Interspecies transfer of biosynthetic cobalamin for complete dechlorination of trichloroethene by *Dehalococcoides mccartyi*

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**ABSTRACT**

Complete dechlorination of trichloroethene (TCE) by *Dehalococcoides mccartyi* is catalyzed by reductive dehalogenases (RDases), which possess cobalamin as the crucial cofactor. However, virtually all *D. mccartyi* isolated thus far are corrinoid auxotrophs. The exogenous addition of commercially available cobalamin for TCE-contaminated site decontamination is costly. In this study, TCE reduction by a *D. mccartyi*-containing microbial consortium utilizing biosynthetic cobalamin generated by interior corrinoid-producing organisms within this microbial consortium was studied. The results confirmed that subcultures without exogenous cobalamin in the medium were apparently unaffected and were able to successively metabolize TCE to nonchlorinated ethene. The 2-bromoethanesulfonate and ampicillin resistance tests results suggested that ampicillin-sensitive bacteria rather than methanogenic archaea within this microbial consortium were responsible for biosynthesizing cobalamin. Moreover, relatively stable carbon isotopic enrichment factor ($\epsilon_{\text{carbon}}$) values of TCE were obtained regardless of whether exogenous cobalamin or selective inhibitors existed in the medium, indicating that the cobalamin biosynthesized by these organisms was absorbed and utilized by *D. mccartyi* for RDase synthesis and eventually participated in TCE reduction. Finally, the Illumina MiSeq sequencing analysis indicated that *Desulfotobacterium* and *Acetobacterium* in this microbial consortium were responsible for the *de novo* cobalamin biosynthesis to fulfill the requirements of *D. mccartyi* for TCE metabolism.

**Key words:** biosynthesis, corrinoid auxotrophs, corrinoid-producing organisms, microbial consortium, TCE reduction

**HIGHLIGHTS**

- TCE degradation to ETH via *D. mccartyi*-containing consortium without exogenous cobalamin is achieved.
- Biosynthesized cobalamin facilitates stable $\epsilon_{\text{carbon}}$ values for TCE.
- Bacteria rather than archaea in this consortium are potential cobalamin synthesizers.
- *Desulfotobacterium* and *Acetobacterium* are responsible for cobalamin biosynthesis.
1. INTRODUCTION

Trichloroethene (TCE) is a widely used industrial solvent owing to its excellent solvent properties (Liu et al. 2017). However, as a result of its improper handling, storage, and disposal, TCE has become one of the ubiquitous chlorinated organic pollutants detected in contaminated groundwater and soil sites in recent decades (He et al. 2007; Men et al. 2014a). Remediation of TCE pollution via microbial anaerobic dechlororespiration is commonly considered to be viable and sustainable (Men et al. 2013). However, partial dechlorination of TCE to less chlorinated intermediates, including isomers of dichloroethylenes (DCEs) and vinyl chloride (VC), by organohalide-respiring bacteria remains problematic because of their greater threat to the environment than TCE (Liu et al. 2018; Anam et al. 2019). Recently, the bacterium *Dehalococcoides mccartyi*, which harbors the ability of completely dechlorinating TCE to nonchlorinated ethene (ETH), has received extensive attention. Manifold reductive dehalogenases (RDases) encoded by the dechlorinating genes *tceA*, *bvcA*, and *vcrA* within *D. mccartyi* are directly in charge throughout the degradation process (Brisson et al. 2012; Liu et al. 2018).

Reductive dechlorination conducted by pure *D. mccartyi* typically requires extremely strict growth conditions (Löffler et al. 2005; Men et al. 2013). The coexistence of *D. mccartyi* with other anaerobic microbes in microbial enrichments is generally more robust than *D. mccartyi* alone and is thus commonly used in dechlorination practices (Brisson et al. 2012; Harding et al. 2013; Men et al. 2014a). Within *D. mccartyi*-containing communities, a variety of organisms, including fermenters, acetogens, and methanogens, are ubiquitously identified and ultimately constitute a complex network with *D. mccartyi* (Li et al. 2019; Hellal et al. 2021). The significances of these microbes on microbial reductive dechlorination have already been confirmed in a small number of researches before (Men et al. 2013; Liu et al. 2017; Li et al. 2019; Wen et al. 2020). In our previous study, it was suggested that nondechlorinating organisms coexisted within a *D. mccartyi*-containing microbial consortium, playing a crucial role in scavenging oxygen to protect the strictly anaerobic *D. mccartyi* from being damaged. TCE dechlorination to ETH by this microbial consortium was achieved, rising oxygen concentrations up to 7.2 mg/L (Liu et al. 2017).

Cobalamin affiliated with corrinoids is an indispensable cofactor that functions in RDases (Brisson et al. 2012). Unfortunately, however, genome sequence analyses have demonstrated that most *D. mccartyi* isolated thus far (i.e., CBDB1, BAV1, 195, VS, and GT) are corrinoid auxotrophs (Men et al. 2013). Ten of the 17 critical upstream corrin ring biosynthesis genes...
for cobalamin de novo synthesis are absent in the genomes of D. mccartyi (Hug et al. 2012). Therefore, de novo corrinoids synthesis by D. mccartyi is impossible (Hug et al. 2012; Men et al. 2013). However, the putative corrinoid salvaging and remodeling genes within the genomes of all sequenced D. mccartyi strains are intact, which means that D. mccartyi strains have versatile capacities to absorb and modify nonfunctional corrinoids to form cobalamin from the surrounding environment and ultimately the requirement of this enzymatic cofactor is satisfied (Hug et al. 2012; Men et al. 2014a). Generally, exogenous cobalamin is regularly supplemented into the medium to fulfill the growth requirements as well as to enhance the dechlorination performance of D. mccartyi for both D. mccartyi in isolation and D. mccartyi-containing enrichments (Men et al. 2014a, 2014b; Jácome et al. 2019). However, the addition of cobalamin, the only cobamide that is commercially available to a TCE-contaminated field site, can be unrealistic on account of its exorbitant price (approximately 1,800 USD per 100 g) (Jácome et al. 2019). Instead, it has long been recognized that D. mccartyi growing in mixed consortia enables the utilization of cobalamin generated by other members via interspecies cobalamin transfer to fulfill the cobamide requirement of D. mccartyi. Many anaerobic bacteria and archaea have been confirmed to share the ability to synthesize this complicated cofactor de novo (Hug et al. 2012; Yan et al. 2013; Jácome et al. 2019). Notably, an alternative solution is to supply D. mccartyi with biosynthetic cobalamin by corrinoid-producing organisms for the successful bioremediation of a TCE-contaminated site. However, the organisms responsible for providing corrinoids in specific communities have not yet been fully studied, and whether the biosynthetic cobalamin sustains the long-term growth of D. mccartyi remains elusive.

In a previous study, a fraction of nondechlorinating organisms coexisted within a D. mccartyi-containing microbial consortium have confirmed to protect D. mccartyi from being damaged by oxygen (Liu et al. 2017). Exploration of the roles of the other organisms within this microbial consortium is also of great environmental significance for its practical application. Therefore, the objective of this study was (i) to investigate the feasibility of TCE dechlorination by a microbial consortium in the absence of exogenous cobalamin, (ii) to compare the variation of TCE reduction by the subculture with specific organisms absent and without exogenous cobalamin available, and (iii) to identify the potential biosynthetic cobalamin manufacturers within this microbial consortium.

2. MATERIALS AND METHODS

2.1. Chemicals and microorganisms cultivation

All chemical reagents including chloroethenes, cobalamin, 2-bromoethanesulfonate (2-BES) and ampicillin were purchased through Sigma-Aldrich (St. Louis, MO, USA) or J&K Scientific (Beijing, China) at the highest purity available. High-purity (≥99.999%, vol/vol) ethylene, methane (CH₄), nitrogen and gas mixtures (80/10/10 nitrogen/carbon dioxide/hydrogen) were obtained from Changchun Xinguang Gas Manufacturing (Changchun, China). A methanogenic TCE-dechlorinating microbial consortium applied in this study was originally provided by Prof. Tielong Li (Nankai University, China), and the consortium capable of complete dechlorination of TCE to ETH was cultivated steadily in our laboratory for more than two years in a 240 mL amber screw-capped bottle with 100 mL of liquid medium, serving 4.56 mmol methanol and 45.60 μmol TCE as the electron donor and acceptor. The enrichment was regularly transferred to freshly prepared medium and statically cultivated at 30 °C in an incubator once TCE was completely dechlorinated to ETH, and the inoculation proportion was 5% (vol/vol). The liquid medium containing 50 μg/L cobalamin was prepared as previously described and stored in an anaerobic chamber until use (Liu et al. 2017).

2.2. TCE dechlorination by the microbial consortium in the absence of cobalamin

Batch experiments were conducted in 240 mL amber screw-capped bottles equipped with a Mininert® valve (Supelco, Bellefonte, PA, USA). An inoculation proportion of 5% (vol/vol) and a constant volume of 100 mL were used throughout the experiments, and each bottle and microbial generation added 45.60 μmol TCE and 4.56 mmol methanol to initiate the reaction. The design of the experiments is shown in Table 1.

The experiments were divided into two phases, and all cultures were cultivated under cobalamin-absent conditions unless otherwise specified. In phase I, the culture (defined as ICob_0) containing filter-sterilized cobalamin (final concentration of 50 μg/L) and the dechlorinating stock culture were used as the initial inoculum. The enrichment of ICob_0 was successively inoculated for three generations (defined as ICob.1, ICob.2, and ICob.3), and a subculture of ICob_0 (defined as ICob_1), cultivated under the cobalamin-existed condition, was used as a positive control. In phase II, ICob.2 was used as the initial inoculum. Considering 2-BES (2 mM) and ampicillin (≤1 g/L) were successfully used as inhibitors to eliminate methanogens and specified bacteria, respectively, from the D. mccartyi-containing microbial consortium (Löfler et al. 2005), ICob.2 was
separately inoculated for three generations (defined as IICob_BES_1, IICob_BES_2, and IICob_BES_3) or two generations (defined as IICob_Amp_1 and IICob_Amp_2) with either 2-BES (final concentration of 2 mM) or ampicillin (final concentration of 1 g/L). At the same time, subcultures of IICob_BES_2 and IICob_Amp_1 cultivated under cobalamin-existed conditions (defined as IICob_BES_3 and IICob_Amp_2) served separately as positive controls of IICob_BES_3 and IICob_Amp_2. All cultivations conducted above were produced in duplicate.

The carbon isotopic enrichment factor ($\varepsilon_{carbon}$) of TCE, calculated by compound-specific isotope analysis (CSIA), allows the elucidation of the microbial transformation pathways (Cichocka et al. 2008; Harding et al. 2013). Additional evidence is provided by comparing the $\varepsilon_{carbon}$ values of TCE among distinct subcultures. To collect the carbon isotope fractionation of TCE during dechlorination by the microbial consortium, cultivated under the specified, abovementioned growth conditions, experiments were conducted in 40 mL vials containing 10 mL of fresh anaerobic medium, as previously described (Liu et al. 2018). To maintain the same liquid phase concentration of TCE as the batch experiments, 11.58 μmol of TCE dissolved in 1.158 mmol methanol was added as the terminal electron acceptor, and for each culture condition, 8 parallel incubations were simultaneously prepared from the same inoculum. At the specified time, one of the incubations was sacrificed for analyzing the concentration and isotope composition of TCE.

### 2.3. DNA isolation, Illumina MiSeq sequencing, assembly and annotation

The microbial pellets for specified conditions were collected at the end of the experiments by sampling 1 mL of enrichment and centrifuging the samples at eight thousand revolutions per minute for 10 min. The obtained pellets were immediately froze to −20 °C and then airlifted to Sangon Biotech, Shanghai, China for 16S rRNA sequencing for both archaea and bacteria. Genomic DNA of the pellets were extracted with E.Z.N.A.® Soil DNA Kit (Omega Bio-Tech, USA) as recommended by the manufacturer. In order to fulfillment the requirements of subsequent amplification and sequencing, the concentration of genomic DNA for each sample had to be greater than 10 ng/μL, the V3–V4 regions of the bacterial and archaeal 16S rRNA gene amplification were completed by using 341F 5'-barcode-ACTCTACGGGAGGCAGCAG-3' and 805R 5'-GGACTACHVGGGTWTCTAAT-3' as the universal primers (Dennis et al. 2013). The barcode is a seven-base sequence unique to each sample. Amplicons of each sample were successively electrophoresed, purified, quantified, and eventually pooled in equimolar and sequenced (2 × 300 bp) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA).

The obtained sequences experienced screening and quality control, after the invalid sequences were excluded from each sample, the entire non-chimeric sequences of all the samples were clustered into operational taxonomic units (OTU) by

### Table 1 | Design information and codes of growth conditions for studying trichloroethene (TCE) dechlorination by a microbial consortium in the absence of exogenous cobalamin

| Codes | Phase | Initial inoculum | Generation | Cobalamin (50 μg/L) | Ampicillin (1 g/L) | 2-BES (2 mM) |
|-------|-------|-----------------|------------|---------------------|-------------------|--------------|
| Icob.1 | I     | Icob.0          | 1          | -                   | ±                 | *            |
| Icob.2 |       |                 | 2          | -                   | =                 | *            |
| Icob.3 |       |                 | 3          | -                   | ±                 | ±            |
| Icob.1 |       |                 | 1          | +                   | =                 | *            |
| IIcob-BES | II  | Icob.2          | 1          | -                   | =                 | +            |
| Icob.BES.2 |   |                 | 2          | -                   | ±                 | ±            |
| Icob.BES.3 |   |                 | 3          | +                   | ±                 | +            |
| Icob.BES.3 |   |                 | 3          | -                   | ±                 | +            |
| Icob.Amp.1 |   |                 | 1          | -                   | ±                 | ±            |
| Icob.Amp.2 |   |                 | 2          | -                   | ±                 | ±            |
| Icob.Amp.2 |   |                 | 2          | +                   | +                 | +            |

Notes.
1) Phases of the experiments. In phase 1, cobalamin is the only variate; in phase 2, cobalamin and an inhibitor (ampicillin or 2-bromoethanesulfonate) are both variates.
2) Cobalamin exists in equimolar and sequenced (2 × 300 bp) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA).
3) ± indicates the presence or absence of the specific substance in the medium.
4) * means not the variate in this subculture.
setting a 0.97 similarity cut off using Uclust (version 1.1.579). The taxonomic assignment of OTUs was performed by Ribosomal Database Project (RDP) classifier at 0.8 confidence threshold applying a Naive Bayesian assignment algorithm. The detailed description of the sequencing data analysis was presented in previous research (Liu et al. 2017).

2.4. Analytical methods
The concentrations of chloroethenes, ETH, and CH₄ were periodically quantified by injecting 100 μL of equalized headspace samples into a GC-FID (GC; Shimadzu, Kyoto, Japan) equipped with a 30-m HP PLOT-Q capillary column with a 0.53-mm inside diameter (Agilent Technologies, Santa Clara, CA, USA). The injector and detector temperatures were set at 150 °C and 200 °C, respectively. The ultra-pure nitrogen was supplied as the carrier gas with a constant flow rate of 8 mL/min. A gradient temperature program that started at 60 °C and held for 1 min, then increased to 200 °C within 2.33 min and held at 200 °C for 9.67 min was used to separation the constituents. For mass balance calculation purpose, the nonstandard units ‘μmol/bottle’ and ‘mmol/bottle’ were used to exhibit the concentrations of chloroethenes, ETH, and CH₄ instead of μmol/L or mmol/L.

The stable carbon isotope compositions of the TCE were determined using gas chromatography combustion isotope ratio mass spectrometer (GC–IRMS; GC, Agilent Technologies, CA, USA; IRMS, Isoprime 100, Elementar, Germany) as described previously (Liu et al. 2018). The 10 mL glass vials were pre-incubated at 60 °C in the auto sampler system prior to subsequent isotope analysis, a 60 m DB-624 capillary column with a 0.25 mm internal diameter was applied to chromatographic separation of the headspace samples, and the oven temperature was 45 °C for 4 min, then ramped to 80 °C within 3.5 min, finally ramped by 15 °C/min to 130 °C and held for 3 min. The carbon isotope ratios (δ¹³C) as well as the carbon isotopic enrichment factors (ɛ-carbon) of TCE were calculated using the equations available in previous research (Van Breukelen 2007; Coplen 2011; Liu et al. 2018).

3. RESULTS AND DISCUSSION

3.1. Microbial consortium description
The composition of this trichloroethene dechlorination microbial consortium is relatively complex, with up to five genera of archaea, mainly affiliated with *Euryarchaeota*, and approximately 50 genera of bacteria, mainly distributed in the phyla of *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*. As shown in Figure 1, the primary archaea genera are *Methanospirillum*, *Methanosarcina*, and *Methanomethylovorans*, which account for more than 99% of the total archaea. Meanwhile, bacteria are more diversified, with the most dominated bacteria in the microbial consortium, including unclassified *Peptococaceae, Acetobacterium, Petrimonas, Paludibacter*, and *Proteiniphilum*.

So far, the full metabolization of TCE to ETH has been reported to have only been achieved by *Dehalogenimonas* and *D. mccartyi* (Anam et al. 2019; Li et al. 2019). The Illumina MiSeq sequencing analysis indicated that *D. mccartyi* existed in this microbial consortium but that *Dehalogenimonas* did not. Although the relative abundance of *D. mccartyi* was low (less than 0.5%), TCE was persistently reduced to ETH by this microbial consortium for a couple of years in our laboratory, emphasizing the significance and special niche of this biodegrader.

Our previous studies showed that the microbial consortium contained two kinds of *D. mccartyi*, which separately contain the *tceA* and *vcrA* genes (Liu et al. 2017, 2018). Moreover, the qPCR results demonstrated that during the TCE degradation process, amplicons of *tceA-D. mccartyi* increased with the decrease in TCE, and the amount of *tceA-D. mccartyi* remained stable once TCE was exhausted; *vcrA-D. mccartyi* then increased with VC decrease and ETH production (Liu et al. 2017). Therefore, it was concluded that *tceA-D. mccartyi* in this microbial consortium was responsible for reducing TCE stepwise to 1,2-cis-DCE and VC, and VC further dechlorinated to ETH was completed by *vcrA-D. mccartyi*.

3.2. TCE dechlorination by the subculture without in vitro exogenous cobalamin dosage
TCE dechlorination by the microbial consortium under standard conditions with 50 μg/L cobalamin in the medium was shown to be efficient, with 45.60 μmol/bottle of TCE both in I Cob,0 and I Cob,1 being quickly consumed within 4 days, and 1,2-cis-DCE, a more toxic intermediate product, was maintained at a very low concentration throughout the dechlorination process (Figure 2). Meanwhile, increasing VC concentration was closely correlated with a decline in TCE concentration, and a maximum value of 35.08 ± 0.40 μmol/bottle was detected on the fourth day of the dechlorination process. The VC concentration subsequently continuously declined, accompanied by rapid generation of the innocuous product, ETH. All TCE amended into the bottle was capable of complete dechlorination to a stoichiometric amount of ETH within 12 days. Simultaneously, CH₄, produced by methanogens, also rose with time and reached a peak value on the 12th day.
Figure 1 | The taxonomic composition of the microbial consortium at (a) phylum level and (b) genus level. ICob.0 represents the initial inoculum of this microbial consortium in phase I cultured in the presence of 50 μg/L cobalamin and ICob.+1 represents the subculture of ICob.0. All taxonomic groups present above 0.1% at phylum level and 0.5% at genus level in any of the samples are included in the proportional representations. The relative abundance of *D. mccartyi* in ICob.0 (0.02%) and ICob.+1 (0.04%) failed to meet the condition of 0.5% thus are absent from Fig. 1b.
Although the dechlorination of TCE by *D. mccartyi* isolates has been confirmed to be relatively unreliable without exogenous cobalamin amended into the medium (Men et al. 2013), the *D. mccartyi*-containing dechlorinating communities in this study exhibited comparable TCE dechlorination ability to the control, and the absence of exogenous cobalamin had a negligible impact on TCE degradation. As shown in Figure 3, the concentration of the fed TCE in ICob.1 and ICob.3 was undetectable on the 4th day, more than 90% of the TCE was translated into ETH by the 12th day, and ETH became the

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**Figure 2** | Time profiles of trichloroethene dechlorination (left y-axis) and methane production (right y-axis) in (a) ICob.0 and (b) ICob.1 with medium amended with 50 μg/L exogenous cobalamin. Concentration data of chlorinated ethenes and methane are plotted as the mean of duplicates and error bars represent standard deviation. — , Trichloroethene (TCE); — , 1,2-cis-dichloroethylene (1,2-cis-DCE); — , vinyl chloride (VC); — , ethene (ETH); — , total chlorinated ethene and ethene (Total); — , methane (Methane).
The yield of CH$_4$ produced in the absence of cobalamin in ICob-1 and ICob-3 at the end of the experiments was also similar to that in the standard condition.

The concentration of exogenous cobalamin in the ICob$_0$ medium was set at $50 \mu$g/L. Considering that the inoculation proportion was persistently maintained at 5% (v/v), exogenous cobalamin introduced from the medium of ICob$_0$ to ICob-1, ICob-2, and ICob-3 theoretically was no more than 2.5, 1.25 $\times 10^{-1}$, and $6.25 \times 10^{-3}$ $\mu$g/L, respectively. Meanwhile,
as shown in Figure 3, both in ICob1 and ICob3, TCE was dechlorinated by D. mccartyi to ETH as the end product, which means that cobalamin was consumed by D. mccartyi in ICob1 and ICob3 for the synthesis of RDases. However, D. mccartyi is unable to de novo synthesize cobalamin, and the reported minimum cobalamin concentration that D. mccartyi is able to absorb in vitro is 3 μg/L (Harding et al. 2013). The exogenous cobalamin within the medium of ICob1 and ICob3 was obviously inadequate. Therefore, some other members within this D. mccartyi-containing community must have participated in the de novo synthesis of this constituent to fulfill the requirements of D. mccartyi for TCE metabolism.

3.3. The 2-BES and ampicillin resistance tests of the exogenous cobalamin-starved subculture

The organisms within this microbial consortium are affiliated with prokaryotic bacteria and methanogenic archaea. Archaea are extremely sensitive to 2-BES, which is a structural analog and competitive inhibitor of 2-mercaptoethanesulfonate (CoM), the unique cofactor of the methyl reductase enzyme (Löfler et al. 1997). Therefore, 2-BES is widely considered a specific inhibitor to selectively eliminate methanogens (Löfler et al. 1997). As exhibited in Figure 4, the dosage of 2 mM of 2-BES into the subcultures that were nourished with cobalamin-absent medium greatly repressed the activities of methanogens. The detected CH₄ in both IICob.BES₁ and IICob.BES₃ was less than the subculture without the addition of 2-BES. Moreover, Illumina MiSeq sequencing targeted to archaea failed to obtain negative amplicons using the extracted genomic DNA of IICob.BES₃ as the PCR template. It was thus speculated that fractional CH₄ was introduced into IICob.BES₁ and IICob.BES₃, by the inoculation process. Simultaneously, it can be concluded that virtually, all methanogens were successfully excluded from this microbial consortium in IICob.BES.₃. From the perspective of the TCE degradation rate, methanogens may be beneficial assistants to TCE respiration for D. mccartyi; more time was necessary in IICob.BES.₃ to deplete uniform TCE to ETH, especially 1,2-cis-DCE stepwise reduction to VC (and subsequently ETH), results which agree with the previous study (Löfler et al. 1997). TCE was completely transformed into ETH in the presence of 2 mM of 2-BES approximately 1 month later. Consequently, 1,2-cis-DCE was accumulated to a considerable quantity as a metabolic intermediate of TCE. Meanwhile, from the perspective of the extent of TCE dechlorination, the absence of methanogens had a faint influence on TCE respiration by D. mccartyi. TCE was successfully metabolized to ETH in IICob.BES₁, IICob.BES₂, and IICob.BES₃, with persistent 2-BES existing in the medium. Methanogens are identified as potential cobalamin synthesizers and have been shown in previous studies to be capable of facilitating TCE degradation (Men et al. 2014b; Wen et al. 2020; Lin et al. 2021). Considering that in IICob.BES.₃ (without exogenous cobalamin), methanogens were completely eliminated and cobalamin was still available for D. mccartyi catalytic dechlorination of TCE to ETH by RDases, the probability that methanogens are the candidates within this microbial community for cobalamin synthesis is minor.

In addition to archaea, the microbial consortium was composed of multitudinous bacteria. Considering that a portion of bacteria (excluding D. mccartyi) are resistant to ampicillin, a broad-spectrum antibiotic that is widely used as a peptidoglycan synthesis inhibitor (Maymó-Gatell et al. 1997; Löfler et al. 2005), a 1 g/L (Löfler et al. 2005) of ampicillin was added into the cobalamin-absent medium to examine the TCE dechlorination ability of this microbial consortium. As shown in Figure 5, the degradation of TCE in IICob.Amp₁ obtained almost indistinguishable results from the positive control. Indeed, 1 g/L of ampicillin in the cobalamin-absent medium appeared to confirm the influence on the activity of D. mccartyi. Nevertheless, complete degradation of TCE to ETH failed in the successively incubated enrichment of IICob.Amp₁ to IICob.Amp₂; 36.10% ± 2.80% of TCE remained intact in these cultures, even when the incubation time was extended to 28 days. A trace amount of ETH was formed throughout the dechlorination process, indicating that enrichment cultures containing D. mccartyi are unable to have a second time into ampicillin-containing medium, a phenomenon that has been confirmed in another study (Maymó-Gatell et al. 1997). Instead, the dechlorination of TCE proceeded extremely well, and all fed TCE was quickly converted to ETH in IICob.Amp₁ to IICob.Amp₂ when the medium was re-amended with 50 μg/L of cobalamin. It can therefore be concluded that certain ampicillin-sensitive bacteria within this microbial consortium contributed to the synthesis of cobalamin, an essential component of RDases for the multiplication of D. mccartyi.

3.4. Carbon isotope fractionation of TCE by the microbial consortium and subcultures

The carbon isotope fractionations of TCE by the microbial consortium and the subcultures in ICob₀, ICob₃, IICob.BES₃, and IICob.Amp₁ were collected, and the results are presented in Figure 6(a). In ICob₀, 86.93% ± 2.80% of the 11.52 ± 0.35 μmol TCE was eventually removed after 85 h of incubation. Correspondingly, the carbon isotope ratios (δ¹³C) of TCE increased from an initial value of −24.50‰ ± 0.04‰ at the beginning of the experiment to −10.46‰ ± 1.17‰ at the 85th hour. Although D. mccartyi strains suffered distinct growth conditions in ICob₃, IICob.BES₃, and IICob.Amp₁, the
concentrations of TCE in all samples declined with time. The residual TCE values at the 95th hour in ICob-3, IICob-BES, and IICob-Amp were 82.58% + 3.32%, 80.84% + 3.69%, and 76.86% + 2.38%, respectively, and the $\delta^{13}$C of TCE was maintained at $-12.23\%_{\text{oo}} \pm 1.92\%_{\text{oo}}$, $-13.51\%_{\text{oo}} \pm 0.21\%_{\text{oo}}$, and $-14.12\%_{\text{oo}} \pm 0.35\%_{\text{oo}}$, respectively.

Furthermore, the $\varepsilon_{\text{carbon}}$ values of TCE in ICob, IICob.BES, and IICob.Amp were calculated using the Rayleigh equation (Liu et al. 2018). As shown in Figure 6(b), the $\varepsilon_{\text{carbon}}$ value of TCE in ICob, 0 was calculated to be $-7.17\%_{\text{oo}} \pm 0.50\%_{\text{oo}}$. The $\varepsilon_{\text{carbon}}$ values of TCE in ICob, IICob.BES, 3, and IICob.Amp, 1, were determined to be $-7.41\%_{\text{oo}} \pm 0.63\%_{\text{oo}}$.

Figure 4 | Time profiles of trichloroethene dechlorination (left y-axis) and methane production (right y-axis) in (a) IICob.BES, 1 and (b) IICob.BES, 3 without exogenous cobalamin (medium contained 5 mM 2-BES). Concentration data of chlorinated ethenes and methane were plotted as the mean of duplicates and error bars represent standard deviation. – – – , Trichloroethene (TCE); – – – , 1,2-cis-dichloroethylene (1,2-cis-DCE); – – – , vinyl chloride (VC); – – – , ethene (ETH); – – – , total chlorinated ethene and ethene (Total); – – – , methane (Methane).

Water Science & Technology Vol 00 No 0, 10

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Figure 5 | Time profiles of trichloroethene dechlorination (left y axis) and methane production (right y axis) in (a) NC3A and (b) NC4A without exogenous cobalamin (medium contained 1 g/L ampicillin), and (c) P4A amended with 50 μg/L exogenous cobalamin and 1 g/L ampicillin, serving as the positive control for NC4A. Concentration data of chlorinated ethenes and methane were plotted as the mean of duplicates and error bars represent standard deviation. •, Trichloroethene (TCE); ▲, 1,2-cis-dichloroethylene (1,2-cis-DCE); △, vinyl chloride (VC); ▼, ethene (ETH); ◆, total chlorinated ethene and ethene (Total); □, methane (Methane).
Figure 6 | (a) Changes in concentration (full lines and solid symbols) and carbon isotope ratios ($\delta^{13}C$, dash lines and hollow symbols) of TCE in ICob$_0$ (─●─ and ◇), ICob.3 (─■─ and □), IICob.BES.3 (─▲─ and △) and IICob.Amp.1 (─▼─ and ▽) and (b) corresponding $\varepsilon$-carbon values of TCE in ICob.$0$ (●), ICob.3 (▲), IICob.BES.3 (∆) and IICob.Amp.1 (▼) plotted using the Rayleigh equation. $\delta^{13}$C was derived using the Vienna Pee Dee Belemnite standard as reference. The concentration and carbon isotope data of TCE were obtained from duplicated batch experiments and error bars represent standard deviation. The mean of the concentration and carbon isotope data of TCE from the duplicates were employed for acquiring $\varepsilon$-carbon of TCE. ICob$_0$ and ICob.3 represent the initial inoculum of this microbial consortium in phase I, cultured in the presence of 50 μg/L cobalamin, and ICob.3 represents the third generation of ICob$_0$, cultured in the absence of cobalamin. IICob.BES.3 and IICob.Amp.1, respectively, represent the third generation and second generation of IICob.2 in phase II, cultured in the presence of 2 mM 2-BES and 1 g/L ampicillin.
−7.34‰ ± 0.72‰, and −7.50‰ ± 0.51‰, respectively. TCE dechlorination by the subcultures with exogenous cobalamin omitted from the culture medium apparently obtained similar $\varepsilon_{\text{carbon}}$ values of TCE to ICob, 0, which were all within the extreme boundaries of the 95% CI of the standard condition by this microbial consortium (−7.24% ± 0.59%) (Liu et al. 2018).

Generally, the $\varepsilon_{\text{carbon}}$ value of TCE is identified as a characteristic parameter for a specific dechlorinator (Harding et al. 2013). This parameter is reported to be consistent for a given dechlorinator under varying growth conditions (Harding et al. 2013).

**Figure 7** | The taxonomic composition of ICob, 0, ICob, 3, II Cob BES, 3, and II Cob Amp, 1 at (a) phylum level and (b) genus level. All taxonomic groups present above 0.1% at phylum level and 0.5% at genus level in any of the samples were included in the proportional representations. ICob, 0 and ICob, 3 represent the initial inoculum of this microbial consortium in phase I, cultured in the presence of 50 μg/L cobalamin, and ICob, 3 represents the third generation of ICob, 0, cultured in the absence of cobalamin. II Cob BES, 3 and II Cob Amp, 1, respectively, represent the third generation and second generation of ICob, 2 in phase II, cultured in the presence of 2 mM 2-BES and 1 g/L ampicillin.
et al. 2013; Liu et al. 2018). Simultaneously, multiple dechlorinators together are responsible for dechlorinating TCE to cis-1,2-DCE, resulting in variable $\varepsilon_{\text{carbon}}$ values of TCE, as previously reported (Cichocka et al. 2008). In this study, the stable $\varepsilon_{\text{carbon}}$ values of TCE among the distinct subcultures suggest that reductive dechlorination of TCE in ICob-$0$, ICob-$3$, IICob-BES-$3$, and IICob-Amp-$1$ was probably solely conducted by one biodegrader, specifically tceA-D. mccartyi. Considering that RDases expressed by tceA-D. mccartyi are directly responsible for TCE reduction and that RDases harbor cobalomin as a cofactor, the insufficiency of exogenous cobalomin in ICob-$3$, IICob-BES-$3$, and IICob-Amp-$1$ seems to be resolved by some crucial organisms within this microbial consortium that are able to biosynthesize cobalamin functionally similar to exogenous cobalamin. The biosynthesized cobalamin is then absorbed by corrinoid auxotrophic D. mccartyi for RDase synthesis and eventually participates in TCE reduction.

3.5. Microbial community composition revealing biosynthetic cobalamin manufacturers

The de novo cobalamin-synthesizing organisms that coexist with D. mccartyi in dechlorinating communities are primarily acetogens, sulfate reducers, and methanogens (Jun et al. 2012; Men et al. 2014b). Among them, the most widely identified and reported organisms include Clostridium, Acetobacterium, Desulfovibrio, Desulfotobacterium, Sporomusa, Geobacter, Methanococcus, and Methanosarcina barkeri (He et al. 2007; Reinhold et al. 2012; Men et al. 2013, 2014a; Wen et al. 2020). In IICob-BES-$3$, even though the methanogens were completely removed, corrinoid auxotrophic D. mccartyi reduction of TCE to ETH was still attainable. In addition, the inhibition of ampicillin-sensitive bacteria in IICob-Amp-$2$ resulted in the failure of TCE to completely degrade to ETH. By contrast, in IICob-Amp-$2$, D. mccartyi dechlorinated TCE to ETH once exogenous cobalamin was supplemented into the medium. In conjunction with the results above, it is likely that in this microbial consortium, bacteria rather than methanogenic archaea participated in biosynthesizing cobalamin, which was fundamental for D. mccartyi’s dechlorination of TCE.

The microbial community compositions of bacteria in representative samples at the genus level and phylum level are shown in Figure 7. It is notable that, except for IICob-Amp-$1$, the categories of bacteria at the genus and phylum levels in all of the samples were almost indistinguishable; one of the exclusive labels to separate them from each other is the relative proportion of these organisms. Probably owning to the fact that certain ampicillin-sensitive bacteria were selectively inhibited in IICob-Amp-$1$, bacterial members of IICob-Amp-$1$ at the genus level and phylum level were greatly different. Considering that the genera Clostridium and Sporomusa, two widely recognized cobalamin synthesizers, were completely absent in all of the sequenced samples, and Desulfovibrio was also sporadically distributed in some of these samples, it is impossible that these organisms were the cobalamin synthesizers in this microbial consortium. Although interspecies cobalamin transfer from Geobacter to D. mccartyi has been demonstrated previously (Jun et al. 2012), it was only largely detected in IICob-Amp-$1$ and was completely absent in other samples, indicating that determining Geobacter as a potential cobalamin synthesizer in this microbial consortium is also untenable. Moreover, other previous research has shown that Geobacter supports D. mccartyi activity only when the lower $\alpha$-ligand of cobamide, 5',6'-dimethylbenzimidazole (DMB), is supplied to the growth medium (Yan et al. 2013). Desulfotobacterium was the only cobalamin-synthesizing organism in this microbial consortium with the variation tendency of the relative proportion conformed with the stresses, indicating that the cobamide synthesized by these bacteria then fluxes to D. mccartyi. Finally, although the relative proportion of another largely reported cobalamin synthesizer, Acetobacterium, among the sequenced samples was irregular, a superior niche of this microbe was detected in this microbial consortium in all tested samples, suggesting that Acetobacterium might be another cobalamin synthesizer responsible for cobalamin synthesis with Desulfotobacterium. The dechlorination of TCE still performed pretty well when Desulfotobacterium was inhibited by ampicillin in NC5A further confirms this hypothesis.

4. CONCLUSION

The findings of this study imply that TCE reduction to ETH was successful in subcultures of a D. mccartyi-containing microbial consortium with exogenous cobalamin omitted from the medium. The nondechlorinating organisms in this consortium are crucial for the biosynthesis of cobalamin to sustain the growth and metabolism of the corrinoid auxotrophic D. mccartyi. The results of the 2-BES and ampicillin resistance tests further suggested that ampicillin-sensitive bacteria rather than methanogenic archaea within this microbial consortium were responsible for biosynthesizing cobalamin. The results of CSIA indicated that cobalamin biosynthesized by these internal organisms within this microbial consortium was functionally similar to exogenous cobalamin and was therefore absorbed by D. mccartyi for RDases synthesis and TCE reduction, eventually generating relatively stable $\varepsilon_{\text{carbon}}$ values for TCE, regardless of whether the medium was amended.
with exogenous cobalamin. According to the Illumina MiSeq sequencing analysis, the genera *Desulfitobacterium* and *Acetobacterium* are both crucial in the regulation of the biosynthesis of this extremely vital component for *D. mccartyi* utilization. The results of this study suggest the potential and feasibility of TCE remediation by this microbial consortium without the need for exogenous cobalamin.

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**DATA AVAILABILITY STATEMENT**

All relevant data are included in the paper or its Supplementary Information.

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