consortium, although the respiratory rate of every event depended on the substrate supplemented. Table 1 summarizes the respiratory rates observed under the distinct redox conditions applied. Denitrification showed the highest respiratory rates for all organic pollutants degraded. Moreover, denitrification was exclusively observed during the first hours of incubation of this consortium for most electron donors studied (Figs. 1, 2, 3). Further analyses revealed transient accumulation of nitrite (up to 0.2 mM) in several nitrate-amended cultures; however, nitrite was not longer detected in any incubation after complete reduction of nitrate (data not shown). Furthermore, nitrous oxide was not detected in all incubations performed.

AQDS reduction generally prevailed over methanogenesis for all substrates provided. However, when phenol was included as an electron donor, the methane production rate was higher than the reduction rate of AQDS (Fig. 2) during the first week of incubation, which was required as a lag phase for quinone-reducing microorganisms. Methane production was not detected in sludge incubations with acetate, propionate, and lactate probably due to the higher AQDS concentration provided (5 mM) compared to the experiments with phenolic compounds.

In most cases, the reduction of AQDS and sulfate started immediately after complete nitrate depletion by the anaerobic sludge. However, during *p*-cresol biodegradation (Fig. 1d), both respiratory processes even occurred simultaneously with denitrification, although the respiratory rate of the latter event was approximately twofold faster compared to the former in terms of electron equivalents transferred (Table 1). Moreover, there was a lag phase (about 1 week) on the reduction of AQDS and sulfate, after nitrate was completely depleted in the *p*-cresol degrading cultures, but these activities were gradually recovered during prolonged incubation (Fig. 1d).

The reduction of both AQDS and sulfate showed similar respiratory rates during the anaerobic biodegradation of phenol and *p*-cresol. When acetate and lactate were supplied as electron donors AQDS reduction was the preferred respiratory pathway over sulfate reduction. Meanwhile, sulfate reduction overcame AQDS reduction when propionate was supplied as an electron donor (Table 1). Furthermore, in propionate-amended cultures, the denitrifying and sulfate-reducing activity obtained, supported the reduction of AQDS by this consortium via acetate accumulated from propionate oxidation. Indeed, there was no AQDS reduction detected by the anaerobic sludge when propionate and AQDS were supplemented as energy source and sole electron acceptor, respectively (Table 1).

Among the organic substrates provided, lactate promoted the highest respiratory rates for denitrification, AQDS reduction and methanogenesis by the anaerobic sludge. Propionate, on the other hand, supported the highest sulfate reduction rate by this consortium.

Respiratory activities of anaerobic wetland sediment under different redox conditions

Incubations performed with wetland sediment generally showed lower AQDS-reducing rates, but higher nitrate

Table 1 Maximum respiratory rates (in μEq/g NOM-h) and extent of biodegradation of different substrates by anaerobic sludge originated from a paper mill wastewater treatment plant under different redox conditions

Conditions	Respiratory rates				Biodegradation[a] (%)
	γ_d	γ_s	γ_q	γ_m	
Acetate	NA	NA	NA	42 ± 3	100 ± 5
Acetate–AQDS	NA	NA	239 ± 22	10 ± 0.8	49 ± 3
Acetate–AQDS–sulfate	NA	52 ± 3	181 ± 15	24 ± 2	65 ± 8
Acetate–AQDS–sulfate–nitrate	368 ± 30	84 ± 7	210 ± 8	ND	69 ± 5
Propionate	NA	NA	NA	12 ± 0.7	47 ± 5
Propionate–AQDS	NA	NA	ND	ND	57 ± 7
Propionate–AQDS–sulfate	NA	176 ± 12	2.3 ± 0.2	ND	45 ± 2
Propionate–AQDS–sulfate–nitrate	581 ± 41	272 ± 60	174 ± 5	ND	82 ± 11
Lactate	NA	NA	NA	591 ± 23	49 ± 7
Lactate–AQDS	NA	NA	332 ± 17	19 ± 1.2	44 ± 6
Lactate–AQDS–sulfate	NA	150 ± 12	336 ± 21	15 ± 3	60 ± 4
Lactate–AQDS–sulfate–nitrate	654 ± 7	26 ± 5	236 ± 7	ND	68 ± 3
Phenol	NA	NA	NA	2.3 ± 0.1	88 ± 4
Phenol–AQDS	NA	NA	5.4 ± 0.7	1.6 ± 0.1	72 ± 12
Phenol–AQDS–sulfate	NA	5.4 ± 0.2	6.2 ± 0.3	1.6 ± 0.2	54 ± 9
Phenol–AQDS–sulfate–nitrate	76 ± 11	9.3 ± 2	8.5 ± 0.2	1.6 ± 0.2	65 ± 10
p-cresol	NA	NA	NA	8.5 ± 0.7	65 ± 3
p-cresol–AQDS	NA	NA	5.4 ± 0.3	0.8 ± 0.1	59 ± 5
p-cresol–AQDS–sulfate	NA	12 ± 1	12 ± 2	0.8 ± 0.1	64 ± 5
p-cresol–AQDS–sulfate–nitrate	23 ± 2	8.5 ± 1	11 ± 1	0.8 ± 0.1	71 ± 8

Initial concentrations: Acetate (16.6 mM), propionate (9 mM), lactate (10.4 mM), phenol (1.5 mM), *p*-cresol (1.5 mM), nitrate (3 mM), sulfate (2 mM), and AQDS (5 mM). When phenol or *p*-cresol were used as electron donors, the initial concentrations of nitrate, sulfate, and AQDS were 1, 0.625, and 2.5 mM, respectively. Results represent average from triplicate incubations ± 1 SD. Endogenous respiration subtracted in all experimental data presented. Inoculum provided at 10 g DW/L. The units μEq/g NOM-h mean micro-electron equivalents transferred per g NOM per hour

DW dried weight, *NOM* natural organic matter, *NA* not applicable, *ND* not detected, *AQDS* anthraquinone-2,6-disulfonate, γ_d denitrifying rate, γ_s sulfate-reducing rate, γ_q AQDS-reducing rate, γ_m methanogenic rate

[a] Extent of biodegradation corrected for the amount of substrates consumed by endogenous activities

and sulfate reduction rates, compared to the anaerobic sludge cultures (compare Tables 1, 2). Methane production was not detected by the anaerobic wetland sediment with any substrate provided. Moreover, negligible or null accumulation of nitrite and nitrous oxide was observed in all nitrate-amended cultures. Sediment incubations with lactate yielded the highest sulfate- and AQDS-reducing rates, while propionate promoted the highest denitrifying activity by this consortium (Table 2). Competition between denitrification and sulfate reduction occurred during the biodegradation of all organic pollutants studied (Figs. 4, 5, 6). In contrast, AQDS reduction remained at a marginal level during the course of denitrification and sulfate reduction. When propionate was supplied as an electron donor in the absence of nitrate, sulfate reduction increased the capacity of the wetland sediment to use AQDS as electron acceptor. However, in the presence of nitrate and sulfate, AQDS reduction did not proceed significantly, with propionate as electron donor, because the reduction of the former electron acceptors out-competed the latter respiratory process (Table 2). Likewise, denitrification increased the sulfate reduction rate by this consortium when lactate was supplemented as energy source. The last scenario allowed similar respiratory rates for all

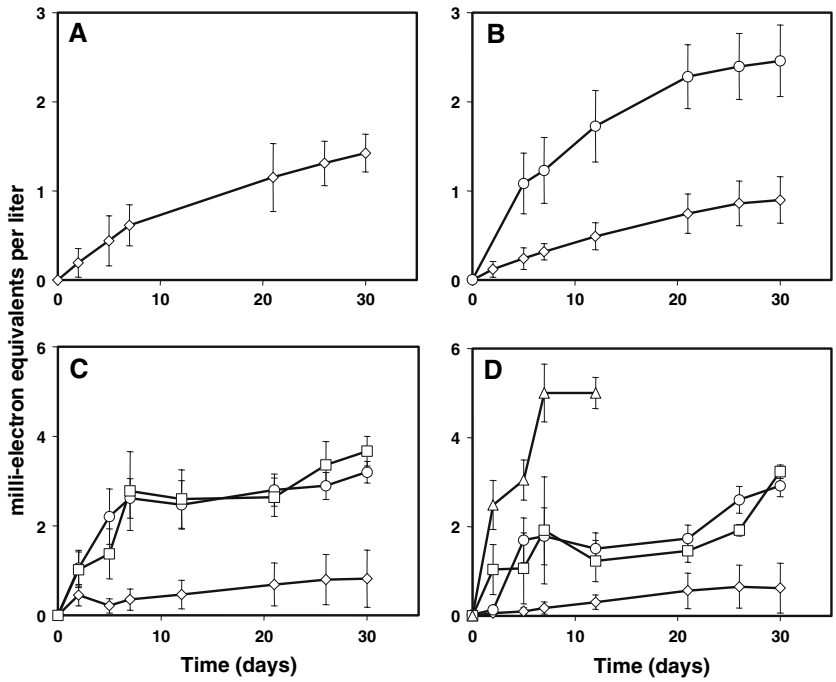

Fig. 1 Respiratory activities during the anaerobic biodegradation of *p*-cresol by anaerobic sludge in the absence of external electron acceptors (**a**), and in the presence of AQDS (**b**), AQDS–sulfate (**c**), and AQDS–sulfate–nitrate (**d**). Concentrations reported refer to the amount of AQDS, sulfate, and nitrate reduced. Production of methane referred to the liquid volume (50 mL). Results represent the average from triplicate incubations and error bars, the SD. Endogenous respiration subtracted in all experimental data presented. ◇, methane; ○, AQDS; □, sulfate; △, nitrate. Initial concentrations: AQDS (2.5 mM), sulfate (0.625 mM), nitrate (1 mM), and *p*-cresol (1.5 mM)

electron acceptors provided (Table 2); although AQDS reduction occurred only after denitrification and sulfate reduction ceased (Fig. 6).

Discussion

In the present study, two different consortia, which previously showed quinone-reducing activity, were evaluated for degrading a number of organic pollutants under distinct redox conditions. To our knowledge, this is the first report on the contribution of quinone-reducing microorganisms to the biodegradation of different organic compounds in the presence of nitrate and sulfate. The results indicate different respiratory patterns depending on the inoculum and substrate provided. Competition of quinone-reducing microorganisms with denitrifying, sulfate-reducing, and methanogenic microorganisms occurred at distinct levels in anaerobic incubations. The results also suggest syntrophic interactions between the different microbial groups studied. The contribution of quinone-reducing microorganisms to anaerobic substrate oxidations, under different redox conditions, may have important implications on the carbon cycle as will be discussed below.

Competition between microbial reduction of quinones and other respiratory processes

Previous reports indicated that AQDS reduction was the preferred physiological process over methanogenesis during the anaerobic oxidation of a wide variety of organic pollutants, including phenolic compounds (Cervantes et al. 2000a, b). The high concentration of AQDS (20–25 mM) used during these studies, which imposed a high redox potential, was suggested as the main reason for inhibiting methane production by the consortia evaluated. Nonetheless, during the same studies (Cervantes et al. 2000a), a toxicity test revealed negligible effects of AQDS on the acetoclastic methanogenesis

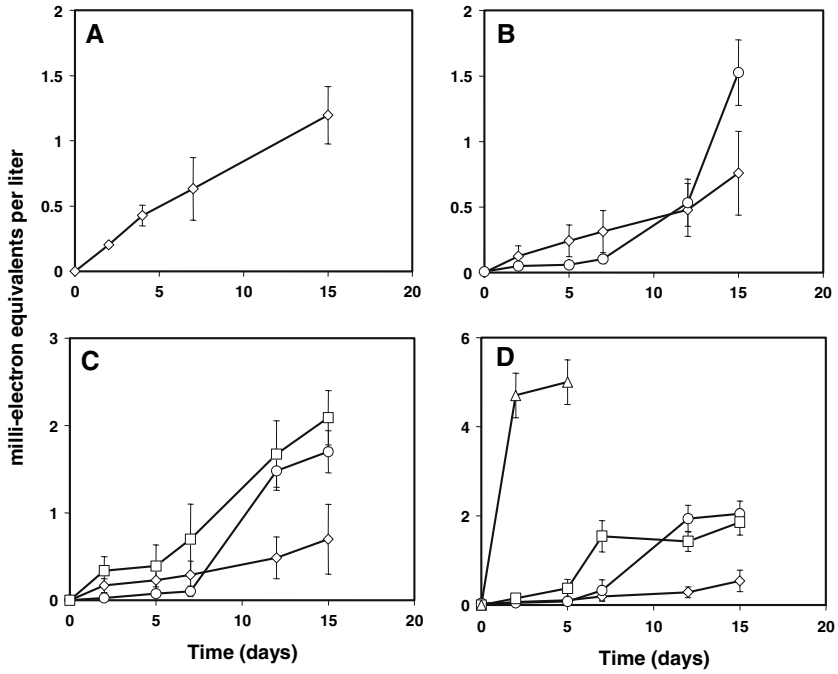

Fig. 2 Respiratory activities during the anaerobic biodegradation of phenol by anaerobic sludge in the absence of external electron acceptors (**a**), and in the presence of AQDS (**b**), AQDS–sulfate (**c**), and AQDS–sulfate–nitrate (**d**). Concentrations reported refer to the amount of AQDS, sulfate, and nitrate reduced. Production of methane referred to the liquid volume (50 mL). Results represent the average from triplicate incubations and error bars, the SD. Endogenous respiration subtracted in all experimental data presented. ◇, methane; ○, AQDS; □, sulfate; △, nitrate. Initial concentrations: AQDS (2.5 mM), sulfate (0.625 mM), nitrate (1 mM), and phenol (1.5 mM)

of an anaerobic granular sludge when applied at the level used in the present experiments (2.5–5 mM). Furthermore, simultaneous reduction of AQDS (12 mM) and methane production were also observed by a different consortium in a lab-scale UASB reactor in which quinone-reducing microorganisms were enriched and immobilized (Cervantes et al. 2003). Thus, competition for the available substrates, rather than toxicity effects, seems to be the mechanism of methanogenesis inhibition in the anaerobic sludge studied here. Further experiments revealed the respiratory preference of the same anaerobic sludge to reduce AQDS, even supplied at the initial concentration of 2 mM, instead of producing methane when lactate, acetate or propionate were incorporated in sludge incubations as energy source (data not shown). The last scenario is surprising considering the methanogenic and sulfate-reducing capacities performed by this consortium in a stable wastewater treatment plant (Oude-Elferink et al. 1998; Roest et al. 2005). However, several preliminary reports indicated the successful competition of different respiratory processes, such as sulfate-, iron-, and AQDS reduction, as well as denitrification, over methanogenesis (Lovley et al. 1982; Lovley 1985; van Bodegom and Stams 1999; Cervantes et al. 2000a; Scholten et al. 2002; Scheid et al. 2003).

The prevalence of quinone reduction over methanogenesis could also be explained by the respiratory shift observed in several methanogenic archaea, which diverted their physiology from methane production toward the reduction of HS or ferric iron (Lovley et al. 2000; Cervantes et al. 2002; van Bodegom et al. 2004).

AQDS reduction was the preferred respiratory pathway also over sulfate reduction by the anaerobic sludge for most substrates provided, except for propionate, which promoted a higher sulfate-reducing activity compared to the former respiratory process (Table 1).

The competition between AQDS- and sulfate reduction showed a different situation in incubations

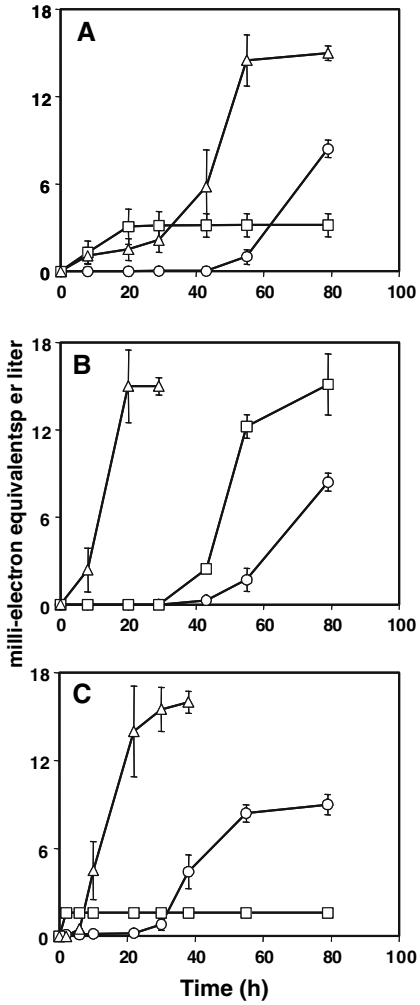

Fig. 3 Respiratory activities during the anaerobic biodegradation of (**a**) acetate (16.6 mM), (**b**) propionate (9 mM), and (**c**) lactate (10.4 mM) by anaerobic sludge in the presence AQDS (5 mM) sulfate (2 mM) and nitrate (3 mM). Concentrations reported refer to the amount of AQDS, sulfate, and nitrate reduced. Results represent the average from triplicate incubations and error bars, the SD. Endogenous respiration subtracted in all experimental data presented. ○, AQDS; □, sulfate; △, nitrate

carried out with anaerobic wetland sediment. For this consortium, sulfate reduction was the preferred respiratory process over quinone reduction and methanogenesis for all electron donors considered in the study (Table 2). This inoculum was exposed to marine streams, which allowed significant sulfate levels throughout the year. Thus, the wetland sediment could be conceived as a proper niche for sulfate-reducing microorganisms, then justifying the respiratory profiles observed.

Therefore, the phylogenetic characteristics of the consortia, as well as the affinity of each microbial group for the electron acceptors available, may be the main factors explaining the respiratory patterns observed. Thermodynamics seems less decisive with this respect since the reduction of AQDS and sulfate show very similar standard free energy change ($\Delta G^{\circ\prime}$, Cervantes et al. 2000a).

AQDS reduction generally occurred after complete nitrate reduction, except during the biodegradation of *p*-cresol by the anaerobic sludge, which showed simultaneous reduction of these two electron acceptors. Besides the high redox potential imposed by nitrate addition and the more favorable thermodynamics of denitrification compared to AQDS reduction (Cervantes et al. 2000a), inhibition of nitrite (accumulated up to 0.2 mM in our incubations) on the latter process could also have determined the prevalence of denitrification.

Syntrophism between quinone-reducing microorganisms and other microbial groups

Syntrophic interactions between denitrifying, sulfate-, and quinone-reducing microorganisms were evident in the two consortia evaluated here. Certainly, denitrifying activities carried out by both inocula promoted the reduction of sulfate and AQDS with different substrates. For instance, sludge incubations amended with propionate as electron donor, showed poor AQDS-reducing capacity in the absence of sulfate and nitrate, which agrees with previous experiments revealing low AQDS reduction rates by different consortia when propionate was provided as electron donor (Cervantes et al. 2000a). However, AQDS reduction by the anaerobic sludge was increased in the presence of sulfate and nitrate (Table 1). The conversion of propionate to acetate, which was detected at significant levels during the course of sulfate reduction, presumably promoted the reduction of AQDS in this case. Indeed, acetate concentration remained below 0.1 mM during the course of denitrification in propionate-amended cultures, but increased up to 0.25 mM during the simultaneous reduction of AQDS and sulfate. Moreover, simultaneous reduction of nitrate, sulfate and AQDS occurred during the anaerobic biodegradation of *p*-cresol by the anaerobic sludge (Fig. 1d). The lag phase observed after complete nitrate reduction in

Table 2 Maximum respiratory rates (in μEq/g NOM-h) and extent of biodegradation of different substrates by anaerobic wetland sediment under different redox conditions

Conditions	Respiratory rates			Biodegradation[a] (%)
	γ_d	γ_s	γ_q	
Acetate–AQDS	NA	NA	194 ± 15	12 ± 3
Acetate–AQDS–sulfate	NA	968 ± 35	97 ± 12	48 ± 5
Acetate–AQDS–sulfate–nitrate	1,926 ± 96	1,315 ± 13	56 ± 4	48 ± 5
Propionate–AQDS	NA	NA	42 ± 3	15 ± 2
Propionate–AQDS–sulfate	NA	889 ± 47	394 ± 22	28 ± 2
Propionate–AQDS–sulfate–nitrate	2,671 ± 107	1,505 ± 75	42 ± 0.4	34 ± 6
Lactate–AQDS	NA	NA	1,014 ± 87	29 ± 7
Lactate–AQDS–sulfate	NA	60 ± 4	1,477 ± 34	60 ± 3
Lactate–AQDS–sulfate–nitrate	1,796 ± 54	1,694 ± 186	1,509 ± 242	68 ± 7
Phenol–AQDS	NA	NA	1.9 ± 0.1	33 ± 2
Phenol–AQDS–sulfate	NA	51 ± 4	0.9 ± 0.1	62 ± 5
Phenol–AQDS–sulfate–nitrate	51 ± 7	42 ± 4	0.9 ± 0.05	31 ± 5
p-cresol–AQDS	NA	NA	0.4 ± 0.05	28 ± 4
p-cresol–AQDS–sulfate	NA	56 ± 3	0.9 ± 0.1	46 ± 7
p-cresol–AQDS–sulfate–nitrate	171 ± 2	51 ± 3	4.6 ± 0.05	67 ± 10

Initial concentrations: Acetate (16.6 mM), propionate (9 mM), lactate (10.4 mM), phenol (1.5 mM), *p*-cresol (1.5 mM), nitrate (3 mM), sulfate (2 mM), and AQDS (5 mM). When phenol or *p*-cresol were used as electron donors, the initial concentrations of nitrate, sulfate, and AQDS were 1, 0.625, and 2.5 mM, respectively. Results represent average from triplicate incubations ± 1 SD. Endogenous respiration subtracted in all experimental data presented. Inoculum provided at 10 g DW/L. The units μEq/g NOM-h mean micro-electron equivalents transferred per g NOM per hour

DW dried weight, *NOM* natural organic matter, *NA* not applicable, *AQDS* anthraquinone-2,6-disulfonate, γ_d denitrifying rate, γ_s sulfate-reducing rate, γ_q AQDS-reducing rate

[a] Extent of biodegradation corrected for the amount of substrates consumed by endogenous activities

these incubations, suggests that the reduction of both AQDS and sulfate was probably linked to the anaerobic oxidation of intermediates, which were produced during *p*-cresol biodegradation coupled to denitrification. Prolonged incubation revealed the capacity of the anaerobic sludge to oxidize the remaining *p*-cresol via reduction of AQDS and sulfate (Fig. 1d).

Incubations with wetland sediment showed syntrophism between sulfate reducers and quinone reducers when propionate was supplied as an electron donor. Certainly, sulfate-reducing activity linked to the anaerobic oxidation of this substrate improved the reduction of AQDS by the wetland sediment (Table 2) due to the accumulation of acetate (up to 0.18 mM). Denitrification also increased the sulfate-reducing activity of the same consortium, when lactate was provided as electron donor (Table 2), as this substrate did not sustain sulfate reduction even in the absence of AQDS and nitrate (data not shown). Acetate produced during lactate utilization by denitrifying microorganisms may explain the promotion of sulfate reduction by this consortium.

The simultaneous occurrence of different respiratory processes (e.g., denitrification, sulfate reduction, quinone reduction, and methanogenesis) observed in several incubations performed in the present study agrees with previous reports illustrating the same scenario when electron donors were supplemented in excess (Holmer and Kristensen 1994; Achtnich et al. 1995; Roy et al. 1997).

Ecological significance

Our results suggest that the microbial reduction of HS may play an important role during the anaerobic

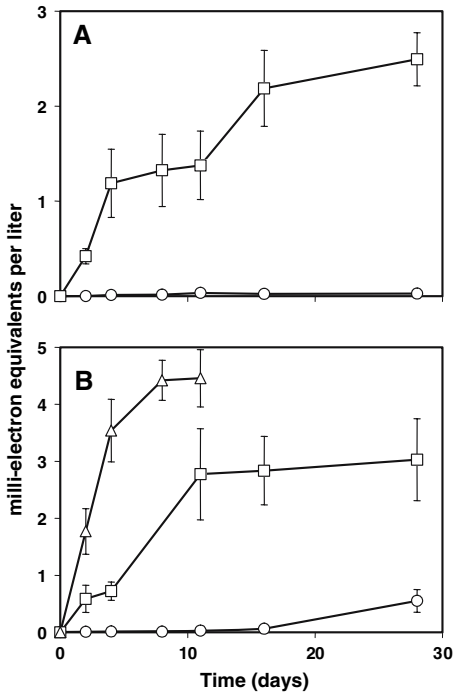

Fig. 4 Respiratory activities during the anaerobic biodegradation of *p*-cresol by anaerobic wetland sediment in the presence of AQDS–sulfate (**a**), and AQDS–sulfate–nitrate (**b**). Concentrations reported refer to the amount of AQDS, sulfate, and nitrate reduced. Results represent the average from triplicate incubations and error bars, the SD. Endogenous respiration subtracted in all experimental data presented. ○, AQDS; □, sulfate; △, nitrate. Initial concentrations: AQDS (2.5 mM), sulfate (0.625 mM), nitrate (1 mM), and *p*-cresol (1.5 mM)

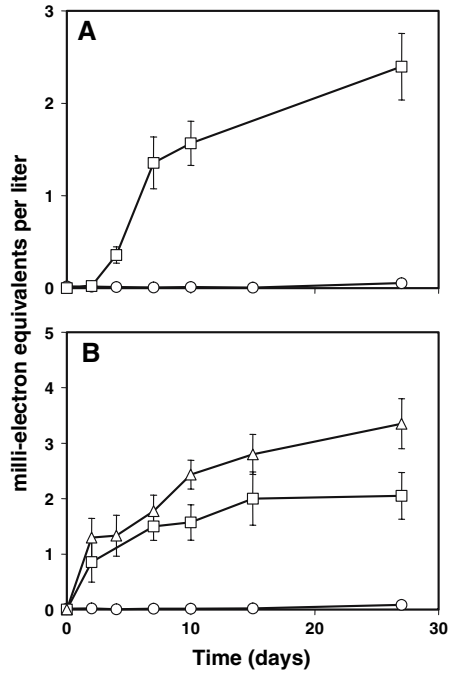

Fig. 5 Respiratory activities during the anaerobic biodegradation of phenol by anaerobic wetland sediment in the presence of AQDS–sulfate (**a**), and AQDS–sulfate–nitrate (**b**). Concentrations reported refer to the amount of AQDS, sulfate, and nitrate reduced. Results represent the average from triplicate incubations and error bars, the SD. Endogenous respiration subtracted in all experimental data presented. ○, AQDS; □, sulfate; △, nitrate. Initial concentrations: AQDS (2.5 mM), sulfate (0.625 mM), nitrate (1 mM), and phenol (1.5 mM)

oxidation of several organic pollutants in anaerobic environments despite the presence of alternative electron acceptors, such as sulfate and nitrate. According to our results, AQDS reduction contributed up to 14% of the substrates degraded by the anaerobic granular sludge in the presence of sulfate and nitrate (Table 1). Meanwhile, the contribution of quinone-reducing microorganisms in wetland sediment incubations was only significant when lactate was provided as an electron donor (up to 33% of lactate degraded, Table 2).

The contribution of HS reducing microorganisms is further emphasized by the large number of organic compounds, including priority pollutants like VC, DCE, and toluene (Bradley et al. 1998; Cervantes et al. 2001), which can be oxidized via quinone respiration. Furthermore, HS-reducing microorganisms are widespread in nature and the role of HS can be enhanced by recycling mechanisms, such as the chemical reaction of reduced HS with metal oxides commonly found in anaerobic habitats (Stone and Morgan 1984; Lovley et al. 1996). However, a greater contribution of metal oxides on the recycling of HS is expected in environments that continuously shift from anoxic to aerobic conditions, such as shallow water bodies, so that metal oxides could be replenished. Reduced HS could also be recycled back to their oxidized form by transferring electrons to electron-withdrawing pollutants, such as azo dyes, nitroaromatics, polyhalogenated compounds, and radionuclides (Field and Cervantes 2005). HS could also serve as electron donors for the microbial reduction of different electron acceptors with a higher standard redox potential, such as nitrate, fumarate, perchlorate, arsenate, and selenate (Bruce et al. 1999; Lovley et al. 1999).

Although the physical characteristics of AQDS and HS are quite different, quinones, such as AQDS,

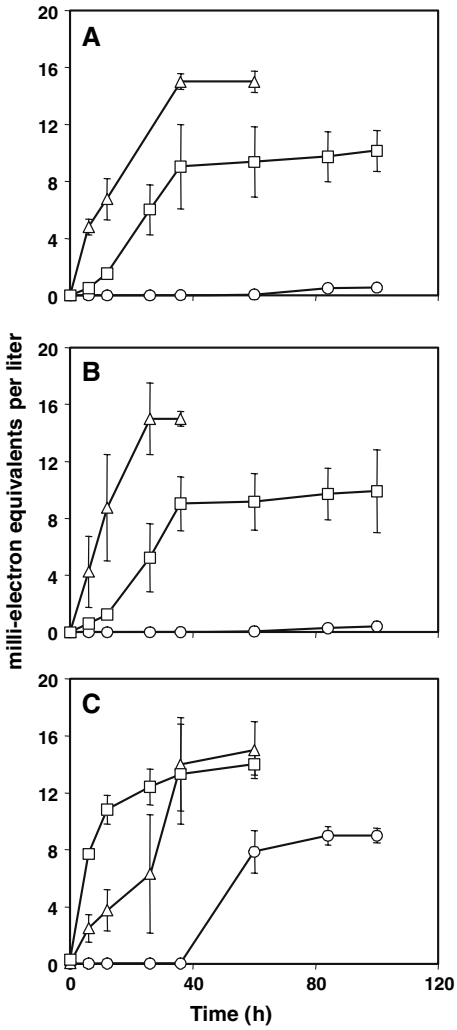

Fig. 6 Respiratory activities during the anaerobic biodegradation of (**a**) acetate (16.6 mM), (**b**) propionate (9 mM), and (**c**) lactate (10.4 mM) by anaerobic wetland sediment in the presence AQDS (5 mM) sulfate (2 mM) and nitrate (3 mM). Concentrations reported refer to the amount of AQDS, sulfate, and nitrate reduced. Results represent the average from triplicate incubations and error bars, the SD. Endogenous respiration subtracted in all experimental data presented. ○, AQDS; □, sulfate; △, nitrate

can be considered as good analogues for the function of HS as a terminal electron acceptor due to the evidence reported in the literature. First, quinones are responsible for the electron accepting capacity of HS during their microbial reduction (Scott et al. 1998; Newman and Kolter 2000). Second, microorganisms recovered as AQDS-reducers showed also the ability to reduce HS (Coates et al. 1998). Third, similar biodegradation rates of different organic pollutants, such as toluene and carbon tetrachloride, were observed when anaerobic consortia had been supplemented either with humic acids or with AQDS (Cervantes et al. 2001, 2004). Furthermore, genetic evidence provided a common biochemical basis for quinone and humus reduction in *Shewanella putrefaciens* MR. The study showed that menaquinone was involved in the electron transport chain of *S. putrefaciens* MR during the reduction of humus and AQDS. Mutants of this organism, lacking the ability to synthesize menaquinone, were unable to reduce AQDS and humus (Newman and Kolter 2000). Thus, quinones are good analogues for the function of humus as a terminal electron acceptor.

The suppression of methanogenesis by quinone respiration, observed in the present study and with several different sediments (Cervantes et al. 2000a), suggests that HS may play a significant role on the diminishment of methane emissions in many different environments, particularly considering the ubiquity and wide diversity of quinone-reducing microorganisms. Furthermore, the methane biosynthesis achieved during the anaerobic mineralization of the carcinogenic solvents, VC, and DCE, by anaerobic sediments, was totally shifted toward CO_2 production when sediments were supplemented with humic acids or AQDS as terminal electron acceptors (Bradley et al. 1998). Further analysis revealed the coupling between VC and DCE mineralization to the reduction of HS. Consequently, HS may have important implications on the global climate preservation, considering the green-house effects of methane.

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