Synergistic Reductive Dechlorination of 1,1,1-Trichloroethane and Trichloroethene and Aerobic Degradation of 1,4-Dioxane

by

Yihao Luo

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Graduate Supervisory Committee:

Bruce E. Rittmann, Chair
Rosa Krajmalnik-Brown
Chen Zhou

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ABSTRACT

Widespread use of chlorinated solvents for commercial and industrial purposes makes co-occurring contamination by 1,1,1-trichloroethane (TCA), trichloroethene (TCE), and 1,4-dioxane (1,4-D) a serious problem for groundwater. TCE and TCA often are treated by reductive dechlorination, while 1,4-D resists reductive treatment. Aerobic bacteria are able to oxidize 1,4-D, but the biological oxidation of 1,4-D could be inhibited TCA, TCE, and their reductive transformation products. To overcome the challenges from co-occurring contamination, I propose a two-stage synergistic system. First, anaerobic reduction of the chlorinated hydrocarbons takes place in a H_2-based hollow-fiber "X-film" (biofilm or catalyst-coated film) reactor (MXfR), where "X-film" can be a "bio-film" (MBfR) or an abiotic "palladium-film" (MPfR). Then, aerobic removal of 1,4-D and other organic compounds takes place in an O_2-based MBfR. For the reductive part, I tested reductive bio-dechlorination of TCA and TCE simultaneously in an MBfR. I found that the community of anaerobic bacteria can rapidly reduce TCE to cis-dichloroethene (cis-DCE), but further reductions of cis-DCE to vinyl chloride (VC) and VC to ethene were inhibited by TCA. Also, it took months to grow a strong biofilm that could reduce TCA and TCE. Another problem with reductive dechlorination in the MBfR is that mono-chloroethane (MCA) was not reduced to ethane. In contrast, a film of palladium nano-particles (PdNPs), i.e., an MPfR, could the simultaneous reductions of TCA and TCE to mainly ethane, with only small amounts of intermediates: 1,1-dichloroethane (DCA) (~3% of total influent TCA and TCE) and MCA (~1%) in continuous operation. For aerobic oxidation, I enriched an ethanotrophic culture that could oxidize 1,4-D with ethane as the

primary electron donor. An O_2-based MBfR, inoculated with the enriched ethanotrophic culture, achieved over 99% 1,4-D removal with ethane as the primary electron donor in continuous operation. Finally, I evaluated two-stage treatment with a H_2-based MPfR followed by an O_2-MBfR. The two-stage system gave complete removal of TCA, TCE, and 1,4-D in continuous operation.

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Yihao Luo

TABLE OF CONTENTS

Page

LIST OF TABLES

TABLE OF FIGURES

FIGURE

1 INTRODUCTION

1.1. Sources and toxicity of the contaminants

For decades, chlorinated solvents have been used for many commercial and industrial purposes, including as degreasers, cleaning solutions, paint thinners, pesticides, and in resins and glues (Pankow and Cherry 1996, Schaefer et al. 2011). 1,1,1-trichloroethane (TCA) and trichloroethene (TCE) are two of the most widely used solvents (Bakke et al. 2007), since they are non-flammable, volatile, colorless, and lipophilic liquids. They also are common groundwater pollutants as the result of improper disposal and accidental spills (Bakke et al. 2007, Scheutz et al. 2011). TCA causes liver, nervous system, and circulatory problems, while TCE causes liver problems and increases the risk of cancer (Johnson et al. 1998, Dumas et al. 2018). The U.S. Environmental Protection Agency (U.S. EPA) has set up the maximum contaminants level for TCA and TCE of 200 and 5 μg/L, respectively (USEPA 2009).

Physicochemical characteristics of TCA and TCE are summarized in Table 1.1. TCA and TCE are denser than water, which causes them to migrate through an aquifer vertically by the force of gravity. They also are volatile (Henry's constant) and moderately hydrophobic (Log Kow).

Table 1. 1 Properties of 1,1,1-TCA, TCE, dioxane, and their transformation products.

properties and characteristics	Chloroethanes			Chloroethenes			1,4-Dioxane	References
	1,1,1-TCA	1,1-DCA	MCA	TCE	cis-DCE	VC		
Density[a]	1.339	1.176	0.898	1.464	1.284	0.911	1.003	(Haynes 2014)
Henry's constant[b]	0.69	1.80	0.85	1.20	2.50	0.39	1980	(Sander 2015)
Log Kow[c]	2.48	1.76	1.43	2.42	1.86	0.6	-0.27	USEPA, 2014

a: unit of g/mL at 20 °C; b unit of mol m^{-3} kPa^{-1};
c Kow = octanol-water partition coefficient at 25 °C, (mol L^{-1} octanol)/(mol L^{-1} water).

1,4-dioxane (1,4-D) is frequently detected as a co-contaminant with the chlorinated solvents, primarily because of its widespread use as a stabilizer in TCA formulations. Furthermore, TCE was used at many of the same industrial site with TCA, which leads to co-contaminations of 1,4-D, TCA, TCE, and their degradation products (e.g., dichoroethene (DCE), dichloroethane (DCA), vinyl chloride (VC), and monochloroethane (MCA)) (Anderson et al. 2012, Adamson et al. 2015, Karges et al. 2018). Distinct from TCA and TCE, dioxane is miscible in groundwater and does not sorb strongly to aquifer solids. Toxicological studies suggest that dioxane has carcinogenic potential (Stickney et al. 2003, Ohri and Fernandes 2016, USEPA 2018), and 1,4-D is classified as a probable human carcinogen by the U.S. EPA, the International Agency for Research on Cancer (IARC), and the Agency for Toxic Substances and Disease Registry (ATSDR).

1.2. Treatment Options

Various treatment methods, including physical, chemical and biological ones, have been investigated and applied to TCA and TCE removal. Typical physical treatments for TCA and TCE are air stripping and adsorption (Miyake et al. 2003, Wei and Seo 2010),

which remove TCA and TCE from the aqueous phase, but do not destroy them. Zero-valent iron (ZVI) is the most used chemical treatment for TCA and TCE (Kim et al. 2010, Cho and Choi 2010). ZVI rapidly reduces TCA and TCE to ethene and ethane (respectively) through chemical hydrodechlorination (HDC). TCA and TCE also can undergo biological HDC (De Bruin et al. 1992, Grostern and Edwards 2006, Ding et al. 2014, Holliger et al. 1998). Various species of bacteria capture the energy generated in the dechlorination process by "dehalorespiration" (Holliger et al. 1998). For TCA, bacteria in the genera of *Dehalobacter* and *Desulfovibrio* are able to reductively dechlorinate TCA to DCA and finally MCA (Grostern and Edwards 2006, Sun et al. 2002). For TCE, various genera of bacteria (e.g., *Dehalococcoides*, *Dehalobacter*, *Desulfitobacterium*, *Dehalospirillum*, *Desulfuromonas*) are able to reduce TCE to cis-DCE using H_2, pyruvate or acetate as electron donors; only *Dehalococcoides mccartyi* is able to reduce cis-DCE to vinyl chloride (VC) and finally to ethene (Holliger et al. 1998, Maymó-Gatell et al. 2001, Mao et al. 2015).

Since 1,4-D has low volatility (Henry's Law constant 5 $*10^{-6}$ atm m^3 mol^{-1} at 20°C) and does not sorb strongly to aquifer solids, the typical physical treatments (air stripping and adsorption) are not suitable for it. Advanced oxidation processes (AOPs) (e.g., O_3/H_2O_2, UV/TiO_2, and electrochemical) are able to achieve fast removal of 1,4-D (Suh and Mohseni 2004, Coleman et al. 2007, Choi et al. 2010), but are hampered by incomplete removal and high cost. Because of its cyclic ether structure, 1,4-D is more stable to biodegradation than most organic compounds (e.g., alcohols, aldehydes, and carboxylic acids). However, 1,4-D can be biodegraded by certain bacterial species. The first species shown to biodegrade dioxane and grow on it as a sole electron and carbon donor is *Pseudonocardia dioxanivorans* CB1190 (Parales et al. 1994, Mahendra et al. 2007).

Subsequently, other bacteria were found able to oxidize dioxane, e.g., *Acinetobacter baumannii, Rhodococcus aetherivorans,* and *Afipia* sp. strain D1 (Huang et al. 2014, Inoue et al. 2016, Isaka et al. 2016). The key common feature for these bacteria is that they utilize monooxygenases to initiate biodegradation of 1,4-D.

1.3. Challenges and strategies for co-occurrence of TCA, TCE, and dioxane

Although separate treatments of TCA, TCE, and 1,4-D are well studied and into field application, treatment for the co-occurrence of the three contaminants remains unsolved. Because of the large physicochemical differences between 1,4-D and TCA/TCE, traditional treatment methods are not able to remove them simultaneously. For example, the structure of the 1,4-D molecule resists reductive-degradation mechanisms (Adamson et al. 2015), and 1,4-D survives the reductive dechlorination. Furthermore, TCA, TCE, and their reduction products can inhibit the aerobic biodegradation of 1,4-D (Mahendra et al. 2013, Zhang et al. 2016). In principle, AOPs have the capability to remove TCA, TCE, and 1,4-D simultaneously, but AOPs often have incomplete removal and always have high cost.

To overcome the challenges presented by co-contamination, I propose the two-stage synergistic strategy illustrated in Figure 1.1: a first reduction stage and a second oxidation stage. In the first stage, I plan to use reductive a dechlorination process (biotic or abiotic) to convert TCA and TCE to ethene, ethane, and chloride. In the second stage, an aerobic biological-oxidation processes removes 1,4-D and any residual reduction products from the first stage. This two-stage synergy ought to achieve complete removal of TCA, TCE,

and 1,4-D, while minimizing costs. The common feature of both stages is utilizing hollow-fiber membranes to supply H_2 gas (in the first stage) and O_2 gas (in the second stage).

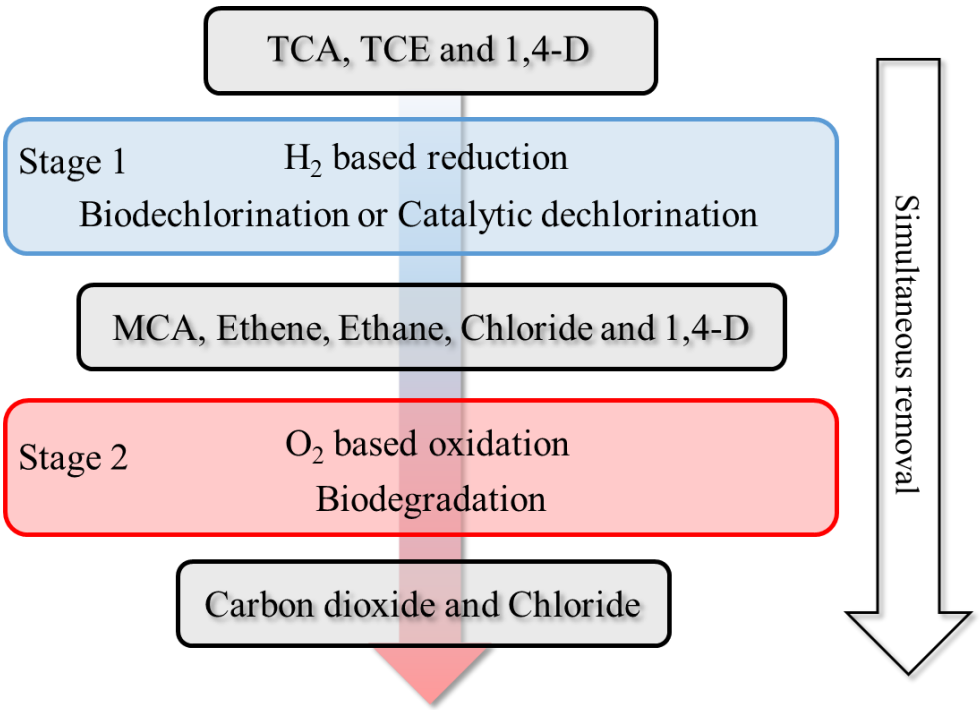

Figure 1. 1 Two-stage strategy for H_2-based simultaneous reduction of TCA, TCE, and O_2-based oxidation of 1,4-D.

2 REDUCTIVE BIO-DECHLORINATION OF 1,1,1-TCA AND TCE

2.1 Background

Research on *in situ* enhanced reductive dechlorination (ERD) has been carried out for decades, and ERD has proved itself as a cost-effective solution toward the removal of TCA, TCE, and their transformation daughter products from contaminated groundwater. Figure 2.1 shows the steps of biological reductive dichlorination of TCE and TCA.

Biological reductive dichlorination of TCE is catalyzed by dehalogenases, which replace chlorine with hydrogen atoms step-wise from TCE to DCE to vinyl chloride (VC) and to ethene. Although many microorganisms partially reduce TCE to DCE or VC -- including the genera *Geobacter*, *Desulfovibrio*, *Desulfuromonas*, *Dehalobacter*, *Desulfitobacterium*, *Dehalobium*, and *Dehalogenimonas* -- *Dehalococcoides mccartyi* is the only known bacterium that can completely dechlorinate TCE to the terminal product ethene. The metabolism of *D. mccartyi* requires a variety of exogenous compounds, such as hydrogen gas, acetate, corrinoids, biotin, thiamine, and vitamin B12, which usually are supplied by coexisting microorganisms within the mixed culture. Hence, *D. mccartyi* is more robust within diverse microbial communities than in pure cultures.

Since the 1980s, anaerobic reductive dechlorination of TCA have been observed in laboratory experiments with marine sediments, methanogenic biofilm reactors, and mixed and pure cultures in batch reactors. Fig. 2.1 shows that TCA is stepwise reduced to DCA to CA and to ethane. A *Dehalobacter* strain TCA1 isolated by Sun et al. (2002) is the first pure culture shown to gain energy and grow during dechlorination of TCA to MCA. Pure

cultures and mixed cultures containing *Dehalobacter* (Dhb) are the most studied for TCA reduction.

Though ERD has been studied for decades, challenges remain, including achieving complete dechlorination of TCA to ethane. Holliger et al. (1990) reported dechlorination of CA to ethane by a *Methanosarcina barkeri* pure culture, but the result has rarely been repeated. Also, *in situ* ERD is a slow process, requiring a long period of time to achieve complete removal of TCE and TCA.

In this chapter, I first enriched a mixed culture that can reduce TCA and TCE simultaneously. Second, I inoculated a H_2-based MBfR with the enriched culture and operated the reactor in batch and continuous modes. Third, I attempted to optimize the operating conditions of the MBfR to minimize the effluent concentration of incomplete-reduction products. Finally, I investigated the possibility of reducing MCA and ethene to ethane.

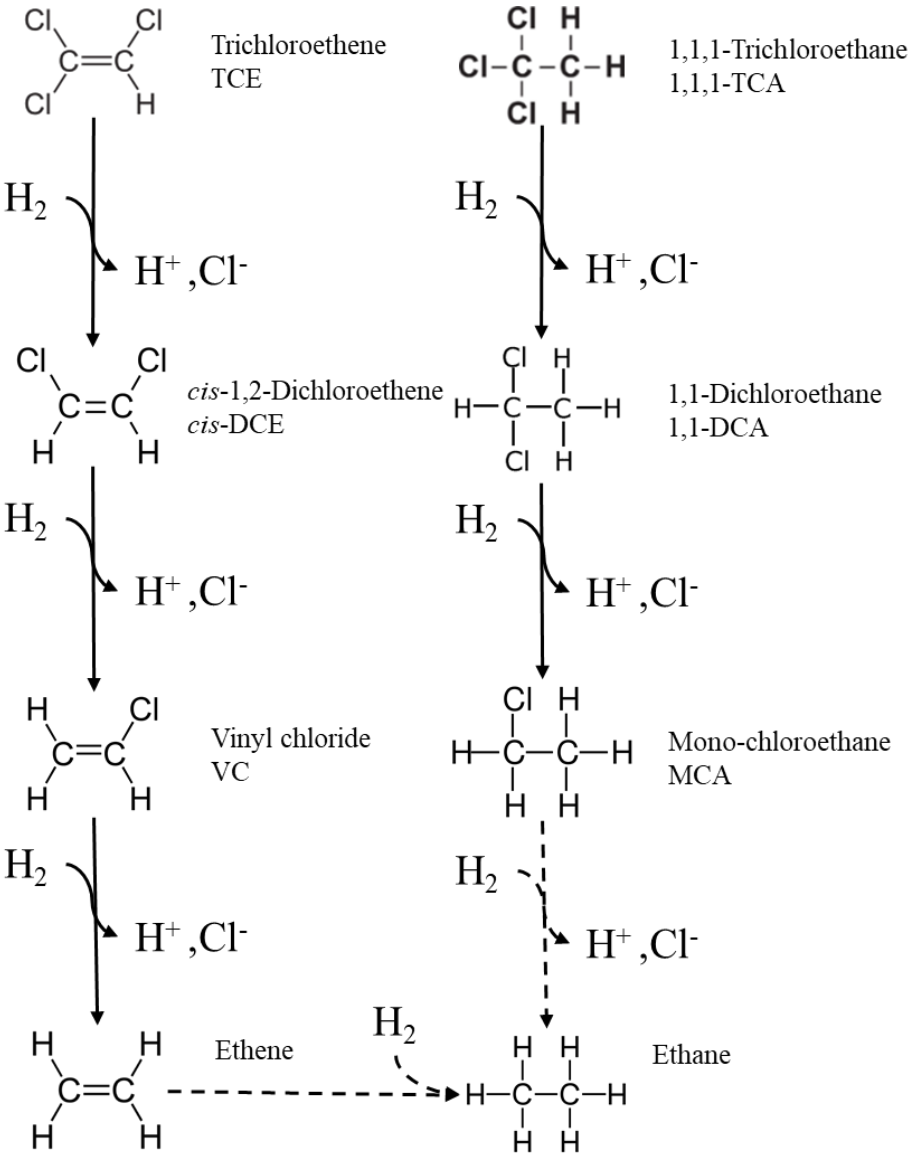

Figure 2. 1 Anaerobic degradation pathways of TCE and TCA. Solid lines show the confirmed bio-reductive dechlorinating pathways of TCE and TCA. The dotted lines show the unproved bio-reductive dechlorinating pathways of ethene to ethane and MCA to ethane.

2.2 Methods

2.2.1 Chemicals, materials and analytics

The feeding substrates TCE, 1,1,1-TCA, and their reduction products were purchased from Sigma-Aldrich®. I assayed the chlorinated ethanes and ethenes, ethene, ethane, and methane in gas samples using direct injection to a gas chromatograph (GC) with an Rt-QSPLOT column (30m×0.32mm×10 mm, Restek®, Bellefonte, PA) equipped with a flame ionization detector (GC-2010, Shimadzu®, Columbia, MD). H_2 was the makeup gas fed at a constant flow rate of 3.0 mL/min, and the temperature conditions for the injector and detector were 240°C. The oven's temperature program was 110 °C for 1 min, heat to 200°C at 50 °C/min, heat to 230°C at 15°C/min, and hold at 230°C for 1 min. Analytical grade chlorinated ethanes and ethenes, ethene, ethane, and methane were added into 100 mL of water in 160-mL bottles to makes standards for calibration curves, which were linear range in the range from 0.1 to 1000 μM ($R^2 \geqslant 0.99$). Dissolved concentrations in the liquid were computed from their Henry's constants.

I filtered 1-mL liquid samples through a 0.22-μm polyvinylidene fluoride membrane syringe filter into 2-mL glass vials for acetate and chloride analysis using anionic ion-exchange chromatography (IC, Dionex® ICS3000).

The cultures employed for this chapter were ZARA (Delgado et al. 2014), DehaloR^2 (Ziv-El et al. 2011), and SDC-9, a mixed culture of TCE-to-ethene dechlorinating consortium containing *Geobacter* and *Dehalococcoides*.

2.2.2 Reactor setup

Batch tests for culture enrichments were set up in 160-mL serum bottle containing 100 mL medium and 60 mL headspace. The medium was prepared in anaerobic conditions, and the headspace was filled with 80% H_2 and 20% CO_2. A schematic and picture of the MBfR used in this chapter are shown in Figure 2.2, and details of the reactor and its characteristics are described by Liu et al. (2018). It consisted of a 380-mL glass bottle, Viton or Teflon tubing, and Teflon stopcocks. The glass bottle contained five bundles of 32 hollow-fiber membranes (Composite bubble-less gas-transfer membrane, Model MHF 200TL Mitsubishi Rayon Co., Ltd, Tokyo, Japan), each 14-cm long; this gave a total membrane surface area of 160 cm^2. The reactor was positioned with an inclination about 40° to keep the effluent at the top and to avoid gas being trapped in the reactor.

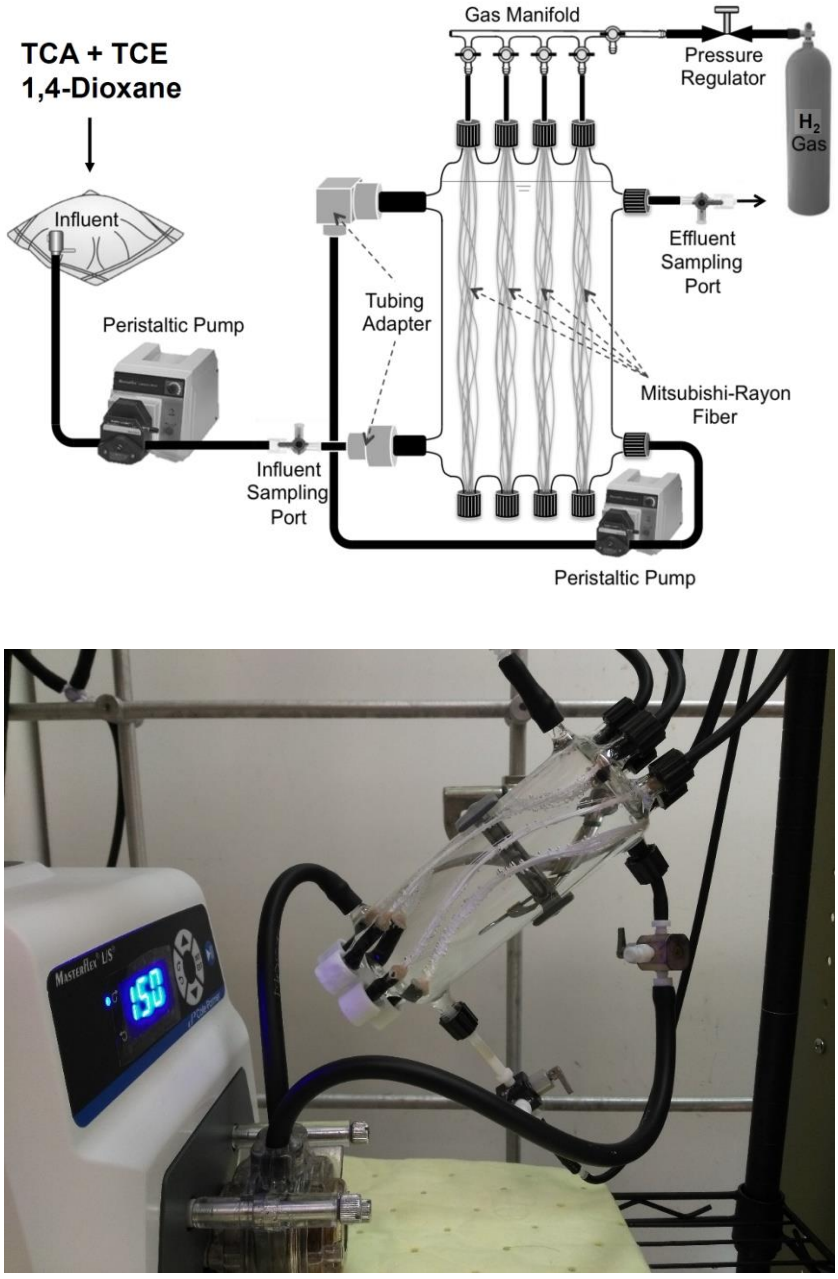

Figure 2. 2 Schematic and picture of the MBfR system for H_2-based dechlorination of TCA and TCE.

2.3 Results and discussion

2.3.1 Enrichment of dechlorinating cultures

Figure 2.3 showed the dechlorination performance of the mixed culture ZARA. The data are presented for the serum bottle giving the fastest TCE removal, although the results were similar for the other bottle. When TCE was added as the only electron acceptor, ZARA initially could reduce 99% of 92 μmole of TCE to cis-DCE within 14 days; after the second respike of 95 μmole, TCE was totally reduced within 9 days (Fig. 2.3A). Further reduction of cis-DCE to VC or ethene was not observed within the 60-day enrichment. When TCA was added as the only electron acceptor, ZARA did not reduce TCA at all in the 50-day enrichment (Fig. 2.3B). When TCA and TCE were added together (Fig. 2.3C), ZARA was still able to reduce TCE to cis-DCE, although the reduction rate was slower (99% of 94 μmole TCE reduced within 20 days) compared to adding TCE alone (Fig. 2.3A). No further reductions of TCE or TCA reduction were observed during the 50-day enrichment.

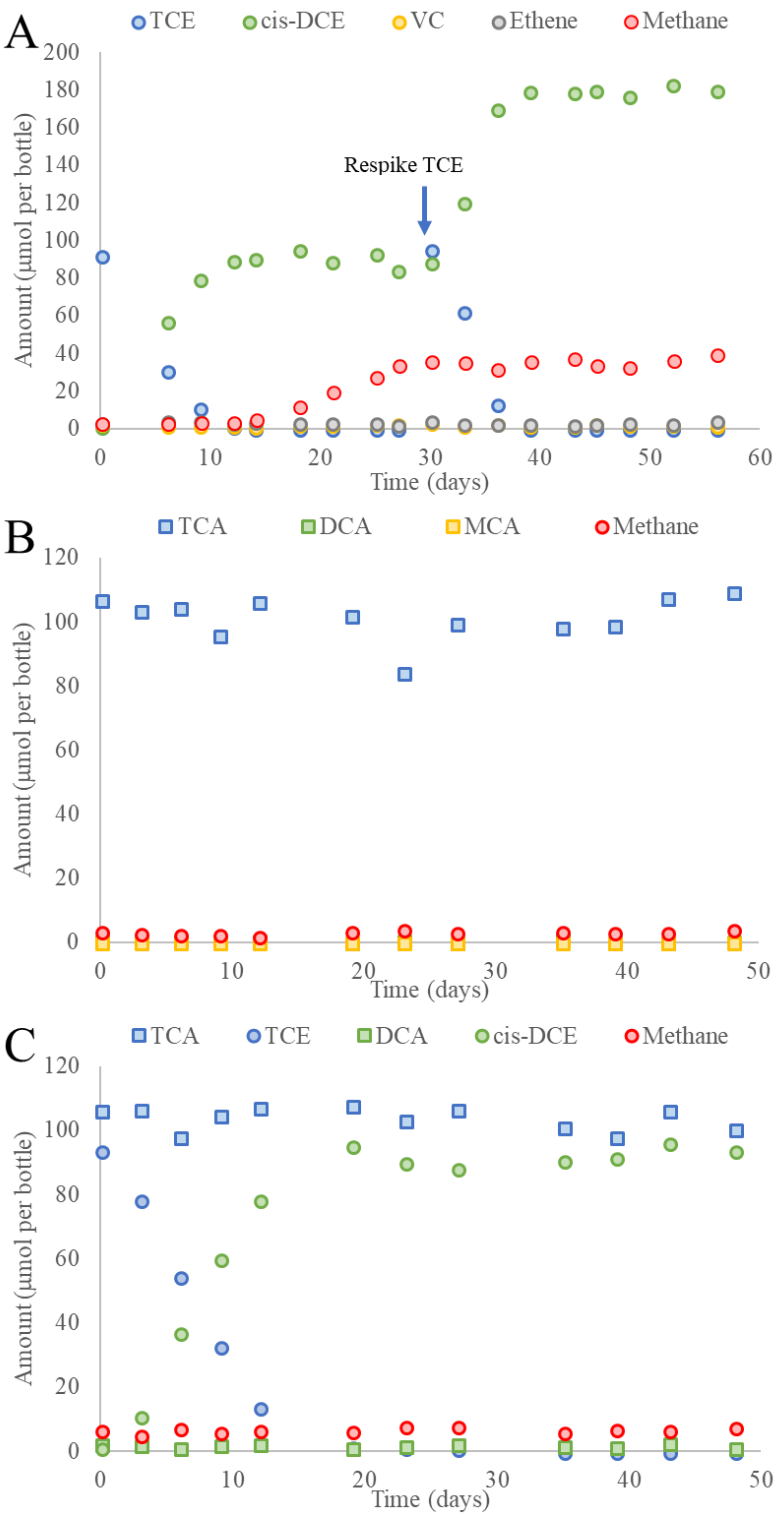

Figure 2. 3 Dechlorinating performance of the mixed culture ZARA. Three different batch tests result with TCE, TCA and TCE + TCA as electron acceptors are show in panels A, B, and C, respectively.

The mixed culture DehaloR^2 had similar results compared to ZARA when TCE and TCA were added separately; Figs. 2.4 A and B show the respective results for the bottle with the faster rates. When TCA and TCE were added together, Fig. 2.4 C, DehaloR^2 had a much slower TCE reducing rate (99% of 101 μmole TCE reduced within 28 days) compared to with TCE alone (99% of 105 μmole TCE reduced within 9 days, Fig. 2.4 A).

Figure 2.5 shows that, for the mixed culture SDC-9, about 0.1 mmol TCE was completely converted to cis-DCE within 12 days, and VC started to accumulate. After I respiked 0.1 mmol TCE into the bottles on the 30th day, TCE was converted to cis-DCE within 7 days, and VC slowly accumulated (280 μM or 14% of the initial TCE in two weeks). 10 mM lactate was added to the bottle on the 43th day, but it did not influence cis-DCE reduction.

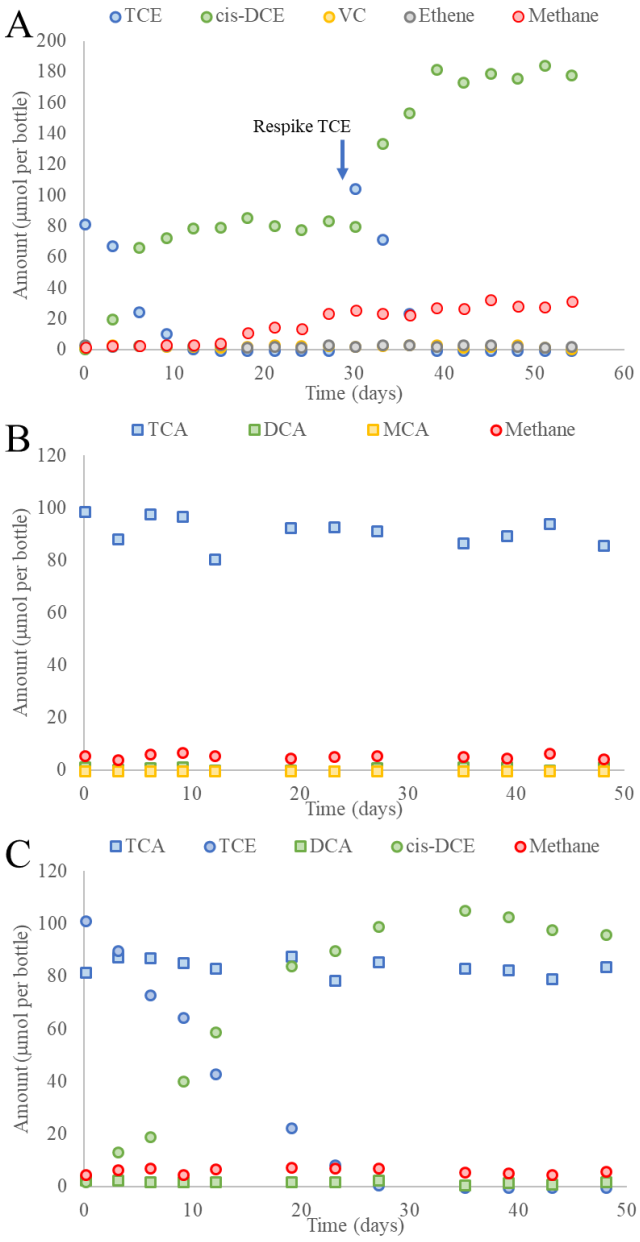

Figure 2. 4 Dechlorinating performance of the mixed culture DehaloR^2. Three different batch tests result with TCE, TCA and TCE + TCA as electron acceptors are shown in panels A, B and C, respectively.

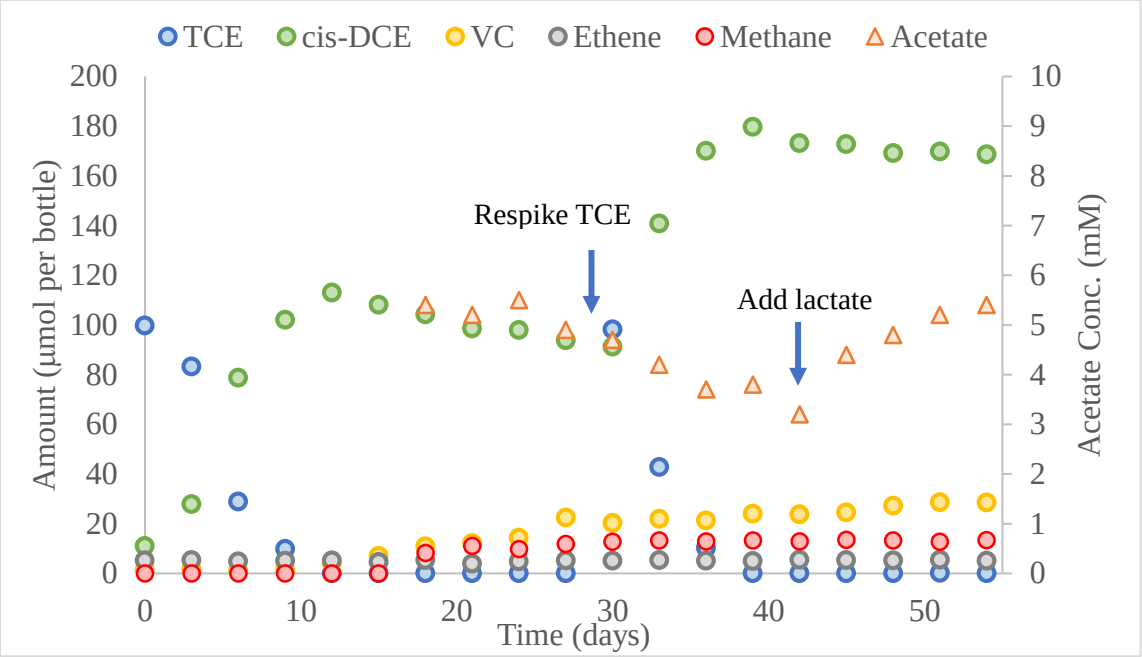

Figure 2. 5 TCE dechlorinating performance of mixed culture SDC-9 for only TCE added.

Along with the batch tests of TCA and TCE reductions, I also carried out tests for ethene and MCA reductions in serum bottles with different inocula: anaerobic-digestor sludge, wetland sediments, and the enriched dechlorinating cultures. All the test bottles showed the same results, shown in Fig. 2.6 for all three mixed cultures: Ethene and MCA were not reduced to ethane with these cultures, but methane was generated.

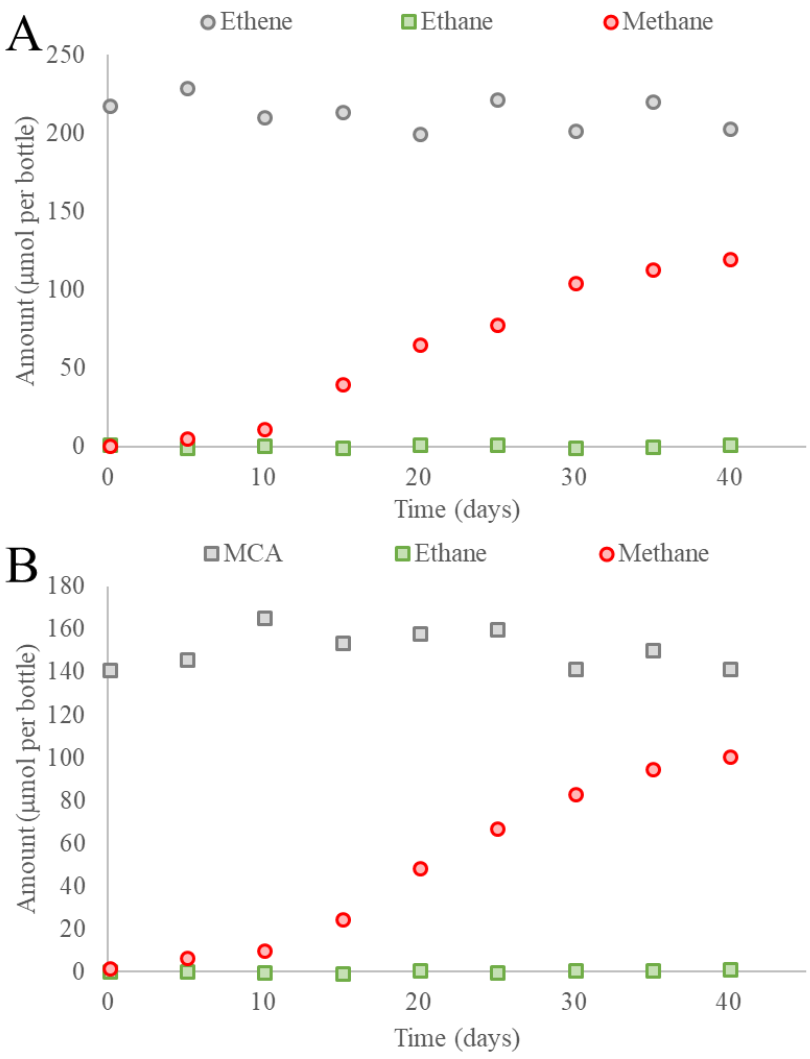

Figure 2. 6 Batch test results testing ethene and MCA bio-reduction for ZARA, other two cultures have same results (data not show).

2.3.2 Growth of a dechlorinating biofilm in the H_2-based MBfR

To begin the experiments with the H_2-based MBfR, I inoculated the dechlorinating culture ZARA into the MBfR together with 0.1 mM each of TCE and TCA as electron acceptors. Fig. 2.7 shows the results for TCE dechlorination in the H_2-based MBfR during batch operation. Dechlorinating activity was negligible for the first 10 days, and no

intermediates were observed. Low dechlorinating activity may have been caused by a low density of biomass active in dechlorination. Then, I re-inoculated the MBfR with dechlorinating culture ZARA at the 10^{th} and 20^{th} days of batch experiment. Reduction intermediates and chloride were not observed in the next 29 days. As the low dechlorinating activity may have been caused TCA inhibition, I changed the medium to be the same as I used in batch bottles and removed TCA, using 0.1 mM TCE as the only electron acceptor. After reinoculating DehaloR^2 on the 35th day, I started to observe TCE dechlorination to cis-DCE from the 40^{th} day. 99% of the 0.1 mM TCE was conversed to cis-DCE after the second respike of TCE within 40 days. Further cis-DCE reduction was not observed out to day 155. To strengthen the dechlorinating biofilm, I re-inoculated the MBfR with dechlorinating culture SDC-9 (10 mL active culture) on the 155^{th} day. TCE was 99% reduced to form cis-DCE, and the VC slowly began to accumulate (to 2 μM in 20 days).

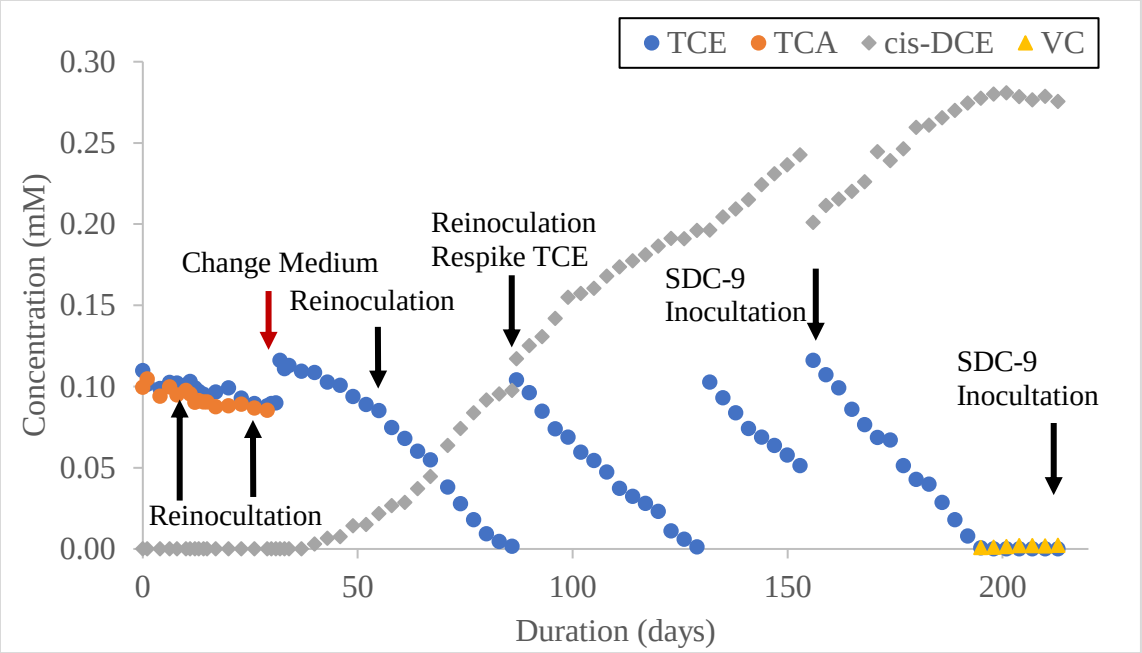

Figure 2. 7 Concentrations of TCE (blue dots), TCA (orange dots), cis-DCE (grey diamond), and VC (yellow triangle) in the H_2-MBfR.

2.3.3 Summary

For batch tests in serum bottles and the H_2-based MBfR, the mixed cultures achieved complete TCE reduction to cis-DCE. However, further reduction of cis-DCE to VC was observed only with the culture of SDC-9. All three cultures are known to be able to reduce TCE to ethene (Delgado et al. 2014, Ziv-El et al. 2011), but only SDC-9 retained the capability of reducing cis-DCE to VC in my experiments. The capability for reducing cis-DCE to ethene probably was lost during storage of the cultures in the refrigerator (4°C), which led to the inactivation of *Dehalococcoides*. ZARA and DehaloR^2 did not reduce TCA to DCA or MCA, which means the culture may not have contained active *Dehalobacter*. Further tests of TCA reduction of SDC-9 are needed.

In summary, the experimental results were not promising for using the H_2-based MBfR for reductive dechlorination of TCE and TCA. Bio-dechlorinations of TCA and TCE to

ethane or ethene were not achieved, the development of a strong dechlorinating biofilm took a long time, when it occurred at all, and TCA seemed to inhibit TCE reduction. A more effective reduction strategy will be needed, and that is the target of the next chapter.

3 PALLADIUM-CATALYTIC HYDRODECHLORINATION OF 1,1,1-TCA AND TCE

3.1 Background

Pd-based catalysis has been applied for a variety of water-treatment strategies. Palladium nano-particles (PdNPs), supported-Pd, and Pd-based bimetallic catalysts can activate hydrogen (H_2) and reductively catalyze the transformation of a number of drinking-water contaminants: e.g., halogenated organics, oxyanions, and nitrosamines (Lowry and Reinhard 1999, Heck et al. 2009, Chaplin et al. 2012). In particular, hydrodechlorination (HDC) of TCE, though widely reported through enzymatic processes, also can be catalyzed by Pd (Lowry and Reinhard 2001, Davie et al. 2008). The reaction replaces one or more carbon-bound chlorine atoms with atomic hydrogen. During the reaction, as illustrated in Figure 3.1, the reactants -- H_2 and TCE -- are adsorbed to the surface of metallic Pd, forming adsorbed atomic hydrogen and alkane with higher reactivities, respectively. The hydrogen atoms then replace all or part of the chlorine atoms on the TCE. Eventually, the selected products, including ethane, MCA, DCA, detach from the Pd surface. In general, ethane is the primary product of TCE HDC in the aqueous phase, while some undesired by-products, like DCE and MCA, may also be formed in parallel. Since Pd-based HDC is a surface reaction, the specific surface area (related to particle size) significantly affects the reaction kinetics and selectivity (Chaplin et al. 2012, Nutt et al. 2005). Besides, environmental conditions (e.g., gas versus aqueous phase, pH, H_2 supply) also are key parameters.

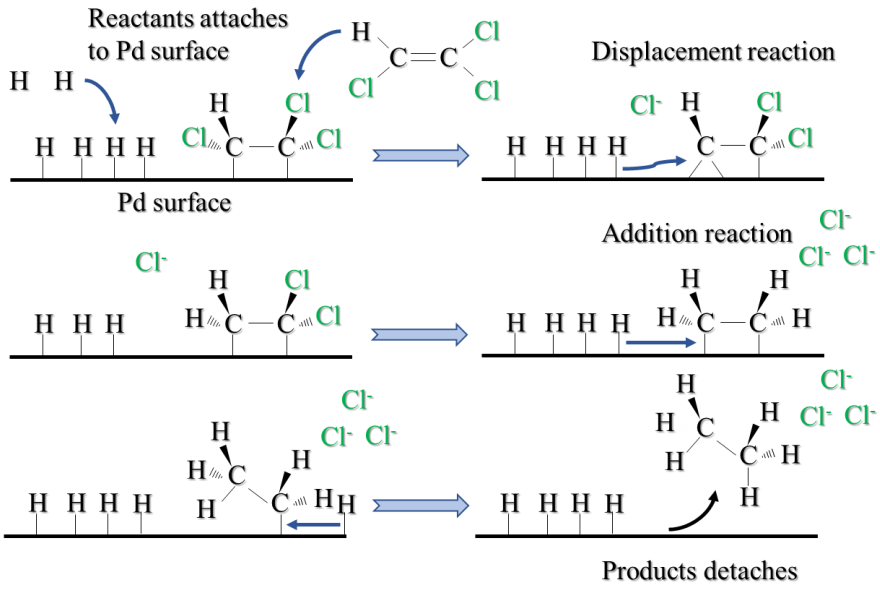

Figure 3. 1 Proposed Schematic of Pd-catalytic hydrodechlorination of TCE.

The goals of this chapter are to test simultaneous TCA and TCE HDC by using PdNPs and build a H_2-based MPfR to continuously convert TCA and TCE to ethane. I first tested TCA and TCE HDC catalyzed by suspended PdNPs. Second, I coated PdNPs onto the surface of hollow-fiber membranes and tested TCA and TCE HDC in a H_2-MPfR. Third, I operated the MPfR in a continuous mode and optimize the operating conditions to maximize H_2 utilization and minimize by-products formation. Fourth, I optimized the method of coating PdNPs coating to minimize the cost of the MPfR. I am preparing a manuscript on these results. The manuscript is tentatively titled "Simultaneously TCE and TCA remove in a novel hydrogen based hollow fiber Pd-catalytic film reactor (H_2-MPfR)".

3.2 Methods

3.2.1 Chemicals, materials and analytics

The sources and measurements of TCE, TCA, and their reductive products are same as Chapter 2. To coat the fibers with PdNPs, I filled the reactor with the medium containing ~10 mg L^{-1} of soluble Pd^{II} added as Na_2PdCl_4. The medium had been autoclaved and deoxygenated before use. The PdNPs spontaneously formed through autocatalytic reduction from Pd^{II} to Pd^0 and attached to the membrane surfaces under continuous H_2 supply within 12 hours. The estimated loading of the PdNPs coated to the membrane was ~65 mg/m^2. Detailed of the MPfR are listed in Table 3.1.

3.2.2 Reactor setup

The preliminary batch test with suspended PdNPs was set in a 160-mL serum bottle, with 100 mL medium and 60 mL headspace. The temperature was 23°C, and the medium pH was ~7 set with 10 mM phosphate buffer. Continuous catalytic HDC was carried out in a H_2-MPfR having the same dual-tube configuration (illustrated in Fig. 3.2) as in a previous study (Zhou et al. 2016a). The MPfR had a total working volume of 80 mL (60 mL liquid phase and 20 mL headspace) and contained two bundles of 30 identical hollow-fiber membranes in two glass tubes (6 mm internal diameter and 27 cm length). I evaluated two types of membranes in separate tests: One was a composite bubble-less gas-transfer membrane (280 μm OD, 180 μm ID, wall thickness 50 μm, Model MHF 200TL Mitsubishi Rayon Co., Ltd, Tokyo, Japan); the other was a nonporous polypropylene fiber (200 μm OD, 100–110 μm ID, wall thickness 50–55 μm, made by Teijin, Ltd., Japan). H_2 gas (>99.9%) was supplied to both ends of each fiber bundle at a pressure controlled by a

pressure regulator. A solute's concentration inside an MPfR was equal to its effluent concentrations due to mixing from a recirculation rate of 150 mL/min created by using a peristaltic pump. A 30-mL serum bottle was set between two tubes to create gas-liquid interface; this enabled the volatile organic compounds (TCA, TCE, reduction products) to reach equilibrium of partitioning between the liquid phase and the headspace where gaseous samples were collected.

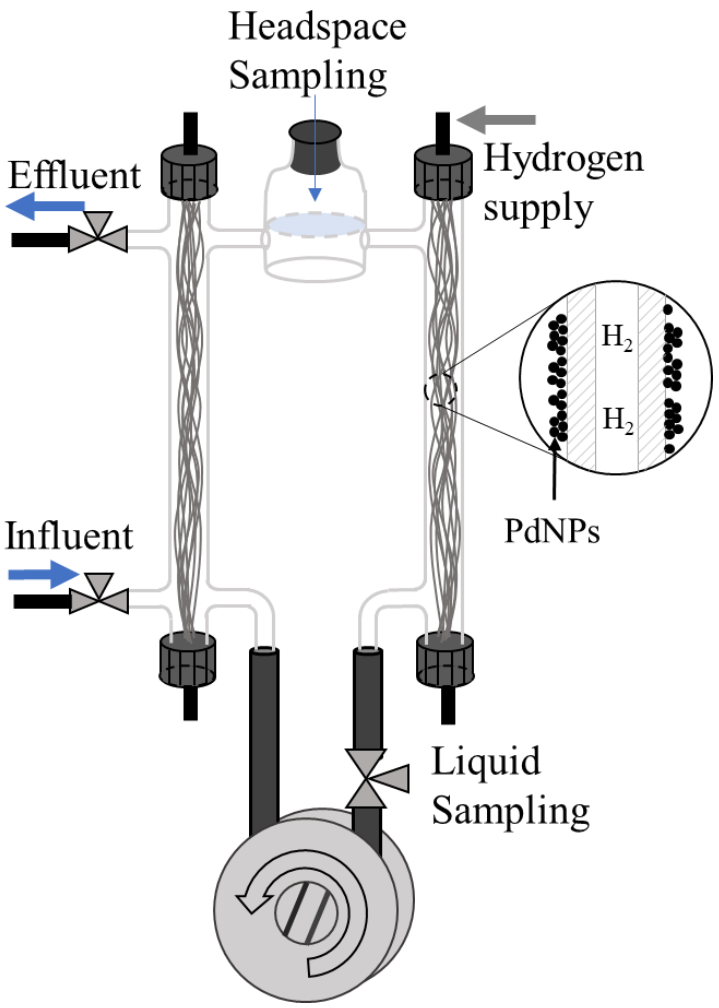

Figure 3. 2 Schematic of the H_2-MPfR setup.

3.2.3. Analytical methods

The concentrations of gaseous TCA, TCE, DCA, MCA, ethene, ethane, oxygen and hydrogen were analyzed routinely by the methods in section 2.2.1.

3.3 Results and discussion

3.3.1 Suspended PdNPs

Two preliminary batch tests were performed to assess abiotic TCE and TCA dechlorination catalyzed by suspended PdNPs. I added 40 μmole TCE into a serum bottle containing 100 ml liquid medium plus 0.5 mM suspended PdNPs and 60 ml headspace featuring 0.4 atm H_2 and 0.8 atm N_2. Figure 3.3 shows that TCE was converted into ethene (6.4 μmole, or 16% of the initial TCE) and ethane (32.1 μmole, or 80% of the initial TCE) within an hour, while other by-products were present at trace levels (MCA, DCA < than 0.1 μmole, or < 0.25% of the initial TCE). With the same conditions, 45 μmole TCA was converted to ethane (43.1 μmole, or 95.8% of the initial TCA) and DCA (1.9 μmole, or 4.2% of the initial TCA). The batch tests confirm that HDC of TCA and TCE can be catalyzed by PdNPs under ambient conditions (23°C, 1 atm, circumneutral pH).

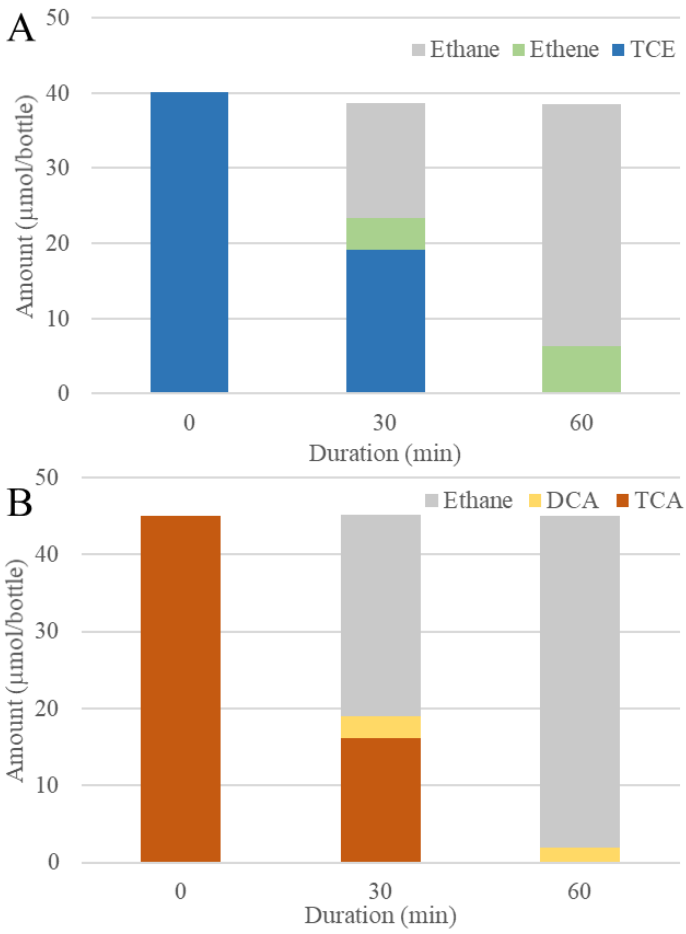

Figure 3. 3 TCE and TCA reductions catalyzed by suspended PdNPs. The missing mass for TCE was MCA and DCA (< 3 µmole each).

3.3.2 TCE and TCA removal in the H_2-MPfR

The first H_2-MPfR was built with the composite bubble-less gas-transfer membranes. I first tested TCE and TCA reductions in this H_2-MPfR in batch mode with a H_2-supply pressure of 3 psig (~1.2 atm total pressure). Figure 3.4A shows that the initial 1 mM TCE was rapidly reduced to ethane (88%) and MCA (10%) within about 70 mins once H_2 was supplied. The maximum rate of TCE reduction was 0.54 µmol/min for the whole reactor, which corresponded to 146 µmol/min·g-Pd. The H_2 accumulation in the reactor meant that

the reduction reaction did not need all the H_2 that could be delivered from the membrane, which means that H_2 was being wasted. Figure 3.4B shows that 1 mM TCA was converted to ethane (75%), DCA (12%), and MCA (12%) within 120 mins. Furthermore, 1 mM TCE and 1 mM TCA together were converted to ethane (75%), DCA (13%), and MCA (12%) within 180 mins (Fig. 3.4C). These tests confirm that TCE and TCA reductions catalyzed by the PdNPs coated on the H_2-delivery membranes were as efficient as the suspended PdNPs.

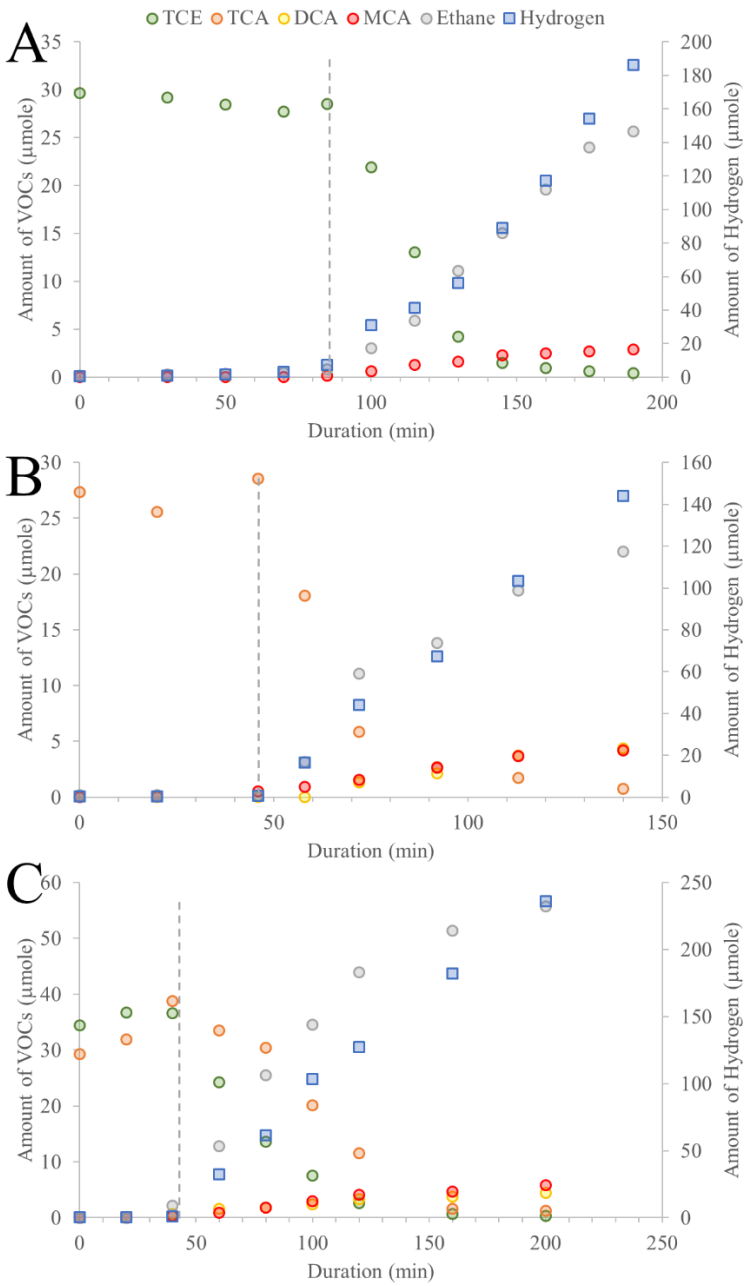

Figure 3. 4 TCE and TCA catalytic reduction batch test of a H_2-MPfR with composite membranes. The gray dash line shows the start of the hydrogen supply. A: TCE only, B: TCA only, C: TCE and TCA.

After the batch tests, the H_2-MPfR was operated in the continuous mode with different hydraulic retention times (HRTs) and H_2-supply pressures. Figure 3.5 presents the results

of the initial 30 days of operation divided into five stages. The influent concentrations of TCA and TCE are 1 mM, which means that the influent Cl 6 μM. In the first stage, the H_2-MPfR was supplied with 3 psig H_2 with an HRT of 12 h. To optimize the reactor operating conditions, I kept decreasing the HRT: from 12 h to 6.8 h, 3.8 h, and finally 2.6 h. Figure 2.5 summarizes the results. The effluent concentrations of TCE (from 10 μM to 20 μM, 40 μM, and 70 μM with decreasing HRT), TCA (from 40 μM to 100 μM, 200 μM and 280 μM), and DCA (from 40 μM to 50 μM, 75 μM and 80 μM) increased, and the effluent chloride concentration decreased from 5.3 μM to 5.0 μM, 4.7 μM, and 4.3 μM. Total Cl in the effluent was stable at around 80% of the influent chlorine throughout. The other 20% of chlorine was lost, since some of the TCA, TCE, and reduced products were stripped out of the reactor by the excess H_2.

The trends with HRT show that, when the HRT was less than 6.8 h, the H_2 supply became insufficient for full reductions of TCE and TCA (H_2 in the headspace was lower than 0.1%). In the last stage, when I increased the H_2 pressure to 5 psig (from 3 psig), the concentrations of all the chlorinated hydrocarbons ions decreased very fast, but so did the concentration of Cl^-; the latter supports that excess H_2 probably stripped TCA and TCE out of the reactor.

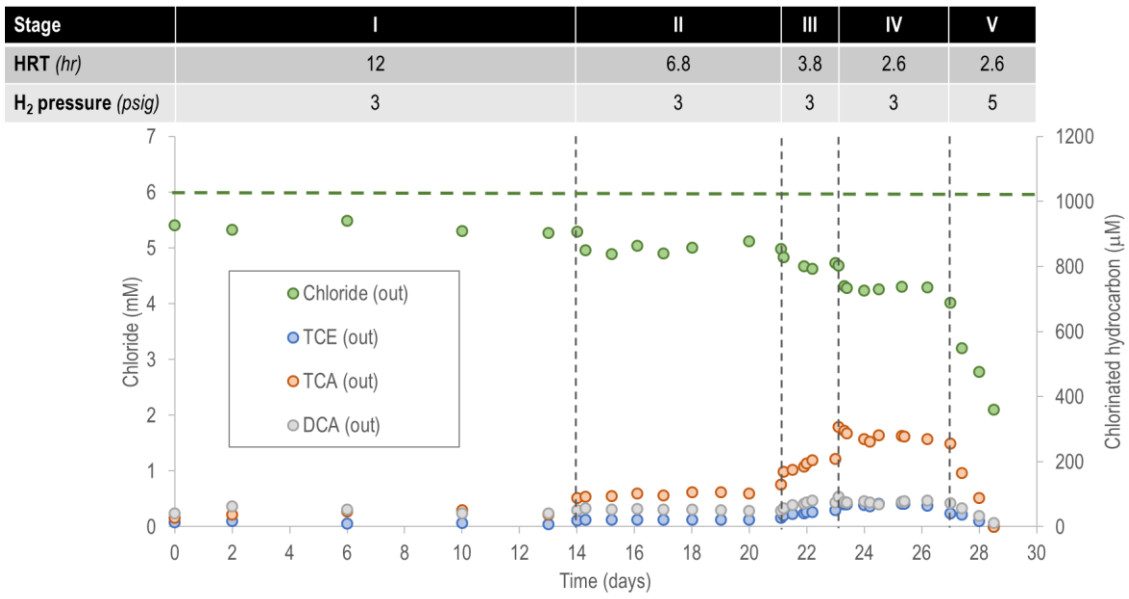

Figure 3. 5 Catalytic reductions of TCA and TCE in the H_2-MPfR with composite membranes in continuous operation. The green horizontal dash line represents the influent concentration of total chlorine in TCE and TCA. The black vertical dash line indicates HRT and pressure changes.

The previous results indicate that the H_2-delivery capacity of the composite membranes was too high to preclude over-supply of H_2, which wasted H_2 and led to stripping of TCE and TCA. Therefore, I changed to the polypropylene membranes, which have a lower delivery rate (about 10% of the composite membrane). Figure 3.6 shows the results of separate TCE- and TCA-reduction batch tests using polypropylene membrane at the hydrogen pressure of 10 psig and initial concentrations of 0.1 mM. TCE was rapidly reduced to ethane (84%) and MCA (12%) within about 100 minutes with the maximum reaction rate of ~20 µmol/min/g-Pd. TCA also was converted to ethane (70%), DCA (16%), and MCA (11%) within 100 mins, with the maximum reaction rate of 17 µmol/min/g-Pd. The total mass of VOCs (black triangles) had 10% mass-balance closure at the beginning

and the end of the batch experiment. The transient loss of VOC mass (as low as 62% for TCE and 75% for TCA) probably was due to reaction intermediates that were transiently attached to the PdNPs or the fibers. Compared to the composite membrane, the H_2 delivered by polypropylene in the first 40 minutes was well matched to the reaction rate, which mean that H_2 was not wasted and did not cause stripping of TCE or TCA.

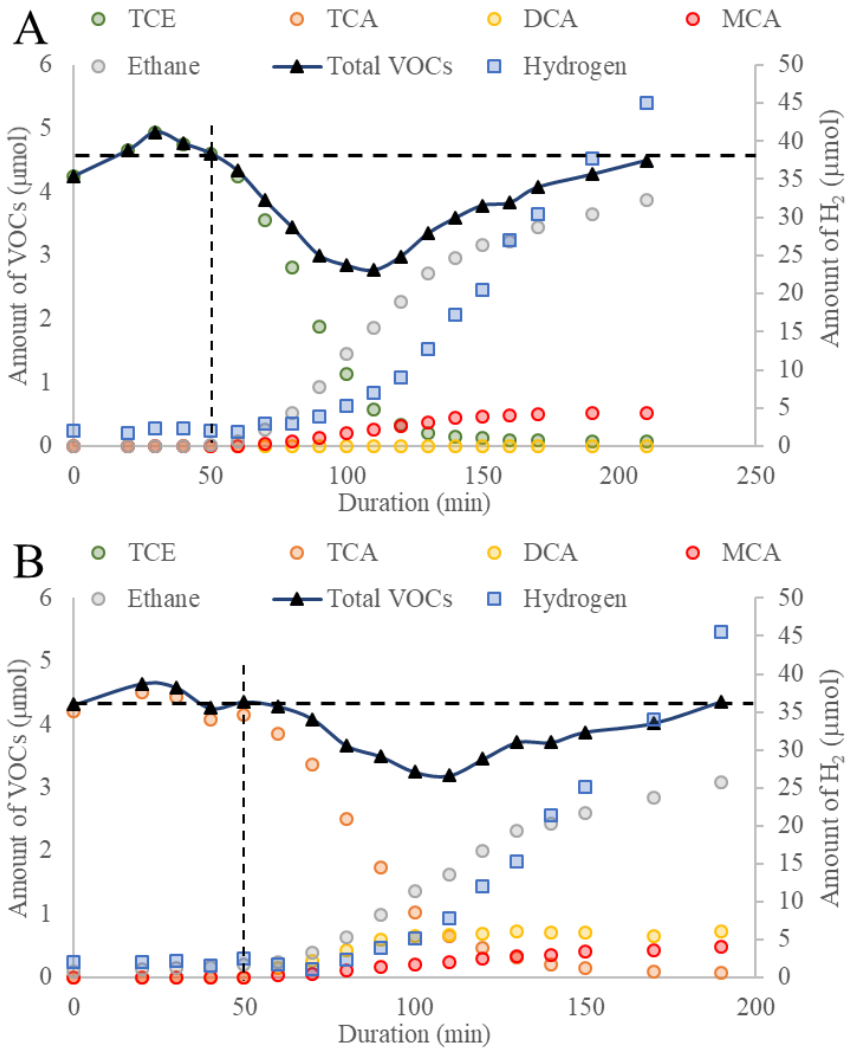

Figure 3. 6 Catalytic hydrodechlorination batch test of a H_2-MPfR with polypropylene membranes. A shows the amount changes of hydrogen, TCE, and its

reduction products in the MPfR with initial dissolved TCE concentration of 100 μM. B shows the amount changes of hydrogen, TCA, and its reductive products in the MBfR with an influent TCA concentration of 100 μM.

Simultaneous TCE and TCA reductions were tested for H_2-supply pressures of 20 and 10 psig and with TCA and TCE initial concentrations of 1 mM and 0.1 mM. Figure 3.7 shows the results for three batch tests with the different H_2 pressure and different initial TCE/TCA concentrations. For the three tests, all the compounds have the same trends, but the reaction rate was higher when the initial concentration and hydrogen pressure are higher: 130 mmol/min·g-Pd for 1 mM / 20 psig; 84 mmol/min·g-Pd for 1 mM /10 psig, and 31 mmol/min·g-Pd for 0.1 mM / 10 psig. In addition, product selectivity was affected by the initial TCE and TCA concentrations. Figure 3.8 summarizes the selectivity for three different batch tests. Increasing the H_2 pressure from 10 to 20 psig did not significantly affect selectivity with an initial concentration of 1mM. When the H_2 pressure was set at 10 psig, the lower initial concentration led to higher selectivity towards ethane (from 57.5% to 68.9%).

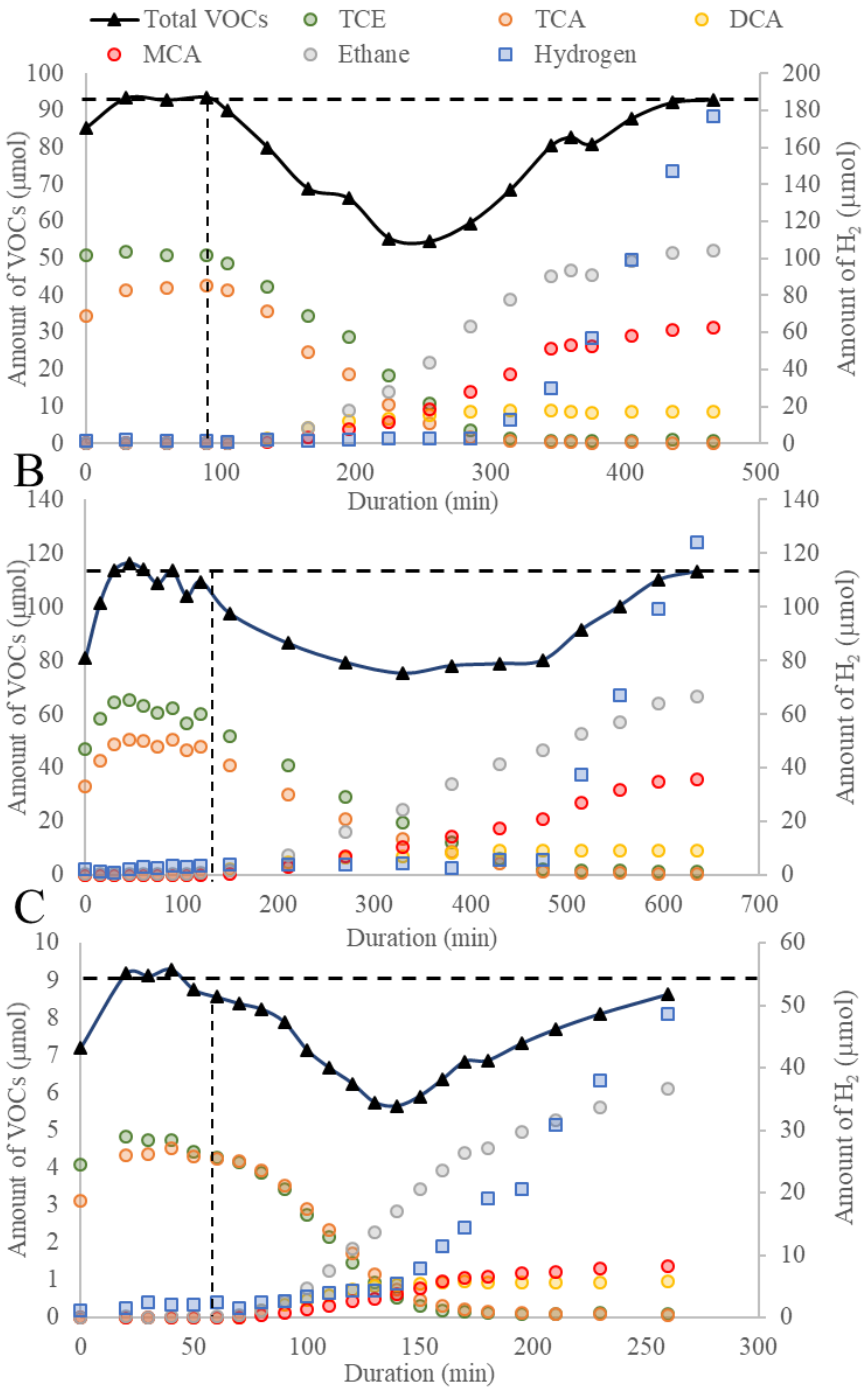

Figure 3. 7 Simultaneous TCA and TCE batch tests of a H_2-MPfR with polypropylene membranes. A had a H_2-supply pressure of 20 psig and initial concentration of 1 mM; B had 10 psig and 1 mM; C had 10 psig and 0.1 mM.

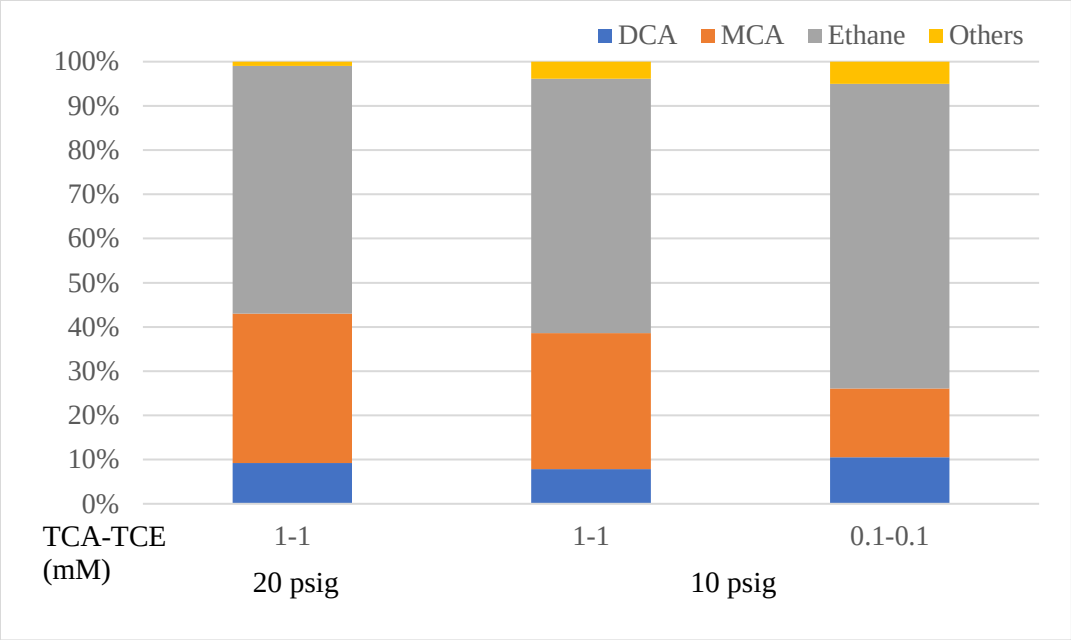

Figure 3.8 Product selectivity (DCA, MCA, and ethane) for the three MPfR batch tests having different H_2 supply pressure and initial TCA concentration.

I ran the polypropylene H_2-MPfR in continuous operation with an HRT of 15 hours and influent TCE and TCA concentrations at 1 mM (Table 3.1), and results are in Figure 3.9. In stage 1, the influent featured TCE, TCA, and DI water only, and the medium pH in the reactor was around 1. TCE and TCA removal exceeded 96% with the HRT of 15 hours and surface loading of 50 mmol/m^2-day. The main by-products in the effluent were DCA and MCA, accounting for about 3% and 4% of total TCE and TCA.

In stage 2, I added phosphate buffer to the medium to control the pH at ~7. The pH change from 1 to 7 did not have a significant effect on TCE and TCA reductions.

In stage 3, I increased the surface loading from 75 mmol/m^2-day with the same influent concentration and H_2 supply by decreasing the HRT to 10 hours. The effluent

concentrations of TCE and TCA did not have significant change, while DCA increased from 60 μM to 160 μM (3% to 8% of total TCE and TCA), and MCA increased from 80 μM to 100 μM (3% to 5% of total TCE and TCA).

Table 3.1 The coating and operating parameter for the H_2-MPfR

MPfR parameters					
		Value	Unit 1	Value	Unit 2
Coating	Palladium Conc.	0.1	mmol/L	10.64	mg/L
	H_2 pressure	3	psi	1.2	atm
	pH	10			
	Membranes type	Polypropylene membrane			
	Membranes Area	0.0115	m^2	115	cm^2
	Coated Pd	0.07	mmol	0.74	mg
		0.60	$mmol/m^2$	64	mg/m^2
Operation	Flow rate	0.10	mL/min	144	mL/d
	H_2 pressure	10	psig	1.69	atm
	TCE influent	100	μmol/L	13.1	mg/L
	TCE loading	1.25	$mmol/m^2$-day	164	mg/m^2-day
	TCE Flux	1.24	$mmol/m^2$-day	163	mg/m^2-day
	TCA influent	100	μmol/L	13.3	mg/L
	TCA loading	1.25	$mmol/m^2$-day	167	mg/m^2-day
	TCA Flux	1.22	$mmol/m^2$-day	163	mg/m^2-day

In the fourth stage, I fed the reactor with the medium used for 1,4-D biodegradation in the O_2-based MBfR; TCE and TCA influent concentrations were 1 mM, and the 1,4-D concentration was 0.5 mM. The additional medium compounds (ammonium, calcium, magnesium, trace metals, and 1,4-D) did not have a significant effect on TCE and TCA reductions. The chlorine mass balance during the continuous was stable around 98 ± 5%.

In summary, HDC using the PdNPs reductively transformed TCA and TCE to ethane with only small concentrations of DCA and MCA. The PdNPs coated well to the gas-

delivering membranes and provided effective HDC in the H_2-based MPfR. The HDC reaction rate in the H_2-based MPfR was mostly controlled by the H_2-supply pressure, and selectivity towards ethane was controlled by the initial concentrations of TCA and TCE. Thus, the H_2-based MPfR generated an effluent suitable for the next stage, 1,4-D biodegradation.

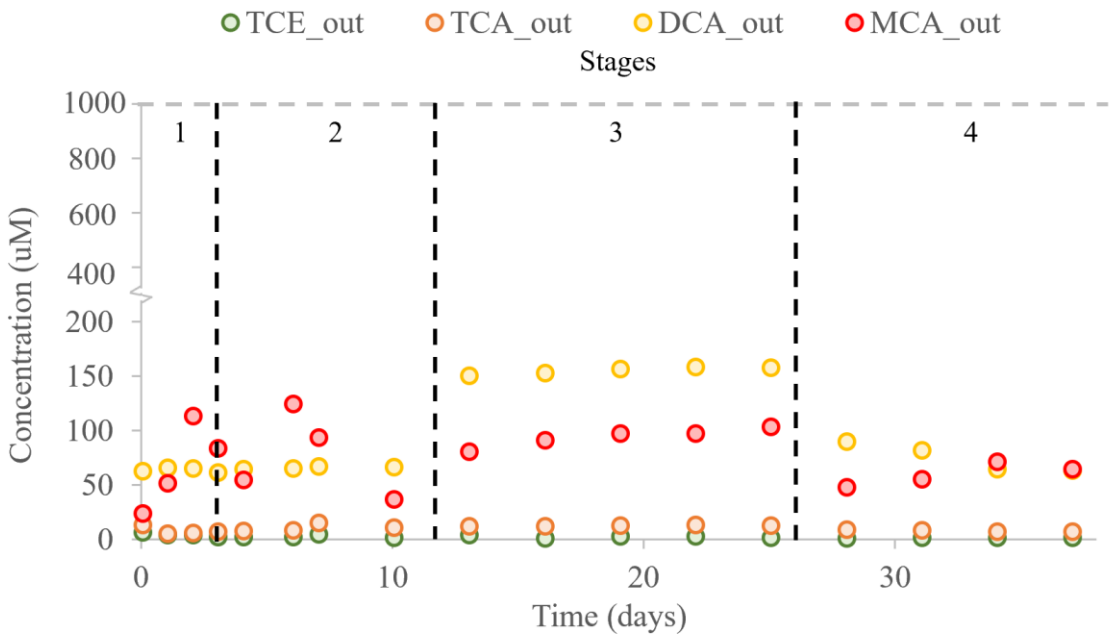

Figure 3.9 TCA and TCE catalytic reduction in the H_2-based MPfR with polypropylene membranes in the continuous mode. The gray horizontal dash line represents the influent concentration of TCE and TCA. The black vertical dash line separates the four stages with different operating conditions.

4 BIODEGRADATION OF 1,4-DIOXANE

4.1 Background

The first species shown to biodegrade 1,4-D and grow on it as a sole electron and carbon donor is *Pseudonocardia dioxanivorans* CB1190 (Parales et al. 1994, Mahendra et al. 2007). On the basis of the intermediates identified during 1,4-D biodegradation, Mahendra et al. (2007) proposed the complete biodegradation pathway shown in Fig. 4.1. I highlight the two steps of monooxygenation, as they are the key steps that convert 1,4-D from a stable cyclic ether to metabolically labile organic intermediates.

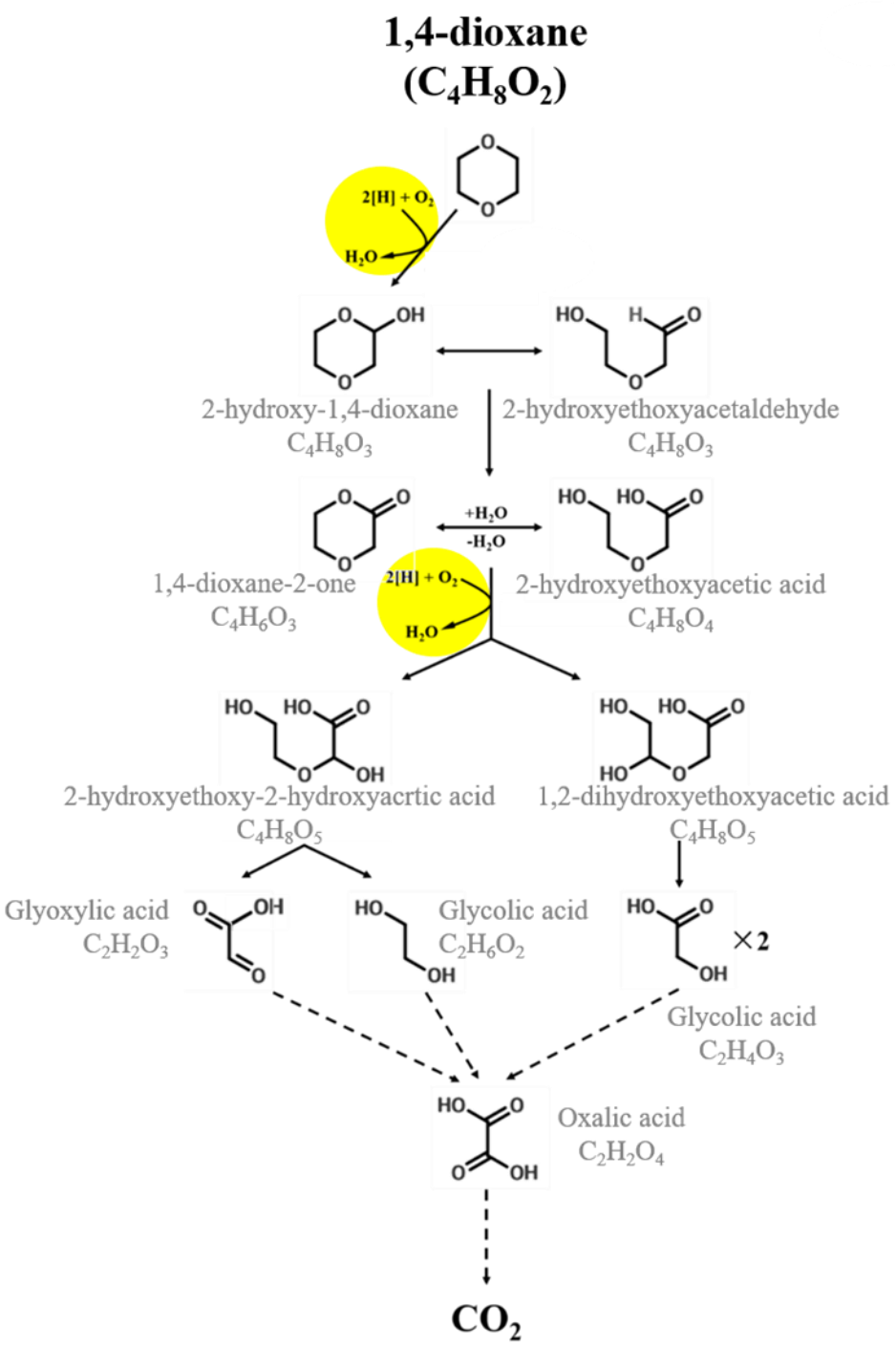

Figure 4. 1 Schematic for 1,4-D aerobic biodegradation. Yellow highlight the initial mono-oxygenation reactions.

Besides *P. dioxanivorans* CB1190, a few other aerobic microorganisms are able to use 1,4-D as sole electron and carbon donor, including *Rhodococcus ruber* strain 219, *Acinetobacter baumannii* DD1, *Xanthobacter flavus* DT8, and *Afipia* sp. strain D1 (Isaka et al. 2016, Bernhardt and Diekmann 1991, Zhou et al. 2016b, Chen et al. 2016). Only a few microorganisms have the capability to metabolically degrade 1,4-D, and most research on 1,4-D biodegradation indicates that it is co-metabolic, requiring a growth-supporting primary substrate, such as tetrahydrofuran (THF), methane, propane, toluene, or ethanol (Burback and Perry 1993, Vainberg et al. 2006, Kim et al. 2009). For co-metabolic 1,4-D biodegradation, the microorganisms have the enzymes to oxidize it, but cannot use 1,4-D as the solo electron donor and carbon source for growth.

In this chapter, I first attempt to enrich a mixed culture for 1,4-D biodegradation from different inocula: wastewater treatment aerobic sludge, landfill leachate, oil-contaminated soil, and wetland sediments. I do the enrichments with and without primary electron donors. Second, I attempt to use a pure culture of *R. ruber* strain 219 to do kinetic experiments for 1,4-D biodegradation. Third, I use the enriched mixed culture or the *R. ruber* strain 219 pure culture as an inoculum to start up an O_2-based MBfR for 1,4-D removal. Finally, I run the O_2-based MBfR with continuous operation and optimize the operating conditions to achieve a high rate of 1,4-D removal.

4.2 Methods

4.2.1 Chemical, materials and analysis

Concentrations of 1,4-D, tetrahydrofuran, and ethanol were monitored in aqueous samples using direct injection into a gas chromatograph (GC) with an Rt-QSPLOT column (30m×0.53mm×10 mm, Restek®, Bellefonte, PA) equipped with a flame ionization detector (GC-2010, Shimadzu®, Columbia, MD). Helium was the makeup gas fed at a constant flow rate of 20 mL/min, and the temperature conditions for injector and detector were 200 and 240 °C respectively. The oven temperature program was hold at 120 °C for 1 min, heated to 230 °C (5 °C/min) and held at 230 °C for 5 min.

4.2.2 Reactor setup

Initial batch tests were conducted in the 250-mL serum bottles, with 100 mL medium and 150 mL headspace. Continuous-mode biodegradation of 1,4-D was achieved in an O_2-based hollow fiber membrane biofilm reactor (O_2-MBfR) of the same dual-tube configuration, illustrated in Fig. 3.2, as in a previous study (Zhou et al. 2016a). The MBfR had a total working volume of 80 mL (60 mL medium and 20 mL headspace) and contained two bundles of 30 hollow-fiber membranes (composite bubble-less gas-transfer membrane, 280 μm OD, 180 μm ID, wall thickness 50 μm; Model MHF 200TL Mitsubishi Rayon Co., Ltd, Tokyo, Japan) in two glass tubes (6 mm internal diameter and 27 cm length). O_2 gas (>99.9%) was supplied to one end of fiber bundles at different pressures controlled by a pressure regulator.

4.3 Results and discussion

4.3.1 Enrichment of 1,4-D oxidizing culture

To enrich a 1,4-D-oxidizing culture, I tried four inocula (a pure culture of *Rhodococcus ruber* 219, WWTPs aerobic sludge, landfill leachate, and wetland sediments) and two electron donors (acetate and ethane).

I initially used batch tests to measure the rates of growth and 1,4-D biodegradation for *R. ruber* 219. Figure 4.2 shows the results for the pure culture of *R. ruber* 219 growing on 5 mM acetate, 5 mM acetate with 0.5 mM 1,4-D, and 1 mM 1,4-D only (with 0.4 mM acetate coming from the transfer source). *R. ruber* 219 growing with acetate as the only electron donor had a generation time about 13 hours. *R. ruber* 219 growth was slower when 0.5 mM 1,4-D added as an additional substance, since monooxygenation of 1,4-D consumed some electron equivalents from acetate oxidation. Less acetate led to a stoppage of growth, presumably when acetate was depleted. Growth without an added electron donor showed almost no growth.

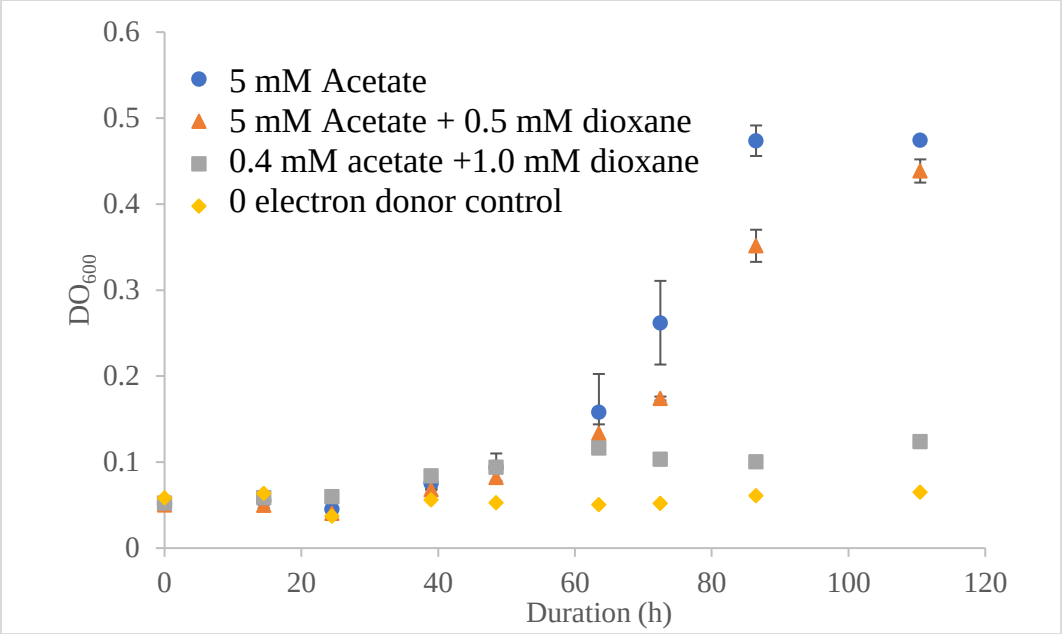

Figure 4. 2 The growth curve of *R. ruber* 219 with the different electron donors.

Figure 4.3A shows that the *R. ruber* 219 pure culture achieved more than 90% removal of 0.4 mM dioxane with acetate as the primary electron donor. Figure 4.3B showed that *R. ruber* 219 did not use ethane as a primary electron donor for growth or 1,4-D degradation through co-metabolism. Also, Figure 4.3B shows that *R. ruber* 219 could not use dioxane as the only electron donor for growth. These batch tests document that *R. ruber* 219 had the capability of 1,4-D degradation with acetate as a primary electron donor, but not with ethane or with 1,4-D alone as the sole electron donor.

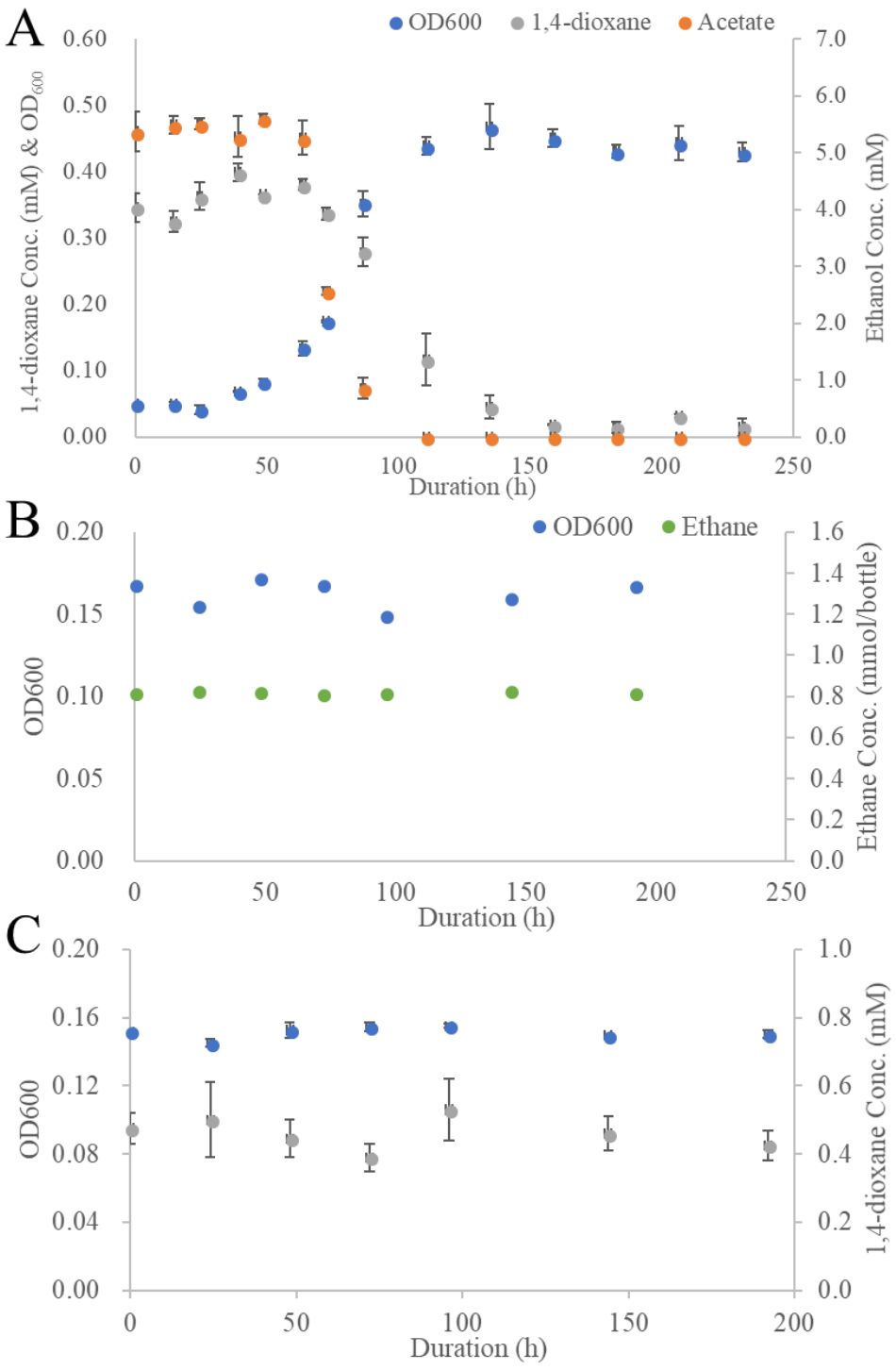

Figure 4. 3 Batch tests for *R. ruber* 219. (A) shows the dioxane oxidation performance of *R. ruber* 219 with acetate as the primary electron donor. (B) and (C) show the *R. ruber* 219 growth with ethane and 1,4-D as the sole electron donor.

I eventually succeeded in enriching mixed an ethanotrophic culture from the wetland sediments inoculum. The enriched ethanotrophic mixed culture was able to rapidly oxidize ethane at a rate of 11 µmol/day, shown in Fig. 4.4. In the presence of ethane as the primary electron donor, the mixed culture also was able to degrade 14-D.

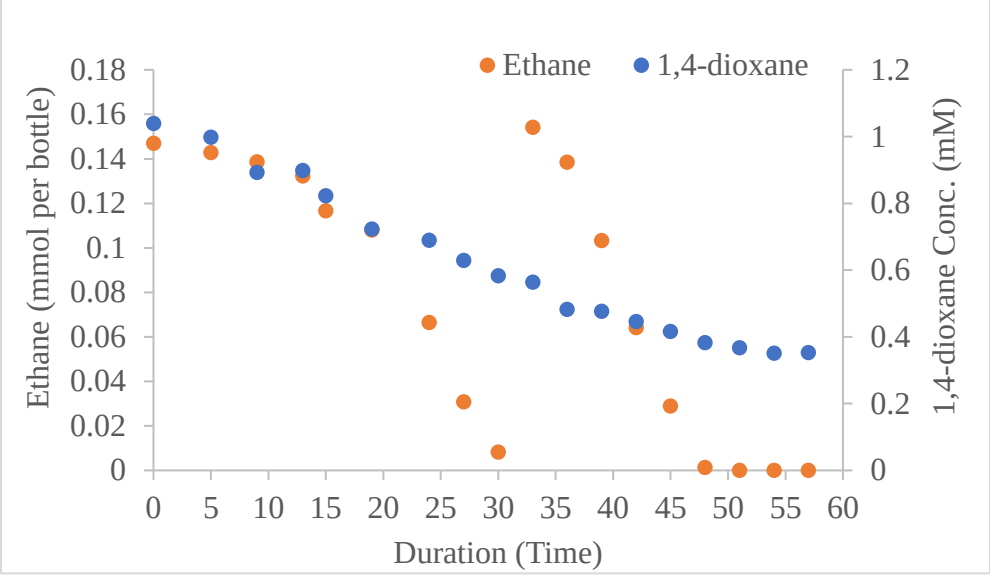

Figure 4. 4 Wetland sediments enriched 1,4-D oxidation culture with ethane as the primary electron donor.

4.3.2 Dioxane removal in the O_2-based MBfR

I set up an O_2-based MBfR (O_2-MBfR) and inoculated it with the pure culture *R. ruber* 219. MBfR operation does not sustain pure cultures (Zhou et al., 2014), and a mixed culture ultimately developed. The configuration of the O_2-MBfR was identical to the H_2-MBfR, except that the fiber lumens were pressurized with gaseous O_2, not H_2. Results are summarized in Figure 4.5. In the initial enrichment stage, the O_2-MBfR was fed with 2.5

mM acetate and set at a long HRT of 24 hour to promote the biofilm growth. The O_2-based MBfR soon attained the capability of complete acetate removal. I then increased the influent concentration to about 12 mM (Fig. 4.5A). In the second stage, I operated the O_2-MBfR in a sequencing-batch mode and started to feed it with acetate and 1,4-D. The trend was similar to the batch bottles: 1,4-D was removed only in the presence of acetate as the primary electron donor (Fig. 4.5B).

Oxidation of 1,4-D slowed after few acetate re-spikes. To test if this biofilm could maintain stable 1,4-D oxidation capacity, I switched the reactor to the continuous mode. In the continuous mode, the influent concentration of acetate was 10 mM, 1,4-D was 200 μM, and the HRT was 48 hours. During continuous operation, I observed that 1,4-D removal was gradually decreasing, probably due to the loss of monooxygenation capability. Another possible reason was that the bacteria capable of 1,4-D oxidation (including *R. ruber* 219, but probably also other species) gradually lost their dominant position in the biofilm or even were washed out, as the high acetate-to-dioxane ratio might have been favorable to other faster growing bacteria that utilized acetate, but not 1,4-D. To decrease the acetate-to-dioxane ratio, I increased the influent 1,4-D concentration from 200 μM to 300 μM and decreased the acetate concentration form 11 mM to 5 mM and then to 2 mM during continuous operation. The negligible decrease of 1,4-D concentration in the effluent for four weeks reveals that the lost capacity of 1,4-D removal was not recovered. Thus, *R. ruber* 219 or other 1,4-D oxidizers had been washed out before I made the concentration change. Overall, the results show that the pure culture *R. ruber* 219 could remove 1,4-D, but did not maintain stable 1,4-D-removal capability in the O_2-MBfR.

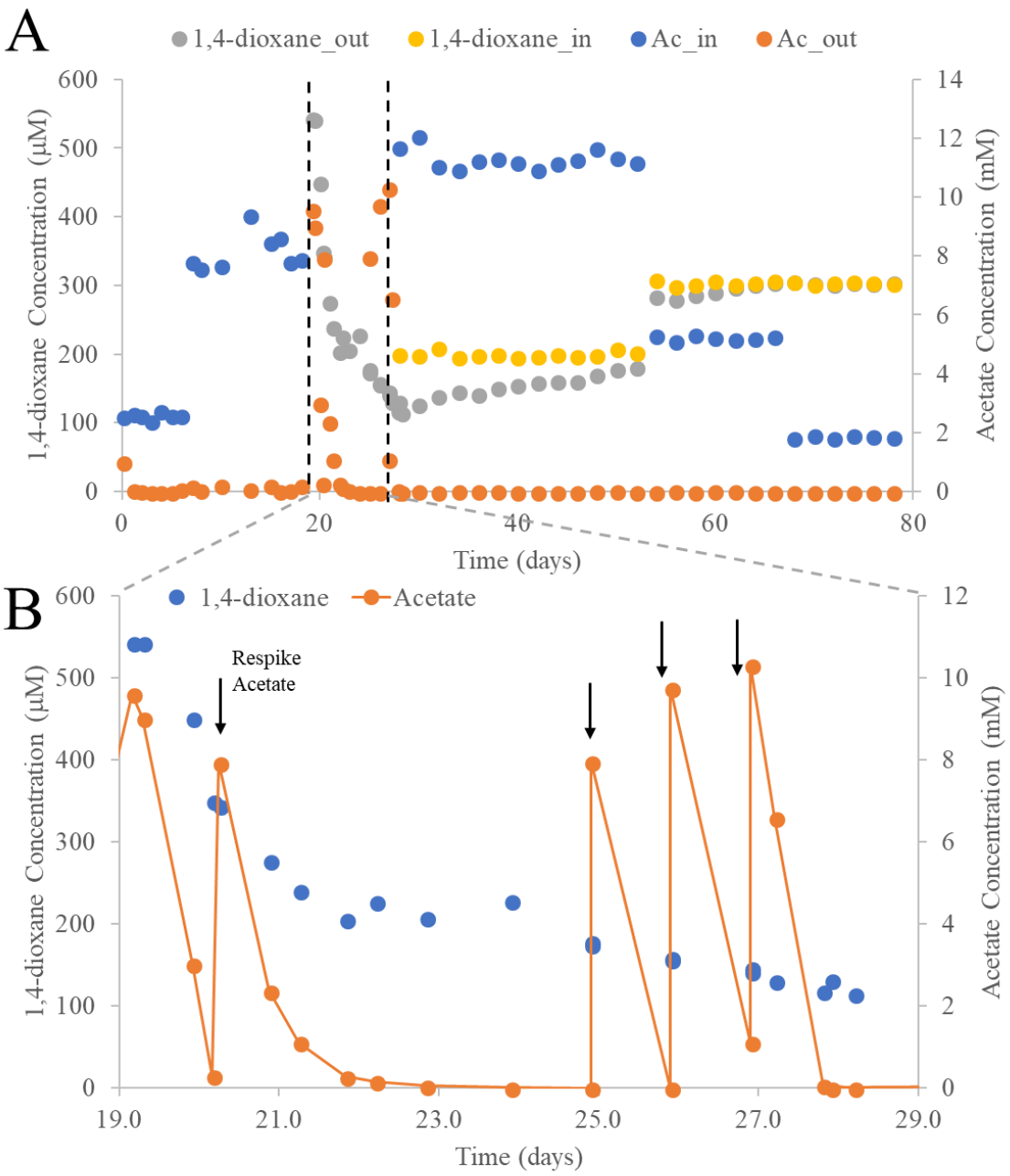

Figure 4. 5 Performance of the O_2-MBfR inoculated with *R. ruber* 219 in continuous (A) and batch (B) modes.

I then operated an O_2-MBfR fed with 1,4-D medium (0.5 mM) saturated with ethane (up to 58 mg/L or 1.9 mM soluble ethane after sparkling with pure ethane gas). The O_2-MBfR was inoculated with wetland sediment. Figure 4.6 presents the results. In the first stage, 1,4-D removal began after 18 days and gradually reached 7% within the following 6 days. Once ethane was fully consumed, 1,4-D also began to be consumed rapidly. By Day 38, 1,4-D removal reached a steady state with > 99% removal, which was stable for over four weeks.

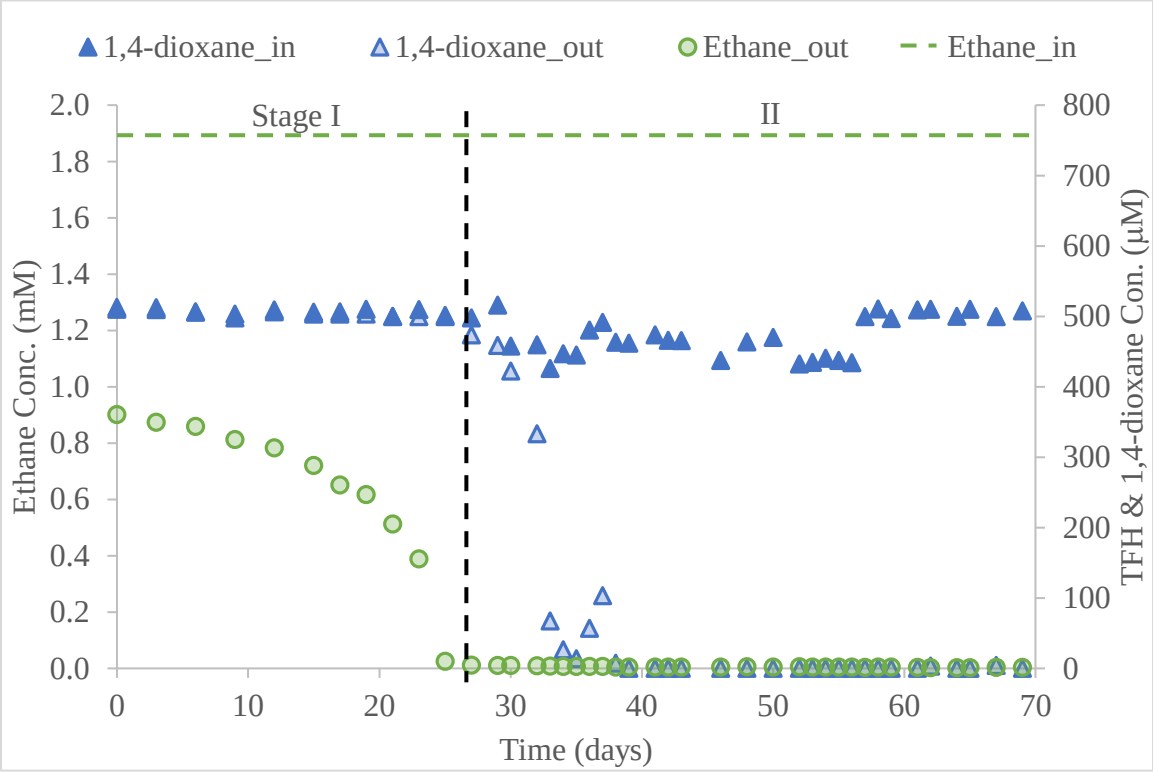

Figure 4. 6 1,4-D concentrations in the O_2-MBfR. The first black dash line indicates the day when the reactor was re-inoculated.

4.3.3 Summary

A pure culture of *R. ruber* 219 had the capability for 1,4-D degradation with acetate as the primary electron donor, but not with ethane or 1,4-dixoane alone. When this pure culture inoculated the O_2-MBfR, the biofilm could remove 1,4-D with acetate as the primary electron donor initially; 1,4-D removal did not persist with acetate feeding, since the 1,4-D oxidizing bacteria were out-competed by other acetate-consuming bacteria. When the O_2-MBfR was inoculated with and enriched ethanotrophic culture and fed with 1,4-D and ethane, an ethanotrophic biofilm grew and achieve stable 1,4-D removal > 99%. Since the H_2-MPfR can reductively transform TCA and TCE to mainly ethane and few byproducts, the ethane and 1,4-D in the effluent of H_2-MPfR are likely to be treated with this subsequence O_2-MBfR. This is the topic of the next chapter.

5 SYNERGISTIC REMOVAL OF TCE, TCA, AND 1,4-D

5.1 Strategies for synergistic removal

The previous experiments proved that 1,4-D is not removed in a reduction stage (biodechlorination and Pd-HDC). Furthermore, one-step aerobic biodegradation is ineffective for TCA and TCE. Promising is synergistic removal that involves reductive dechlorination for TCE ad TCA and aerobic oxidation for 1,4-D and by-products of reductive dechlorination.

For the reduction stage, based on my experiments and the literature, Pd-catalyzed reductive dechlorination can produce an effluent containing MCA (one product of TCA reduction), ethene (main terminal products of TCE reduction), and ethane (main product of MCA dichlorination and ethene hydrogenation). Continuous operating results show that an H_2-MPfR can have an effluent containing mostly ethane, MCA, DCA, all of which can be oxidized in the O_2-MBfR.

In this chapter, I first operate the H_2-MPfR and O_2-MBfR in sequence. Then, I optimize the operating conditions to minimize undesired products and system inputs.

5.2 Methods

Since the H_2-MPfR can reduce TCE and TCA to ethane simultaneously and the O_2-MBfR can remove 1,4-D with ethane as the primary electron donor, the two reactors were ready to be connected in series. I connected the two reactors by linking the effluent tube of H_2-MPfR to the influent tube of O_2-MBfR; this is illustrated in Fig. 5.1. VOCs in the gas phases of the H_2-MPfR and O_2-MBfR were measured through gas-sampling points 1 and 2, and their dissolved concentrations were calculated using Henry's law. The concentration of 1,4-D and chloride concentration were measured by GC and IC for samples taken through the two liquid-sampling points.

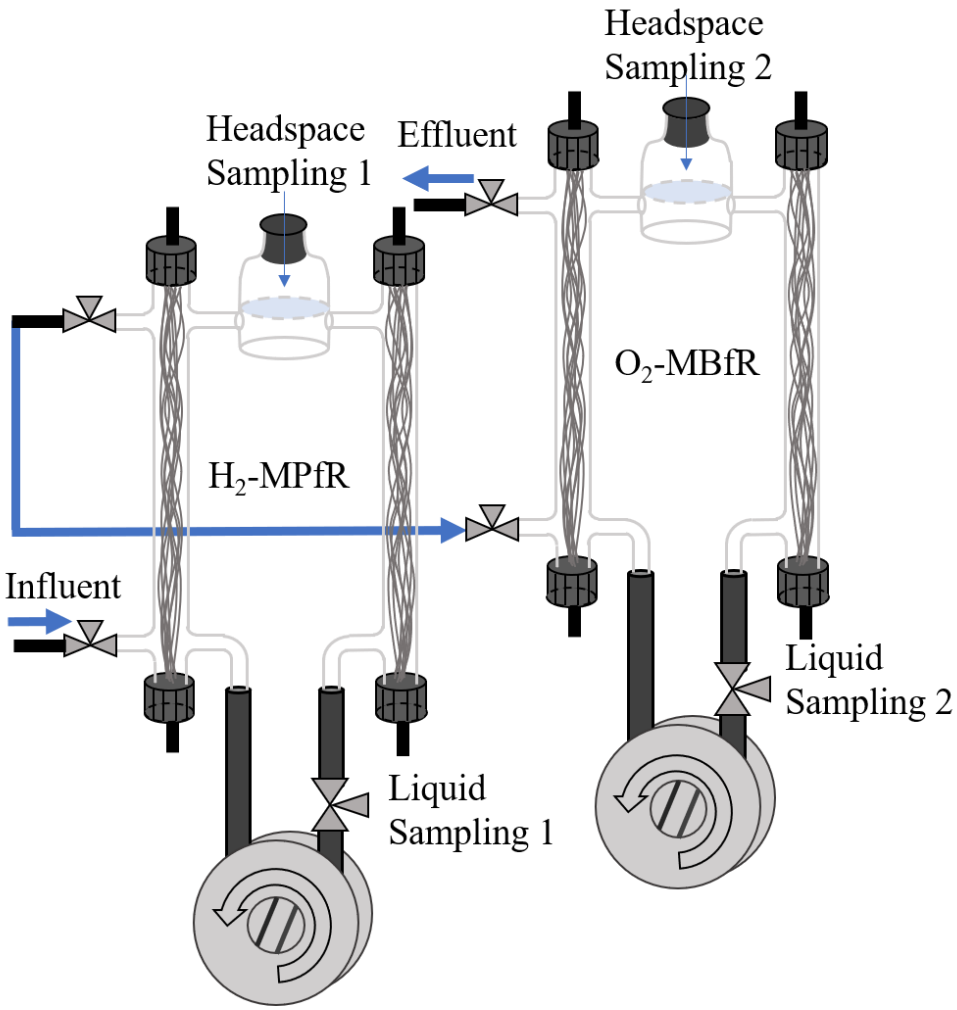

Figure 5. 1 Schematic of the H_2-MPfR and O_2-MBfR operated in sequence.

5.3 Results and discussion

Figures 5.2 and 5.3 profile concentrations of the major (ethane and 1,4-D) and minor (chlorinated hydrocarbons) electron donors in the O_2-MBfR receiving the effluent from the H_2-MPfR during the 130 days of sequential operation. In Stage 1, once the two reactors were connected and operated in sequence, the degradation of 1,4-D was strongly inhibited (Fig. 5.2). Zhang et al. (2016) investigated the inhibition of 1,4-D degradation

by 1,1-DCE, cis-DCE, TCE, and TCA, and they showed that 1,1-DCE had the strongest inhibition effect on 1,4-D biodegradation. Though they did not test DCA and MCA, I hypothesize that these by-products from the H_2-MPfR also inhibited 1,4-D biodegradation in the O_2-MBfR. To attenuate the inhibitive effect, I first increased the H_2 supply from 20 to 30 psig (Days 72 to 85). This did not decrease the production of TCA, DCA, and MCA in the H_2-MPfR. Then, I increased the O_2 supply from 10 to 15 psig (Days 85 to 94) in the O_2-MBfR. Removal of neither 1,4-D nor chlorinated hydrocarbons was enhanced; this indicates that O_2 was not a limiting factor in oxidizing the chlorinated hydrocarbons to eliminate their inhibition on 1,4-D. Table 5.1 lists the measured concentrations of chlorinated solvents in the O_2-MBfR. Most of the chlorinated solvents from the effluent of MPfR were further oxidized to less than 5 μM in the O_2-MBfR, but 1,1-DCA was recalcitrant to oxidation and remained as high as 36 μM (only 60% removal of the effluent from the H_2-MPfR). Since 1,1-DCA has the most similar structure to 1,1-DCE, I further hypothesized that 1,1-DCA probably inhibited 1,4-D oxidation.

I did a batch test in the O_2-MBfR to test if the biofilm could remove 1,4-D with a low ethane concentration. The results in Figure 5.4 show that the biofilm retained the capacity of 1,4-D biodegradation with or without ethane as the primary electron donor, and the reaction rates were similar (83 and 90 mM hr^{-1} with and without ethane, respectively). The results indicate that the inhibition of 1,4-D was reversible, and the biofilm was able to remove 1,4-D even with a low ethane concentration.

Based on the previous results, I decreased the influent concentration of TCA and TCE from 1.0 mM to 0.1 mM in Stage II to minimize the inhibition of TCE, TCA, and their reductive transformation products to 1,4-D oxidation. The influent concentration of 1,4-D also was decreased from 500 μM to 200 μM to maintain a reasonable TCE/TCA-to1,4-D ratio (Anderson et al. 2012). In the H_2-MPfR, the effluent concentrations of TCA/TCE and all the products were significantly decreased (Fig. 5.3). In the O_2-MBfR, these chlorinated hydrocarbons were further removed to undetectable levels. In particular, the 1,1-DCA concentration gradually dropped to 6.8 μM within 10 days. Accordingly, the capacity of 1,4-D biodegradation was recovered from 8% to 97%. This observation further confirms that the presence of chlorinated hydrocarbons, especially 1,1-DCA at a concentration of above 200 μM in our study, inhibited 1,4-D biodegradation, but the inhibition could be removed.

5.3 Summary

For the two-stage synergistic system, I operated the H_2-MPfR and the O_2-MBfR in sequence. When the influent concentrations of TCA and TCE were about 1.0 mM, the H_2-MPfR could achieve 96% TCA removal and 99% TCE removal, along with ethane as the main product, with 36 μM DCA and 3 μM MCA as the minor byproducts. The remaining chlorinated solvents from the H_2-MPfR to the O_2-MBfR strongly inhibited the oxidation of 1,4-D removal in the O_2-MBfR (decreased from >99% removal to 8%). By decreasing the influent concentrations of TCA and TCE to 0.1 mM, the inhibition of 1,4-D oxidation was minimized. The detailed performance results are present in Table 5.1. The removal of 1,4-D recovered to over 97%, with minimal chlorinated solvents in the effluent as well. Thus, the two-stage synergistic system successfully achieved

simultaneous removals of TCA and TCE by Pd-catalytic reductive dechlorination and subsequently 1,4-D removal by aerobic biodegradation.

Table 5.1 Concentrations of dissolved VOCs at steady state during Stages I and II in H_2-MPfR and the O_2-MBfR operated continuously in sequence.

Dissolved VOCs (μM)		TCE	TCA	DCA	MCA	Ethane	Sum
	Influent	780	600	0	0	0	1380
	MPfR	9.7	42.4	60.3	25.3	312	459
Stage I	MBfR	2.1	4.3	36.0	2.9	27.3	73
	Influent	98	103	0	0	0	201
	MPfR	4.1	15.5	6.2	10.5	61.5	98
Stage II	MBfR	1.2	6.4	3.64	0.14	2.4	134

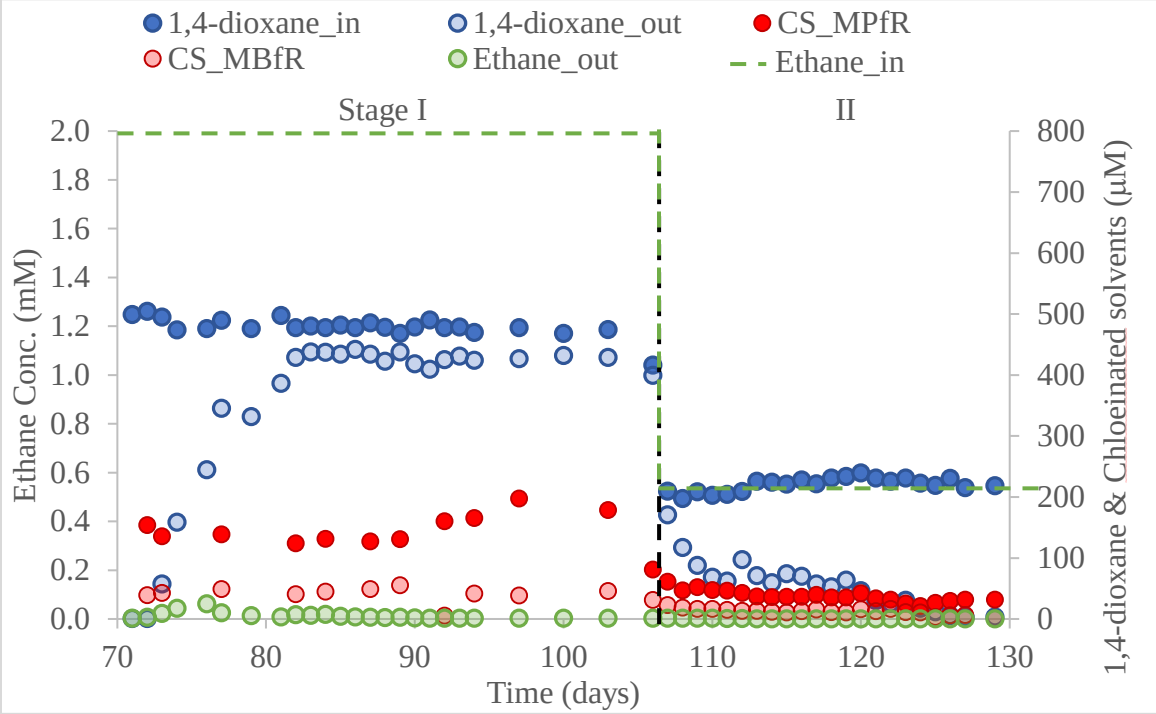

Figure 5. 2 The 1,4-D concentration in the O_2-MBfR fed with the effluent from the H_2-MPfR during the 130 days of sequential operation. The vertical black dash line indicates the day when the influent concentrations of all the three substrates were decreased. The red dots indicate the total chlorinated solvent (CS) concentration in both H_2-MPfR and O_2-MBfR.

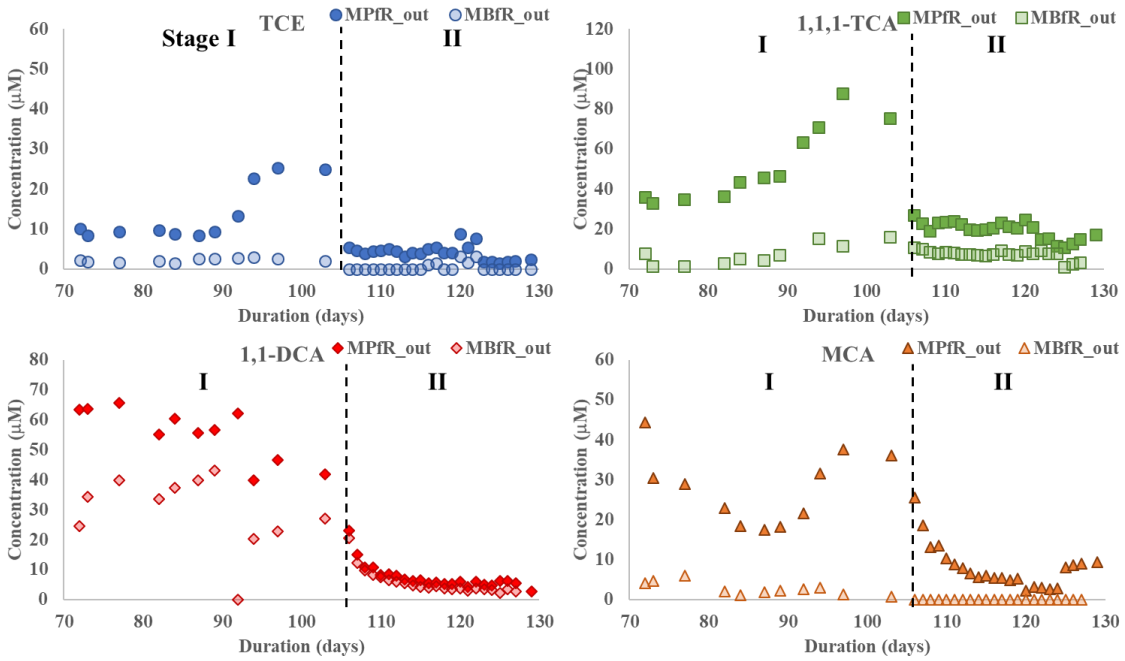

Figure 5. 3 Concentration of chlorinated solvents in H_2-MPfR and O_2-MBfR when operated in series. The vertical back dashed line indicates the stage changing from I to II.

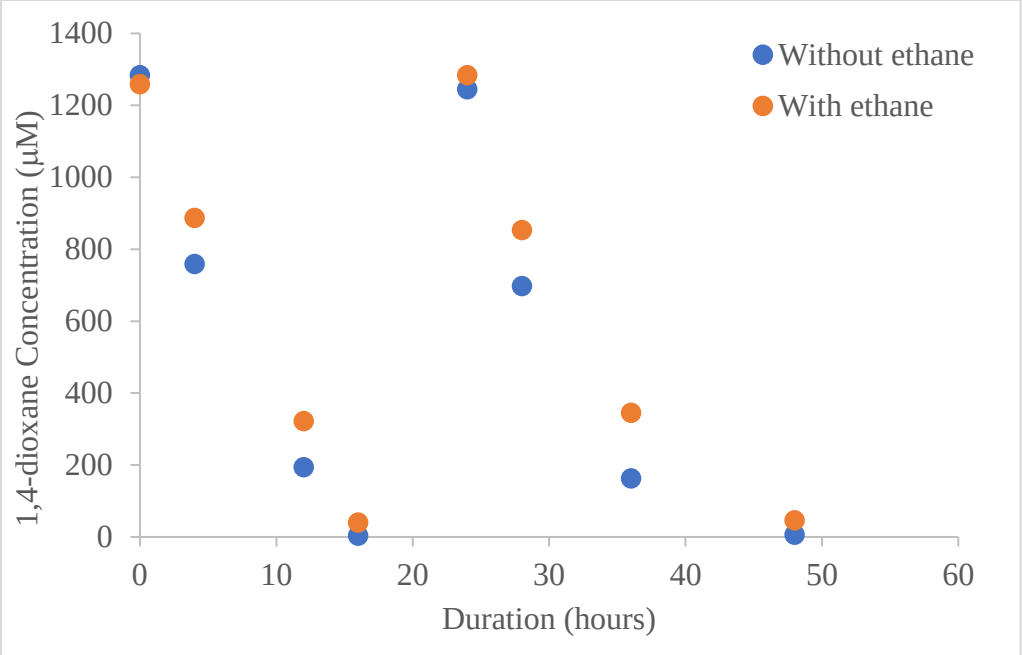

Figure 5. 4 A short batch to test the 1,4-D degrading activity of the O_2-MBfR without ethane as primary electron donor.

6 CONCLUSIONS AND FUTURE WORK

PdNPs coated well to the gas-delivering membranes and provided effective HDC in the H_2-based MPfR. Batch and continuous studies in the H_2-based MPfR proved that TCA and TCE could be reduced to ethane, with DCA and MCA as the main by-products. The HDC reaction rate in the H_2-based MPfR was mostly controlled by the H_2-supply pressure, and selectivity towards ethane was controlled by the initial concentrations of TCA and TCE. Thus, the H_2-based MPfR generated an effluent suitable for the next stage, aerobic biodegradation of 1,4-D.

When the O_2-based MBfR was inoculated and enriched with ethanotrophic culture and fed with 1,4-D and ethane, an ethanotrophic biofilm grew and achieved stable 1,4-D removal > 99%. Since the H_2-based MPfR was able to reductively transform TCA and TCE to mainly ethane and few byproducts, the ethane and 1,4-D in the effluent of the H_2-based MPfR can be treated with an O_2-based MBfR in series.

By operating the H_2-based MPfR and O_2-based MBfR in sequence, this two-stage synergistic system could successfully treat co-contaminants TCA, TCE and 1,4-D. When the system was operating with high influent TCA and TCE concentrations (about 1 mM), the H_2-based MPfR was able to remove over 96% of TCA and 99% of TCE, but the concentrations of the partial-reduction products (DCA and MCA, 60 μM and 25 μM respectively) in the effluent were remain high strongly inhibited the biodegradation of 1,4-D in the O_2-MBfR (only 8% removal of 0.5 mM). However, decreasing the influent concentrations of TCA and TCE to 0.1 mM, which are more field-relevant concentrations, eliminated inhibition of 1,4-D biodegradation. Then, the synergistic

system achieved simultaneous removals of TCA, TCE, and 1,4-D removal in continuous operation with influent concentration of TCA and TCE at 0.1 mM and 1,4-D at 0.2 mM.

Although the two-stage synergistic system achieved >95% removals of TCA, TCE, and 1,4-D, their effluent concentrations still were higher than their MCLs: effluent concentration of TCA, TCE, and 1,4-D of 1.2 μM, 6.4 μM, and 3 μM. Thus, future work should focus on means to achieve still lower effluent concentrations of TCA, TCE and 1,4-D. To pursue better performance of the two-stage synergistic system, I suggest that deeper understanding of mechanisms are needed for Pd-based catalytic reductive dechlorination, PdNPs performance on the gas-delivery membranes, monooxygenation of 1,4-D, and the inhibition of chlorinated solvents to the 1,4-D oxidation bacteria.

Specifically, I recommend the following future studies:

1. Detailed kinetic studies of Pd-based catalytic reductive dechlorination for TCA and TCE, focusing on the effects of H_2 supply pressure, pH, and PdNPs size and loading.

2. Characterization of the PdNPs on the membrane surface: e.g., Pd-coverage, Pd-size, and Pd-crystal structure.

3. Detailed kinetic characterization for the O_2-based MBfR, focusing on O_2 supply pressure, pH, 1,4-D surface loading, and the impacts of inhibition from TCA, TCE, DCA, MCA, and VC).

4. Comprehensive evaluation of the biofilm's microbial-community structure and function in the O_2-MBfR. Special attention should be given to the abundance and expression of monooxygenases.

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