Can Tho University Journal of Science
website: sj.ctu.edu.vn

DOI: 10.22144/ctu.jen.2018.040

Syntrophic interaction in organochlorine bioremediation: A review

Chau Thi Anh Thy
College of Agriculture, Can Tho University, Vietnam
Correspondence: Chau Thi Anh Thy (email: ctathy@ctu.edu.vn)

Article info.

Received 12 Dec 2017
Revised 04 Jun 2018
Accepted 30 Nov 2018

Keywords

Anaerobic respiration, bioremediation, organochlorine, syntrophy

ABSTRACT

Organochlorine compounds are released in the environment by many sources such as industrial pollution (polychlorinated biphenyls-PCBs), naturally in marine sponge's metabolism (halomethanes, chloroethenes, chloroacetic acids, chlorophenols) or agricultural application (organochlorine pesticides). These compounds can have carcinogenic and lethal effects on human. Hence, it becomes significant concern, especially in Vietnam where high concentration of organochlorine residue has been detected in environment. Recently, interest in the microbial biodegradation of pollutants has intensified as effective applications to find sustainable ways to clean up contaminated environments. Understanding how microbial communities metabolize and respond to contaminants is the key to predicting contaminant fate at contaminated sites in bioremediation. The organohalide respiration process under anaerobic conditions involves a consortium of many microorganisms working together with complex relationships known as syntrophy. Syntrophic relationship, such as those observed between fermentative bacteria and methanogens, is an obligate form of mutualism in which both partners are dependent on each other. Syntrophic interactions are a unique niche in nature and play an important role in carbon cycling under anoxic conditions. Associations of syntrophic fermentative organisms and partners that consume fermentation products contribute to the anaerobic biodegradation of organochlorines. Addition of substrates that ferment to H_2 to stimulate reductive dechlorination has been demonstrated to be effective in bioremediation applications. However, due to changes in community structure and difficulties in studying the function of individual populations in defined culture, understanding of syntrophic interactions is still limited.

Cited as: Thy, C.T.A., 2018. Syntrophic interaction in organochlorine bioremediation: A review. Can Tho University Journal of Science. 54(8): 70-80.

1 INTRODUCTION

Halogenated hydrocarbons have been applied in industry and agriculture as solvents, pesticides and preservatives with high amount and long term led to their accumulation in the environment. Organochlorine compounds are released in the environment by many sources such as industrial waste, naturally in marine sponge's metabolism or agricultural application (pesticides) (Max and Bossert, 2003). Organochlorine compounds have divergent and useful chemical properties and are widely used as solvents (e.g. chloroform, dichloromethane, dichloroethene, trichloroethene (TCE), and tetrachloroethene [perchloroethene; PCE]); polymers (e.g. polyvinylchloride); insulators (e.g. polychlorinated

biphenyls [PCBs]); and pesticides (e.g. pentachlorophenol [PCP], and hexachlorobenzene) (Mohn and Tiedje, 1992; Max and Bossert, 2003). In addition, some compounds such as polychlorinated dibenzo- p -dioxins (PCDDs) and dibenzo- p -furans (PCDFs) are formed as unwanted by-products of manufacturing and incineration processes (Meharg and Osborn, 1995). These compounds can have carcinogenic and lethal effects on human through an ability to concentrate in biota and magnify in the food chain (Reineke *et al*., 2001). These chemicals also have considerable persistence in soils and sediment (Loganathan and Kannan, 1994). They are water insoluble, strongly associated with organic carbon and fine sediment, and have long half-lives. It has been shown that they exhibit a high persistence against aerobic biodegradation with half-live ranging from 5 to 20 years in soil such as HCB, PCBs, dioxin (Barber *et al*., 2005; Torsten and Gabriele, 2016). The formation of organochlorine in terrestrial environments is known to be tightly linked to the decomposition of organic matter and processes of soil humus formation (Leri *et al*., 2007). Organochlorine compounds have been classified among 150 priority hazardous substances by the US Comprehensive Environmental Response, Compensation and Liability Act (CERLA) (ATSDR, 2015). According to Ruth and Paul (2001), organochlorine compounds have adverse effects on reproductive and immune systems as well as human carcinogens due to their bioaccumulation in the food chain. Persistent organochlorine pesticides and PCBs were widely used in Vietnam since the 1960s in agriculture, industry and public health (Sinh *et al*., 1999; MONRE, 2006). Though these compounds are officially banned in Vietnam, they are still detected at high concentration in various environment (Sinh *et al*., 1999). Based on their use history, persistent and physicochemical properties, organochlorine compounds have been considered as an interesting chemical to study, especially in exploring how to accelerate their degradation in environment by both physicochemical and biological methods.

In recent years, interest in the microbial biodegradation of pollutants has intensified as effective applications to find sustainable ways to clean up contaminated environments. Enhancing the growth of microbes might already be living at the contaminated site and to add specialized microbes have ability to degrade the contaminants become a common technique known as bioremediation. Reductive dehalogenation is a rapid and effort way to detoxify chlorinated compounds. The complete degradation of organochlorine contaminants under anaerobic conditions involves a consortium of many microorganisms working together with complex interrelationships (McCarty, 1997). Hence, this review focuses on organochlorine dechlorination in complex interaction among bacterial community in contaminated environment.

2 ORGANOHALIDE DEHALOGENATION PROCESS

For many halogenated compounds, reductive dehalogenation is the initial step in anaerobic biodegradation. There are two types of organohalide dehalogenation including hydrogenolysis and dihaloelimination (Figure 1) (Dolfing, 2016). Carbon–chlorine bond breakage is mediated by dehalogenating enzyme systems i.e. dehalogenases, and three main mechanisms have been discovered: hydrolytic dehalogenases, oxygenolytic dehalogenases and reductive dehalogenases atom. The former two enzymes replace the halogen substituent with a hydroxyl group derived from water and oxygen, whilst the latter replaces the halogen substituent with a hydrogen atom (Adrian and Löffler, 2016). Anaerobic respiration that uses halogenated hydrocarbons as terminal electron acceptors is an electron transport-based energy conservation process known as organohalide respiration (Hug *et al*., 2013). It has been known that molecular hydrogen, or formate, and acetate as electron donor and a halogenated compound as terminal electron acceptor apparently couple reductive dehalogenation to electrogenic energy conservation in organohalide respiration process (Figure 2) (Holliger and Schumacher, 1994).

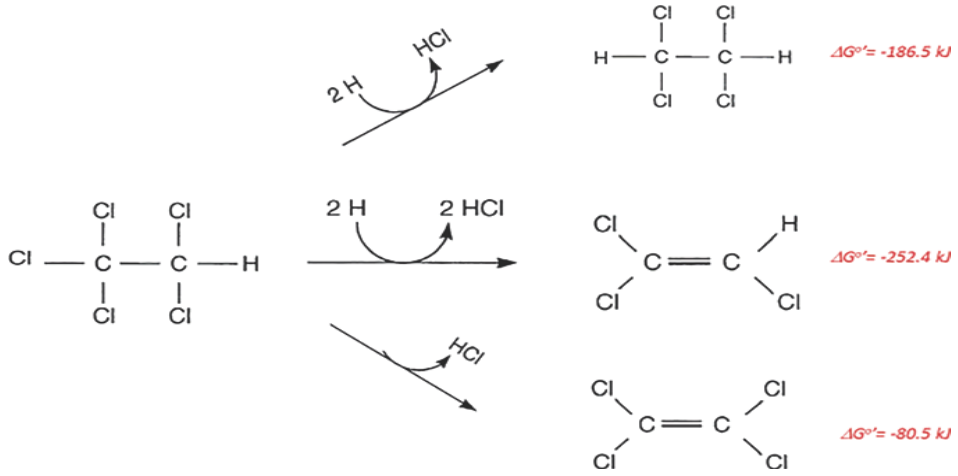

Fig. 1: Examples of reductive dehalogenation of pentachloroethane to tetrachloroethane (Dofling, 2016)

There are hydrogenolysis of pentachlorethane to tetrachloroethane (*upper pathway*) and dihaloelimination of pentachloroethane to trichloroethene (*middle pathway*). In theory, both reactions can be coupled to energy conservation via organohalide respiration. The *lower pathway* shows dehydrohalogenation of pentachloroethane to tetrachlorethene. This reaction does not consume reducing equivalents, is not a reductive dehalogenation reaction, and can therefore not be coupled to organohalide respiration.

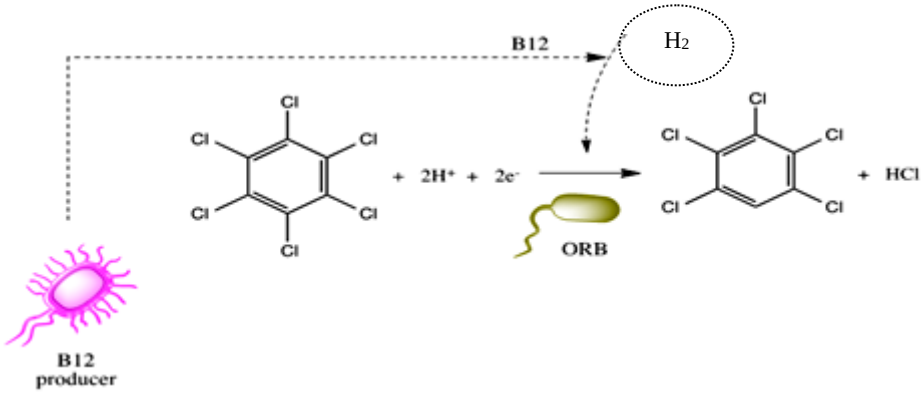

Fig. 2: Hexachlorobenzene respiration process in mixed cultures (Alfán-Guzmán, 2016)

In mixed cultures, organic electron donors, like lactate or ethanol yield H_2 and volatile fatty acids such as acetate and propionate. These can then be used by organohalide-respiring bacteria (ORB) to perform reductive dechlorination (H_2) and build biomass (acetate). organochlorines serve as electron acceptors. Reductive dechlorination generally involves the sequential replacement of chlorine atoms with hydrogen atoms. In reductive dechlorination, ORB use chlorinated hydrocarbons as electron acceptors in energy-producing redox reactions.

3 ORGANOHALIDE-RESPIRING BACTERIA

Organohalide-respiring bacteria are microbes capable of using an electron transport-based energy for growth from dehalogenation of halogenated compounds (Figure 2). These bacteria can reduce or eliminate toxicity or render the compounds more biodegradable, making this process important for the bioremediation of contaminated sites. ORB have been identified from many phyla including *Proteobacteria, Firmicutes* and *Chloroflexi*. While *Proteobacteria* and *Firmicutes* group are non-obligated ORB, the *Chloroflexi* phylum has been confirmed to contain several isolates that have been shown to be related to obligate organohalide respiration (Hug *et al*., 2013). The *Chloroflexi* phylum seems to be the most studied genus because of ability to degrade organochlorine compounds which have high toxicity and persistence in soil and sediments. *Dehalococcoides mccartyi* strains, strictly anaerobic bacteria, which are well-known bacteria can obtain energy by reductive dehalogenation of organic chlorinated compounds within the phylum

Chloroflexi. All described *Dehalococcoides* strains use hydrogen as an electron donor, acetate as carbon sources and halogenated aliphatic or aromatic compound as respiratory electron acceptor. For examples, strain 195 uses perchloroethene (PCE), 1,2,3,4-tetrachlorodibenzo-p-dioxin and hexachlorobenzene (HCB) as electron acceptor in respiration process (Maymo-Gatell *et al*., 1999; Fennell *et al*., 2004; Adrian *et al*., 2007) or strain CBDB1 has been shown to grow with HCB and dioxins (Adrian *et al*., 2000; Bunge *et al*., 2003) (Table 1).

Table 1: Some well-known *Dehalococcoides mccartyi* isolates (modified from Löffler *et al*., 2013, Atashgahi *et al*., 2016)

Strain	Origin	Electron acceptor*	Major end products	Electron acceptor range*	RDase genes with assigned function catalytic activity	Reference
Dehalococcoides mccartyi 195	Contaminated aquifer	PCE, TCE, cis-DCE, 1,1-DCE	VC, ethene	1,2-DCA, 1,2-dibromoethane, PCBs, PCDDs, chlorinated naphthalenes, chlorobenzenes chlorophenols	*pceA* PCE → TCE *tceA* TCE → VC	Maymo-Gatell *et al*. (1997) Fennell *et al*. (2004) Adrian *et al*. (2007)
Dehalococcoides mccartyi CBDB1	Saale River sediment	1,2,3,4-TeCB 1,2,3-TCB and 1,2,4-TCB	1,2,4-TCB 1,3-DCB 1,4-DCB	Hexachlorobenzene, chlorobenzenes, chlorophenols, PCDDs, PCBs	*cbrA* (1,2,3,4-TeCB → 1,2,4-TCB 1,2,3-TCB → 1,3-DCB)	Adrian *et al*. (1998, 2000, 2007, 2009) Bunge *et al*. (2003) Hölscher *et al*. (2003)
Dehalococcoides mccartyi BAV1	Contaminated aquifer	cis-DCE, trans-DCE, 1,1-DCE, VC	ethene	1,2-DCA, vinyl Bromide	*bvcA* DCEs, VC → ethene	He *et al*. (2002)

** DCA, Dichloroethane; DCB, dichlorobenzene; DCE, dichloroethene; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzodioxin; PCE, tetrachloroethene; TCB, trichlorobenzene; TCE, trichloroethene; TeCB, tetrachlorobenzene; VC, vinyl chloride*

4 DEPENDENCIES IN MICROORGANISM INTERACTION

The organohalide respiration process under anaerobic conditions involves a consortium of many microorganisms working together with complex relationships (McCarty, 1997). There are two types of dependencies in microorganism interaction including commensalism and mutualism. While commensalism is a relationship between two organisms where one organism benefits but the other gets neither harm nor benefit, mutualism is a beneficial relationship to both partners of different species. Syntrophic relationship, such as those observed between fermentative bacteria and methanogens, is an obligate form of mutualism in which both partners are dependent on each other. In all cases, syntrophic activities refer to the fact that the relationship is obligate and is different from what could occur when each microbe acts separately (Morris *et al*., 2013).

The term of syntrophy was previously used to describe microbial cross-feeding as a cooperation that both partner involve in metabolic activity and cannot overcome by adding a co-substrate or any type of nutrient (Schink, 1997), or a nutrient situation that two or more organisms catalyze a substrate that cannot be catalyzed by either one of them alone (Plugge *et al*., 2009). Syntrophic interactions are a unique niche in nature and play an important role in carbon cycling under anoxic conditions. Understanding how microbial communities respond to and metabolize contaminants is a key to predicting contaminant fate at contaminated sites in bioremediation and to optimizing engineered bioremediation approaches (Becker *et al*., 2005). However, due to the changes of communities' structure and difficult study in function of individual population in defined culture, understanding of interdependency interactions is restricted. Moreover, syntrophic interactions have developed unstoppably as a course of evolution – specialized

biochemical mechanisms changing to adapt to specific environment condition (Morris *et al*., 2013). For instance, in media without an electron acceptor, *Desulfovibrio vulgaris* can still survive by transferring H_2, a waste product of lactate fermentation, to *Methanococus maripaludis* and in return benefited from a chemical environment (a low H_2 concentration) in which lactate fermentation was thermodynamically favourable (Zhou *et al*., 2011). The genomic insight showed that syntrophic microorganisms can contain multiple copies of specific reductases, acyl-CoA synthases, and hydrogen or formate-evolving/producing dehydrogenases. Depending on environmental conditions, these bacteria may be able to grow partner-free by fatty acid fermentation or disproportionation, or partner-dependent by production of reduced electron carrier (McInerney *et al*., 2008).

Microbial interactions are always complicated and challenging for researchers to approach biological system. Although individual strains can be well characteristic and highly potential in cleaning up contaminated compounds, the interactive mechanisms within microorganism's community play an important role in bioremediation. Therefore, a deep knowledge of interactions will need to be elucidated to have the best bioremediation method in contaminated sites. This review aims to provide an overview of the basic principle of dependencies in microbes and specific examples of metabolic cooperation in reductive organochlorine reaction in contaminated sites.

5 SYNTROPHIC INTERACTION

5.1 Classical examples of syntrophic interaction with different organic compounds

The classical example of syntrophy is the microbe interaction in mixture of S-organism of *Methanobacillus omelianskii* culture on oxidizing ethanol to acetate and methane, which was investigated on biochemistry of methanogenesis by many researchers (Bryant *et al*., 1967). *M. omelianskii* was first isolated and described by Baker (1940). It was proposed that *M. omelianskii* obtained energy from oxidizing ethanol to acetate. However, Bryant *et al*. (1967) revealed that *M.omelianskii* was unable to ultilize ethanol for growth or producing methane under hydrogen partial pressure of 0.5 atm. They proposed that two organisms involved in this process in which a methanogen (*Methanobacterium bryantii* strain M.O.H) that utilizes H_2 and CO_2 for methane formation and an S-organism (stand for syntrophy) that oxidizes ethanol to acetate and H_2. The first term of syntrophic relationship was mentioned in this research as microbial associations in which H_2-producing organisms can grow only in the presence of H_2-consuming organisms. Similarly, other studies confirmed the syntrohic ethanol oxidizing process by *Pelobacter* strains and methanogen (Eichler and Schink, 1985). Figure 3 presents the overall electron flow in anaerobic degradation of organic matters under methanogenic and organohalide reducing conditions, as well as in microbial fuel cells.

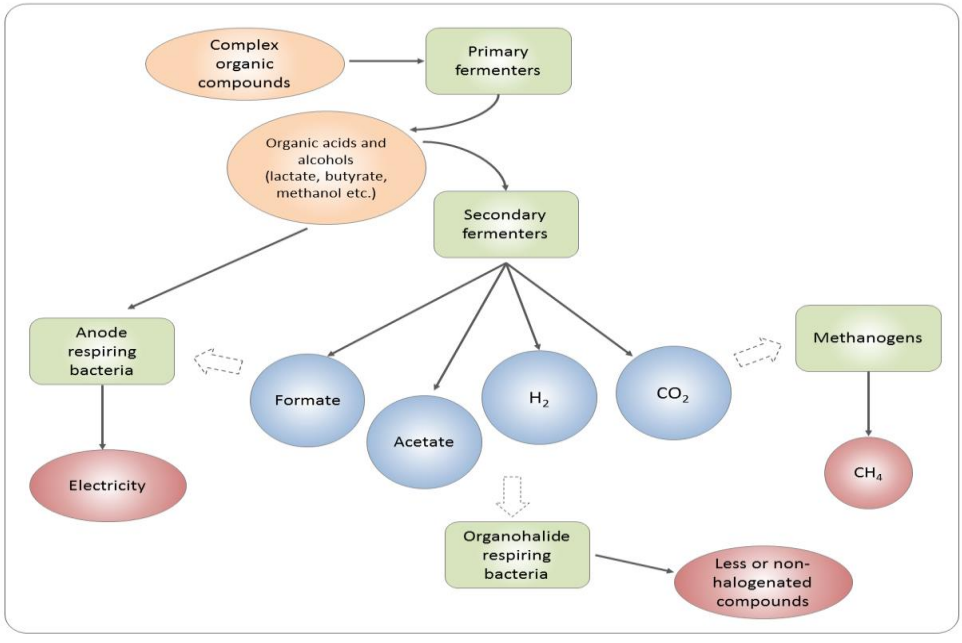

Fig. 3: Electron flow in anaerobic degradation of organic matter under different conditions

During dehalorespiration, bacteria couple reductive dehalogenation to a respiratory process for the generation of cellular energy. This energy yielding process utilizes the halogenated organic compound as the terminal electron acceptor while often molecular hydrogen (H_2) or a short chain organic compound is used as the electron donor. This process occurs under anaerobic conditions and dehalorespiring bacteria compete for electrons with other terminal electron accepting processes such as methanogenesis and sulfate reduction (modified from Max *et al*., 2012).

Biebl and Pfennig (1978) coined the term syntrophy in a study of the interaction between phototrophic green sulfur bacteria and sulfur reducing bacteria during anaerobic photosynthesis. In this research, mix culture between *Chlorobium* strains and *Desulfuromonas acetoxidans* or *Desulfovibrio desulfuricans* with different organic compounds as acetate, ethanol and propanol were carried out. The sulfur or sulfate reducing bacteria reduce sulfur or sulfate to sulfide which subsequently serves as an electron donor for the green sulfur bacterium.

The first syntrophic interaction identified in microbial reductive dehalogenation was 3-chlorobenzoate revealed by Mohn and Tiedje (1992) (Figure 4). Benzoate fermentation was shown to stall in the absence of two hydrogenotrophic microbes, one of which was the *Desulfomonile tiedjei*, and the other was a methanogen belonging to *Methanospirillum* genus. The syntrophic interaction between *Desulfomonile tiedjei* and bacterium BZ-2 was maintained only when there was low hydrogen partial pressure. No competition for hydrogen between the *Methanospirillum* genus and ORB was found. Only in combination could hydrogen formation from benzoate fermentation be stimulated (Mohn and Tiedje, 1992). Up to date, some researches revealed relationship between ORB and partners with different substrates in which fermenters played a role as syntrophic partners in delivering hydrogen which was taking away by ORB used for dechlorination activity (Table 2).

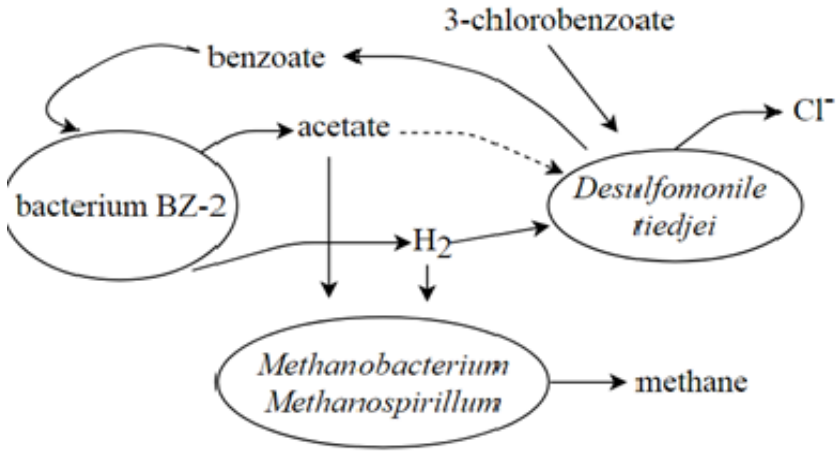

Fig. 4: Interaction between organohalide respiring bacterium *Desulfomonile tiedjei* and *Methanospirillum* sp. in dechlorination of 3-chlorobenzoate (Mohn and Tiedje, 1992)

Table 2: Some examples of syntrophic growth of organohalide-respiring bacteria and their partners during reductive dehalogenation of organohalide compounds

Electron donor microbe	Electron acceptor microbe	Substrate/Electron acceptor	Reference
Desulfomonile tiedjei	Benzoate-fermenting rod BZ2	Benzoate/3-chlorobenzoate	Shelton and Tiedje (1984)
Desulfovibrio desulfuricans	Dehalococcoides mccartyi strain 195 (formerly Dehalococcoides ethenogenes strain 195)	Lactate/trichloroethene	He et al. (2007)
Acetobacterium woodii	Dehalococcoides mccartyi strain 195	Lactate/trichloroethene	He *et al.* (2007)
Desulfovibrio vulgaris	*Dehalococcoides mccartyi* strain BAV1 and FL2	Lactate/trichloroethene	Men *et al.* (2011, 2012)
Geobacter sp.	Dehalococcoides mccartyi strain 195	Fumarate/tetrachloroethene	Yan et al. (2012)
Syntrophomonas wolfei	Desulfitobacterium frappieri TCE1	Butyrate/trichloroethene	Mao et al. (2015)
Desulfovibrio fructosivorans	PCB-dehalorespiring KFL culture contain Dehalobacter sp.	Lactate/tetrachloroethene	Drzyzga et al. (2001)
Clostridium sp. strain Ma13	Dehalobacter sp.	Starch-based plastic/4,5,6,7-tetrachlorophthalide	Naoko et al. (2013)
Sporomusa ovata Clostridium aceticum	Desulfuromonas sp. strain BB1	Acetate, tetrachloroethene, Ftrichloroethene, dichloroethene	He et al. (2002)
Desulfovibrio sp. strain SULF1	Desulfitobacterium frappieri TCE1	Lactate/ trichloroethene	Drzyzga et al. (2001)

5.2 Enhanced dechlorination in co-culture and omics related to syntrophic growth

The presence of syntrophic partners in organochlorine respiring cultures can enhance dehalorespiration rates up to 2 to 3 folds over that of ORB in isolation such as co-culture of ORB *Dehalococcoides mccartyi* strain 195 (DE195) with *Desulfovibrio vulgaris* (Men *et al*., 2012), *D. mccartyi* strain BAV1 and FL2 with *Geobacter* spp. (Yan *et al*., 2012), DE195 with *Desulfovibrio desulfuricans* and/or *Acetobacterium woodii* (He *et al*., 2007). Men *et al*. (2012) observed 102 significantly up or down regulated gene by transcriptomic analysis and 120 proteins expressed differently in the co-culture of DE195 and *D. vulgaris* compared with DE195 isolation. The robust growth of DE195 in co-culture may result in hydrogen and acetate which were fermented by *D. vulgaris*, along with potential benefit from proton translocation, cobalamin-salvaging and amino acids biosynthesis (Men *et al*., 2012). Zhuang *et al*. (2014) carried out the study on impact of incomplete "Wood-Ljungdahl" pathway, a crucial pathway for microbial energy conservation under anaerobic condition, found in *D. mccartyi* genome. The results indicated that *D. mccartyi* strain 195 was inhibited in pure culture by CO accumulating as a by-product from acetyl-CoA cleavage because of the lack of CO dehydrogenase in *D. mccartyi* strain 195. By using ^{13}C-labeling and bioinformatics analysis, they found out that bacteria and Achaea exist in consortia as CO-oxidizing organisms capable of gaining additional energy from coexist with *D. mccartyi* while enhancing the growth of *D. mccartyi* (Zhuang *et al*., 2014). Therefore, an unusual syntrophic association was established. This study seems elucidated how other microorganism including sulfate-reducers, hydrogen-producers, acetogen, etc. robust the growth of *D. mccartyi* in co-culture and consortia in the aspect of energy conservation.

Recent advances in genomics, transcriptomics and proteomics approaches have shed insights into the potential involvement of key genes and proteins in syntrophic interactions of mixed communities, further enabling exploration of improved method for rapid and robust growth of ORB. Omics-type analyses have widely been used in various ORB, such as members of *Dehalococcoides* (Morris *et al*., 2013; Lee *et al*., 2012; Schiffmann *et al*., 2014; Kublik *et al*., 2015), *Dehalobacter* (Rupakula *et al*., 2014), *Desulfitobacterium* and *Sulfurospirillum* genera (Goris *et al*., 2015; Kruse *et al*., 2015). These studies mainly deal with elucidating possible roles of the membrane associated and cytoplasmic protein/protein complexes and electron carriers in

organohalide respiration process as well as other cellular key metabolisms, such as endogenous corrinoid biosynthesis pathway and carbon fixation.

Transcriptomics and proteomics have been applied to various syntrophically grown bacteria to gain insights into their metabolism. Model syntrophic organisms, such as *Syntrophomonas wolfei*, have been investigated in several proteomic studies (Schmidt *et al*., 2013; Sieber *et al*., 2014, 2015). The reverse quinone loop in *S. wolfei* cells grown with *M. hungatei* on butyrate for syntrophic reverse electron transfer model was previously reported and supported by proteomic level observations using the techniques such as denaturing 1D- and 2D-PAGE or blue native PAGE followed by peptide fingerprinting-mass spectrometry. During the syntrophic growth, greater than 100-fold upregulation of a membrane-bound Hyd2 hydrogenase (Sieber *et al*., 2014) and the presence of a membrane bound formate dehydrogenase complex as well as a membrane-bound, iron-sulfur protein that may function as an EtfAB: menaquinone oxidoreductase and EtfAB2 (Schmidt *et al*., 2013) were revealed to give a broader view on an electron flow scheme during syntrophic butyrate oxidation. A further proteomic analysis employing a similar approach revealed that the most abundant proteins detected were GroEL and GroES chaperonins, a small heat shock protein, hydrogenases, proteins involved in beta-oxidation, and ATP synthesis (Sieber *et al*., 2015). The abundance of an uncharacterized, membrane-bound iron-sulfur oxidoreductase and EtfAB2 was proposed to be associated with electron transfer between acyl-CoA dehydrogenases and membrane redox carriers. Furthermore, a zinc-dependent dehydrogenase with a GroES domain and other transcriptional regulators were revealed and postulated to be involved in the cellular responses to environmental stimuli or the physiological status (Sieber *et al*., 2015).

Transcriptomic/proteomics-driven investigation of ORB on their syntrophic cooperation was somehow lacking, except the recent report by Men and co-workers (Men *et al*., 2012). In their work, syntrophic interaction of *Dehalococcoides ethenogenes* strain 195 with *Desulfovibrio vulgaris* Hildenborough as a co-culture, as well as *D. vulgaris* Hildenborough plus hydrogenotrophic methanogen *Methanobacterium congolense* as a tri-culture grown on lactate and TCE was investigated via both proteomic and transcriptomic approaches (Men *et al*., 2012). In the microarray transcriptomics and the two-dimensional nanoES 2d LC-MS/MS proteomics analyses of the co-culture compared to pure strain 195 revealed 102 genes and 120 proteins differentially expressed, respectively. It was concluded that lactate fermentation of *D. vulgaris* Hildenborough as well as proton translocation, cobalamin-salvaging and amino acid biosynthesis was beneficial for improved growth of strain 195. The versatility of *D. vulgaris* in terms of its adaptation to various electron acceptors was previously studied using qRT-PCR and genome-wide microarray transcriptomics (Scholten *et al*., 2007; Plugge *et al*., 2010). Scholten *et al*. (2007) revealed a set of three genes that played an important role in lifestyle shift of *D. vulgaris* from syntroph to sulfate reducer including DVU2103, DVU2104 and DVU 2108. They also found out that almost all bacteria species known to have syntrophic relation with methanogen had homologies to this gene set such as *Desulfuromonas acetoxidans*, *Pelobacter carbinolicus*, *Syntrophobacter fumaroxidans*, *Syntrophus aciditrophicus*, *Thermoanaerobacter ethanolicus*, and *Thermotogamaritima* (Scholten *et al*., 2007). The transcriptional changes of the *D. vulgaris* cells grown syntrophically with a lactate-oxidizing *Methanosarcina barkeri* in response to sulfate addition revealed 132 differentially expressed genes of which cell envelope and energy metabolism associated genes with the highest regulation, such as genes encoding ATPase and Ech hydrogenase (Plugge *et al*., 2010). Moreover, in this study reported were several down-regulated lipoproteins and membrane-bound proteins-encoding genes and novel c-type cytochrome-encoding genes.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

Bacteria involved in syntrophy seem to be highly adapted to a cooperation lifestyle including reduced genomic inventories and unique multiple membrane complexes (McInerney *et al*., 2007). To improve the ability to form a partnership from archaeal to bacterial partners in their environmental niche, there must be some metabolic and regulatory advantages for them as transferring and maintaining certain genes involved in different lifestyles (Scholten *et al*., 2007).

In the anaerobic biodegradation of chlorinated contaminants, syntrophic interaction between fermentative organisms and partners plays key roles. The involvement of these syntrophic populations is essential for mineralization of chlorinated aromatic compounds under anoxic conditions. However, the exact biochemical mechanisms in which these bacteria overcome the energetic barriers are not yet fully understood. Genome sequence information of ORB and their partners can be used to discover how these organochlorine compounds regulate

their syntrophic metabolism. Syntrophic relationship in organochlorine bioremediation remains a major challenge in the elucidation of key biosynthetic metabolic pathway. Cooperation in degradation of aromatic compounds, especially organochlorine compounds, under anoxic conditions continues to be an interesting area of modern research.

REFERENCES

Adrian, L. & Loffler, F. E., 2016. Organohalide-respiring bacteria. First edition. Springer-Verlag Berlin Heidelberg publishing, Berlin. 632 pages.

Adrian, L., Hansen, S. K., Fung, J. M., Gorisch, H. & Zinder, S. H., 2007. Growth of *Dehalococcoides* Strains with Chlorophenols as Electron Acceptors. Environmental Science & Technology. 41(7): 2318-2323.

Adrian, L., Szewzyk, U., Wecke, J. & Gorisch, H., 2000. Bacterial dehalorespiration with chlorinated benzenes. Nature. 408(6812): 580-583.

ATSDR, Agency for Toxic Substances and Disease Registry, 2015. Toxicological profile for hexachlorobenzene. Available from https://www.atsdr.cdc.gov/toxprofiles/tp90.pdf.

Alfan-Guzman, R., 2016. Anaerobic biodegradation of chlorinated benzenes. PhD thesis, University of New South Wales, Australia.

Baker, H. A., 1940. Study upon the methane fermentation. Martin Dworkin, Stanley Falkow and Eugene Rosenberg (Eds.). *In*: The isolation and culture of *Methanobacillus omelianskii* (3rd edition). Springer Singarpore. pp. 20-31.

Barber, J. L., Sweetman, A. J., Van Wijk, D. & Jones, K.C., 2005. Hexachlorobenzene in the global environment: emissions, levels, distribution, trends and processes. Science of the total environment, 349(1-3): 1-44.

Barber, J. L., Sweetman, A. J., Van Wijk, D., & Jones, K. C.. 2005. Hexachlorobenzene in the global environment: emissions, levels, distribution, trends and processes. Science of the total environment, 349(1-3), 1-44.

Becker, J. G., Berardesco, G., Rittmann, B. E. & Stahl, D. A., 2005. The role of syntrophic associations in sustaining anaerobic mineralization of chlorinated organic compounds. Environmental Health Perspect. 113(3): 310-316.

Biebl, H. & Pfennig, N., 1978. Growth yields of green sulfur bacteria in mixed cultures with sulfur and sulfate reducing bacteria. Archive Microbiology. 117(1): 9–16.

Bryant, M.P., Wolin, E.A., & Wolin, M.J., 1967. *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria. Archive Microbiology. 59: 20-31.

Bunge, M., Adrian, L., Kraus, A., *et al*., 2003. Reductive dehalogenation of chlorinated dioxins by an anaerobic bacterium. Nature. 421(6921): 357-360.

Dolfing, J., 2016. Energetic considerations in organohalide respiration. *In*: Adrian, L., Löffler F.E. (Eds.). Organohalide-respiring bacteria. Springer-Verlag Berlin Heidelberg publishing. Berlin, pp. 31-50.

Drzyzga, O., 2001. Coexistance of sulfate reducing *Desulfovibro* species and the halorespiring TCE1. Environmental Microbiology. 3(2): 92-99.

Eichler, B. & Schink, B., 1985. Fermentation of primary alcohols and diols and pure culture of syntrophically alcohol-oxidizing anaerobes. Archive Microbiology. 143(1): 60-66.

Fennell, D. E., Nijenhuis, I., Wilson, S. F., Zinder, S. H. & Haggblom, M. M., 2004. *Dehalococcoides ethenogenes* Strain 195 Reductively Dechlorinates Diverse Chlorinated Aromatic Pollutants. Environmental Science & Technology. 38(7): 2075-2081.

Goris, T., Schiffmann, C. L., Gadkari, J., *et al*., 2015. Proteomics of the organohalide-respiring Epsilonproteobacterium Sulfurospirillum multivorans adapted to tetrachloroethene and other energy substrates. Sci Rep, 5(1): 13794.

He, J., Holmes, V. F., Lee, P. K. & Alvarez-Cohen, L., 2007. Influence of vitamin B12 and cocultures on the growth of *Dehalococcoides* isolates in defined medium. Applied Environmental Microbiology. 73(9): 2847-53.

He, J., Sung, Y., Dollhopf, M. E., Fathepure, B. Z., Tiedje, J. M. & Loffler, F. E. 2002. Acetate versus Hydrogen as Direct Electron Donors To Stimulate the Microbial Reductive Dechlorination Process at Chloroethene-Contaminated Sites. Environmental Science & Technology, 36: 3945-3952.

Holliger, C. & Schumacher, W., 1994. Reductive dehalogenation as a respiratory process. Antonie van Leeuwenhoek, 66(1): 239 – 246

Hug, L. A., Maphosa, F., Leys, D., *et al*., 2013. Overview of organohalide-respiring bacteria and a proposal for a classification system for reductive dehalogenases. Philosophical Transactions of the Royal Society of London. 368(1616): 2012-2022.

Kruse, T., Van De Pas, B. A., Atteia, A. *et al*., 2015. Genomic, proteomic, and biochemical analysis of the organohalide respiratory pathway in *Desulfitobacterium dehalogenans*. Journal of Bacteriol. 197(5): 893-904.

Kublik, A., Deobald, D., Hartwig, S., *et al*., 2015. Identification of a multiprotein reductive dehalogenase complex in Dehalococcoides mccartyi strain CBDB1 suggests a protein-dependent respiratory electron transport chain obviating quinone involvement. Environmental Microbiology. 18(9): 3044-3056.

Lee, M., Low, A., Zemb, O., *et al.*, 2012. Complete chloroform dechlorination by organochlorine respiration and fermentation. Environmental Microbiology. 14(4): 883-894.

Leri, A.C., Marcus, M.A. & Myneni, S.C.B., 2007. X-ray spectromicroscopic investigation of natural organochlorine distribution in weathering plant material.

Geochimica et Cosmochimica Acta. 71(23): 5834-5846.

Loganathan, B. G., & Kannan, K., 1994. Global organochlorine contamination trends: an overview. Ambio. 23: 187–191.

Mao, X., Stenuit, B., Polasko, A. & Alvarez-Cohen, L., 2015. Efficient Metabolic Exchange and Electron Transfer within a Syntrophic Trichloroethene-Degrading Coculture of *Dehalococcoides mccartyi* 195 and Syntrophomonas wolfei. Applied Environmental Microbiology. 81(6): 2015-2024.

Max, H. & Bossert, I. D., 2003. Halogenated Organic Compounds - A Global Perspective. *In:* Häggblom, M. M. & Bossert, I. D. (Eds.). Dehalogenation: Microbial Processes and Environmental Applications. Boston, MA: Springer US.

Max, H. Donna, F., Lisa, R. N. & Lee, K., 2012. Quantifying Enhanced Microbial Dehalogenation Impacting the Fate and Transport of Organohalide Mixtures in Contaminated Sediments. SERDP Project ER-1492.

Maymo-Gatell, X., Chien, Y.-T., Gossett, J. M. & Zinder, S. H., 1997. Isolation of a Bacterium That Reductively Dechlorinates Tetrachloroethene to Ethene. *Science*, 276(5318): 1568-1579.

McInerney, M. J., Struchtemeyer, C. G., Sieber, J., *et al.* 2008. Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism. Annals of the New York Academy of Sciences, 1125**,** 58-72.

McCarty, P. L., 1997. Breathing with chlorinated solvents. Science, 276(5318): 1521-1532.

Meharg, A.A. & Osborn, D., 1995. Dioxins released from chemical accidents. Nature. 375: 353–354

Men, Y., Feil, H., Verberkmoes, N. C., *et al.*, 2012. Sustainable syntrophic growth of *Dehalococcoides ethenogenes* strain 195 with *Desulfovibrio vulgaris Hildenborough* and *Methanobacterium congolense*: global transcriptomic and proteomic analyses. ISME J, 6: 410-21.

Men, Y., Johnson, D. R., Andersen, G. L. *et al*., 2011. Sustainable syntrophic growth of *Dehalococcoides ethenogenes* strain 195 with *Desulfovibrio vulgaris* Hildenborough and *Methanobacterium congolense*: global transcriptomic and proteomic analyses. International Society for Microbial Ecology (ISME) 1751-7362: 1-12.

Mohn, W.W. & Tiedje, J.M., 1992. Microbial reductive dehalogenation. Microbiology Review. 56(2): 482–507.

MONRE, Ministry of Natural Resources and Environment, 2006. Vietnam National Implementation Plan: For Stockholm Convention on Persistent Oragnic pollutants. http://www.pops.int/documents/implementation/nips/submissions/NIP_Vietnam.pdf

Morris, B. E., Henneberger, R., Huber, H. & Moissl-Eichinger, C. 2013. Microbial syntrophy: interaction for the common good. FEMS Microbiology Review. 37(3): 384-406.

Naoko, Y., Lizhen, L., Fengmao, L. & Zhiling, L., 2013. Evaluation of biodegradable plastics as solid hydrogen donors for the reductive dechlorination of fthalide by *Dehalobacter* species. Bioresourses Technology. 130(1): 478-485.

Plugge, C. M., Jiang, B., De Bok, F. A., Tsai, C. & Stams, A. J., 2009. Effect of tungsten and molybdenum on growth of a syntrophic coculture of *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei*. Archive Microbiology. 191(1): 55-61.

Plugge, C. M., Scholten, J. C., Culley, D. E., Nie, L., Brockman, F. J. & Zhang, W., 2010. Global transcriptomics analysis of the *Desulfovibrio vulgaris* change from syntrophic growth with Methanosarcina barkeri to sulfidogenic metabolism. *Microbiology*, 156(9): 2746-56.

Reineke, W., Mandt, C., Kaschabek, S. R. & Pieper, D. H., 2001. Chlorinated Hydrocarbon Metabolism. *eLS.* John Wiley & Sons, Ltd.

Rupakula, A., Lu, Y., Kruse, T., *et al*., 2014. Functional genomics of corrinoid starvation in the organohalide-respiring bacterium Dehalobacter restrictus strain PER-K23. Frontier in Microbiology. 5(1): 751-68.

Ruth, S. & Paul, J., 2001. Industry Chlorine manufacture. In: Chlorine and the Environment: An Overview of the Chlorine Industry. Springer Netherlands, pp. 1-24.

Schiffmann, C. L., Jehmlich, N., Otto, W., *et al*., 2014. Proteome profile and proteogenomics of the organohalide-respiring bacterium *Dehalococcoides mccartyi* strain CBDB1 grown on hexachlorobenzene as electron acceptor. *J* Proteomics. 98: 59-64.

Schink, B., 1997. Energetics of syntrophic cooperation in methanogenic degradation. Microbiology and Molecular Biology Reviews. 61(2): 262-282 .

Schmidt, A., Muller, N., Schink, B. & Schleheck, D., 2013. A proteomic view at the biochemistry of syntrophic butyrate oxidation in Syntrophomonas wolfei. *PLoS One*, 8(2): e56905.

Scholten, J. C., Culley, D. E., Brockman, F. J., Wu, G. & Zhang, W., 2007. Evolution of the syntrophic interaction between *Desulfovibrio vulgaris* and *Methanosarcina barkeri*: Involvement of an ancient horizontal gene transfer. Biochemical and Biophysical Research Community. 352(1): 48-54.

Shelton, D. R & Tiedje, J. M., 1984. Isolation and Partial Characterization of Bacteria in an Anaerobic Consortium That Mineralizes 3-Chlorobenzoic Acid. Applied and Environmental Microbiology. 48(4): 840-848.

Sieber, J. R., Crable, B. R., Sheik, C. S., *et al*., 2015. Proteomic analysis reveals metabolic and regulatory systems involved in the syntrophic and axenic lifestyle of Syntrophomonas wolfei. Frontier in Microbiolog. 6(2): 115 - 124.

Sieber, J. R., Le, H. M. & Mcinerney, M. J., 2014. The importance of hydrogen and formate transfer for syntrophic fatty, aromatic and alicyclic metabolism. *Environmental Microbiology.* 16(1): 177-88.

Sinh, N.N., Thuy, L.T.B., Kinh, N.K., & Thang, L.B., 1999. The persistent organic pollutants and their management in Vietnam. In: Proceedings of the Regional Workshop on the Management of Persistent Organic Pollutants – POPs, UNEP, Hanoi, Vietnam, pp. 385-406.

Torsten, S. & Gabriele D. 2016. Comparative Biochemistry of Organohalide Respiration. In: Adrian, L., Löffler F.E. (Eds.). Organohalide-respiring bacteria. Springer-Verlag Berlin Heidelberg publishing. Berlin, p397-427.

Yan, J., Ritalahti, K. M., Wagner, D. D. & Loffler, F. E., 2012. Unexpected Specificity of Interspecies Cobamide Transfer from *Geobacter* spp. to Organohalide-Respiring *Dehalococcoides mccartyi* Strains. Applied and Environmental Microbiology. 78(18): 6630-6636.

Zhou, J., He Q., Hemmer C. L. *et al*., 2011. How sulphate-reducing microorganisms cope with stress: lessons from systems biology. Review in Nature. 9: 452-466

Zhuang, W. Q., Yi, S., Bill, M., *et al.*, 2014. Incomplete Wood-Ljungdahl pathway facilitates one-carbon metabolism in organohalide-respiring *Dehalococcoides mccartyi*. Proceedings of the National Academy of Sciences of the United States of America. 111(17): 6419-6424.