Completely dechlorinating of trichloroethene by a Dehalococcoides mccartyi-containing microbial consortium in the absence of cobalamin

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Abstract

Completely dechlorinating of trichloroethene (TCE) by Dehalococcoides mccartyi (D.mccartyi) is catalyzed by reductive dehalogenases (RDases) which possess cobalamin as the crucial cofactor, whereas virtually all pure D.mccartyi strains isolated thus far are corrinoid auxotrophs. Exogenous addition of commercially available cobalamin for real TCE-contaminated site decontamination is deemed to be unrealistic. In this study, TCE reduction by a D.mccartyi-containing microbial consortium utilizing biosynthetic cobalamin generated by interior corrinoid-producing organisms within this mixed consortia was studied. The results confirmed that subcultures with exogenous cobalamin omitting from the medium apparently were impervious and enabled to successively metabolize TCE to non-chlorinated ethene. The 2-bromoethanesulfonate and ampicillin resistance tests results suggested that bacteria (particularly certain ampicillin-sensitive ones) rather than methanogenic archaea within this microbial consortium were responsible for biosynthesizing cobalamin. Moreover, relative stable m-carbon values of TCE among treatments in disregard of whether exogenous cobalamin or selective inhibitors were existed in the medium also speculated that cobalamin biosynthesized by these organisms was enable to uptake and utilize by D.mccartyi for RDases synthesis and eventually participated in TCE reduction. Finally, the Illumina MiSeq sequencing analysis indicated that Desulfotobacterium and Acetobacterium in this microbial consortium probably both were in charge of de novo cobalamin biosynthesis to fulfillment the requirements of D.mccartyi for TCE metabolism.

1. Introduction

Trichloroethene (TCE) is a widely used industrial solvent owing to its excellent solvent properties(Liu et al., 2017). As a result of improper handling, storage and disposal, TCE has become one of the ubiquitous chlorinated organic pollutants detected in groundwater and soil contaminated sites in recent decades(He et al., 2007; Men et al., 2014a; Révész et al., 2006). In comparison with the other physical and chemical approaches, remediation of TCE pollution by microbial anaerobic dechlororespiration is commonly considered to be more viable and sustainable(Liu et al., 2020; Men et al., 2013; Molenda et al., 2020). Meanwhile, partial dechlorination of TCE to less chlorinated intermediates including isomers of dichloroethylenes (DCEs) and vinyl chloride (VC) by organohalide-respiring bacteria is still problematic since their greater threat to environment than TCE generated(Anam et al., 2019; Liu et al., 2018). Nowadays, genus of D.mccartyi is a starlit TCE dechlororespirator ever isolated that is specialized in completely metabolizing TCE to non-chlorinated ethene (ETH), and manifold RDases encoded by dechlorinating genes (i.e., tceA, bvcA and vcrA) within these D.mccartyi are directly in charge of the throughout degradation process(Brisson et al., 2012; Liu et al., 2018; Rong et al., 2018).

However, pure D.mccartyi is barely applied for TCE contaminated site remediation, extremely strict growth requirements to support reductive dechlorination by pure D.mccartyi has been extensively documented in literatures(Brisson et al., 2012; Löffler et al., 2005; Li et al., 2019; Men et al., 2013). The growth of D.mccartyi in isolation has reported to be unreliable and dechlorination of TCE is resulted in stall if any of the redox condition, electron donor, and nutrition is unsuitable(Men et al., 2013). On the contrary,
D.mccartyi co-existed in the means of microbial enrichments with other anaerobic microbes is generally more effective and robust (Brisson et al., 2012; Harding et al., 2013; Men et al., 2014a). Within D.mccartyi-containing communities, a variety of non-dechlorinators such as fermentors, acetogens, and methanogens are ubiquitously identified and ultimately constitute a complex network with D.mccartyi (Imfeld et al., 2020; Li et al., 2019). The significances of these microbes on microbial reductive dechlorination have already been confirmed in a handful of researches before (Li et al., 2019; Liu et al., 2017; Men et al., 2013; Wang et al., 2019; Wen et al., 2020). In our previous study, it is suggested that the non-dechlorinating organisms co-existed within a D.mccartyi-containing microbial consortium playing a crucial role in scavenging oxygen to protect the strict anaerobic D.mccartyi from being damaged, TCE dechlorinating to ETH by this microbial consortium was achieved at last even rising oxygen concentrations up to 7.2 mg/L (Liu et al., 2017). Meanwhile, research emphases of D.mccartyi-containing communities generally are still mainly concentrating on D.mccartyi, the functions of the non-dechlorinating organisms within microbial enrichments are still eager to ascertain.

Cobalamin affiliated with corrinoids is an indispensable cofactor that function in RDases, unfortunately, genome sequence analysis demonstrate that most D.mccartyi isolated thus far (i.e., CBDB1, BAV1, 195, VS, and GT) are corrinoid auxotrophs, 10 of the 17 critical upstream corrin ring biosynthesis genes for cobalamin de novo synthesis are absent in genomes of D.mccartyi and therefore synthesizes corrinoids de novo by D.mccartyi is impossible (El-Athman et al., 2019; Hug et al., 2012; Men et al., 2013). The putative corrinoid salvaging and remodeling genes, whereas are intact within the genomes of all sequenced D. mccartyi strains, meaning that D.mccartyi strains have versatile capacities to uptake and modify the 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; Yi et al., 2012).

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 (Jácome et al., 2019; Men et al., 2014a; Men et al., 2014b; Rowe et al., 2008). While addition of cobamides, the only cobamide that is commercially available to a TCE-contaminated field site can be unrealistic on account of its exorbitant price (around 1,800 USD per 100 g) (Jácome et al., 2019). Instead, it has long been recognized that D.mccartyi growing in mixed consortia enables to utilize cobalamin generated by other members via interspecies cobalamin transfer to fulfill the cobamide requirement of D. mccartyi, and many anaerobic bacteria and archaea until now have confirmed to share the ability to synthesize this complicated cofactor de novo (Hug et al., 2012; Jácome et al., 2019; Wen et al., 2020; Yan et al., 2013). Notably, it is probably an alternative solution to supply D. mccartyi with biosynthetic cobalamin by corrinoid-producing organisms for the successful bioremediation of an actual TCE-contaminated site. However, the organisms responsible for providing corrinoids in specific communities has not yet been fully studied, whether the biosynthetic cobalamin sustained long-term growth of D. mccartyi also remains elusive.

In this study, a methanogenic, TCE-dechlorinating microbial consortium abidingly cultured in medium supplemented with 50 µg/L exogenous cobalamin was primarily successively subcultured to the cobalamin-absent medium to examine TCE dechlorination abilities of these subcultures. Methanogens
and fractional bacteria of the subculture were then selectively inhibited by 2-bromoethanesulfonate (2-BES) and ampicillin to identify the influences of these two families on TCE reduction in the absence of cobalamin. Moreover, compound-specific isotope analysis (CSIA) was employed to furnish extra evidences that were favourable for profoundly comprehending the whole degradation process. In addition, Illumina MiSeq sequencing analysis was likewise applied to compare the variation of microbial community structure in specified subcultures and further to speculate the potential cobalamin biosynthesis organisms within this microbial consortium.

2. Materials And Methods

2.1 Chemicals and microorganisms cultivation

All chemical reagents including chloroethenes, cobalamin, 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 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 was cultivated steadily in our laboratory for more than two years in a 240 mL reactor with 100 mL of liquid medium, serving 4.56 mmol methanol and 45.6 µmol TCE severally as the electron donor and acceptor. After TCE was completely dechlorinated to ETH, 5% (vol/vol) of the enrichment was regularly transferred to freshly prepared medium and statically cultivated at 30°C in an incubator. 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

The batch experiments were conducted in 240-mL amber screw-capped bottles equipped with a Mininert® valve (Supelco, Bellefonte, PA, USA), each bottle and microbial generation added 45.6 µmol TCE and 4.56 mmol methanol to initiate the reaction. The primary cultivation (Defined as P0) was established by dosing 95mL of the autoclaved, cobalamin-contained (50 µg/L) liquid medium and inoculating the bottle with 5 mL (5%, v/v) of the dechlorinating stock culture. Then, the enrichment of P0 was sub-cultured (5 %, vol/vol) into 95 mL fresh medium without cobalamin dosed (Defined as NC1), a sub-culture (Defined as P1) cultivated under the cobalamin-existed condition was used as a control. After a subsequent sub-cultured of NC1 under the same cobalamin-absent conditions (Defined as NC2), sub-cultures were split into three enrichment conditions, namely (1) cobalamin-absent in the liquid medium (Defined as NC3), (2) cobalamin-absent in the liquid medium and simultaneously 2-BES was dosed at a finally concentration of 5 mM (Defined as NC3B), and (3) cobalamin-absent in the liquid medium and simultaneously ampicillin was dosed at a finally concentration of 1 g/L (Defined as NC3A). NC3B and NC3A were then sub-cultured under the corresponding conditions for another 2 generation (Defined as NC4B and NC5B) and 1 generation (Defined as NC4A), separately. At the same time, sub-cultures of NC4B
and NC3A cultivated under cobalamin-existed condition which defined as P4A and P5B were served as contrast to NC4A and NC5B. All the cultivation conducted above were performed in duplicate. Graphical design of the experiments was available in Fig. S1. To collect carbon isotope fractionation of the microbial consortium cultivated under the specified, above-mentioned growth conditions, experiments were conducted in 40 mL vials containing 10 mL of fresh anaerobic medium, in order to maintain the same liquid phase concentration of TCE as the batch experiments, 11.58 µmol of TCE dissolved in 1.158 mmol methanol were added as the terminal electron acceptor, and for each culture condition, eight to ten parallel incubations were simultaneously prepared from the same inoculum.

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 8,000 rpm 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 fulfill 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-ACTCCTACGGGAGGCAGCA-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 (2X300 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 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.
The stable carbon isotope compositions of the TCE were determined using gas chromatography combustion isotope ratio mass spectrometer (GC–C–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 ($\delta^{13}$C) as well as the carbon isotopic enrichment factors ($\beta$-carbon) of TCE were calculated using the equations available in previous research (Breukelen and Boris, 2007; Coplen, 2011; Liu et al., 2018).

3. Results And Discussion

3.1 Microbial consortium description

The composition of trichloroethene-dechlorination microbial consortium is relatively complicated, up to 5 genera of archaea mainly affiliated with Euryarchaeota and about 50 genera of bacteria mainly distributed in phyla of Firmicutes, Bacteroidetes as well as Proteobacteria are totally identified and coexisted in the system. As shown in Fig. 1, the primary archaea genera detected are Methanospirillum, Methanosarcina as well as Methanomethylovorans, which account for more than 99% of the total archaea. Meanwhile, bacteria are more diversified, except for the unclassified, the most dominated bacteria exist in the microbial consortium including unclassified Peptococcaceae, Acetobacterium, Petrimonas, Paludibacter, and Proteiniphilum. Interestingly, although the microbial consortium exhibits stable TCE dechlorination ability for a couple of years in our laboratory, the functional bacteria D.mccartyi within this microbial consortium is not conformed to anticipation. The relative abundance of this microbe is less than 1% of the total bacteria, which is obviously unconspicuous in this microbial consortium. It is speculated that steady feeding this mixed culture with methanol and TCE at a molar ratio of 100 probably is the reasonable explanation of this phenomenon. However, during TCE degradation process, a PCR approach targets on D.mccartyi 16S rRNA gene and functional genes (tceA and vcrA) easily obtained positive amplicons (Liu et al., 2017), meaning a significant role of this member.

3.2 TCE dechlorination by the subculture without in vitro exogenous cobalamin dosage

TCE dechlorination by the microbial consortium under standard condition with 50 µg/L cobalamin in the medium is efficient, 45.60 µmol/bottle TCE both in P0 and P1 are quickly consumed within 4 days, 1,2-cis-DCE, a more toxic intermediate product, maintains at a very low concentration throughout the dechlorination process (Fig. 2). Meanwhile, increasing of VC concentration is closely correlated with TCE concentration declining, and a maximum value of 35.08 ± 0.40 µmol/bottle is detected on the 4th day of the dechlorination process. The concentration of VC continuous declines subsequently, accompanying by rapid generation of the innocuous products, ETH. All the TCE amended into the bottle capable of
completely dechlorinating to stoichiometric amount of ETH within 12 days. Simultaneously, CH₄ produced by methanogens also rises with time and reaches a peak value on the 12th day.

Although the dechlorination of TCE by *D.mccartyi* in isolation is confirmed to be relatively unreliable without exogenous cobalamin amended into the medium (Men et al., 2013), *D.mccartyi*-containing dechlorinating communities in this study exhibit comparable TCE dechlorination ability to the control, the absent of exogenous cobalamin has negligible impact on TCE degradation. As shown in Fig. 3, the concentration of the fed TCE in NC1 and NC3 both are undetectable on the fourth day, and more than 90% of TCE are translated into ETH on the 12th day, ETH becomes the unique product of TCE on the 16th day. The yield of CH₄ produced in the absence of cobalamin in NC1 and NC3 at the end of the experiments are also similar to the standard condition.

The concentration of cobalamin in the medium of P0 is set at 50 µg/L, hypothesizing none of the cobalamin is biosynthesized by the non-dechlorinating members, the theoretical cobalamin concentration within NC1, NC2 and NC3 are 2.5, 1.25 × 10⁻¹ and 6.25 × 10⁻³ µg/L, which all are below 3 µg/L, a documented minimum cobalamin concentration that *D.mccartyi* is enable to uptake (Harding et al., 2013). Considering TCE is almost indiscriminately respired in P0, NC1 and NC3 with diminishing cobalamin available in the medium, some non-dechlorinating members within this *D.mccartyi*-containing community obviously are participated in *de novo* synthesizing of this constituent to fulfill the requirements of *D.mccartyi* for TCE metabolism.

### 3.3 The 2-BES and ampicillin resistance tests of the cobalamin-starving subculture

The non-dechlorinators within this microbial consortium are apparently affiliated with prokaryotic bacteria and methanogenic archaea. The archaea are extremely sensitive to 2-BES, which is a structural analog and competitive inhibitor of 2-mercaptoethanesulfonate (CoM), the unique cofactor of methyl reductase enzyme, hence 2-BES is widely considered as a specific inhibitor of methanogenesis (Loffler et al., 1997; Loffler et al., 2013). As exhibited in Fig. 4, the dosage of 5 mM of 2-BES into subculture which are nourished in cobalamin absent medium greatly repress the activities of methanogens, the production of CH₄ continues to decrease in NC3B and NC5B. Simultaneously, Illumina MiSeq sequencing targets on archaea failed to obtain negative amplicons using the extracted genomic DNA of NC5B as the PCR template (data not shown), meaning that methanogens are successfully excluded from this microbial consortium. From the perspective of the velocity of TCE dechlorination, methanogens may beneficial assistants to TCE respiring for *D.mccartyi*, more time are necessary in NC5B to deplete uniform TCE to ETH, especially 1,2-cis-DCE stepwise reduces to VC and subsequently to ETH, which is uniform with the previous studys (Chiu and Lee, 2001; Loffler et al., 1997). TCE completely translated into ETH in the presence 5 mM of 2-BES is obtained about 1 month later, and obviously, 1,2-cis-DCE is accumulated to a considerable quantity as metabolic intermediates of TCE. Meanwhile, from the perspective of the extend of TCE dechlorination, the absence of methanogens has faintly influence on TCE respiring by *D.mccartyi*, all fed TCE successfully metabolized to ETH in NC3B, NC4B and NC5B with persistent 2-BES existing in
the medium. Consequently, methanogens albeit are identified as potential cobalamin synthesizers and are capable of facilitating TCE degradation in other studies (Lin et al., 2021; Men et al., 2014b; Wen et al., 2020), the functions of methanogens in this microbial community must be something else other than the candidate of cobalamin manufacturer.

Apart from archaea, the microbial consortium is composed of multitudinous bacteria. Considering that majority of bacteria except for *D. mccartyi* are resistless to ampicillin, a broad-spectrum antibiotic that is widely used as peptidoglycan synthesis inhibitor (Löffler et al., 2005; Maymo-Gatell et al., 1997), a 1 g/L of ampicillin is added into the cobalamin absent medium to examine the TCE dechlorination ability by this microbial consortium. As shown in Fig. 5, the degradation of TCE in NC3A obtained almost indistinguishable results from the positive control, a 1 g/L of ampicillin in the cobalamin absent medium appears to have confined influence on the activity of *D. mccartyi*. Nevertheless, TCE completely degrades to ETH is failed when successively incubates enrichment of NC3A to NC4A, 36.10 ± 2.80% of TCE is still intact even extends incubation time to 28 days, trace amount of ETH is formed throughout the dechlorination process, indicating enrichment culture containing *D. mccartyi* is unable to transferred a second time into ampicillin-containing medium, a phenomenon that has also confirmed in other studies (Maymo-Gatell et al., 1997). Instead, the dechlorination of TCE proceeds extremely well and all fed TCE is quickly converted to ETH once sub-culturing NC3A to P4A, which re-amending the medium with 50 µg/L cobalamin. It is thus convinced that certain ampicillin-sensitive bacteria within this microbial consortium have contributed to the synthesis of cobalamin, an essential nutrition component for the multiplying 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 P0, NC3, NC5B and NC3A are collected and presented in Fig. 6. 86.93 ± 2.80% of the 11.52 ± 0.35 µmol TCE in P0 was eventually removed after 85 hours 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 have suffered distinct growth conditions in NC3, NC5B and NC3A, the concentrations of TCE in all samples decline with time, the residual TCE at the 95th hour in NC3, NC5B and NC3A are 82.58 ± 3.32%, 80.84 ± 3.69% and 76.86 ± 2.38%, and the δ¹³C of TCE are maintained at -12.23 ± 1.92‰, -13.51 ± 0.21‰ and -14.12 ± 0.35‰, respectively.

Ulteriorly, The carbon isotopic enrichment factors (δ-carbon) of TCE in P0, NC3, NC5B and NC3A are calculated using the Rayleigh equation. As shown in Fig. 8, the δ-carbon value of TCE in P0 is calculated to be -7.17 ± 0.50‰, the δ-carbon values of TCE in NC3, NC5B and NC3A likewise are determined to be -7.41 ± 0.63‰, -7.34 ± 0.72‰ and -7.50 ± 0.51‰, respectively. TCE dechlorination by the subcultures with exogenous cobalamin omitting from the culture medium apparently obtained similar δ-carbon values of TCE with P0, which are 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). In consideration of RDases harbouring cobalamin as cofactor within *D. mccartyi* are directly responsible for TCE reduction, the comparable δ-carbon values of TCE in P0, NC3, NC5B and NC3A provide definitive evidence that certain cobalamin biosynthesis organisms within this microbial consortium are crucial to *D. mccartyi* to resist the starvation of exogenous cobalamin. It is also acceptable that cobalamin biosynthesized by these internal members is functionally similar to exogenous cobalamin, enabling to uptaked by *D. mccartyi* for the RDases synthesis and eventually is participated in TCE reduction.

### 3.4 Microbial community composition revealing biosynthetic cobalamin manufacturers

Currently, the *de novo* cobalamin-synthesizing organisms coexists with *D. mccartyi* in dechlorinating communities primarily are members of acetogens, sulfate reducers and methanogens (Jun et al., 2012; Men et al., 2014b). Moreover, the most widely identified and reported organisms among them include *Clostridium, Acetobacterium, Desulfovibrio, Desulfitobacterium, Sporomusa, Geobacter, Methanococcus* and *Methanosarcinaarkeri* (He et al., 2007; Men et al., 2013; Men et al., 2014a; Reinhold et al., 2012; Wen et al., 2020). In conjunction with all results above, it is most likely that bacteria rather than methanogenic archaea within this microbial consortium are participated in biosynthesizing cobalamin that is fundamental for *D. mccartyi* dechlorinating of TCE. The microbial community compositions of bacteria in representative samples at the genus level and phylum level are shown in Fig. 7. It is markedly that except for NC3A, categories of bacteria at the genus level and phylum level in all of the rest samples are almost indistinguishable, one of the exclusive labels to separate them from each other is the relative proportion of these organisms. Probably owning to certain ampicillin-sensitive bacteria are selectively inhibited in NC3A, bacterial members of NC3A at the genus level and phylum level are greatly changed. Considering that genera of *Clostridium* and *Sporomusa*, two widely recognized cobalamin synthesizers are completely absent in all of the sequenced samples, *Desulfovibrio* is also sporadically distributed in some of these samples, notably it is impossible that these organisms are the cobalamin synthesizers in this microbial consortium. Although interspecies cobalamin transfer from *Geobacter* to *D. mccartyi* was demonstrated, while the fact that it is only largely detected in NC3A but absolute beingless in other samples meaning that determing *Geobacter* as potential cobalamin synthesizers in this microbial consortium is also untenable, Moreover, recently published literature points out that *Geobacter* is support *D. mccartyi* activity only when the lower α-ligand of cobamide, 5’,6’-dimethylbenzimidazole (DMB) was supplied to the growth medium (Yan et al., 2013). *Desulfitobacterium* in this microbial consortium is the only cobalamin-synthesizing organism that variation tendency of the relative proportion is conformed with the suffering stresses, indicating that cobamide synthesised by this bacteria then fluxes to *D. mccartyi* is reasonable. Finally, albeit variation of the relative proportion of another largely reported cobalamin synthesizers, *Acetobacterium*, among sequenced samples is irregular, whereas a superior niche of this microbe in this microbial consortium in all tested samples suggesting that *Acetobacterium* may be another cobalamin synthesizer together in charge of cobalamin synthesis with *Desulfitobacterium*, the dechlorination of TCE when *Desulfitobacterium* was inhibited by ampicillin in NC3A still performs pretty well further confirmed this hypothesis.
4. Conclusion

It is widely acknowledged that TCE dechlorination conducted by pure *D.mccartyi* strains in the absence of cobalamin is always listless. Cobalamin is considered as one of the essential nutrients which needs to supplement from the surrounding environment as cofactor for *D.mccartyi* synthesizing of RDases. Generally, cobalamin is exogenous added into the medium to meet the requirements of *D.mccartyi* for TCE metabolism. The aim of this study was to evaluated *de novo* synthesizing of this constituent by cobalamin-synthesizing organisms coexists with *D. mccartyi* in dechlorinating communities to sustain completely and successively decontaminating of TCE to innocuous ETH. The findings of this study imply that TCE reduction proceeding pretty well in the subcultures by a *D.mccartyi*-containing microbial consortium with exogenous cobalamin omitting from the medium. The *in vitro* cobalamin concentration of the subcultures during serial cultivating are stepwise diluted to lower than 1 µg/L, which is lower than the reported minimum requirement needed to support *D.mccartyi* growth and metabolism, cobalamin thus indeed was biosynthesized by the non-dechlorinating organisms in this consortium. Moreover, TCE metabolises to ETH remains available, albeit accomplished more slowly when 5 mM of 2-BES was further dosed into the cobalamin absent medium to inhibit methanogens, whereas TCE reduction was incomplete once replacement of 2-BES with 1g/L ampicillin, indicating that prokaryotic bacteria rather than methanogenic archaea within this microbial consortium are participating in biosynthesizing cobalamin. In addition, in conjunction with CSIA, it is concluded that cobalamin biosynthesized by these internal organisms within this microbial consortium is functionally similar to exogenous cobalamin therefore are uptaked by *D.mccartyi* for the RDases synthesis and TCE reduction, and eventually generating relative stable δ-carbon values of TCE regardless of whether mediums were amended with exogenous cobalamin. According to the Illumina MiSeq sequencing analysis, it is speculated that genera of *Desulfitobacterium* and *Acetobacterium* both are crucial to regulate the biosynthesis of this extremely vital nutrition for *D.mccartyi* utilization.

Declarations

**Ethics approval and consent to participate** Not applicable

**Consent for publication** Not applicable

**Availability of data and materials** All data generated or analyzed during study are included in this published article and its supplementary information files.

**Competing interests** The authors declare that they have no competing interests

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Authors' contributions All authors contributed to the study conception and design. Investigation, experiment design, and data collection were performed by Haijun Li, Shanming Wei and Yalu Du. The first draft of the manuscript was written by Haijun Li, Na Liu and Guantao Ding commented and revised on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1
The taxonomic composition of the microbial consortium at phylum level (a) and genus level (b). P0 represents the primary culture of this microbial consortium and P1 represents the subculture of P0. 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.

Figure 2
Time profiles of trichloroethene dechlorination (left y-axis) and methane production (right y-axis) in P0 (a) and P1 (b) with medium amended with 50 μg/L exogenous cobalamin. Concentration data of chlorinated ethenes and methane were plotted as the mean of duplicates and error bars represent standard deviation.

Figure 3

Time profiles of trichloroethene dechlorination (left y-axis) and methane production (right y-axis) in NC1 (a) and NC3 (b) with exogenous cobalamin omitting from the medium. Concentration data of chlorinated
ethenes and methane were plotted as the mean of duplicates and error bars represent standard deviation.

Figure 4

Time profiles of trichloroethene dechlorination (left y-axis) and methane production (right y-axis) in NC3B (a) and NC5B (b) without exogenous cobalamin whereas containing 5 mM 2-BES in the medium. Concentration data of chlorinated ethenes and methane were plotted as the mean of duplicates and error bars represent standard deviation.
Figure 5

Time profiles of trichloroethene dechlorination (left y-axis) and methane production (right y-axis) in NC3A (a) and NC4A (b) without exogenous cobalamin whereas containing 1 g/L ampicillin in the medium, and P4A (c) amended with 50 μg/L exogenous cobalamin and 1 g/L ampicillin into the medium was served as the positive control of NC4A. Concentration data of chlorinated ethenes and methane were plotted as the mean of duplicates and error bars represent standard deviation.
Figure 6

Changes in concentration and carbon isotope ratios (δ13C) of TCE in P0, NC3, NC5B and NC3A (a) and corresponding δ13C of TCE in P0, NC3, NC5B and NC3A plotted using the Rayleigh equation (b). δ13C were 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 δ-carbon of TCE.

Figure 7

The taxonomic composition of P0, NC3, NC5B and NC3A at phylum level (a) and genus level (b). 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.
Supplementary Files

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- Fig.S1.jpeg