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RESEARCH ARTICLE

Coexistence of two distinct Sulfurospirillum populations respiring tetrachloroethene—genomic and kinetic considerations

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One sentence summary: Long-term coexistence of closely related populations in a bacterial consortium respiring the groundwater pollutant tetrachloroethene relies on their significantly different kinetic parameters.

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‡Julien Maillard, http://orcid.org/0000-0002-7498-7202

ABSTRACT

Two anaerobic bacterial consortia, each harboring a distinct Sulfurospirillum population, were derived from a 10 year old consortium, SL2, previously characterized for the stepwise dechlorination of tetrachloroethene (PCE) to cis-dichloroethene (cis-DCE) via accumulation of trichloroethene (TCE). Population SL2-1 dechlorinated PCE to TCE exclusively, while SL2-2 produced cis-DCE from PCE without substantial TCE accumulation. The reasons explaining the long-term coexistence of the populations were investigated. Genome sequencing revealed a novel Sulfurospirillum species, designated ‘Candidatus Sulfurospirillum diekertiae’, whose genome differed significantly from other Sulfurospirillum spp. (78%–83% ANI).

Genome-wise, SL2-1 and SL2-2 populations are almost identical, but differences in their tetrachloroethene reductive dehalogenase sequences explain the distinct dechlorination patterns. An extended series of batch cultures were performed at PCE concentrations of 2–200 μM. A model was developed to determine their dechlorination kinetic parameters. The affinity constant and maximal growth rate differ between the populations: the affinity is 6- to 8-fold higher and the growth rate 5-fold lower for SL2-1 than SL2-2. Mixed cultivation of the enriched populations at 6 and 30 μM PCE showed that a low PCE concentration could be the driving force for both functional diversity of reductive dehalogenases and niche specialization of organohalide-respiring bacteria with overlapping substrate ranges.

Keywords: organohalide respiration; Sulfurospirillum; reductive dehalogenase; tetrachloroethene; kinetics; numerical modeling
INTRODUCTION

Organohalide respiration (OHR) is a key bacterial metabolic pathway for the biodegradation of a wide range of halogenated compounds such as tetrachloroethene (PCE), a major groundwater pollutant (Adrian and Löfler 2016). Organohalide-respiring bacteria (OHRB) are spread among many phylogenetic groups and share the capability to produce reductive dehalogenases, the catalytic enzymes directly involved in the dehalogenation reaction (Atashgahi, Lu and Smidt 2016). The major known PCE-respiring bacteria belong to the genera Dehalococcoides, Dehalobacter, Desulfotobacterium and Sulfurospirillum, of which the two latter are facultative OHRB. The range of organohalide substrates converted by these genera varies extensively within Dehalococcoides (Zinder 2016) and Dehalobacter (Maillard and Holliger 2016), exhibiting a wider range than for Desulfotobacterium (Futagami and Furukawa 2016) and Sulfurospirillum (Goris and Diekert 2016). This broader organohalide substrate range is also reflected by the number and diversity of reductive dehalo- nase homologous genes (irdA) present in the available genomes. Although the sequences of identified PCE reductive dehalo- nases (PceA) vary significantly, all of them except one are able to dechlorinate PCE to cis-dichloroethene (cis-DCE) or further to vinyl chloride. Two notable exceptions are PceA of Desulfitobacterium sp. PCE1 (van de Pas et al. 2001) and PceA of D. maccartyi 11a5 (Zhao, Ding and He 2016), both of which dechlorinate PCE to TCE. Sulfurospirillum multivorans, one of the most studied OHRB, is able to dechlorinate PCE to cis-DCE (Goris and Diekert 2016). PceA of S. multivorans is extensively characterized and its crystal structure was solved (Bommer et al. 2014). The kinetic parameters associated with OHRB can be ascertained by models of various levels of complexity. The state of the art, as described in (Chambon et al. 2013), are numerical models that use Monod kinetics to describe the multiple guilds present in OHR ecosystems, where importance must be placed on the bacterial function rather than the individual species (Wade et al. 2016). Furthermore, numerical models can account for physical processes that may affect the bacterial activity and degradation of chlorinated ethenes (Aeppli et al. 2009; Kournetsova et al. 2010; Buchner et al. 2017). An anaerobic bacterial consortium, named SL2-PCEb, has been maintained for more than a decade in our laboratory. This consortium, which primarily consists of two different but coexisting Sulfurospirillum populations, was obtained from a fixed-bed bioreactor treating PCE contaminated groundwater (Rouzeau-Szynalski, Maillard and Holliger 2011). This consortium is characterized by the ability to perform stepwise dechlo- rination of PCE to trichloroethene (TCE) and cis-DCE (Mail- lard et al. 2011). Two consortia were enriched from SL2-PCEb, each harboring a unique Sulfurospirillum population and each exhibiting distinct dechlorination potential. Consortium SL2- PCEc, harboring population SL2-1, dechlorinates PCE to TCE only. Consortium SL2-TCE, which was selected on TCE and harbors population SL2-2, has kept the potential to dechlorinate both PCE and TCE to cis-DCE. The reductive dehalogenase gene of both populations was identified as pceACcis and pceACciscis. Their respective gene products were shown to use PCE only in the case of PceAcis from population SL2-1, or PCE and TCE (PceAcis from SL2-2) (Buttet, Holliger and Maillard 2013). However, we think that the ability of population SL2-2 to dechlorinate TCE, in con- trast to population SL2-1, cannot fully explain the long-term co- existence of both SL2 populations in the parental consortium.

PCE dechlorination occurs via the successive elimination of chlorine atoms. PceA of S. multivorans catalyzes the reduc- tion of PCE to cis-DCE without substantial accumulation of TCE (Neumann, Scholz-Muramatsu and Diekert 1994), which sug- gests that PCE to TCE dechlorination is the rate-limiting step during successive dechlorination. Bacterial consortia containing several OHRB that compete for PCE, such as the two SL2 populations in this study, constitute an ideal case study by which more complex ecological systems can be better understood. Therefore, the first aim of the present work was to identify the factors that enable the long-term coexistence of the Sulfurospirillum populations SL2-1 and SL2-2 in the consortium SL2-PCEb. The second aim was to investigate the population-specific properties that may explain the populations’ successive appearance in the parent consortium (please note that in this study, successive appearance is distinct from the ecological concept of suc- cession). The genomes of the SL2 populations were sequenced in order to identify genetic features possibly responsible for the difference in their dechlorination potential and to compare the predicted metabolic pathways of the new Sulfurospirillum popula- tions with those of S. multivorans. Next, the dechlorination parameters of both populations were monitored in batch cultures, and then the growth kinetics were determined with a numerical model that was specifically developed for the culture conditions involving two liquid phases and one gas phase. Lastly, additional culture experiments were performed by mixing both Sulfurospirillum populations to highlight their sequence in dechlorination depending on the initial PCE concentration.

MATERIAL AND METHODS

All chemicals were analytical grade and used without purification. The gases (N₂, CO₂ and H₂) were purchased from SGL gas (Sauerstoffwerk Lenzburg, Switzerland).

Bacterial strains and growth conditions

The bacterial consortia SL2-PCEc and SL2-TCE were cultivated anaerobically in 125-mL serum bottles sealed with rubber- stoppers in 50 mL of medium as described previously (Buttet, Holliger and Maillard 2013), if not stated differently. Formate was added as electron donor to a final concentration of 20 mM. PCE stock solutions (10 mM to 1 M) were prepared in hexadecane (Holliger et al. 1998) and were used in a system with two liq- uid phases. The hexadecane phase allows the addition of sub- stantial amounts of chlorinated compounds to a batch system without affecting growth. The expected initial PCE concentra- tion in the aqueous phase ([PCEaq]) was calculated with the partition coefficient between hexadecane and water (log K OW) of 3.7 (~5000 part in hexadecane for 1 part in the aqueous phase) (Hol- liger et al. 1998). The total available amount of PCE in all culture flasks was kept at 500 μmol by varying both the volume of added organic phase and the PCE concentration in hexadecane ([PCEorg]). The consortia were cultivated anaerobically at 37°C in Luria–Bertani (LB) liquid medium or agar plates containing 100 μg mL⁻¹ of ampi- cillin when needed.
**Table 1. Bacterial strains and consortia, and plasmids used in this work.**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL2-PCEc</td>
<td>Bacterial consortium selected from SL2-PCEB containing a <em>Sulfurospirillum</em> population displaying PCE to TCE dechlorination</td>
<td>Maillard et al. (2011); Rouzeau-Szylnalski, Maillard and Holliger (2011); Buttet, Holliger and Maillard (2013)</td>
</tr>
<tr>
<td>SL2-TCE</td>
<td>Bacterial consortium selected on TCE from SL2-PCEB containing a Sulfurospirillum population displaying PCE to cis-DCE dechlorination</td>
<td>Maillard et al. (2011); Rouzeau-Szylnalski, Maillard and Holliger (2011); Buttet, Holliger and Maillard (2013)</td>
</tr>
<tr>
<td>E. coli DH5α</td>
<td>F′::endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG80d lacZΔM15 Δ(lacZYA-argFDU169, hsdR17(k- mK− rK−) λ−)</td>
<td>Invitrogen</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTIP-TQ</td>
<td>pGEM-T harboring a fragment of <em>pceATCE</em></td>
<td>Buttet, Holliger and Maillard (2013)</td>
</tr>
<tr>
<td>pTIP-DQ</td>
<td>pGEM-T harboring a fragment of <em>pceADCE</em></td>
<td>Buttet, Holliger and Maillard (2013)</td>
</tr>
<tr>
<td>pRPOB</td>
<td>pGEM-T harboring a fragment of <em>Sulfurospirillum rpoB</em></td>
<td>This study</td>
</tr>
</tbody>
</table>

**Table 2. Oligonucleotides used in this study.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Target gene</th>
<th>Sequence (5′→3′)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1-PTQ-f</td>
<td><em>pceATCE</em></td>
<td>CTTTGGAGGATACCTTGTAGTTA</td>
<td>Buttet, Holliger and Maillard (2013)</td>
</tr>
<tr>
<td>T1-PTQ-r</td>
<td><em>pceATCE</em></td>
<td>CTTTGGAGGATACCTTGTAGTTA</td>
<td>Buttet, Holliger and Maillard (2013)</td>
</tr>
<tr>
<td>T1-PDQ-f</td>
<td><em>pceADCE</em></td>
<td>GTCATTACGACTGTAAGTACC</td>
<td>Buttet, Holliger and Maillard (2013)</td>
</tr>
<tr>
<td>T1-PDQ-r</td>
<td><em>pceADCE</em></td>
<td>CATACCATGACCTGAAAGGA</td>
<td>Buttet, Holliger and Maillard (2013)</td>
</tr>
<tr>
<td>rpoB-f</td>
<td><em>rpoB</em></td>
<td>GATGCCTGGAATTTATTTGA</td>
<td>This study</td>
</tr>
<tr>
<td>rpoB-r</td>
<td><em>rpoB</em></td>
<td>AAGCCTCTAAAGTTAACAC</td>
<td>This study</td>
</tr>
</tbody>
</table>

**Chemical analyses**

The aqueous chloroethylene concentration was measured in all batch cultures. One milliliter of the aqueous phase was collected at defined time intervals, filtered (0.2 μm), and stored at −20°C. Chloroethylene concentration was analyzed with ion chromatography (ICS-90, IonPac AS14A-5 mm/3 × 150 mm column, lower; ACRS 500 2 mm, Dionex, Life Technologies, Zug, Switzerland) with a mix of 8 mmol L⁻¹ Na₂CO₃ and 1 mmol L⁻¹ NaHCO₃ as the eluent and 50 mmol L⁻¹ H₂SO₄ as the regenerating solution.

Chloroethenes were analyzed by gas chromatography with the Agilent Technologies 7890B system equipped with an Optima 624 LB (30 μm by 0.32 mm; M&N GmbH & Co. KG) coupled to a flame ionization detector. The carrier gas was helium, utilized at a flow rate of 1.8 mL min⁻¹. The column was kept at the initial temperature of 30°C for 5 min, raised to 60°C at a rate of 10°C min⁻¹, and then raised to 200°C at a rate of 40°C min⁻¹, and lastly the column was kept at the final temperature of 200°C for 5 min. Each culture was analyzed by collecting 1.5 mL of the gas phase from the culture flask in sterile conditions. Percentile proportions of PCE, TCE, and cis-DCE were calculated from the area of corresponding peaks obtained in each chromatograms that were divided by a response factor relative to the area of PCE (5.8 for TCE, and 21.8 for cis-DCE, as determined from analyzing known concentrations of individual chloroethenes in the same conditions).

**Quantitative PCR (qPCR)**

For qPCR, plasmids containing fragments of the target genes (*rpoB*, *pceATCE*, and *pceADCE*) were used as standards (Table 1). Primers to clone the *rpoB* gene, encoding the B subunit of the RNA polymerase and used here as a reference gene to follow *Sulfurospirillum* population size in the SL2 consortia, were designed based on the genomes of *S. multivorum* (Genbank CP007201.1), *Sulfurospirillum deleyianum* (CP001816.1) and *Sulfurospirillum halorespirans* (CP017111.1) (Table 2). The cloning procedure and preparation of qPCR standards were completed as previously described (Maillard et al. 2003; Buttet, Holliger, and Maillard 2013). Reaction mixtures were prepared as previously described (Buttet, Holliger, and Maillard 2013) and run in an MIC apparatus (Bio Molecular Systems, Labgene, Châtel-St-Denis, Switzerland). The thermocycling program for qPCR was as follows: an initial denaturation of 2 min at 95°C; then 45 cycles of 10 s at 95°C, 30 s at 55°C (rpoB) or 60°C (*pceATCE* and *pceADCE*), and 20 s at 72°C, after which the acquisition of fluorescence took place using the SYBR detection channel. A melting curve ranging from 72°C to 95°C was performed at 0.1°C s⁻¹. For each run, triplicates of samples and standards were run concurrently and the concentration of samples was calculated from the derived standard curve. The MicPCR v1.6.0 software was used for data analysis. Average run performances are given in Table S1 (Supporting Information). Replicates with the standard deviation of less than 15% of the average value were kept for analysis.

**Genomic DNA extraction and library preparation**

For genomic DNA extraction, two 200 μL batch cultures of the SL2-PCEc and SL2-TCE consortia were cultivated. The biomass was collected by centrifugation at 3300 × g for 10 min at 4°C. Biomass pellets were washed in 1 mL of 50 mM Tris-HCl buffer (pH 8.0) and collected by centrifugation at 8800 × g for 5 min. Biomass pellets (18.6 mg and 19.7 mg for SL2-PCEc and SL2-TCE, respectively) were flash-frozen in liquid N₂ and stored at −80°C. DNA extraction was performed with the DNeasy Blood & Tissue kit following the recommendations of the manufacturer (Qiagen, Hombrechtikon, Switzerland). Preparation of the genomic libraries was carried out according to Diaby et al. (2015). DNA samples were purified with magnetic beads (AxyPrep Mag PCR Clean-Up, Axygen, Fisher Scientific, Reinach, Switzerland) and fragmentated using the enzyme mix provided in the Ion Xpress Plus Fragment Library Kit according to the manufacturer instruc-
Comparing with those of other oxidoreductases were checked manually by comparing all eight assembled contigs. By comparing all eight assembled contigs, overlapping contigs were merged. By comparing all eight assembled contigs.
corresponding region in the genomes of S. diekertiae between 99.3 and 99.7% (Fig. 2). The largest difference is a high degree of sequence identity, with pairwise alignments of the gene clusters compared to that of rdh gene cluster responsible for the biosynthesis of corrinoids. DNA sequence alignment of the S. diekertiae cluster revealed that we propose to designate of a new species Sulfurospirillum diekertiae’ (shortened as S.diekertiae) that we propose to designate a new species Sulfurospirillum diekertiae’ (shortened as S.diekertiae) that we propose to designate a new species Sulfurospirillum diekertiae’ (shortened as S.diekertiae) that we propose to designate a new species Sulfurospirillum diekertiae’ (shortened as S.diekertiae) that we propose to designate a new species Sulfurospirillum diekertiae’ (shortened as S.diekertiae) that we propose to designate a new species Sulfurospirillum diekertiae’ (shortened as S.diekertiae) that we propose to designate a new species Sulfurospirillum diekertiae’ (shortened as S.diekertiae) that we propose to designate a new species Sulfurospirillum diekertiae’ (shortened as S.diekertiae) that we propose to designate a new species Sulfurospirillum diekertiae’ (shortened as S.diekertiae) that we propose to designate a new 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The designation of a new Sulfurospirillum species invited us to investigate gene clusters outside the OHR region; comparing these gene clusters to those from other members of this genus may be useful to characterize the physiology of this new species. The genomes of both S. diekertiae populations encode proteins for the oxidation and reduction of a large variety of organic and inorganic substrates. Similar to most other Sulfurospirillum spp., arsenate is likely to be reduced by this species with an arsenate reductase (SL2-1 loci Sdiek1_1006-1008); additionally, both populations contain an arsenite oxidase for the catabolic oxidation of arsenite (Sdiek1_1026-1028). Nitrate could be reduced by the Nap/Nir-system, and several molybdopterin oxidoreductases are found that might be involved in the oxidation and reduction of e.g. sulfur compounds (Goris and Diekert 2016). Similar to S. halorespirans (Sdiek1_967-982), suggesting the reduction of the greenhouse gas nitrous oxide by S. diekertiae. Five hydrogenase gene clusters are found in the S. diekertiae genomes, one periplasmic membrane-bound hydrogenase used for the oxidation of H₂ as an energy source (Sdiek1_1468-1471) and two cytoplasmic hydrogenases (Sdiek1_1364-1369; Sdiek1_1466-1467) with unknown function (Krus et al. 2017). The fourth hydrogenase (Sdiek1_2191-2201) gene cluster is likely to encode a membrane-bound H₂-evolving enzyme facing the cytoplasmic side similar to that of S. multivorans (Krus et al. 2017). Interestingly, the fifth hydrogenase (Sdiek1_1064-1073) is most likely part of a formate hydrogen lyase (FHL) complex, as it is co-localized with genes encoding a cytoplasmic formate dehydrogenase (Sdiek1_1063) and a formate channel protein (Sdiek1_1074). For E. coli, an FHL complex was proposed to diminish intracellular formate produced by a pyruvate formate lyase during fermentation (Sawers 1994). Since the latter enzyme is not encoded in the genome of S. diekertiae, an intracellular accumulation of formate is unlikely. Instead, the S. diekertiae FHL complex might contribute to growth on formate as found for syntrophic Moorella spp. or specialized Archaea (Dolfing et al. 2008; Kim et al. 2010).

Dechlorination as a measure for growth of SL2 consortia

Since the genomes of both SL2 populations were found to be extremely similar, the reason for their long-term coexistence was then investigated at the level of their dechlorination kinetics. To this purpose, batch cultures of both SL2 consortia were performed and monitored under various initial aqueous PCE concentrations. When chlorinated compounds are used as the sole terminal electron acceptor, chloride release is often used as a proxy for the growth of OHRB (Holliger 2003; Spormann and McCarty 2003; Prat et al. 2011). This was verified for the two Sulfurospirillum populations present in the consortia SL2-PCEc and SL2-TCE. To this purpose, rpox, the β subunit of the RNA polymerase, was chosen to monitor growth, as both populations harbor a single copy of this gene with full sequence conservation in their genomes. Indeed, chloride concentration and rpox gene copy number were monitored in triplicate cultures, and the data obtained show that the increase of rpox gene copy number of both SL2 populations aligns with the chloride release (Fig. S3, Supporting Information). The apparent yield on PCE (gene copy number per mole of PCE consumed) is approximately the same for both populations. However, the gene copy number yield per mole of chloride released of SL2-1 is approximately twice that of SL2-2, since the latter dechlorinates TCE further to cis-DCE. This unexpected result was further examined using the numerical model (see below).

PCE dechlorination kinetic parameters of individual S. diekertiae SL2 populations

To evaluate the kinetic parameters of individual S. diekertiae SL2 populations and determine whether one population presents a distinct advantage over the other in growth rate and/or affinity for PCE, more than 70 batch cultures (including replicates) with initial aqueous PCE concentrations of 2-200 μM were monitored for chloride release. In a first attempt to estimate the dechlorination-based growth rates (μ_{CL}) of individual batch cultures, the obtained chloride data were fitted with the Gompertz equation (Zwietering et al. 1990) (see Table S3, Supporting Information, for the complete fitting parameters), and plotted against the initial aqueous PCE concentration (Fig. 3). The apparent growth rate of the SL2-1 population clearly increased faster than that of SL2-2 in the initial part of the plot, suggesting that the former shows a higher affinity for PCE than the latter. However, above 50 and 80 μM PCE, SL2-1 and SL2-2 populations displayed a decrease in growth rate, respectively, indicative for inhibitory effects of PCE or of daughter products. This observation is to be compared to what has been reported for S. multivorans, which can dechlorinate PCE at concentrations lower than 330 μM but not at concentrations above 540 μM (Amos et al. 2007), suggesting that S. diekertiae is slightly more sensitive than S. multivorans to inhibition by chloroethenes. Lineweaver–Burk and Hanes–Woolf linearization procedures of the growth rates obtained at non-inhibitory PCE concentrations allowed the estimation of the apparent maximal growth rates ($μ_{max,CL}$) and affinity constants for PCE (K_{S,PCE}) for both SL2 populations (Fig. S4 and Table S4, Supporting Information). While the two populations seem to display a similar maximal growth rate of approximately 0.1 h⁻¹, the apparent affinity for PCE of S. diekertiae SL2-1 is 5 to 6-fold higher compared to SL2-2. The difference in affinity for PCE in both populations could explain why TCE accumulation was observed in the parental consortium. Indeed, when cultivated at PCE concentrations lower than 50 μM, the SL2-1 population is able to dechlorinate PCE at a faster pace relative to SL2-2 (Table S3, Supporting Information), resulting in the production of TCE, which can in turn be dechlorinated to cis-DCE by SL2-2.
Table 3. Kinetic parameters of S. diekertiae SL2 populations obtained after simulation of the measured dechlorination data.

<table>
<thead>
<tr>
<th>Population</th>
<th>Batch #</th>
<th>Initial aqueous PCE conc. (μM)</th>
<th>Vol. of organic phase (mL)</th>
<th>$k_{max,PCE}$ (gene cn·(μmol PCE h)$^{-1}$)</th>
<th>$K_{S,PCE}$ (μM)</th>
<th>Chloride NRMSE (%)</th>
<th>Gene cn NRMSE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL2-1</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>2.12·10$^{-09}$</td>
<td>0.96</td>
<td>2.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20</td>
<td>5</td>
<td>1.70·10$^{-09}$</td>
<td>7.84</td>
<td>2.51</td>
<td>4.15</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>30</td>
<td>3.33</td>
<td>2.61·10$^{-09}$</td>
<td>15.42</td>
<td>2.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>50</td>
<td>2</td>
<td>1.22·10$^{-09}$</td>
<td>1.47</td>
<td>4.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>100</td>
<td>1</td>
<td>9.15·10$^{-10}$</td>
<td>8.29</td>
<td>3.23</td>
<td></td>
</tr>
<tr>
<td>SL2-2</td>
<td>6</td>
<td>20</td>
<td>5</td>
<td>8.88·10$^{-09}$</td>
<td>65.82</td>
<td>2.20</td>
<td>7.45</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>50</td>
<td>2</td>
<td>2.24·10$^{-09}$</td>
<td>64.51</td>
<td>4.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>50</td>
<td>2</td>
<td>3.17·10$^{-09}$</td>
<td>63.01</td>
<td>2.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>100</td>
<td>1</td>
<td>2.46·10$^{-09}$</td>
<td>89.60</td>
<td>2.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>150</td>
<td>0.67</td>
<td>1.27·10$^{-09}$</td>
<td>84.59</td>
<td>2.96</td>
<td></td>
</tr>
</tbody>
</table>

*NRMSE: normalized root mean squared error.

A numerical model was also developed to describe the batch system and determine the kinetic parameters of dechlorination. Unlike the Gompertz model, the numerical model considers the distribution and transport of PCE and daughter products in the three-phase anaerobic batch system (two-liquid phases and the gaseous head space) and the effect of PCE removal from the system through successive sampling events. The model was tested on chloride datasets of 10 batches, selected in order to span the range of initial aqueous PCE concentrations used in the experiments. Batches #2 and #6 (Table 3), used to validate chloride release as a proxy for growth and displaying both chloride and rpoB gene copy number time series data, were included in the numerical simulations. The model was able to capture the development in biomass and chloride for all 10 datasets on which it was tested. Simulations for batches #2 and #6 are presented in Fig. 4 and show very good agreement between the measured and the simulated data for both populations. The goodness of fit is also substantiated by the normalized root mean squared error (NRMSE), which was calculated as a quantitative metric for all the tested batches (Table 3). In Fig. 4, the dechlorination rate is visualized, which shows when PCE is transformed most effectively by SL2-1 and SL2-2 populations in the two selected systems. The detailed simulated results for the selected datasets corroborate the $K_{S,PCE}$ values determined for both SL2-1 and SL2-2 for PCE dechlorination to their respective end product, with approximately an 8-fold difference. The best fit for each was determined to be 7.84 and 65.82 μM for SL2-1 and SL2-2, respectively, with ranges of 0.96–15.42 μM and 63.01–89.60 μM (Table 3). These values differ slightly from the $K_{S,PCE}$ values obtained using the Gompertz equation and the linearization procedures (Table S4, Supporting Information), but most probably better reflect the complexity of the three-phase system and the limitation imposed by mass transfer at low substrate concentrations, which is explicitly considered in the three-phase numerical model (see Section S2 of Supporting Information). Furthermore, dechlorination rates were found to decrease with increasing initial aqueous PCE concentration.

Growth yield factor and additional insights from the numerical modeling of dechlorination data

In addition to understanding of the kinetic parameters, the numerical model can provide insights into other physical and biological parameters of the three-phase batch culture system. The apparent similar growth yield of both SL2 populations cultivated on PCE was estimated and a yield factor of 1.32·10$^9$ rpoB gene cn·(μmol PCE)$^{-1}$, in agreement with published data on Sulfoispirillum spp. (Scholz-Muramatsu et al. 1995; Aeppli et al. 2009), was used to fit all dechlorination data successfully. To dissect the observed yield factor of population SL2-2 for individual dechlorination steps, two duplicate batches of population SL2-2 were cultivated with TCE rather than PCE as the electron acceptor. Fitting with the numerical model (for details see Supporting Information) revealed that dechlorination of TCE to cis-DCE accounted for approximately half of the biomass produced by SL2-2 when PCE was provided (Table S5 and Fig. S5, Supporting Information). The comparison with the yield factor of SL2-1 on PCE (one step dechlorination) suggests that single dechlorination steps in SL2-2 only generate half of the corresponding SL2-1 biomass. This observation needs further investigation that goes beyond the scope of the present study.

The numerical modeling exercise allowed us to quantitatively interpret some important aspects of the dynamic interplay between physical, chemical and biodegradation processes in the experimental three-phase system (see the description in Section S2 of Supporting Information). For instance, via the model simulations, the PCE concentration below which the system is mass-transfer limited could be elucidated (Fig. S6, Supporting Information) and the effect of sample size on the change in phase volume could be visually depicted (Fig. S7, Supporting Information). The model was also used to explore the possible bacterial growth patterns with similar kinetic parameters for each strain. If only the affinity for PCE ($K_{S,PCE}$) had differed in the two populations, but the maximum specific dechlorination rate kept identical, the coexistence of the two strains would have been due only to their different electron acceptor spectra (Fig. S8, Supporting Information).

The succession of S. diekertiae SL2-1 and SL2-2 depends on the initial PCE concentration

Knowing that each SL2 population has a different affinity for PCE, batch cultures with both populations together were established to show the effect of the initial PCE concentration on the sequence of SL2-1 and SL2-2 populations in PCE and TCE dechlorination, respectively. The two separate consortia were first cultivated at 6 and 30 μM [PCEaq], and then a mix of equal population proportion was used as inoculum to start cultures amended with the same initial concentrations of PCE. Care was taken to avoid PCE concentrations leading to inhibition. The cultures were monitored for the relative concentration of chlorinated ethenes, chloride release, and the abundance of population-specific pceA genes (Fig. 5). At an aqueous PCE concentration of 6 μM, a clear accumulation of TCE was observed between 48 and
Figure 4. Data, and simulation thereof, of representative batch cultures of *S. diekertiae* SL2-1 and SL2-2 populations. The evolution of measured chloride concentration (A and D) and rpoB gene copy number (B and E) are displayed together with the corresponding simulated data for the consortia SL2-PCEc (harboring *S. diekertiae* SL2-1) (A and B) and SL2-TCE (*S. diekertiae* SL2-2) (D and E) cultivated at 20 μM initial aqueous PCE concentration. The simulated PCE consumption rate is also displayed for SL2-PCEc (panel C) and SL2-TCE (panel F). The fitting parameters obtained from the model for both batch cultures are given in Table 3.

96 h (Fig. 5A), while the SL2-1 population initially grew at a faster rate than SL2-2 (Fig. 5B). Once most PCE was dechlorinated, the population size of SL2-1 stalled and then decreased, while SL2-2 took over and was responsible for TCE dechlorination. Starting at 30 μM aqueous PCE, TCE did not significantly accumulate (Fig. 5C) and both populations grew at approximately the same rate during the first dechlorination step (PCE to TCE), thus reflecting the simultaneous action of both populations (Fig. 5D). After 40 h of incubation, cis-DCE started to appear and was accompanied by a significant increase in SL2-2 population size. The two growth patterns observed here clearly highlight the distinct and concentration-dependent PCE dechlorination rate by the individual SL2 populations. The coexistence of both populations in SL2-PCEb was made possible by the relatively low frequency of culture transfer allowing the SL2-2 population to maintain a substantial population size in SL2-PCEb. These kinetic considerations together with the relaxed time frequency of culture transfers maintained throughout the years explain the long-term coexistence of both SL2 populations in the parental consortium.

**CONCLUSIONS**

During several years of cultivation of the SL2-PCEb bacterial consortium that dechlorinates PCE successively to TCE and cis-DCE, two distinct populations of PCE-dechlorinating *S. diekertiae* were able to coexist. Such a long-term coexistence raised questions about the nature and details of the interplay between these two closely related populations. As demonstrated in the first part of this study, both *S. diekertiae* populations are highly similar at the genome sequence level. Only their respective *pceA* genes show significant differences and appear as a hotspot for mutations, which may represent a possible driving force for the evolution and fine-tuning of *Sulfurospirillum* OHR metabolism. Amino acid changes in the sequence of PceATCE (present in population SL2-1) could be one of the reasons for the specialization of the
Figure 5. Growth pattern of *S. diekertiae* SL2-1 and SL2-2 populations at two different initial PCE concentrations. The two consortia were mixed together and cultivated at 6 μM \([\text{PCE}_{\text{aq}}]\) (A and B) or 30 μM \([\text{PCE}_{\text{aq}}]\) (C and D). The dechlorination of PCE (diamonds) to TCE (triangles) and cis-DCE (black circles) is shown alongside with the chloride release (white circles) in panels A and C. The gene copy number of *pceA\text{_{TCE}}* (SL2-1, triangles), *pceA\text{_{DCE}}* (SL2-2, circles), is presented in panels B and D. Please note the different time scale of experiments in both cultures. The other replicate cultures followed the same trend (data not shown).

The ecological implications of these findings remain to be investigated, but it could be imagined that similar OHRB interplay is likely to occur in environments that display PCE concentration gradients. In order to predict the dechlorination rate of relevant OHRB at polluted sites, it is therefore of great importance to identify the kinetic parameters of individual cultures and their reductive dehalogenases. In addition, (self-)inhibition of dechlorination by PCE and daughter products should be further investigated to expand the applicability of the proposed model.

**SUPPLEMENTARY DATA**

Supplementary data are available at [FEMSEC](https://academic.oup.com/femsec/article-abstract/94/5/fiy018/4923013) online.

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**Conflicts of interest.** None declared.

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