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The role of humic substances in the anaerobic reductive dechlorination of 2,4-dichlorophenoxyacetic acid by *Comamonas koreensis* strain CY01

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ABSTRACT

The role of the humic model compound, anthraquinone-2,6-disulfonate (AQDS), in the anaerobic reductive dechlorination of 2,4-dichlorophenoxyacetic acid (2,4-D) by the Fe(III)- and humic substances (HS)-reducing bacterium, *Comamonas koreensis* strain CY01 was investigated. The results taken as a whole indicated that (i) strain CY01 could couple glucose oxidation to 2,4-D reductive dechlorination; (ii) reductive dechlorination of 2,4-D by strain CY01 was greatly stimulated by the addition of AQDS; (iii) the transfer of electrons from biogenic AH_2QDS to 2,4-D was an abiotic process which can take place in the absence of microorganisms; and (iv) AH_2QDS was reoxidized during the chemical reaction, AQDS can serve again as electron acceptor for microorganisms, thus acting as electron shuttles. All the results suggested that 2,4-D reductive dechlorination by CY01 strain was a biochemical process that oxidizes the electron donors and transfers the electron to the acceptors through redox mediator, AQDS. We proposed the possible mechanism for the HS dependent reduction of 2,4-D. Our results suggested that microbial reduction of HS and subsequent chemical reduction of organic pollutants represent an important path of electron flow in anoxic natural environments. This work is a necessary preliminary step for better understanding the biodegradation of 2,4-D in subsurface soil.

1. Introduction

2,4-Dichlorophenoxyacetic acid (2,4-D) is the third-most widely used herbicide in North America and the most widely used herbicide in the world [1]. It is the active ingredient in several formulations of herbicides recommended for the control of broadleaf weeds. It has major uses in agriculture crops, forestry, turf, non-crop and aquatic weeds [2]. Continuous use and improper disposal may cause soil percolation and groundwater contamination. It causes toxicity in receiving waters and inhibition of biological treatment systems even at low concentrations. The central nervous system is a target organ for the effects of this herbicide in different animal species [3]. Even though human exposure to these herbicides has been associated with numerous clinical manifestations, such as nervous system, liver and kidney damage [4], their continued use is still widely practiced. In situations where these compounds serve as environmental hazards, bioremediation appears to be a potentially powerful clean-up tool [5].

2,4-D does not persist for long in the environment, because it is readily susceptible to microbial degradation [6]. Microbial degradation of 2,4-D has been extensively studied, the degradative pathway for this herbicide has been elucidated and the enzymes involved have been characterized. As a result of this considerable attention, a number of bacterial genera, such as *Arthrobacter*, *Alcaligenes*, *Achromobacter*, *Bacillus*, *Bortedella*, *Burkhoderia*, *Pseudomonas*, *Ralstonia*, *Sphingomonas*, *Sarcina*, *Comamonas* (*Delftia*) and *Sporocytophaga*, are known to degrade 2,4-D, both in mixed and pure cultures [6–9]. Although some studies described 2,4-D degradation by selected bacteria, there have been no reports, to date, on the degradation of 2,4-D improved by humic substances (HS) by *Comamonas* strain. Mechanisms of microbially catalyzed reductive dechlorination of 2,4-D is not well understood and may be species-dependent [10].

Aerobic biotransformation of 2,4-D has been observed in both pure and mixed microbial cultures [11]. However, anaerobic biodegradation of 2,4-D has not been as thoroughly elucidated. It is known that the biological removal of halogen from halogenated compounds under anaerobic conditions occurs by reductive dehalogenation [12]. Reductive dehalogenation reactions catalyzed by anaerobic bacteria are either co-metabolic processes or linked to respiration; a process termed dehalorespiration [13]. In the process of dehalorespiration, an anaerobic bacterium utilizes a

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halogenated compound as a terminal electron acceptor, the reduction of which is coupled to ATP production [14]. Dechlorination is the most commonly reported reductive dehalogenation process, due to the widespread pollution of chlorinated compounds. In the cases of 2,4-D, reductive dechlorination has been observed in sewage sludge [15], pond sediment [16] and a methanogenic Aquifer [17].

HS and quinines [e.g. anthraquinone-2,6-disulfonate (AQDS)], representative of structural moieties in humus, have also been shown to mediate the abiotic reductive dechlorination of polychlorinated pollutants by inorganic electron donors [18]. HS has been also reported to act as an electron mediator to enhance the reduction efficiency of chlorinated aliphatic compounds in aqueous solutions containing bulk reductant [19] and to facilitate the microbially mediated anaerobic dechlorination of chlorinated hydrocarbons under iron-reducing conditions [20]. Although many abiotic experiments have shown that HS catalyzed reductive dechlorination reactions, there has been much less evidence that HS is involved in biological dechlorination [21]. It has been recognized that HS may play an important role in the anaerobic biodegradation and biotransformation of organic as well as inorganic compounds [22]. Biological reductive dechlorination are believed to be the popular approach for the dechlorination of chlorinated ethenes. As little studies concerning the roles of HS in anaerobic biodegradation of 2,4-D by bacteria, it is still not clear so far whether HS are involved in the dechlorination processes of 2,4-D by *Comamonas*. Therefore, knowledge of the role of HS in the dechlorination of 2,4-D is thus required.

Although a capacity for biodegradation of 2,4-D has been found in *Comamonas* microorganisms, the precise mechanism of anaerobic reductive dechlorination is still unknown. In this work, we report 2,4-D reductive dechlorination mediated via HS by *Comamonas koreensis*. A facultative anaerobic bacterium, designated CY01, was isolated successfully from an ancient forest sample collected from Guangzhou, China. It was identified as a strain of *C. koreensis*. CY01 had been tested for its ability to degrade 2,4-D and the kinetics of 2,4-D degradation by *C. koreensis* was explored. The results indicated that Fe(III) and HS-reducing microorganisms, *C. koreensis* CY01, may have a more important role in conversion of 2,4-D in the presence of AQDS than previous considered.

2. Materials and methods

2.1. Bacterial strains

An efficient Fe(III) and HS-reducing bacterium, *C. koreensis* strain CY01, used in the study was isolated from the submerged forest sediment in Sihui City, China. Strain CY01 was tested for a number of key characteristics by using standard procedures [23] and was determined according to Bergey's Manual of Determinative Bacteriology (9th edition, 1994).

2.2. Culture conditions

The strain CY01 was cultured aerobically at 30 °C with shaking at 180 rpm in Luria–Bertani (LB) medium or anaerobically in a defined medium (per liter of distilled water: 2.5 g $NaHCO_3$, 0.25 g NH_4Cl, 0.6 g NaH_2PO_4, 0.1 g KCl, 0.2 g yeast extract, vitamin solution, and mineral solution). After autoclaving and cooling under an atmosphere of N_2/CO_2 (80/20, vol/vol), 5 mmol l^{-1} glucose and 1 mmol l^{-1} AQDS as electron donor and acceptor were added into the defined medium. Strict anaerobic techniques were used throughout the study [24]. All gases were passed through a filter prior to use.

Microbial reduction of 2,4-D, cultures of CY01 were grown overnight in LB medium. The cells were harvested under aerobic conditions and centrifuged for 10 min at 8000 rpm at 4 °C, washed twice, and resuspended in ultrapure water. The microbial reduction of 2,4-D was conducted in 20 ml solutions of 2,4-D (20 ppm), glucose (5 mmol l^{-1}), AQDS (3 mmol l^{-1}), and 1 ml of the cell solution. The solutions (initial pH, 6.5) were dispensed into 25-ml serum vials, bubbled with N_2/CO_2 (80:20) and filtered (0.22 μm filters) before incubation. Then the serum vials were stoppered with butyl rubber bungs and crimped with aluminium caps at a constant temperature (30 °C) in an anaerobic station (Manufactured In USA By Sheldon Manufacturing Inc. 300N, 26Th Cornelius, or 97113). The control experiments were performed in the same manner except that no electron donor, glucose were added to the 2,4-D solutions [25].

The production of Cl^-, the degradation of 2,4-D and the consumption of glucose was monitored simultaneously to examine the 2,4-D reductive dechlorination activity of CY01 strain. The sample without glucose, electron donor, was control. During the 25-day cultivation period, under anaerobic condition, CY01 was added into the defined medium, including 5 mmol l^{-1} glucose and 20 ppm 2,4-D as electron donor and electron acceptor.

3 mmol l^{-1} AQDS was added into the cell suspensions, including glucose and 2,4-D to examine if HS had capacity to accelerate the reductive dechlorination of 2,4-D by CY01 strain in anaerobic condition.

The concentration of Cl^- and 2,4-D of samples which cells of CY01 were killed by autoclaving (121.3 °C for 20 min) or the cells were omitted within a 25-day incubation period to examine if enhanced 2,4-D reductive dechlorination was the result of abiotic process of AQDS.

The biogenic AH_2QDS can be obtained after autoclaving. After the cells with glucose and AQDS were incubated for 2 days (For 40 h incubation, 3 mM AQDS was completely reduced by strain CY01, date not shown, reported in another paper), the samples were autoclaved to move the cells, and then 2,4-D was added into the samples.

2.3. Reagents

Methanol and acetic acid of high-performance-liquid chromatography (HPLC)-gradient grade was purchased from Shanghai Reagent Co., China. Chemical reagents such as AQDS, 2,4-D, 2,4-dichlorophenol (2,4-DCP) and D(+)-glucose used in the experiment were of analytical grade and used without further purification. Standards of these reagents were purchased from Sigma–Aldrich. Solutions were prepared using ultrapure water and NaOH or HCl in proper amounts was used to get the suitable pH value.

2.4. Analysis techniques

All the samples were centrifuged at 4000 rpm for 15 min and filtered through 0.22 μm syringe filter; the filtrates were stored for all immediate analysis.

The concentration of Cl^- was determined by ion chromatography (Dionex ICS-90) with an ion column (IonPac AS14A 4 mm × 250 mm). A mobile phase consisting of Na_2CO_3 (8.0 mM) and $NaHCO_3$ (1.0 mM) solution was operated at a flow rate of 1.0 ml min^{-1}.

Glucose was determined spectrophotometrically using the method described by Miller [26].

The concentrations of 2,4-D and main aromatic intermediates, resulting from the degradation of 2,4-D were analyzed using a high-performance-liquid chromatography apparatus (waters 1527/2487, made in USA). HPLC conditions were: injector volume, 1 ml;

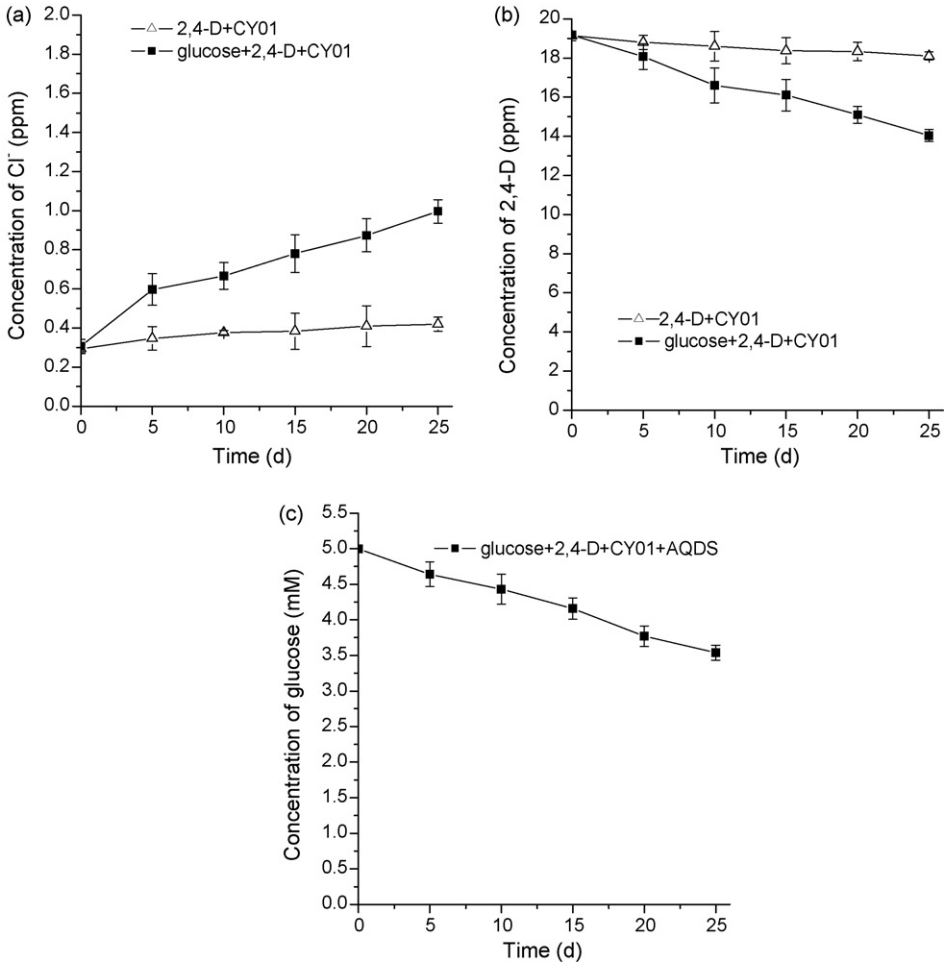

Fig. 1. The effect of *Comamonas koreensis* CY01 on the production of Cl^- (a) and the biodegradation of 2,4-dichlorophenoxyacetic acid (b) and glucose (c). The experiments were performed at 30 °C by addition of 5 mmol l^{-1} glucose, 20 ppm 2,4-D and 1 ml cell solution (1.3×10^8). Bars represent standard deviations of three independent experiments with three replicates: values are means of triplicate ± S.E.

mobile phase flow rate, 1 ml min^{-1}; UV detector wavelength 285 nm; reverse-phase C_{18} column (4.6 mm × 250 mm) and an isocratic mobile phase (vol/vol) (methanol:ultrapure water:acetic acid = 60:38:2).

All the experiments run were conducted in duplicate and analysis of each parameter was done triplicate for each run. Comparison analyse was performed by SPSS10.0 statistical package.

3. Results and discussion

3.1. Isolation and identification of C. koreensis CY01

In anoxic habitats, ferric-iron and humics-reducing microorganisms presumably play an important role in the oxidation of organic matter [27]. Given the abundance of HS in some soils and sediments, electron transfer to HS might also be important if a diversity of microorganisms is capable of this form of respiration [28]. Further understanding of the potential importance of microbial HS reduction requires knowledge about the distribution and diversity of microorganisms that might be responsible for this reduction in sedimentary environments [29].

In this study, a facultative anaerobic bacterium, designated CY01, was isolated from the submerged forest sediment in Sihui City, Guangdong Province, China. The isolate was identified as a strain of *C. koreensis* based on its biochemical, physiological and morphological characteristics as well as the analysis of 16S rDNA sequence and the DNA G + C content. CY01 strain was a Gram-negative, non-motile, non-flagella, rod-shaped (1.2–1.5 μm long, 0.3–0.4 μm wide), oxidase- and catalase-positive (under aerobic growth conditions) bacterium. The G + C content of the DNA was 64.8%. Analysis of 16S rDNA of CY01 indicated that the isolate formed a monophyletic clade with the members of the genus *Comamonas*. The closest phylogenetic relative among the valid species was *C. koreensis*, with 98% 16S rDNA similarity [30]. The optimal temperature and pH value for cultivation was 30–32 °C and 6.5–7.0, respectively. Considering the results reported here, all the experiments were conducted at 30 °C and pH 6.5.

Generally, a wide phylogenetic diversity of microorganisms capable of Fe(III) reduction is also able to reduce HS [28]. Strain CY01 showed the highest Fe(III) and AQDS reduction activity of all the strains isolated in this study. AQDS is a model compound for quinine moieties in humic substances (it is a functional analogue, not a structural analogue or a model humic acid as claimed frequently in the literature). Therefore, AQDS has been used to research HS-reducing instead of HS [27]. CY01 could not only reduce Fe(III) and AQDS, but also 2,4-D with glycerol, glucose, citric acid and sucrose as electron donors under anaerobic growth conditions.

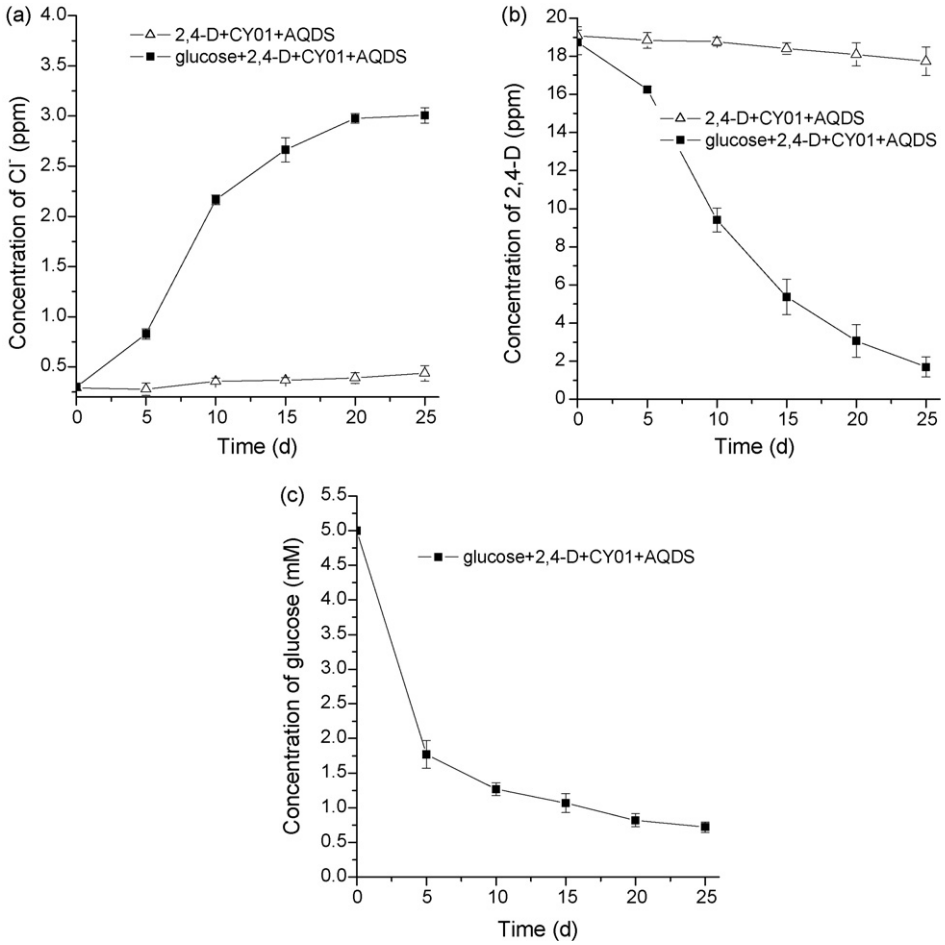

Fig. 2. The effect of AQDS on the production of Cl^- (a) and the biodegradation of 2,4-dichlorophenoxyacetic acid (b) and glucose (c) in the presence of *Comamonas koreensis* CY01. The experiments were performed at 30 °C by addition of 5 mmol l^{-1} glucose, 20 ppm 2,4-D, 3 mmol l^{-1} AQDS and 1 ml cell solution (1.3×10^8). Bars represent standard deviations of three independent experiments with three replicates: values are means of triplicate ± S.E.

3.2. Reductive dechlorination of 2,4-D by C. koreensis CY01 under anaerobic condition

During the 25-day cultivation period, under anaerobic condition, the addition of CY01 to the defined medium, respectively, resulted in a slight degradation of 2,4-D and glucose as well as the production of Cl^- in triplicate samples (the slight levels in the presence of CY01 was to be statistically significant, $P < 0.05$), whereas nearly no degradation of 2,4-D (Fig. 1b), consumption of glucose (Fig. 1c) and production of Cl^- (Fig. 1a) were observed of the control. The dechlorination rate of CY01 was 13.3%. The results indicated that (i) CY01 strain has the ability to reduce 2,4-D directly, whereas the 2,4-D-reducing ability of the microorganism is relatively weak; and (ii) the reduction of 2,4-D by CY01 strain must depend on the presence of glucose, when glucose was used as the electron donor. Under anaerobic or reducing conditions, several chlorinated organic compounds have been shown to be dechlorinated by anaerobic and facultative microorganisms [5]. Our results also demonstrated that 2,4-D could be microbially reduced under anaerobic condition.

3.3. Effect of AQDS on the biodegradation of 2,4-D by CY01 strain

HS are known to stimulate chlorinated organic compounds reduction by serving as electron shuttles between the cells and chlorinated organic compounds [18]. Previous studies demonstrated that *Comamonas* was involved in the process of 2,4-D degradation, but the mechanisms involved were not elucidated during this process [29]. Therefore the role of HS in the anaerobic reductive dechlorination of 2,4-D was next examined. As can be seen from Fig. 2, under anaerobic condition, 2,4-D was reduced significantly by CY01 strain. Concomitant with the decrease of glucose, the amount of Cl^- increased up to ~3 ppm, because of the decomposition of 2,4-D. The dechlorination rate of CY01 was increased by four times (the dechlorination rate of CY01 was up to 50%) in the presence of AQDS. under anaerobic condition. Glucose was oxidized using 2,4-D as electron acceptor by CY01.The positive correlation between the oxidation of glucose and the reduction of 2,4-D. The results suggested that (i) the higher efficiency dechlorination of 2,4-D by the CY01 strain was dependent on the introduction of AQDS which were reduced during the assay. (ii) CY01 can couple glucose oxidation to 2,4-D reductive dechlorination, with AQDS to mediate the course. The stimulation of 2,4-D reductive dechlorination caused by CY01 in the presence of AQDS clearly demonstrated that this particular quinine can serve as an electron shuttle between CY01 and 2,4-D. Quinones in humus, once microbially reduced, can transfer their reducing equivalents to chlorinated compounds [31].

It is accounted that the complete dechlorination of 20 ppm 2,4-D could produce 6 ppm Cl^-. However, during the 25-day incubation, in the presence of AQDS, about 85% 2,4-D was reduced and 80% of glucose was consumed; only about 3 ppm Cl^- was produced. Considerably less dechlorination occurred in incubations lacking glucose. In the presence of AQDS, the removal of Cl^- was less than 50% after 25 day of cultivation, but the biodegradation rate of 2,4-D

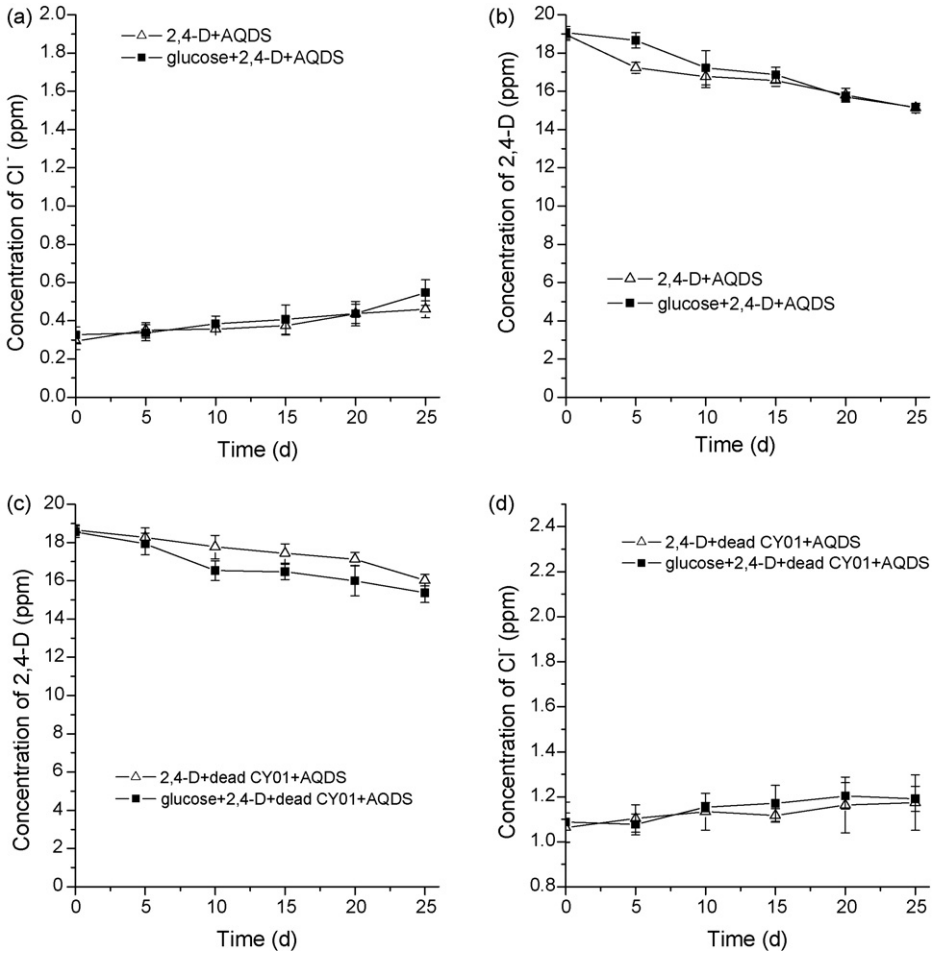

Fig. 3. The production of Cl^- (a, c) and the biodegradation of 2,4-D (b, d) without *Comamonas koreensis* CY01 or with dead *Comamonas koreensis* CY01 cell in the presence of AQDS. The experiments were performed at 30 °C by addition of 5 mmol l^{-1} glucose, 20 ppm 2,4-D, 3 mmol l^{-1} AQDS or 1 ml autoclaved cell solution (1.3×10^8). Bars represent standard deviations of three independent experiments with three replicates: values are means of triplicate ± S.E.

and glucose was ~80–85%. This was an indication that some of the intermediates derived from 2,4-D decomposition remained, mainly as chlorinated organic compounds, such as 2,4-dichlorophenol, 2,4-dichloroanisol (2,4-DCA) and 4,6-dichloroanisolresorcinol, etc. [1]. Within a 25-day period, it appears that the dechlorination of 2,4-D is the result of CY01 action and that the addition of AQDS enhances dechlorination activity. *C. koreensis* CY01 and 2,4-D via HS accelerates the microbial reductive dechlorination of 2,4-D.

AQDS reduction is a good predict of the capacity for HS reducing [28]. Using an intermediate is an effective strategy to accelerate degradation of some environmental pollutants [32]. It was generally considered that HS can serve as redox mediators for microbial reductive dechlorination. This is because reduced HS can directly transfer the electrons gained from microbial reduction to chlorinated compounds. HS are reoxidized during this chemical reaction; HS can serve again as electron acceptors for microorganisms, thus acting as electron shuttles [33–35].

3.4. Effect of AQDS on 2,4-D reductive dechlorination without CY01 strain

Because the addition of AQDS enhanced the conversation of 2,4-D by increasing the rate and extent of dechlorination of this pollutant by *C. koreensis* CY01, we next examined if enhanced 2,4-D reductive dechlorination was the result of abiotic process of AQDS. The results showed the higher background concentration of Cl^- of controls with dead CY01 strain (Fig. 3d) than that of controls without CY01 (Fig. 3a). Cl^- concentration of controls with dead CY01 strain was about 1.1–1.2 ppm, whereas that of controls without CY01 strain was about 0.2-0.3 ppm. The observations can be explained as below: when cells were killed by autoclaving, the component of cytoplasm spilled from the dead cells (including Cl^-), thus Cl^- concentration increased in the controls with dead CY01 strain.

As also seen from Fig. 3, without CY01 cells or with dead CY01 cells, the addition of AQDS hardly affected the production of Cl^- (Fig. 3a and d) and the decreasing of 2,4-D (Fig. 3b and c). The negligible dechlorination achieved in no cells (the dechlorination rate was about 0.63%) or autoclaved cell samples (the dechlorination rate was about 0.34%) in the presence of AQDS indicated that 2,4-D reductive dechlorination was a biological process and not a chemical reaction as reduced AQDS was not able to reduce 2,4-D in the absence of CY01 strain, or when cells were killed by autoclaving. The absence of *C. koreensis* CY01 strain cancelled the stimulating effect of AQDS on 2,4-D-dechlorination in anaerobic condition.

3.5. Effect of the biologically reduced AQDS on reductive dechlorination of 2,4-D

The above results provided evidence that *C. koreensis* CY01 and AQDS were responsible for the enhanced conversion of 2,4-D in anaerobic condition. To further investigate the possible mechanism

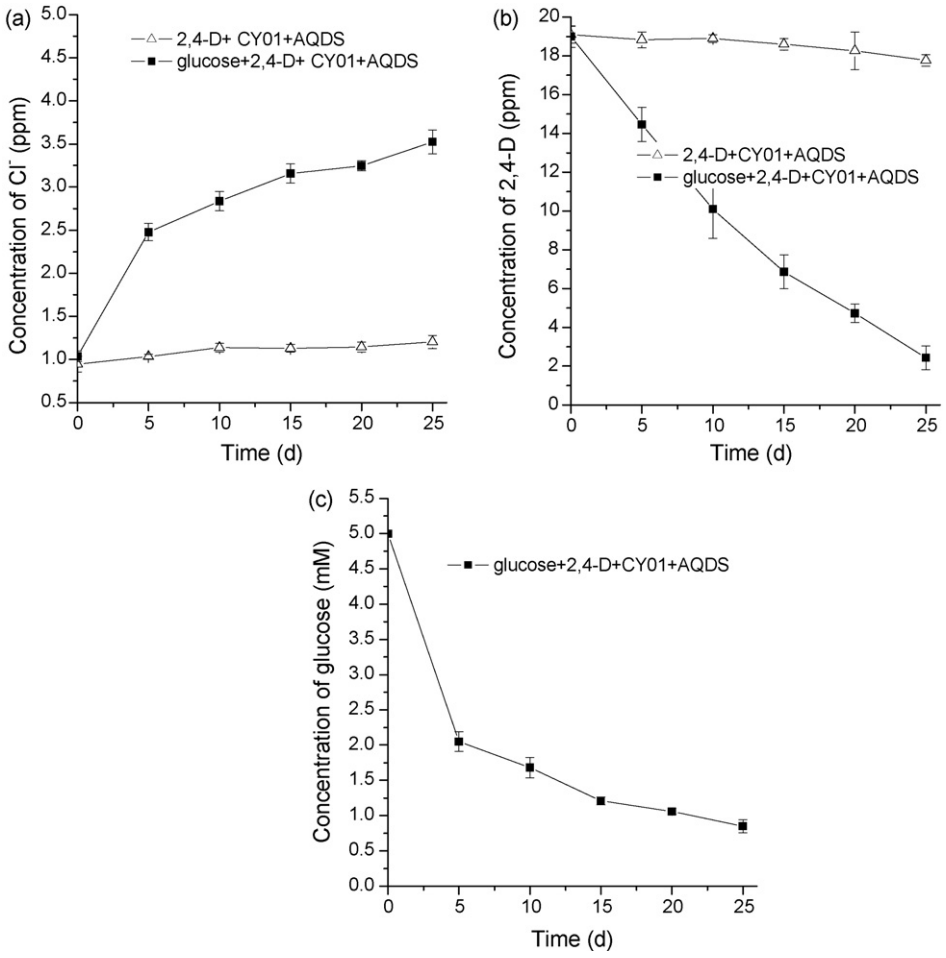

Fig. 4. The effect of biogenic AH_2QDS on the production of Cl^- (a) and the biodegradation of 2,4-dichlorophenoxyacetic acid (b) and glucose (c). The experiments were performed at 30 °C by addition of 5 mmol l^{-1} glucose, 20 ppm 2,4-D, 3 mmol l^{-1} AQDS and 1 ml cell solution (1.3×10^8). Bars represent standard deviations of three independent experiments with three replicates: values are means of triplicate ± S.E.

of microbially catalyzed reductive dechlorination process of 2,4-D in the presence of AQDS, the further research on the effect of biogenic AH_2QDS may be necessary. By autoclaving to exclude the dechlorination activity of strain CY01, and only that of biologically reduced AQDS was investigated. As shown from Fig. 4, controls with dead CY01 strain served as calibration standard, the net production of Cl^- (Fig. 4a) can be calculated as about 2.3–2.7 ppm (the dechlorination rate was about 38.3–45%) during 25 day. The biodegradation rate of 2,4-D and glucose (Fig. 4b and c) almost reached the level of samples without autoclaving (Fig. 2b and c). The biogenic AH_2QDS was shown to directly cause the chemical reduction of 2,4-D when the microorganisms were moved by autoclaving from samples. Once oxidized by 2,4-D, AQDS may again accept electrons from humics and Fe(III)-reducing strain, CY01. The results suggested that 2,4-D reductive dechlorination by CY01 strain is a biochemical process that oxidizes the electron donors and transfers the electron to the acceptors through redox mediator, AQDS. Quinones in humus, once microbially reduced, can transfer their reducing equivalents to chlorinated compounds [21]. Thus, in environments, even small quantities of humics could be important in electron transfer as they could be recycled as electron acceptors numerous times [20].

The proposed mechanism for the HS dependent reduction of 2,4-D is: AQDS is reduced to the corresponding hydroquinone (AH_2QDS) by CY01 strain, then the biogenic AH_2QDS can transfer electrons directly to 2,4-D in a purely chemical reaction, however,

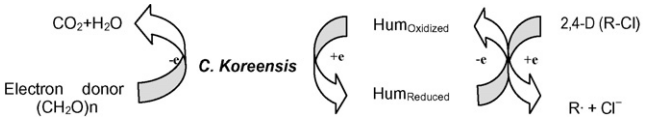

Fig. 5. The hypothetical mechanism describing the enhanced reductive dechlorination of 2,4-D by *C. koreensis* in the presence of humic substances (Hum).

there may be biological interaction between microbial anaerobic humic reduction and 2,4-D reductive dechlorination, which was little known. Because AH_2QDS were reoxidized during the chemical reaction, AQDS can serve again as electron acceptors for microorganisms, thus acting as electron shuttles. This allows an indirect reducing of 2,4-D without direct contact to the bacterial cells in the presence of AQDS, thus enhancing the rates of microbial reduction of 2,4-D. This was proved indirectly by Kappler et al. [36] and Lovely. The proposed mechanism for the HS dependent reduction of 2,4-D is outlined in Fig. 5.

4. Conclusions

In this study, the influence of HS on the dechlorination of 2,4-D by *C. koreensis* CY01 was examined. The results indicated that HS and Fe(III)-reducing bacterium may have more important role in the conversion of 2,4-D in the presence of HS than previously considered. To our knowledge, the present study constitutes the

first report for the anaerobic biodegradation of 2,4-D linked to HS reduction.

Strain CY01 has the ability to reduce 2,4-D directly, whereas the 2,4-D-reducing ability of the microorganism is relatively weak. However, when the humic model compound, AQDS was introduced, the dechlorination activity of strain CY01 was enhanced under the same conditions. The reduction of 2,4-D by CY01 strain with or without AQDS must depend on the presence of glucose, when glucose was used as the electron donor. These results suggested that strain CY01 can couple glucose oxidation to 2,4-D reduction, with AQDS to mediate the course. Electron shuttling between *C. koreensis* CY01 and 2,4-D via HS accelerates the anaerobic biodegradation of 2,4-D. It was believed that CY01 may transfer electrons via HS to 2,4-D. The negligible dechlorination achieved in no cells or autoclaved cell samples in the presence of AQDS indicated that 2,4-D reductive dechlorination was a biological process in anaerobic condition. The biogenic AH_2QDS was shown to directly cause the chemical reduction of 2,4-D when the microorganisms were moved by autoclaving from samples. Higher efficiency of 2,4-D reductive dechlorination depends on the combination of the biologically reduced AQDS and chemical reduction of 2,4-D. AQDS can serve as electron shuttles.

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References

[1] C. Badellino, C.A. Rodrigues, R. Bertazzoli, Oxidation of pesticides by in situ electrogenerated hydrogen peroxide: study for the degradation of 2,4-dichlorophenoxyacetic acid, J. Hazard. Mater. 137 (2006) 856–864.
[2] J.S. Busa, L.E. Hammond, Regulatory progress, toxicology, and public concerns with 2,4-D: where do we stand after two decades? Crop Prot. 26 (2007) 266–269.
[3] A.A. Bortolozzi, A.M.E. Duffard, R. Duffard, M.C. Antonelli, Effects of 2,4-dichlorophenoxyacetic acid exposure on dopamine D2-like receptors in rat brain, Neurotoxicol. Teratol. 26 (4) (2004) 599–605.
[4] I.R. Gonzaĩlez, E.R. Leal, R.F. Cerrato, F.E. Garcıĩa, N.R. Seijas, H.M.P. Varaldo, Bioremediation of a mineral soil with high contents of clay and organic matter contaminated with herbicide 2,4-dichlorophenoxyacetic acid using slurry bioreactors: effect of electron acceptor and supplementation with an organic carbon source, Process Biochem. 41 (2006) 1951–1960.
[5] J.G. Leahy, R.R. Colwell, Microbial degradation of hydrocarbons in the environment, Microbiol. Mol. Biol. Rev. 54 (1990) 305–315.
[6] T. Vroumsia, R. Steiman, F.S. Murandi, J.L.B. Guyod, Fungal bioconversion of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4-dichlorophenol (2,4-DCP), Chemosphere 60 (2005) 1471–1480.
[7] J.O. Ka, W.E. Holben, J.M. Tiedje, Genetic and phenotypic diversity of 2,4-dichlorophenoxyacetic acid (2,4-D) degrading bacteria isolated from 2,4-D-treated field soils, Appl. Environ. Microb. 60 (1994) 1106–1115.
[8] W. Dejonghe, J. Goris, S.E. Fantroussi, M. Hofte, P.D. Vos, W. Verstraete, Effect of dissemination of 2,4-dichlorophenoxyacetic acid (2,4-D) degradation plasmids on 2,4-D degradation and on bacterial community structure in two different soil horizons, Appl. Environ. Microbiol. 66 (2000) 3297–3304.
[9] E.M. Montiel, N.R. Ordaz, C.R. Granados, C.J. Ramiĩrez, C.J.G. Mayer, 2,4-D-degrading bacterial consortium isolation, kinetic characterization in batch and continuous culture and application for bioaugmenting an activated sludge microbial community, Process Biochem. 41 (2006) 1521–1528.
[10] F.W. Picardal, R.G. Arnold, H. Couch, A.M. Little, M.E. Smith, Involvement of Cytochromes in the anaerobic biotransformation of tetrachloromethane by *Shewanella putrefaciens* 200, Appl. Environ. Microbiol. 59 (1993) 3763–3770.
[11] J.W. Neilson, K.L. Josephson, S.D. Pillai, I.L. Pepper, Polymerase chain reaction and gene probe detection of the 2,4-dichlorophenoxyacetic acid degradation plasmid, pJP4, Appl. Environ. Microbiol. 58 (1992) 1271–1275.
[12] B.V. Chang, J.Y. Liu, S.Y. Yuan, Dechlorination of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid in soil, Sci. Total Environ. 215 (1998) 1–8.
[13] C. Holliger, W. Schumacher, Reductive dehalogenation as respiratory process, Antonie Van Leeuwenhoek 66 (1994) 239–246.
[14] A.W. Boyle, V.K. Knight, M.M. Häggblom, L.Y. Young, Transformation of 2,4-dichlorophenoxyacetic acid in four different marine and estuarine sediments: effects of sulfate, hydrogen and acetate on dehalogenation and side-chain cleavage, FEMS Microbiol. Ecol. 29 (1999) 105–113.
[15] M.D. Mikesell, S.A. Boyd, Reductive dechlorination of the pesticides 2,4-D and 2,4,5-T and pentachlorophenol in anaerobic sludges, J. Environ. Qual. 14 (1985) 337–340.
[16] S.A. Gibson, J.M. Suflita, Extrapolation of biodegradation results to groundwater aquifers: reductive dehalogenation of aromatic compounds, Appl. Environ. Microbiol. 52 (1986) 681–688.
[17] S.A. Gibson, J.M. Suflita, Anaerobic biodegradation of 2,4,5-trichlorophenoxyacetic acid in samples from a methanogenic aquifer: simulation by short-chain organic acids and alcohols, Appl. Environ. Microbiol. 56 (1990) 1825–1832.
[18] J.A. Field, F.J. Cervantes, Z.F. Van der, G. Lettinga, Role of quinones in the biodegradation of priority pollutants: a review, Water Sci. Technol. 42 (2000) 215–222.
[19] G.P. Curtis, M. Reinhard, Reductive dehalogenation of hexachloroethane, carbon tetrachloride, and bromoform by anthrahydroquinone disulfate and humic acid, Environ. Sci. Technol. 28 (1994) 2393–2401.
[20] D.R. Lovley, J.D. Coates, E.L. Blunt-Harris, E.J.P. Phillips, J.C. Woodward, Humic substances as electron acceptors for microbial respiration, Nature 382 (1996) 445–448.
[21] F.J. Cervantes, L.Vu-Thi-Thu.G. Lettinga, J.A. Field, Quinone-respiration improves dechlorination of carbon tetrachloride by anaerobic sludge, Appl. Microbiol. Biotechnol. 64 (2004) 702–711.
[22] Y.G. Hong, J. Guo, Z.C. Xu, M.Y. Xu, G.P. Sun, Humic substances act as electron acceptor and redox mediator for microbial dissimilatory azoreduction by Shewanella decolorationis S12, J. Microbiol. Biotechnol. 17 (2007) 428–437.
[23] P. Gerhardt, R.G.E. Murray, W.A. Wood, N.R. Krieg, Methods for General and Molecular Bacteriology, American Society for Microbiology, Washington, DC, 1994.
[24] J.M. Vargas, K. Kashefi, E.L. Blunt-Harris, D.R. Lovely, Microbiological evidence for Fe(III) reduction on early Earth, Nature 395 (1998) 65–67.
[25] L. Apper, D. Mcknight, J. Robinfulton, E.B. Harris, K. Nevin, D. lovely, P. Hatcher, Fulvic acid oxidation state detectionusing fluorescence spectroscopy, Environ. Sci. Technol. 36 (2002) 3170–3175.
[26] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 31 (1959) 426–428.
[27] L.S. Kristina, K. Andreas, S. Bernhard, Enrichment and isolation of ferric-iron- and humic-acid-reducing bacteria, Methods Enzymol. 397 (2005) 58–70.
[28] D.R. Lovley, J.L. Frage, E.L. Blunt-Harris, L.A. Hayes, E.J.P. Phillips, J.D. Coates, Humic substances as a mediator for microbially catalyzed metal reduction, Acta Hydroch. Hydrob. 26 (1998) 152–157.
[29] J.D. Coates, D.J. Ellis, E.L. Blunt-Harris, C.V. Gaw, E.E. Roden, D.R. Lovley, Recovery of humic-reducing bacteria from a diversity of environments, Appl. Environ. Microb. 64 (1998) 1504–1509.
[30] Y.H. Chang, J.I. Han, J. Chun, K.C. Lee, M.S. Rhee, Y.B. Kim, K.S. Bae, *Comamonas koreensis* sp. nov., a non-motile species from wetland in Woopo, Korea, Int. J. Syst. Evol. Microbiol. 52 (2002) 377–381.
[31] F.J. Cervantes, L. Vuthith, G. Lettinga, J.A. Field, Quinone-respiration improves dechlorination of carbon tetrachloride by anaerobic sludge, Environ. Biotechnol. 64 (2004) 702–711.
[32] M.L. Fultz, R.A. Durst, Mediator compounds for the electrochemical study of biological redox systems, Anal. Chim. Acta 140 (1982) 1–18.
[33] D.R. Lovley, Microbial reduction of iron. Manganese and other metals, Adv. Agron. 54 (1995) 175–231.
[34] D.R. Lovley, Bioremediation of organic and metal contaminants with dissimilatory metal reduction, J. Ind. Microbiol. 14 (1995) 85–93.
[35] D.R. Lovley, I.D. Coates, D.A. Saffarini, D. Lonergan, Dissimilatory iron reduction, in: G. Winkelman, C.I. Carrano (Eds.), Iron and Related Transition Metals in Microbial Metabolism, Harwood Academic Publishers, Switzerland, 1997, pp. 187–215.
[36] A. Kappler, M. Benz, A. Brune, B. Schink, Electron shuttling via humic acids in microbial iron(III) reduction in a freshwater sediment, FEMS Microbiol. Ecol. 47 (2004) 85–92.

Glossary

2,4-D: 2,4-dichlorophenoxyacetic acid
AQDS: anthraquinone-2,6-disulfonate
HS: humic substances
LB: Luria–Bertani
AH_2QDS: anthrahydroquinone-2,6-disulfonate
HPLC: high-performance-liquid chromatography