Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2020.25727

191 (2020) 417–425
July

Sodium phthalate-2-sulfonate improves the biodegradation of dye reactive blue 13 and pentachlorophenol by *Pseudomonas* sp. in anaerobic conditions

Yongxing Qian[a,b,c,d], Kefeng Zhang[a,c,d], Huixia Jin[a,c,d], Lecheng Lei[b], Huining Zhang[a,c,d], Jianqing Ma[a,c,d,*]

[a]*Ningbo Institute of Technology, Zhejiang University, Ningbo 315000, China, Tel. (+86-574)88130283; Fax: (+86-574)88130283; emails: majq@nit.net.cn (J. Ma), qianyx312@nit.net.cn (Y. Qian), kfzhang@nit.zju.edu.cn (K. Zhang), hxjin@nit.zju.edu.cn (H. Jin), lclei@zju.edu.cn (L. Lei), zhanghn@nit.zju.edu.cn (H. Zhang)*
[b]*Key Laboratory of Biomass Chemical Engineering of Ministry of Education, Zhejiang University, Hangzhou 310027, China*
[c]*Ningbo Key Laboratory of Urban and Rural Water Pollution Control Technology, Ningbo 315000, China*
[d]*Ningbo Research Institute, Zhejiang University, Ningbo 315100, China*

Received 21 August 2019; Accepted 21 February 2020

ABSTRACT

Electron transfer between a microbe and a pollutant is often the rate-limiting stage of pollutant degradation. Strategies to increase the rate of electron transfer are thus of fundamental importance to accelerating the rate of biodegradation. This study investigated the impact of the redox mediator sodium phthalate-2-sulfonate (AQS) on *Pseudomonas* sp.-mediated degradation of reactive blue 13 (RB13) and pentachlorophenol (PCP) under anaerobic conditions. In a system containing 10 mg/L PCP and 100 mg/L RB13, at an optimized AQS concentration of 0.3 mM, the rates of dechlorination of PCP and biodecolorization of RB13 were increased 2- and 4-fold, respectively. The kinetic constants V_{max} and K_m were obtained from dynamic experiments and were respectively calculated to be 5.16 mg/L h, 33.75 mg/L in the presence of AQS and 4.2 mg/L h, 90.82 mg/L in the absence of AQS. When the concentration of AQS was less than 0.5 mM, there was no significant effect on bacterial growth. The dosing method of AQS had a large influence on the change in degradation; separate addition (bacteria and then AQS) improved the removal of pollutants more than simultaneous addition. Finally, the intermediate degradation products of the pollutants were investigated.

Keywords: Reactive blue 13; Pentachlorophenol; Extracellular polymer substances; Redox mediator

1. Introduction

Wastewater released from dye manufacturing plants contains dyes and a host of other chemicals, many of which are categorized as persistent organic pollutants [1]. Therefore, the disposal of these wastes directly into the environment is harmful and can kill many aquatic organisms.

The methods for removing wastes from manufacturing effluents include coagulation, flotation, adsorption, oxidation, filtration, and biotreatment [2]. Among these methods, biotreatment technology is the most low-cost and environmentally friendly method and has thus been extensively studied [3,4]. However, microbial degradation of pollutants is generally a time-consuming process [5,6]. The transfer of electrons between the microbe and pollutant is the rate-limiting step of pollutant degradation, a finding that has been well established in the literature [7,8]. Therefore, the question of how to increase the rate of electron transfer between microbes and pollutants is of central importance to accelerating the overall rate of biodegradation [9,10].

* Corresponding author.

Electron shuttling materials, that is, redox mediators (RMs), have been extensively studied as catalysts to promote reactions. RMs are organic substances that can be reversibly oxidized and reduced and can be used as an electron carrier in redox reactions. RMs accelerate the reaction by lowering the activation energy of the total reaction. In some cases, RMs are required for a reaction to proceed in real-world conditions. In general, RMs include humic substances [11] and quinoid compounds (such as guanidine-2,6-disulfonic acid, 1,4-benzoquinone, 1,4-naphthoquinone, 4-naphthoquinone, menaquinone and lawsone) [12], and promote electron transport and redox reactions between many inorganic and organic substances. Under optimal conditions, the rate of biological degradation can be increased by several orders of magnitude [12].

The use of RMs to improve the biodecolorization of dyes has been a topic of extensive research [12–14]. Our prior publication showed that sodium phthalate-2-sulfonate (AQS) can improve the rate of biodegradation of reactive blue 13 (RB13) by *Pseudomonas* sp. [15]. However, there is not much published data on the ability of RMs to improve the biodegradation of pentachlorophenol (PCP), an industrial product that was once used worldwide and remains in the environment for a long time [16]. Tong et al. found that the addition of humic acids led to a 2.2-fold improvement in the degradation rate of PCP by microorganisms [17]. Zhang and Katayama [18] reported that humans are very important for the reductive dechlorination of PCP by microbial communities found in soil. However, other RMs, such as anthraquinone-2,6-disulfonate (AQDS), 1,4-naphthoquinone, hydroquinone, and vitamin B12, have been found to have no dechlorination activity. This surprising finding is possibly due to the intricacies of the microbial communities used in the various studies; it is possible that PCP is only degraded by particular bacterial strains [19,20]. Chen et al. [21] investigated the effect of AQDS on soil microbes' anaerobic transformation of PCP and found that AQDS accelerates the transformation of chlorinated contaminants by increasing the relative abundance of indigenous AQDS-utilizing *Geobacter* sp. in paddy soil. Of note, all of these previous studies indicated that RMs can improve PCP dechlorination to some degree under anaerobic conditions. However, the ability of RMs to improve bacterial degradation of other pollutants remains an under-studied topic to date.

In a preliminary experiment, we found that PCP could inhibit the degradation of RB13 by *Pseudomonas* sp. in a co-pollutant system. Indeed, the RB13 biodecolorization time increased from 60 to 360 h as the amount of PCP was increased from 0 to 20 mM. We hypothesized that the introduction of RMs could improve the efficiency of bacterial degradation of contaminants, namely that AQS could improve the rate of biodegradation of RB13 by *Pseudomonas* sp. [15]. In this work, we tested the role of AQS in the anaerobic biodegradation of RB13 and PCP by *Pseudomonas* sp.

2. Materials and methods

2.1. Bacterial maintenance and growth

The *Pseudomonas* sp. were collected from an aerobic tank of the SiBao sewage treatment plant in Hangzhou, China [22]. Bacteria used in all batch experiments were cultivated in serum bottles (510 mL), and the medium composition was as follows: glucose (500 mg/L), $(NH_4)_2SO_4$ (96 mg/L), KH_2PO_4 (15 mg/L), K_2HPO_4 (10 mg/L), $MgSO_4$ (12.5 mg/L), $FeSO_4$ (12.5 mg/L), and was maintained at pH ~7.0. The serum bottles were sealed with a rubber septum by a screw cap and filled with N_2 to maintain anaerobic conditions. Glucose was used as a substrate. To adjust pH, 1 M NaOH or 1 M HCl solution was used as necessary. All incubations were carried out in a temperature-controlled incubator at 33°C and pH 7 (the optimal condition for RB13 decolorization by *Pseudomonas* sp. [22]).

2.2. Experimental procedures

The degradation studies were performed in 500 mL of medium with a 5 mL bacterial inoculum and were kept sealed in dark conditions at 33°C. After the bacteria had grown for 24 h, RB13 and PCP were injected into these bottles to initial concentrations of RB13 and PCP of 100 mg/L and 0–20 mg/L, respectively. The RMs AQS was purchased from Sinopharm Chemical Reagents, (Beijing). The AQS stock solution was prepared as follows: 1 g of AQS solution was weighed and dissolved in a 1,000 mL brown volumetric flask, and the AQS working solutions used in experiments were diluted from the AQS stock solution. In AQS conditions, the AQS was added to the bacterial cultures at the same time as RB13 or PCP. Conditions with AQS added to the system were defined as the "AQS presence" condition (AQS+), and conditions without AQS were defined as the "AQS absence" condition (AQS-). One milliliter samples were taken at regular intervals and centrifuged at 8,000 g for 10 min. The supernatants were then analyzed for different parameters. All experiments were carried out in triplicate.

2.3. Extraction of PCP from different parts of bacteria

A modified heat extraction method [23] was used to isolate the loosely bound bacterial extracellular polymeric substances (LB-EPS) and tightly-bound extracellular polymeric substances (TB-EPS). The bacteria were first pelleted by centrifugation at 8,000 g for 10 min and then were resuspended in 15 mL 0.05% NaCl solution that was pre-heated to 70°C to ensure the bacterial suspension reached an immediate temperature of at least 50°C. Next, the bacterial suspension was vortexed for 1 min, centrifuged at 8,000 g for another 10 min, and then the supernatant was filtered through a 0.22 μm cellulose acetate membrane to obtain the LB-EPS.

The TB-EPS was collected by further treating the remaining pelleted bacteria as follows: (1) the bacteria without LB-EPS were resuspended again in 15 mL of 0.05% NaCl solution; (2) the suspension was heated to 60°C in a water bath for 30 min; (3) the bacterial mixture was then centrifuged at 12,000 g for 30 min, and the supernatant was filtered through a 0.22 μm cellulose acetate membrane to generate the TB-EPS. The remaining pellet was considered to be bacteria without EPS. The PCP was extracted from LB-EPS, TB-EPS, and cells without EPS with ethyl acetate (99.5%) and acetonitrile high-performance liquid chromatography (HPLC grade) for further analysis [24]. Previous

experiments indicated that a 70/30 ($V_{ethanol}/V_{water}$) mixed solvent was effective for extracting RB13 from bacteria solvent [25]. RB13 was extracted from bacteria without EPS using this mixed solvent.

2.4. Analytical methods

The residual RB13 concentration in the supernatant was analyzed by measuring the absorbance at 570 nm with a UV-Visible spectrophotometer. However, this analysis wavelength for RB13 was too close to that of bacterial content (measured at 600 nm), so Walker's method was to account for the interference of RB13 on the determination of OD600 [26]. The specific methods used were as follows: first, OD600 of the sample was measured, and then the bacteria were pelleted out via high-speed centrifugation (8,000 g) for 5 min. The OD600 of the resulting supernatant was again measured to determine the absorbance due to RB13 or other interference, and the OD600 value of bacteria was considered to be the difference between the two values. Thus, OD600 = OD600 (before centrifugation)–OD600 (after centrifugation).

Samples were periodically collected, centrifuged, and then analyzed to determine the residual RB13 concentration at λ_{max} (570 nm). The residual PCP concentration was determined using (HPLC, Agilent 1260, USA) at λ_{max} (220 nm). The metabolites of PCP were determined by HPLC after extraction by ethyl acetate (99.5%) and acetonitrile (HPLC grade), the detailed steps of which have been previously described [24].

The glucose concentration was measured by the phenol sulfuric acid method [27]. The concentration of chloride ions was detected using an ion chromatograph (DIONEX ICS-1100, USA) equipped with an SI-90 4E column and a conductivity detector. The dissolved oxygen (DO) concentration and the oxidation–reduction potential (ORP) were measured with a DO analyzer and redox-potential meter, respectively.

3. Results and discussion

3.1. Selection of RMs

Two water-soluble RMs were chosen for follow-up experiments. One was AQS, with a molecular formula of $C_{14}H_7NaO_5S$, and a molecular weight of 310 g/mol. It is a yellow crystal, soluble in hot water, and slightly soluble in cold water; the other was riboflavin (RF) which has a water solubility of 120 mg/L at 27.5°C and is resistant to oxidation. However, RF undergoes irreversible decomposition after exposure to light or ultraviolet irradiation.

In previous experiments, the decolorization of RB13 by bacteria in the presence of RMs was studied, and those results are shown in Fig. 1. Both AQS and RF clearly increased the extent of dye decolorization. However, many previous studies have shown that AQS is more effective at removing azo dyes than other RMs. For example, Kudlich et al. [28] compared RMs such as AQS, lawsone (LAW), AQDS, benzyl viologen (BV), methyl viologen (MV), flavin adenine dinucleotide (FAD), and ethyl viologen (EV). In tests of the ability to cause decolorization of acid red 27, AQS was the most effective. The RMs, sorted by effect size, were AQS (10.5 times) > LAW (10 times) > AQDS (6.5 times) > BV (2 times) > MV (1.5 times) = FAD (1.5 times) > EV (no effect). However, considering that RF produces a pronounced yellow color when dissolved in water that persists even after degradation, AQS was chosen for subsequent experiments.

Next, we compared the impact of adding AQS to a system with continuous addition of 100 mg/L RB13. As shown in Fig. 2, the decolorization rate was only 60% without AQS (bottles #1 and 2), however, the rate of decolorization of RB13 was significantly improved with the addition of 0.05 mM AQS, leading to nearly 100% decolorization (bottle #3). This finding indicated that, although there have been prior reports that the intermediate product can be toxic to *Pseudomonas* sp. [22], it did not completely inactivate the bacteria. When AQS was added, electron transfer was

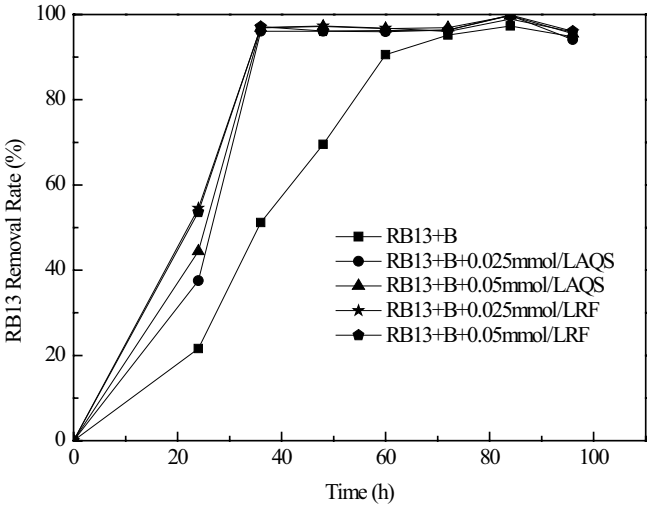

Fig. 1. Effect of AQS and RF on the biodecolorization of 100 mg/L RB13.

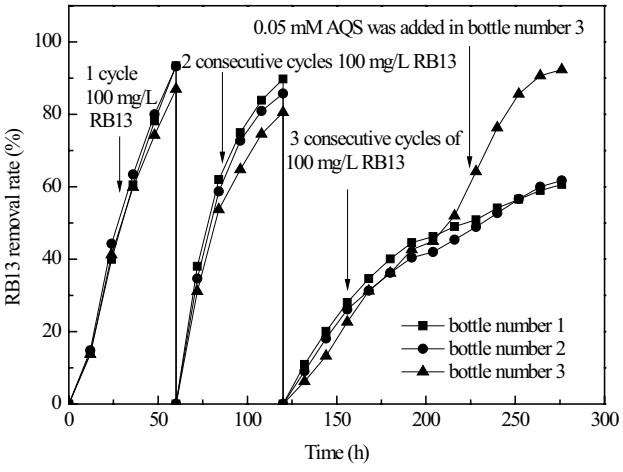

Fig. 2. Impact of AQS on biodegradation during a continuous 100 mg/L RB13 addition process. (100 mg/L RB13 was consecutively added to the serum bottles for biodegradation for 3 cycles).

promoted, thereby accelerating the decolorization rate of RB13.

3.2. Effect of AQS concentration on the biodecolorization of RB13

The impact of AQS dosing method on the decolorization of RB13 was investigated. These studies showed that the dosing method had a significant influence on the decolorization of RB13 (Figs. 3a and b). Stepwise addition of bacteria and then AQS led to more rapid decolorization than the simultaneous addition of AQS and bacteria. This may be related to the delayed generation of electrons, for which there is a lag while the bacteria initially consume glucose (the electron donor). As the bacteria grow, glucose is depleted within 12 h, providing enough electrons for the bacteria to grow to a maximum concentration within 24 h. Therefore, at this 24 h timepoint, electrons are more easily transferred from the bacteria to RB13 (the electron acceptor) after the addition of AQS. In the presence of an increased number of electrons, it is more likely that AQS will contact those electrons and transfer them to RB13, thereby achieving faster biodecolorization (Fig. 3b). Fig. 3b also shows the effect of AQS concentration on the decolorization of RB13. It can be seen that with increasing doses of AQS, the decolorization of RB13 first increases and then stabilizes, however, the higher range of AQS concentrations were not conducive to the decolorization of RB13 [29]. The optimal concentration of AQS was 0.3 mM. Therefore, all subsequent experiments used a fixed concentration of AQS of 0.3 mM.

3.3. Effect of AQS concentration on bacterial growth

To investigate whether AQS itself has adverse effects on the growth of bacteria, bacteria were grown in the presence of various concentrations of AQS (Fig. 4). When the concentration of AQS was in the range of 0–0.4 mM, the density of bacteria (OD600) was consistently above 0.3 after 24 h of growth, indicating that AQS in the selected range had little effect on the bacterial growth.

3.4. Effects of AQS on anaerobic biodegradation of RB13 and PCP by Pseudomonas sp.

3.4.1. Effect of PCP concentration on RB13 biodecolorization in the presence of AQS

In the presence or absence of PCP, 0.3 mM of AQS was added to study the impact on the decolorization of RB13 by bacteria. The initial concentration of RB13 was 100 mg/L, and the initial concentration of PCP ranged from 0–20 mg/L. The results are shown in Fig. 5. After adding AQS, *Pseudomonas* sp. can rapidly decolorize RB13 in the presence of PCP. The catalytic effects of AQS were strongest with lower initial concentrations of PCP. In the case of an initial concentration of PCP less than 8 mg/L, RB13 decolorization as completed within 40 h, which is at least 5 times faster than that without the addition of AQS. However, with an increasing concentration of PCP, the RB13 decolorization effect was notably slower. However, it was completely degraded within 120 h, which was still higher than without the addition of AQS at all (300 h).

Next, we conducted a study to describe the traditional Michaelis-Menten kinetics of bacterial dye degradation in the presence of AQS [30]. This experiment was conducted with various initial dye and PCP concentrations in the presence of 0.3 mM AQS, and was performed in 110 mL serum bottles with a 100 mL inoculum. The Lineweaver–Burk (LB) linear fitting method was used to calculate the kinetics parameters [31]. The equation is as follows,

$$\frac{1}{V}=\left[\frac{K_m}{V_{\max}}\right]\frac{1}{S}+\frac{1}{V_{\max}} \tag{1}$$

where V (mg/L h) is the RB13 removal rate, S (mg/L) is the concentration of RB13, $V_{\max}$ (mg/L h) is the maximum apparent RB13 degradation rate, and K_m (mg/L) is Michaelis–Menten constant.

The experimental data were plotted against 1/V and 1/S according to the LB linear equation, and the corresponding parameter values were obtained according to the obtained

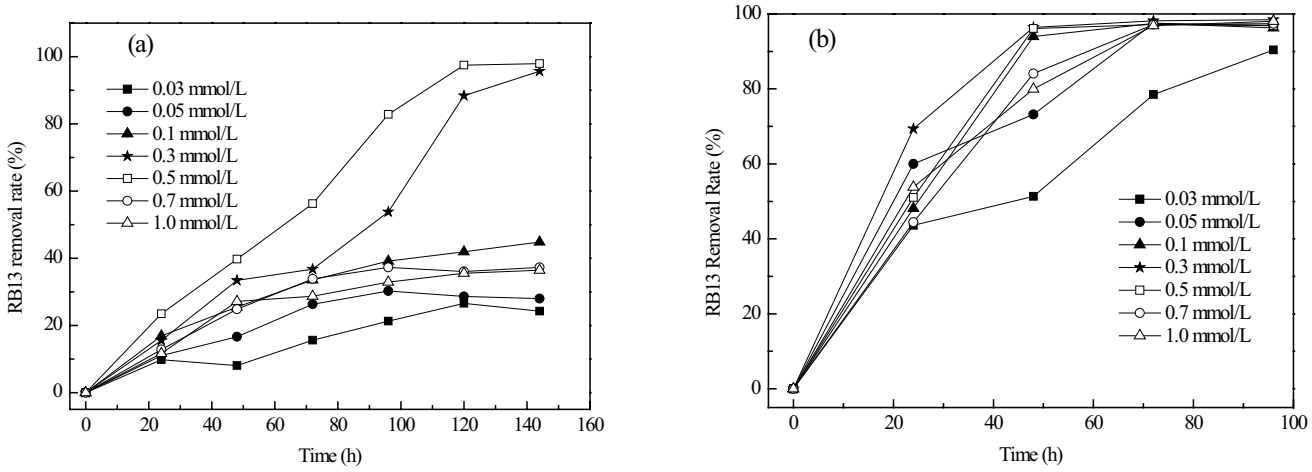

Fig. 3. Effect of AQS dose timing on the biodecolorization of 100 mg/L RB13 (a) simultaneous addition with bacteria and (b) addition after 24 h of bacterial growth.

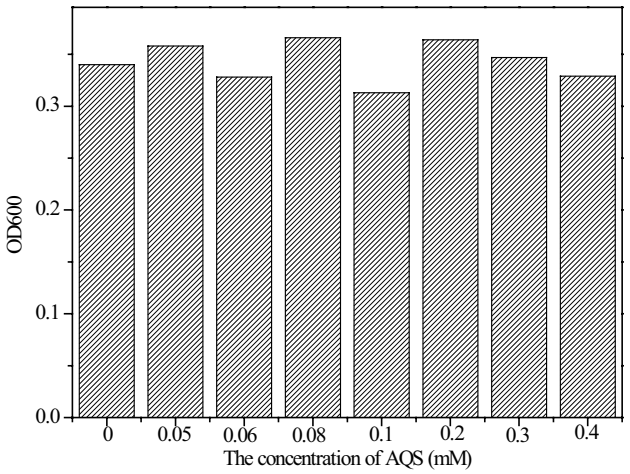

Fig. 4. AQS does not significantly impact bacterial growth after 24 h cultivation in the range of 0–0.4 mM.

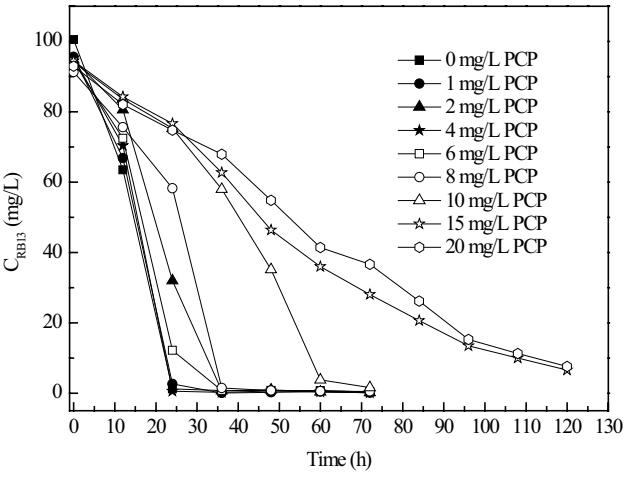

Fig. 5. Effect of PCP concentration on 100 mg/L RB13 decolorization in the presence of 0.3 mM AQS.

slope and intercept values and compared with the condition without AQS.

The decolorization rate of RB13 was accelerated in the presence of AQS (Table 1). For example, the value of V_{max} was increased from 4.20 mg/L h without AQS to 5.16 mg/L h with AQS without PCP in solution. When AQS was added to a solution with 20 mg/L PCP, the decolorization rate V_{max} increased from 0.58 to 0.75 mg/L h. In the presence of AQS, the value of K_m was smaller than without AQS. For example, in the absence of PCP, the K_m was 90.82 mg/L without AQS, but 33.75 mg/L with AQS. The K_m is a parameter that characterizes the affinity of the enzyme to the substrate, with lower values indicating a higher affinity [31]. From this data, the addition of AQS increases the affinity of the bacteria to the dye RB13, thereby facilitating bacterial decolorization of RB13. Furthermore, the K_m values at different PCP concentrations were all reduced in the presence of AQS. A similar tendency toward a simultaneous decrease in K_m and V_{max} was previously reported by Radha et al. [32]. That group found that the K_m and V_{max} values of degradation of methyl violet and congo red by *P. chrysosporium* were simultaneously decreased when immobilized bead diameters increased from 2 to 6 mm.

Table 1
Determination of the kinetic parameters of RB13 biodecolorization at different PCP concentrations in the presence or absence of 0.3 mM AQS

C_{PCP} (mg/L)	K_m (mg/L) AQS+ (AQS-)	V_{max} (mg/L h) AQS+ (AQS-)	R^2 AQS+ (AQS-)
0	33.75 (90.82)	5.16 (4.20)	0.98 (0.97)
5	28.01 (36.70)	2.21 (1.82)	0.97 (0.99)
10	20.20 (30.66)	1.53 (0.91)	0.95 (0.98)
20	5.80 (20.61)	0.75 (0.58)	0.95 (0.99)

3.4.2. Effect of PCP concentration on the growth of bacteria in the presence of AQS

The growth of bacteria was evaluated by a corrected OD600, as described above. Although there was significant variability in the data, it was clear that bacteria could grow in the presence of both RB13 and PCP using glucose as the carbon and electron source. However, the growth rate decreased as the concentration of the PCP increased. Similar observations were reported by Karn et al [33]. Fig. 6 shows the number of bacteria at different PCP concentrations in the presence of AQS. When AQS was not added, an increase in the concentration of PCP reduced the number of bacteria. The OD600 value fluctuated between 0.2 and 0.4, in good agreement with the condition without AQS (fluctuation between 0.15 and 0.4), indicating that the addition of AQS did not significantly affect the growth of bacteria in two contaminants coexist system.

3.4.3. Effect of PCP concentration on glucose-utilization rate of bacteria in the presence of AQS

The metabolism of glucose can be used as an indicator of bacterial activity [34]. In these next studies, the glucose-utilization rate was adopted to directly show the toxic effect of PCP on bacteria metabolism. This experiment examined the effects of different concentrations of PCP on the metabolism of glucose in the presence of AQS. As shown in Fig. 7, glucose consumption was nearly identical in the presence of different concentrations of PCP (0–20 mg/L PCP) with 0.3 mM AQS. This result indicates that the metabolic activity of the bacteria was not significantly affected by PCP in the presence of AQS; up to 95% of the glucose in the media was used by 12 h, and there was no measurable glucose in the media at 24 h. The maintenance of normal metabolic activity could be ascribed to the bacteria itself. *Pseudomonas* sp. can resist PCP toxicity much better than other bacteria, such as acidogenic bacteria and methanogenic bacteria [35].

3.4.4. Fate of PCP in the presence of AQS

The ability of bacteria to remove PCP was tested in a series of conditions of varying initial PCP concentrations,

each of which contained RB13 at a concentration of 100 mg/L. The PCP was degraded while RB13 was decolorized (Fig. 8a). The release of chloride ions (Cl^-) over the course of these experiments provided evidence of PCP degradation. When the concentration of PCP was decreased from 20.92 to 6.73 mg/L, the amount of Cl^- in solution increased from 0 to 3.68 mg/L. Although RB13 releases Cl^- when dissolved in water, the Cl^- released by PCP was calculated by the total released Cl^- minus the Cl^- released by the RB13, which was constant across conditions. The molar ratio of PCP and released Cl^- was nearly 1:2, suggesting that the degradation byproduct of PCP is typically trichlorophenol (TCP).

HPLC was used for further analysis of the PCP degradation intermediates. The HPLC chromatograms showed two peaks during PCP degradation with retention times of 2.701 and 3.971 min, whereas the initial solutions had only 1 peak at 3.97 min (Table 2). The presence of the second peak suggests the formation of an intermediate structure during the degradation process. The identity of that intermediate was determined through a comparison of the chromatographic peaks and retention times of a library of standard compounds. The retention times for 2,3,6-trichlorophenol, 2,4,6-trichlorophenol, and 2,4,5-trichlorophenol are 2.531, 2.702, and 2.594 min, respectively. Thus, the peak of the new compound (2.701) was nearly the same as 2,4,6-trichlorophenol (2.702), and, when a known amount of 2,4,6-trichlorophenol was added into the degradation solution, the signal from the unknown peak was increased. These findings are consistent with prior work by Hendriksen et al. [36] and Tsuno et al. [37], but different from several reports that the intermediate of PCP degradation was dichlorophenol rather than TCP [38,39].

When AQS was added to the system, it accelerated the decolorization of RB13 and promoted the degradation of PCP (Fig. 8b). Indeed, the addition of AQS increased the removal rate of PCP by nearly 2-fold. Measurements of the concentration of Cl^- suggested that the extent of dechlorination was similar regardless of the presence of AQS. For example, in the condition with an initial PCP concentration of 20 mg/L, the PCP concentration decreased to 11.51 mg/L after 108 h, and the Cl^- concentration in the solution increased from 0 to 2.23 mg/L. Based on the molar ratio of PCP lost and Cl^- produced, this product was also determined to likely be trichlorophenol. HPLC was again used to characterize the PCP degradation byproducts, and the main product was verified to be 2,4,6-trichlorophenol. This indicated that the addition of AQS only accelerated the degradation of PCP, but did not change its degradation pathway or intermediate products.

The concentration of DO and the ORP in the solution were measured in order to determine the reduction conditions in the presence and absence of AQS. The experimental results are shown in Table 3. The DO values did not change on the basis of AQS content, remaining around 0 mg/L. However, the ORP was increased from about –550 mV in the absence of AQS to –390 mV in the presence of AQS. When the ORP of the RMs is between –440 and –50 mV, the catalytic effect is strongest [40]. The addition of AQS to the system led to a final ORP around –390 mV, within the range for strong catalytic effects. This ORP range was previously shown to be

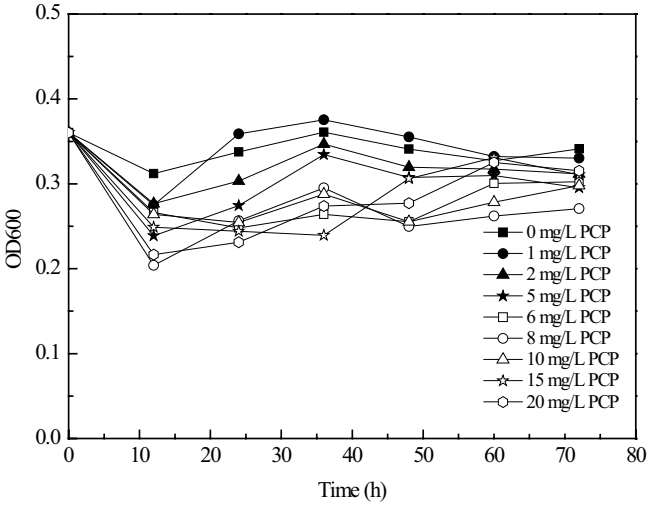

Fig. 6. Effect of PCP concentration on bacterial growth in the presence of 100 mg/L RB13 and 0.3 mM AQS.

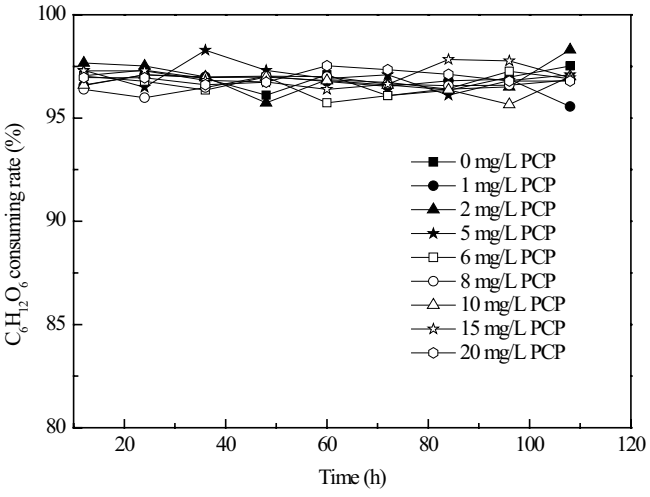

Fig. 7. Effect of PCP concentration on glucose-utilization in the presence of 100 mg/L RB13 and 0.3 mM AQS.

Table 2
HPLC measurements from our study and standard chemical reference compounds

Sample	Untreated water	Treated water		Different kinds of trichlorophenol (TCP)		
				2,3,6-TCP	2,4,6-TCP	2,3,5-TCP
Peak time	3.97	2.701	3.971	2.531	2.702	2.594

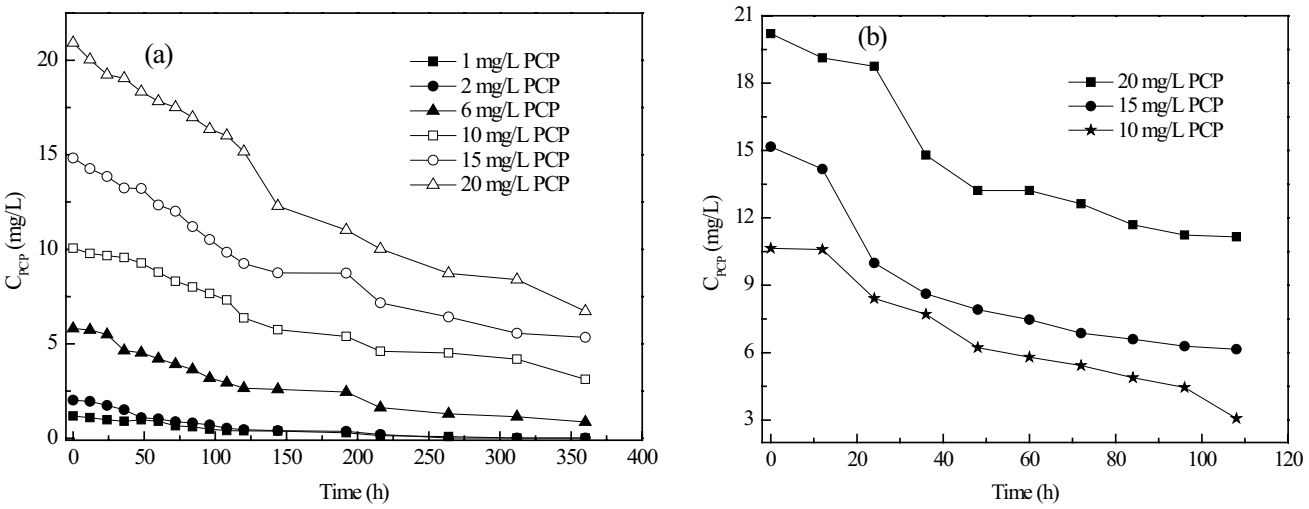

Fig. 8. Effect of AQS on the degradation of different concentrations of PCP (containing 100 mg/L RB13) (a) no AQS and (b) 0.3 mM AQS.

Table 3
Oxidation–reduction potential and dissolved oxygen in solutions with varying concentrations of PCP

	0	1	2	4	6	8	10	15	20	AQS-
E_{redox} (mv)	–390	–396	–404	–390	–401	–380	–394	–388	–391	–541
DO (mg/L)	0	0	0	0	0	0	0	0	0	0.07

beneficial to the dechlorination of PCP and decolorization of RB13 [41].

In order to confirm the role of biosorption in the removal of pollutants, the retained bacteria were collected after the degradation process, and the concentration of PCP in the EPS and bacterial cell wall were tested [42]. The concentrations of both PCP and RB13 were below the limit of detection in both the EPS and bacterial cell wall samples. This result indicated that, regardless of the presence of AQS, both PCP and RB13 were primarily removed by biodegradation rather than biosorption.

4. Conclusions

This study evaluated the impact of AQS on the ability of *Pseudomonas* sp. to degrade RB13 and PCP in anaerobic conditions. The addition of AQS to led to an increase in the rate of *Pseudomonas*-induced RB13 decolorization and PCP dechlorination. In test conditions with 10 mg/L PCP and 100 mg/L RB13 with an AQS concentration of 0.3 mmol/L, the rates of PCP dechlorination and RB13 decolorization were increased 2- and 4-fold, respectively. The kinetic constants V_{max} and K_m were obtained by dynamic experiments, and the values were calculated to be 5.16 mg/L h, 33.75 mg/L in the presence of AQS and 4.2 mg/L h, 90.82 mg/L without AQS. At a concentration of 20 mg/L PCP, the kinetics of degradation was much slower, at 0.75 mg/L h, 5.80 mg/L with AQS and 0.58 mg/L h, 20.61 mg/L without AQS, respectively. Importantly, the addition of low concentrations of AQS (<0.5 mmol/L) had no noticeable effects on microbial growth in culture with various pollutants. However, the timing of the addition of AQS had a large influence on the degradation rates; adding AQS after the addition of bacteria was significantly better than adding both simultaneously. Of note, the pollutants in these experiments were essentially fully degraded, and nearly no pollutants were remained in bacterial EPS or bacterial cell walls.

Acknowledgments

We gratefully acknowledge the financial assistance provided by the National Natural Science Foundation of China (No. 21808200), The Ningbo Natural Science Foundation of China (No. 2018A61028) and the Ningbo major social development projects (No. 2017C510006).

References

[1] X.H. Peng, X.H. Pan, X. Wang, D.Y. Li, P.F. Huang, G.H. Qiu, K. Shan, X.Z. Chu, Accelerated removal of high concentration *p*-chloronitrobenzene using bioelectrocatalysis process and its microbial communities analysis, Bioresour. Technol., 249 (2018) 844–850.

[2] N. Azbar, T. Yonar, K. Kestioglu, Comparison of various advanced oxidation processes and chemical treatment methods for COD and color removal from a polyester and acetate fiber dyeing effluent, Chemosphere, 55 (2004) 35–43.

[3] D.L. Huang, C.J. Hu, G.M. Zeng, M. Cheng, P. Xu, X.M. Gong, R.Z. Wang, W.J. Xue, Combination of Fenton processes and biotreatment for wastewater treatment and soil remediation, Sci. Total Environ., 574 (2017) 1599–1610.

[4] X.Y. Yang, H.Y. Wei, C.X. Zhu, B. Geng, Biodegradation of atrazine by the novel *Citricoccus* sp. strain TT3, Ecotoxicol. Environ. Saf., 147 (2018) 144–150.

[5] M. Fedeila, Z. Hachaichi-Sadouk, L.F. Bautista, R. Simarro, F. Nateche, Biodegradation of anionic surfactants by *Alcaligenes faecalis*, *Enterobacter cloacae* and *Serratia marcescens* strains isolated from industrial wastewater, Ecotoxicol. Environ. Saf., 163 (2018) 629–635.
[6] M.J. Qu, N. Li, H.D. Li, T.W. Yang, W. Liu, Y.P. Yan, X.H. Feng, D.W. Zhu, Phytoextraction and biodegradation of atrazine by *Myriophyllum spicatum* and evaluation of bacterial communities involved in atrazine degradation in lake sediment, Chemosphere, 209 (2018) 439–448.
[7] S.H. Cheng, N. Li, L. Jiang, Y.T. Li, B.H. Xu, W.Z. Zhou, Biodegradation of metal complex Naphthol Green B and formation of iron-sulfur nanoparticles by marine bacterium *Pseudoalteromonas* sp. CF10–13, Bioresour. Technol., 273 (2019) 49–55.
[8] L. Fu, Y.N. Bai, Y.Z. Lu, J. Ding, D.D. Zhou, R.J.X. Zeng, Degradation of organic pollutants by anaerobic methane-oxidizing microorganisms using methyl orange as example, J. Hazard. Mater., 364 (2019) 264–271.
[9] J.D. Rodgers, N.J. Bunce, Treatment methods for the remediation of nitroaromatic explosives, Water Res., 35 (2001) 2101–2112.
[10] F.P. van der Zee, R.H. Bouwman, D.P. Strik, G. Lettinga, J.A. Field, Application of redox mediators to accelerate the transformation of reactive azo dyes in anaerobic bioreactors, Biotechnol. Bioeng., 75 (2001) 691–701.
[11] A. Stolz, Basic and applied aspects in the microbial degradation of azo dyes, Appl. Microbiol. Biotechnol., 56 (2001) 69–80.
[12] F.P. van der Zee, F.J. Cervantes, Impact and application of electron shuttles on the redox (bio) transformation of contaminants: a review, Biotechnol. Adv., 27 (2009) 256–277.
[13] J. Rau, B. Maris, R. Kinget, C. Samyn, G. Van den Mooter, A. Stolz, Enhanced anaerobic degradation of polymeric azo compounds by *Escherichia coli* in the presence of low-molecular-weight redox mediators, J. Pharm. Pharmacol., 54 (2002) 1471–1479.
[14] J. Rau, H.J. Knackmuss, A. Stolz, Effects of different quinoid redox mediators on the anaerobic reduction of azo dyes by bacteria, Environ. Sci. Technol., 36 (2002) 1497–1504.
[15] Y.X. Qian, B. Yang, Z.J. Li, L.C. Lei, X.W. Zhang, Improving the biodecolorization of reactive blue 13 by sodium anthraquinone-2-sulfonate immobilized on modified polyvinyl alcohol beads, Chin. J. Chem. Eng., 23 (2015) 1194–1199.
[16] Y.X. Chen, H.L. Chen, Y.T. Xu, M.W. Shen, Irreversible sorption of pentachlorophenol to sediments: experimental observations, Environ. Int., 30 (2004) 31–37.
[17] H. Tong, M.J. Chen, F.B. Li, C.S. Liu, B. Li, J.I. Qiao, Effects of humic acid on pentachlorophenol biodegrading microorganisms elucidated by stable isotope probing and high-throughput sequencing approaches: the effect of HA on PCP degradation, Eur. J. Soil Sci., 69 (2018) 380–391.
[18] C.F. Zhang, A. Katayama, Humin as an electron mediator for microbial reductive dehalogenation, Environ. Sci. Technol., 46 (2012) 6575–6583.
[19] L. Bosso, G. Cristinzio, A comprehensive overview of bacteria and fungi used for pentachlorophenol biodegradation, Rev. Environ. Sci. Biotechnol., 13 (2014) 387–427.
[20] E. Lopez-Echartea, T. Macek, K. Demnerova, O. Uhlik, Bacterial biotransformation of pentachlorophenol and micropollutants formed during its production process, Int. J. Environ. Res. Public Health, 13 (2016) 1146–1167.
[21] M.J. Chen, H. Tong, C.S. Liu, D.D. Chen, F.B. Li, J.T. Qiao, A humic substance analogue AQDS stimulates *Geobacter* sp. abundance and enhances pentachlorophenol transformation in a paddy soil, Chemosphere, 160 (2016) 141–148.
[22] J. Lin, X.W. Zhang, Z.J. Li, L.C. Lei, Biodegradation of Reactive blue 13 in a two-stage anaerobic-aerobic fluidized beds system with a *Pseudomonas* sp. isolate, Bioresour. Technol., 101 (2009) 34–40.
[23] X.Y. Li, S.F. Yang, Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge, Water Res., 41 (2007) 1022–1030.
[24] S. Singh, R. Chandra, D.K. Patel, V. Rai, Isolation and characterization of novel *Serratia marcescens* (AY927692) for pentachlorophenol degradation from pulp and paper mill waste, World J. Microbiol. Biotechnol., 23 (2007) 1747–1754.
[25] A.R. Binupriya, M. Sathishkumar, D. Kavitha, K. Swaminathan, Aerated and rotated mode decolorization of a textile dye solution by native and modified mycelial biomass of *Trametes versicolor*, J. Chem. Technol. Biotechnol., 82 (2007) 350–359.
[26] G.M. Walker, L.R. Weatherley, Biodegradation and biosorption of acid anthraquinone dye, Environ. Pollut., 108 (2000) 219–223.
[27] H.C. Xu, H.L. Jiang, G.H. Yu, L.Y. Yang, Towards understanding the role of extracellular polymeric substances in cyanobacterial *Microcystis* aggregation and mucilaginous bloom formation, Chemosphere, 117 (2014) 815–822.
[28] M. Kudlich, A. Keck, J. Klein, A. Stolz, Localization of the enzyme system involved in anaerobic reduction of azo dyes by *Sphingomonas* sp. strain BN6 and effect of artificial redox mediators on the rate of azo dye reduction, Appl. Environ. Microbiol., 63 (1997) 3691–3694.
[29] J.A. Field, J. Brady, Riboflavin as a redox mediator accelerating the reduction of the azo dye mordant yellow 10 by anaerobic granular sludge, Water Sci. Technol., 48 (2003) 187–193.
[30] S. Moosvi, H. Keharia, D. Madamwar, Decolorization of textile dye Reactive Violet 5 by a newly isolated bacterial consortium RVM, World J. Microbiol. Biotechnol., 21 (2005) 667–672.
[31] T.P. Thao, H.C. Kao, R.S. Juang, J.C.W. Lan, Kinetic characteristics of biodegradation of methyl orange by *Pseudomonas putida* mt2 in suspended and immobilized cell systems, J. Taiwan Inst. Chem. Eng., 44 (2013) 780–785.
[32] K.V. Radha, I. Regupathi, A. Arunagiri, T. Murugesan, Decolorization studies of synthetic dyes using *Phanerochaete chrysosporium* and their kinetics, Process Biochem., 40 (2005) 3337–3345.
[33] S.K. Karn, S.K. Chakrabarty, M.S. Reddy, Characterization of pentachlorophenol degrading *Bacillus* strains from secondary pulp-and-paper-industry sludge, Int. Biodeterior. Biodegrad., 64 (2010) 609–613.
[34] X.W. Liu, R. He, D.S. Shen, Studies on the toxic effects of pentachlorophenol on the biological activity of anaerobic granular sludge, J. Environ. Manage., 88 (2008) 939–946.
[35] K.A. Mcallister, H. Lee, J.T. Trevors, Microbial degradation of pentachlorophenol, Biodegradation, 7 (1996) 1–40.
[36] H.V. Hendriksen, S. Larsen, B.K. Ahring, Anaerobic degradation of PCP and phenol in fixed film reactors: the influence of an additional substrate, Water Sci. Technol., 24 (1991) 431–436.
[37] H. Tsuno, M. Kawamura, I. Somiya, Anaerobic degradation of pentachlorophenol (PCP) in biological expanded-bed reactor, Water Sci. Technol., 34 (1996) 335–344.
[38] S.J.B. Duff, K.J. Kennedy, A.J. Brady, Treatment of dilute phenol/PCP wastewaters using the upflow anaerobic sludge blanket (UASB) reactor, Water Res., 29 (1995) 645–651.
[39] M.H.R.Z. Damianovic, E.M. Moraes, M. Zaiat, E. Foresti, Pentachlorophenol (PCP) dechlorination in horizontal-flow anaerobic immobilized biomass (HAIB) reactors, Bioresour. Technol., 100 (2009) 4361–4367.
[40] A.B. Dos Santos, I.A.E. Bisschops, F.J. Cervantes, J.B. Van Lier, Effect of different redox mediators during thermophilic azo dye reduction by anaerobic granular sludge and comparative study between mesophilic (30°C) and thermophilic (55°C) treatments for decolourisation of textile wastewaters, Chemosphere, 55 (2004) 1149–1157.
[41] J. Lian, J.B. Guo, G. Feng, G.F. Liu, J.L. Yang, C. Liu, Z.X. Li, L. Yue, L.J. Zhao, Development of bioreactor systems with functional bio-carrier modified by disperse turquoise blue S-GL for disperse scarlet S-BWFL decolorization, Bioresour. Technol., 102 (2001) 11239–11243.
[42] Y.X. Qian, K.F. Zhang, H.X. Jin, L.C. Lei, H.H. Gan, H.N. Zhang. The removal of methyl violet from dye wastewater by *Pseudomonas* sp.: the effect of extra- and intra-cellular substances, Desal. Water Treat., 132 (2018):329–339.

Supplementary information

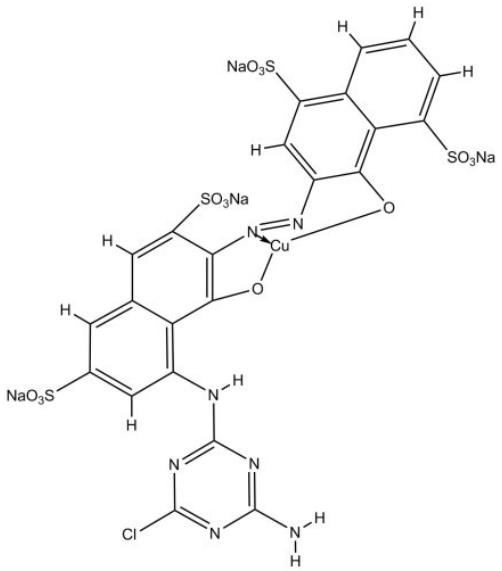

Fig. S1. Molecular structure of RB13.