EFFECTS OF PHYSIOLOGICAL VARIABLES ON GROWTH OF ORGANOHALIDE RESPIRERS

By

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Abstract: There is a group of bacteria that is capable of the reductive dechlorination of chlorinated compounds, such as PCEs, by energetically using the chlorines in the compounds as terminal electron acceptors when oxygen is not present. Most experiments are conducted with the basic growth conditions that are needed to grow *Dehalococcoides* spp., because of its ability to completely dechlorinate PCE compounds to ethane, and other similar organohalide respiring bacteria and the terminal electron acceptor, the pollutant, is what is being tested and changed, but there has not been much research on optimizing or testing the growth conditions of these organohalide respiring bacteria. For this particular experiment, three different variables were changed: salinity, carbon source, and temperature, as well as looking at the differences between the effects of adding oak or pine based organochlorines. Methanol seems to be a better carbon source to use when trying to enrich organohalide respiring bacteria quickly and the carbon source that seemed to enrich the bacteria the least was acetate. Also, the temperature of 30°C seems to be the best temperature in which to enrich the bacteria as well, shown both by the results here and by the studies observed. There is a fair amount of OTUs present that are unique to the microcosms that were grown at 45°C. The ARISA results show an effect from the amendment of organochlorines compared to the non-chlorinated controls implying that organohalide respiring bacteria where enriched at those certain conditions.

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CHAPTER I

INTRODUCTION

Perchloroethylene (PCE) and its daughter products, trichloroethene (TCE), cis-dichloroethene, (cis-DCE), trans-dichloroethene (trans-DCE), and vinyl chloride (VC) are toxic compounds that are common in groundwater (Friis, et. al., 2007). They can cause a range of health effects including kidney dysfunction, coordination impairment, dizziness, mood and behavioral changes, and they are considered a potential carcinogen (Ruder, 2006, Maymo-Gatell, et. al., 1999, and U.S. EPA, 2012). PCEs will quickly sink through the soil and contaminate the groundwater making physical remediation difficult so bioremediation is a better option (Samuel, C., 1996 and Perina, 1997). Naturally occurring organochlorines has been used to enrich organohalide respiring bacteria (Krzmarzick, et. al., 2012). They are found everywhere and are naturally produced (Oberg, G., 2001 and Myneni, S. C. B., 2002). These compounds can be enzymatically produced by an enzyme called chloroperoxidase (CPO), which can be useful when considering the applications of producing organohalide repiring bacteria that can be grown quickly on the less toxic compounds and then bioaugmented to contaminated sites.

The group of organisms that can dechlorinate these chlorinated compounds are referred to as organohalide respiring bacteria. They are able to dechlorinate them by

using them as terminal electron acceptors in an anaerobic environment (Hug, et. al., 2013). This removal of chlorines makes the compounds less toxic. However most of the research performed on these organohalide respirers is done by varying the electron acceptor (Yang, et. al., 2005, Duhamel, et. al., 2002, Holliger, et. al., 1998, Wang, et. al., 2013, and Maness , et. al., 2012). This leads to the idea that varying other parameters could potentially produce other organohalide respiring organisms or reveal parameters in which they can be grown more quickly or efficiently.

In this study, organochlorides were produced with the enzyme CPO and used to enrich organohalide respiring bacteria anaerobically in different environments. Three parameters were tested and varied: carbon source, salinity, and temperature. The difference between organochlorines produced with organic matter extracted from soil obtained from under oak trees and from soil from under pine trees was also tested in the salinity and temperature experiments. Quantitative real-time PCR and automated ribosomal intergenic spacer analysis were performed to see what types of microorganisms were enriched and by how much in each environment.

CHAPTER II

REVIEW OF LITERATURE

2.1 Perchloroethylene (PCE)

Perchloroethylene, or PCE, is an organochloride which means that it is an organic compound that contains at least one covalently bonded atom of chlorine. PCE is a colorless liquid that is volatile, stable, and non-flammable. Because it is an excellent solvent for organic materials and non-flammable, it is used mostly in dry cleaning, but also to degrease metal parts in cars and in other metal working industries as well as paint strippers and spot removers (Lash, et. al., 2001, Ruder, 2006, Maymo-Gatell, et. al., 1999, and Seshadri, et. al., 2005). PCE, or sometimes called tetrachloroethylene, as well as its daughter products, trichloroethene, or TCE, *cis*-dichloroethene, or *cis*-DCE, *trans*-dichloroethene, or *trans*-DCE, and vinyl chloride, or VC, are toxic compounds and vinyl chloride is a liver carcinogen (Ruder, 2006 & Maymo-Gatell, et. al. 1999). They have been found to increase the chance for reproductive failure in both exposed females and males as well as longer pregnancy and spontaneous abortion. Some other effects that correlate with exposure are pattern memory and recognition and blue and yellow color confusion (Ruder, 2006). The EPA has listed a set of acute and chronic effects of PCEs. The acute effects include irritation of the upper respiratory tract and eyes, kidney

dysfunction, and neurological effects such as reversible mood and behavioral changes, impairment of coordination, dizziness, headache, sleepiness, and unconsciousness. The chronic effects listed are impaired cognitive and motor neurobehavioral performance (U.S. EPA, 2012). With these toxic and carcinogenic effects, PCE and its daughter products are considered a dangerous pollutant (Lash, et. al., 2001, Ruder, 2006, Maymo-Gatell, et. al., 1999, Hug, et. al., 2013, & Wang, et. al., 2013). PCEs are mobile in water and can quickly contaminate the groundwater. PCEs fate and transport depends on the environment of the contaminated site, such as soil type, organic matter content, wind speed, and many other factors, but mostly they are mobile in water and will quickly sink through the soil and contaminate the groundwater. They are also volatile and can travel through the air. Because of this mobility and the fact that PCEs are heavier than water, meaning they will sink, physical remediation is difficult and bioremediation is the better option (Samuel C., 1996 & Perina, 1997).

2.2 Naturally Occurring Organochlorines

Naturally occurring organochlorines is a group of compounds that contain carbon, hydrogen, and chlorine (Koeing, J., et. al., 2014). There are thousands of naturally produced chlorinated compounds and a vast array of organisms, such as phytoplankton, wood-rotting fungi, microorganisms, and plants, that are able to convert chloride to organic chlorine by producing a certain enzyme called chloroperoxidase (CPO) (Oberg, G., 2001 and Myneni, S. C. B., 2002). These organochlorines can be enzymatically produced by CPO and used to enrich microorganisms (Krzmarzick, et. al., 2012). A correlation was also observed by Krzmarzick, et. al., between organohalide respiring microorganisms and the total organic carbon present as organochlorines in an

uncontaminated environment (Krzmarzick, et. al., 2012). This could potentially be very useful in that organisms that respire naturally occurring organochlorines might also be able to dechlorinate the more toxic and abundant pollutants such as PCEs. The contaminated areas could be bioaugmented with organohalide respirers grown quickly on the less toxic naturally occurring organochlorines.

2.3 Organohalide Respiring Bacteria

There is a group of bacteria that is capable of the reductive dechlorination of chlorinated compounds, such as PCEs, by energetically using the chlorines in the compounds as terminal electron acceptors when oxygen is not present. The removal of these chlorines makes the compounds less toxic and more able to be degraded by microorganisms (Hug, et. al., 2013,). These bacteria can be put into two groups: obligate or non-obligate organohalide respirers, and they can be found in a few separate phyla: Proteobacteria, Firmicutes, and Chloroflexi. Some non-obligate organohalide respiring bacteria are *Geobacter*, *Desulfuromonas*, *Anaeromyxobacter*, *Sulfurospirillum*, and *Desulfitobacterium*. Organohalide respiring bacteria from the Proteobacteria pylum include *Geobacter lovleyi* strain SZ and *Anaeromyxobacter dehalogenans* strains 2CP-C and 2CP-1. From the Firmicutes phylum there is *Desulfitobacterium hafniense* strains Y51 and DCB-2, *Desulfitobacterium dehalogens* (NC_018017), *Desulfitobacterium dichloroeliminans* (NZ_AGJE00000000), and two *Dehalobacter* strains. Lastly, from the Chloroflexi phylum there are five *Dehalococcoides mccartyi* strains: *Dehalobium chlorcoercia*, DF-1, bacterium o-17, phylotypes SF-1 and DH-10, and *Dehalogenimonas lykanthroporepellens* strains BL-DC-9 and BL-DC-8 (Seshadri, et. al., 2005, Hug, et. al., 2013, & Yang, et. al., 2005).

Dehalococcoides mccartyi, or Dhc, is one of the most studied genus and species because it has the ability to completely dechlorinate PCE compounds to ethane, which is nontoxic and easily degradable by microorganisms (Maymo-Gatell, et. al., 1999, Seshadri, et. al. 2005, & Duhamel, 2002). Therefore, most experiments are conducted with the basic growth conditions that are needed to grow *Dehalococcoides* and other similar organohalide respiring bacteria and the terminal electron acceptor, the pollutant, is what is being tested and changed, but there has not been much research on optimizing or testing the growth conditions of these organohalide respiring bacteria, such as temperature or carbon source (Yang, et. al., 2005, Duhamel, et. al., 2002, Holliger, et. al., 1998, Wang, et. al., 2013, Maness, et. al., 2012).

2.4 Experimental Environmental Growth Conditions

All bacteria have certain environmental factors that they need to survive and certain parameters that will affect the community. These factors include the carbon source, the amount of oxygen available, the pH, the temperature, light, water content, salinity, the inorganic electron donors, and nutrient availability. This study looked at varying salinity, carbon source, and temperature so these are the variables discussed here.

2.4.1 *Dehalococcoides mccartyi*

Dehalococcoides mccartyi is one of the most studied bacteria when it comes to organohalide respiring bacteria because of its ability to reduce PCE all the way to VC. Most studies have grown *Dehalococcoides mccartyi* successfully by using acetate as a carbon source (Maymo-Gatell, et. al., 1999, Seshadri, 2005). The salinity levels of the microcosms in most studies are limited to basic mineral medias made up of essential salts and nutrients. In a study by Maymo-Gatell, et. al., they used a “basal salts medium”

containing NH_4Cl, K_2HPO_4, $MgCl_2*6H_2O$, $CaCl_2*2H_2O$, resazurin, and trace metals (Maymo-Gatell, et. al., 1999, and Maymo-Gatell, et. al., 1995). Another study by Aulenta, et. al., used a Reduced Anaerobic Mineral Medium, or RAMM. This medium contains: NH_4Cl, K_2HPO_4, $MgCl_2*6H_2O$, $CaCl_2*2H_2O$, resazurin, and trace metals, which is the exact medium used in the last study (Zinder, S. H., et. al., 1987, Aulenta, et. al., 2005). In another study, however, by Wang and He, they used a bicarbonate-buffered mineral salts medium. This includes trace elements, vitamins, and salts. Looking at salts specifically, their medium had NaCl, $MgCl_2 \cdot 6H_2O$, KH_2PO_4, NH_4Cl, KCl, and $CaCl_2 \cdot 2H_2O$. This media included 1 gram of NaCl as well as .3 grams of KCl which weren't included in the last two media solutions mentioned above. The temperatures in which *Dehalococcoides mccartyi* were grown are usually room temperature (Maymo-Gatell, et. al., 1999, Aulenta, et. al., 2004, Seshadri, et. al., 2005, and Wang and He, 2013). In the experiment conducted by Maymo-Gatell, et. al., the microcosms were kept at 35°C, Aulenta, et. al., kept their microcosms at 18°C - 22°C, and the experiment conducted by Wang and He were kept at "ambient temperature."

2.4.2 *Dehalobium chlorocoercia* DF-1

Dehalobium chlorocoercia DF-1 was isolated because of its ability to dechlorinate chlorobenzenes as well as polychlorinated biphenyls (PCBs) (Wu, et. al., 2002 and May, et. al., 2008). The carbon source most used when growing this bacteria strain is acetone (Payne, et. al., 2013, Kjellerup, et. al., 2012, Lombard, et. al., 2014, Payne, et. al., 2011, and May, et. al., 2008). The level of salinity these bacteria are mostly grown in are also limited to the amount of salts added by the mineral media just as *Dehalococcoides mccartyi* was. The media of choice for this bacteria seems to be an

estuarine mineral medium named ECl. This medium is composed of NaCl, $MgCl_2*6H_2O$, KCl, and $CaCl_2*2H_2O$ (Berkaw, et. al., 1996). Which is similar to the salt solutions used for *Dehalococcoides mccartyi*, however, they have listed it as estuarine and marine type media solutions. The NaCl added was 8.4 g/L. The temperature that *Dehalobium chlorocoercia* DF-1 was grown at in the experiments conducted by Payne, et. al., Kjellerup, et. al., Lombard, et. al., amd May, et. al. were all 30°C (Payne, et. al., 2013, Kjellerup, et. al., 2012, Lombard, et. al., 2014, Payne, et. al., 2011, and May, et. al., 2008).

2.4.3 *Dehalogenimonas spp.*

Dehalogenimonas spp. are related to the *Dehalococcoides* strains and also organohalide respiring bacteria (Maness, et. al., 2012). In the experiments conducted by Maness, et. al., Mukherjee, et. al., and Martin-Gonzalez, et. al., they used acetate as their carbon source (Maness, et. al., 2012, Mukherjee, et. al., 2014, Martin-Gonzalez, et. al., 2015). The salinity concentrations are based on mineral media compositions. These medias are referred to as "Basal medium" in Maness's study and in Mukherjee's study as "bicarbonate-buffered anaerobic liquid medium" which was based on Maness's media. This media wasn't clearly laid out, but these terms had been used before in the medias mentioned before and are being used similarly. For example, a "basal medium" containing NaCl, KH_2PO_4, NH_4Cl, $MgCl_2*6H_2O$, KCl, and $CaCl_2*2H_2O$, was used in the studies done by Martin-Gonzalez, et. al. and Adrian, et. al. when dechlorinating with *Dehalogenimonas spp*. (Martin-Gonzalez, et. al., 2015, and Adrian, et. al., 1997). This, again, is the same mineral media used in the previous studies. In a study performed by Manchester, et. al., the media used was made up of KH_2PO_4, K_2HPO_4, NH_4Cl,

$CaCl_2*6H_2O$, $FeCl_2*4H_2O$, and trace metals (Manchester, et. al., 2012, and Edwards, et. al., 1993). The microcosms in the studies by Maness, et. al. and Mukherjee, et. al., were conducted at 30°C. The ones conducted by Martin-Gonzalez, et. al. were done at 25°C and the studies by Manchester, et. al. were conducted at "room temperature" (Maness, et. al., 2012,, Murkherjee, et. al., 2014, Martin-Gonzalez, et. al., 2015, and Manchester, et. al., 2012).

2.4.4 *Dehalobacter spp.*

Dehalobacter spp. are also organohalide respiring bacteria that are related to *Desulfitobacterium spp.* in the Firmicutes phylum (Wang, et. al., 2013). They too are mostly grown with acetate as their carbon source (Wang, et. al., 2013 and Tang and Edwards, 2013). The mineral medium used in the studies done by Wang, et. al., was defined in their previous study where they grew *Dehalococcoides spp*. as mentioned earlier (Wang, et. al., 2013 and Wang, et. al. 3013). The medium used by Holliger, et. al., contains K_2HPO_4, $NaH_2PO_4*2H_2O$, NH_4HCO_3, $CaCl_2*2H_2O$, $MgCl_2*6H_2O$, $NaHCO_3$, Na_2S*9H_2O, resazurin, and trace elements (Hollinger, et. al., 1997). The media used in the studies of Tang and Edwards consists of KH_2PO_4, K_2HPO_4, NH_4Cl, $CaCl_2*6H_2O$, $FeCl_2*4H_2O$, and trace minerals (Tang and Edwards, 2013 and Edwards and Grbic-Galic, 1993). The temperatures the microcosms were kept at in the Wang, et. al. and Hollinger, et. al. studies was 30°C. A study done by Zemb, et. al., stated the temperature at which the microcosms were kept was "room temperature" (Zemb, et. al., 2010).

2.4.5 *Desulfitobacterium spp.*

Desulfitobacterium spp. is a close relative to *Dehalobacter* but is notable for the many halogenated compounds it is able to breakdown (Ding, et. al., 2014). This bacteria

is usually grown using pyruvate as its carbon source (Fletcher, et. al., 2010, Futagami, et. al., 2013, Fletcher, et. al., 2008, and Peng, et. al., 2012). The mineral media used in the Fletcher, et. al. experiments contains NaCl, $MgCl_2$,*$6H_2O$, KH_2PO_4, NH_4Cl, KCl, $CaCl_2$*$2H_2O$, resazurin, and trace elements (Fletcher, et. al., 2010 and Loffler, et. al., 1996). The NaCl added was 1 g/L. In the Futagami, et. al. experiments, the mineral media used consisted of K_2HPO_4, KH_2PO_4, sodium citrate, $MgSO_4$*$7H_2O$, yeast extract, and resazurin sodium salt (Futagami, et. al., 2013). In the studies performed by Peng, et. al., the mineral media used was composed of K_2HPO_4, KH_2PO_4, sodium citrate, $MgSO_4$*$7H_2O$, and yeast extract (Peng, et. al., 2012). The temperature of the microcosms in the Fletcher, et. al. experiment in 2008 was 24°C. The temperature of the microcosms of the Fletcher, et. al. experiment in 2010 was "room temperature" (Fletcher, et. al., 2008, Fletcher, et. al., 2010). The temperature of the microcosms in the Futagami, et. al. study and in the Peng, et. al., study was 30°C (Futagami, et. al., 2013 and Peng, et. al., 2012).

2.4.6 *Geobacter lovleyi*

Geobacter lovleyi is a unique member of the *Geobacteraceae* because this species can couple the degradation of PCE to *cis*-DCE with energy conservation and growth (Wagner, D., et. al., 2012). This species is usually grown with acetate as its carbon source (Strycharz, et. al., 2008, Sung, et. al., 2006, Amos, et. al., 2007, and Yan, et. al., 2012). This too is the same as all before mentioned bacteria except *Desulfitobacterium*. The salt solution used in the Strycharz, et. al., experiment was made up of KCl, NH_4Cl, NaH_2PO_4, a vitamin mix, and trace minerals (Strycharz, et. al., 2008 and Bond and Lovley, 2002). The media used in the Sung, et. al., and Amos, et. al., experiments was a bicarbonate-buffered mineral salts media that is composed of $MgSO_4$*$6H_2O$, NH_4SO_4, K_2SO_4,

$CaSO_4*H_2O$, Na_2S*9H_2O, L-cysteine, $NaHCO_3$, resazurin, and a trace element solution (Sung, et. al., 2003 and Amos, et. al., 2007). The experiment performed by Yan, et. al. grew *Geobacter lovleyi* in a medium composed of NaCl, $MgCl_2$,$*6H_2O$, KH_2PO_4, NH_4Cl, KCl, $CaCl_2*2H_2O$, resazurin, and trace elements (Yan, et. al., 2012 and Loffler, et. al., 1996). Again, they added 1 g/L of NaCl. The temperatures the bacteria was grown at was 25°C, "ambient temperature," and 30°C for the Strycharz, et. al., Sung, et. al., Amos, et. al., and Yan, et. al. experiments respectively (Strycharz, et. al., 2008, Sung, et. al., 2003, Amos, et. al., 2007, and Yan, et. al., 2012).

2.4.7 *Desulfomonile spp.*

Desulfomonile spp. is a bacteria that reductively dehalogenates halobenzoates and also reduces sulfate, sulfite, and thiosulfate as electron acceptors (DeWeerd, et. al., 1990). *Desulfomonile spp*. has been grown with pyruvate as its carbon source (Sun, et. al., 2001, DeWeerd, et. al., 1990, Ni, et. al., 1995, and Louie and Mohn, 1999). The mineral media in the DeWeerd, et. al. experiments was made with NaCl, NH_4Cl, KCl, KH_2PO_4, $MgCl*6H_2O$, $CaCl_2*2H_2O$, $NaHCO_3$, and a trace metal solution (DeWeerd, et. al., 1990). The media used in the experiments by Ni, et. al., 1995, was composed of NH_4Cl, KCl, KH_2PO_4, $MgCl_2*6H_2O$, $CaCl_2*2H_2O$, NaCl, $NaHCO_3$, K_2(PIPES), yeast extract, tryptone, trace minerals, resazurin, and vitamins (Ni, et. al., 1995). Lastly, the media used by Louie and Mohn, was composed of sodium lactate, Na_2SO_4, $NaHCO_3$, NaCl, KCl, $MgCl_2*6H_2O$, $CaCl_2*2H_2O$, NH_4Cl, KH_2PO_4, Na_2S, and yeast extract (Louie and Mohn, 1999). The amount of NaCl in these experiments was 1.5 g/L, 1.17 g/L, and 17mM, respectively. An experiment done by Sun, et. al. used a "marine sediment" that achieves an Na^+ concentration of 0.46 M, which approximates that of seawater. The

mineral salts included in the solution are as follows: NaCl, $MgCl_2$, KH_2PO_4, NH_4Cl, KCl, $CaCl_2$, and a trace elements solution (Sun, et. al., 2000 and Sun, et. al., 2001). The amount of NaCl included in this media was 25 g/L. This is particularly interesting because of the higher salt concentrations, *Desulfomonile spp*. grew at ranges of NaCl of .32-2.5%, with an optimum growth at 1.25% NaCl (Sun, et. al., 2001). The temperatures *Desulfomonile spp*. was grown at were 37°C, 37°C-40°C, and 30°C for Sun, et. al., Ni, et. al., and Louie and Mohn respectively (Sun, et. al., 2001, Ni, et. al., 1995, and Louie and Mohn, 1999).

2.4.8 *Desulfovibrio spp.*

Desulfovibrio spp. is a sulfate reducing bacteria that is considered to have a syntrophic association with dehalorespiring bacteria (Drzyzga, et. al., 2001). It has been grown using sodium lactate, acetate, and pyruvate as a carbon source (Cabrera, et. al., 2005, Tarasov, et. al., 2015, Ilhan-Sungar, et. al., 2014, and Colombo, et. al., 2013). The mineral media used in the Cabrera, et. al., experiments contained KH_2PO_4, NH_4Cl, Na_2SO_4, $CaSO_4*2H_2O$, $MgSO_4*7H_2O$, yeast extract, ascorbic acid, Thioglycolic acid, and $FeSO_4*7H_2O$ (Cabrera, et. al., 2005). The mineral media used in the Tarasov, et. al. experiments contained NaCl, KCl, NH_4Cl, K_2HPO_4, $MgCl_2*6H_2O$, Na_2SO_4, $CaCl_2*2H_2O$, yeast extract, and resazurin (Tarasov, et. al., 2015). The amount of NaCl added was 20 g/L; this media was made to simulate a marine salinity. This is why the amount of NaCl is high. The media used by Ilhan-Sungar, et. al. contained KH_2PO_4, NH_4Cl, Na_2SO_4, $CaCl_2*6H_2O$, $MgSO_4*7H_2O$, yeast extract, resazurin, $FeSO_4*7H_2O$, $C_2H_3O_2SNa$, and $C_6H_5O_7Na_3*2H_2O$ (Ilhan-Sungar, et. al., 2014). Lastly, the experiment conducted by Colombo, et. al. used a media containing MOPS, KH_2PO_4, NH_4Cl, KCl,

$MgCl_2$, $CaCl_2$, NaCl, vitamins, minerals (Colombo, et. al., 2013). The amount of NaCl added was 257 mM. The temperatures *Desulfovibrio spp*. were grown at were 30°C, 35°C, 30°C, and 28°C for the Cabrera, et. al., Tarasov, et. al., Ilhan-Sungar, et. al., and Colombo, et. al. experiments, respectively (Cabrera, et. al., 2005, Tarasov, et. al., 2015, Ilhan-Sungar, et. al., 2014, and Colombo, et. al., 2013).

2.4.9 *Sulfurospirillum spp.*

Sulfurospirillun spp. is a strictly anaerobic bacteria that is able to conserve energy via the reductive dehalogenation of chloroethenes like *Desulfitobacterium*, *Dehalococcoides*, and *Dehalobacter* (Scholz-Muramatsu, et. al., 1994). Two studies found use acetate as a carbon source, and another two use a method by Scholz-Muramatsu, et. al. which uses pyruvate as a carbon source (Maurice, et. al., 2003, Lohmayer, et. al., 2014, Keller, et. al., 2014, Cichocka, et. al., 2007, and Scholz-Muramatsu, et. al., 1994). The experiments performed by Maurice, et. al., had a salinity solely from their medium which they called a, "phosphate-/bicarbonate-buffered medium with a low chloride concentration" which contains K_2HPO_4, $NaH_2PO_4*2H_2O$, NH_4HCO_3, $CaCl_2*2H_2O$, $MgCl_2*6H_2O$, $NaHCO_3$, Na_2S*9H_2O, resazurin, trace element solution, and a vitamin solution (Maurice, et. al., 2003 and Holliger, et. al., 1993). The two studies that used pyruvate as a carbon source were modeled after the experiments performed by Scholz-Muramatsu, et. al., so the mineral media used was a basal medium that was composed of Na_2SO_4, KH_2PO_4, NH_4Cl, NaCl, $MgCl_2*6H_2O$, KCl, $CaCl_2*2H_2O$, trace elements, and a vitamin solution (Keller, et. al., 2014, Cichocka, et. al., 2007, and Scholz-Muramatsu, et. al., 1994). The NaCl added was 1 g/L. The studies performed by Lohmayer, et. al. had a media that was made up of KH_2PO_4, NH_4Cl, $CaCl_2*2H_2O$, and

$MgSO_4*7H_2O$ (Lohmayer, et. al., 2014). In the Keller, et. al., Cichocka, et. al., and Lohmayer, et. al. experiments the temperature at which the Sulfurospirillum spp. was grown (Keller, et. al., 2014, Cichocka, et. al., 2007, Lohmayer, et. al., 2014).

2.4.10 Gopher Group

The "Gopher group" is a group of bacteria that was heavily enriched when dechlorinating a broad class of natural organochlorines called chlorinated xanthones in a study by Krzmarzick, et. al. (Krzmarzick, et. al., 2014). They were grown using acetate as the carbon source and the salinity of the solution was because of a mineral media containing KH_2PO_4, K_2HPO_4, NH_4Cl, $CaCl_2*2H_2O$, $MgCl_2*6H_2O$, $FeCl_2*4H_2O$, $NaHCO_3$, resazurin, and trace metals (Krzmarzick, et. al., 2014 and Shelton, et. al., 1983). They were also grown at "room temperature" (Krzmarzick, et. al., 2014). This setup of parameters is pretty typical and the "basal salts" medium has been used in many experiments to grow organohalide respiring bacteria.

2.4.11 *Acinetobacter spp.*

Acinetobacter spp. can dehalogenate under both aerobic and anaerobic conditions (Kobayashi, et. al., 1997). In one study, 4-chlorobenzoic acid (4-CBA) was used as the sole carbon source for the enrichment of *Acinetobacter* (Kobayashi, et. al., 1997). In another, they tested the use of guaiacol as the sole carbon source (Gonzalez, et. al., 1993). They were only able to degrade certain positions (4-, 5-, and 4,5-dichloroguaicol) and not others (any time the Cl was at the 6 position) (Gonzalez, et. al., 1993). Another study tested the use of pentachlorophenol (PCP) as the sole carbon source which grew *Acinetobacter sp*. ISTPCP-3 (Sharma, et. al., 2009). The salinity of the solutions in each of the studies are from the mineral medias used. In the study by Kobayashi, et. al., they

used a basal PAS medium which consisted of K_2HPO_4, KH_2PO_4, NH_4Cl, $MgSO_4$, $MnSO_4*H_2O$, $FeSO_4*7H_2O$, $CaCl_2*2H_2O$, and L-ascorbic acid (Kobayashi, et. al., 1997 and Kobayashi, et. al., 1996). For the experiments by Gonzalez, et. al., the mineral media is a phosphate buffer with KH_2PO_4, Na_2HPO_4, NH_4SO_4, $MgSO_4*7H_2O$, and trace elements (Gonzalez, et. al., 1993 and Krockel and Focht, 1987). In the studies by Sharma, et. al., the mineral salts medium was comprised of KH_2PO_4, Na_2HPO_4, $MgSO_4*7H_2O$, $CaCl_2*2H_2O$, NH_4Cl, and a trace metal solution (Sharma, et. al., 2009). The temperature that Acinetobacter spp. grew at in all three studies was 30°C (Kobayashi, et al., 1997, Gonzalez, et. al., 1993, and Sharma, et. al. 2009). The Gonzalez, et. al. study looked at optimal growth conditions and determined 30°C to be optimal growth for *Acinetobacter spp*.

2.4.12 *Sedimentibacter spp.*

Sedimentibacter spp. has been correlated with the reductive dechlorination of PCBs (Gomes, et. al., 2014). It has been grown using acetate as a carbon source, like many other dechlorinating species mentioned, in the studies performed by Doesburg, et. al. (Doesburg, et. al., 2004). However, it has also been grown on ethanol and sodium formate as well as lactate in studies by Gomes, et. al., Oba, et. al., and Cheng, et. al., respectively (Gomes, et. al., 2014, Oba, et. al., 2013, and Cheng, et. al., 2009). The mineral media in the Doesburg, et. al. experiments was called a "WCB medium or a methanogenic mineral medium" and it was composed of K_2HPO_4, $NaH_2PO_4*2H_2O$, NH_4HCO_3, $CaCl_2*2H_2O$, $MgCl_2*6H_2O$, $NaHCO_3$, Na_2S*9H_2O, resazurin, trace element solution, and a vitamin solution (Holliger, et. al., 1993 and Doesburg, et. al., 2004). The medium used in the Gomes, et. al. experiments was made up of NH4Cl, NaCl,

MgCl2*6H2O, CaCl2*2H2O, K2HPO4*3H2O, resazurin, and a trace metal solution (Gomes, et. al., 2014 and Angelidaki, et. al., 1990). The NaCl added 10 g/L. The media used by Oba, et. al. includes NH_4Cl, KH_2PO_4, $MgCl_2*6H_2O$, $CaCl_2*2H_2O$, trace mineral element solution, a vitamin solution, resazurin, and $NaHCO_3$ (Oba, et. al., 2013). Cheng, et. al. used the bicarbonate-buffered mineral medium used by Loffler, et. al. described in the *Desulfitobacterium spp.* section above (Cheng, et. al., 2009 and Loffler, et. al., 1996). The temperatures used to grow *Sedimentibacter spp*. were 30°C except for the Cheng, et. al. experiments where it was grown at "room temperature" (Doesburg, et. al., 2004, Gomes, et. al., 2014, Oba, et. al., 2013, and Cheng, et. al., 2009).

2.4.13 *Acetobacterium spp.*

Acetobacterium spp. was mostly reported to dechlorinate chlorinated ethenes and ethanes in an undefined mixed culture (Wildeman, et. al., 2003). It has been grown using acetate as a carbon source as well (Wildeman, et. al., 2003). In the studies by Ding, et. al., it was enriched by using lactate, pyruvate, and acetate as a carbon source (Ding, et. al., 2013). Another study used fructose as a carbon source (Stromeyer, et. al., 1992). The media solution used by Jinenez-Salgado, et. al. that grew *Acetobacterium spp*. was K_2HPO_4, KH_2PO_4, $MgSO_4*7H_2O$, $CaCl_2*2H_2O$, $Na_2MoO_4*2H_2O$, and $FeCl_2*6H_2O$ (Jinenez-Salgado, et. al., 1997). The experiments done by Wildeman, et. al., used the same mineral media that was used by Scholz-Muramatsu, et. al. and mentioned before for the growth of *Sulfurospirillum spp*. (Wildeman, et. al., 2003 and Scholz-Muramatsu, et. al., 1995). Ding, et. al. used the bicarbonate-buffered mineral salts medium used by Wang and He and mentioned before for the growth of *Dehalococcoides spp*. above (Ding, et. al., 2013 and Wang and He, 2013). In the study by Stromeyer, et. al., the media

consisted of $NaHCO_3$, potassium phosphate, NH_4Cl, $MgSO_4$, Na_2S, L-cysteine, and resazurin (Stromeyer, et. al., 1992). The temperatures that the studies were run at were all very close, they were 29°C, 28°C, 30°C, and 30°C by Jinenez-Salgado, et. al., Wildeman, et. al., Ding, et. al., and Stromeyer, et. al., respectively (Jinenez-Salgado, et. al., 1997, Wildeman, et. al., 2003, Ding, et. al., 2013, and Stromeyer, et. al., 1992).

2.4.14 *Clostridia spp.*

Clostridia spp. is a group of sulfate-reducing bacteria found in saline waters (Gales, et. al., 2011). In the Gales, et. al. study they used glucose as a carbon source (Gales, et. al., 2011). Kuppardt, et. al. used toluene as a carbon source (Kuppardt, et. al., 2014). Gales, et. al. was able to enrich *Clostridia spp*. in a very saline solution. It was best grown at a 10% NaCl solution, it had .1 g/L of NaCl. The mineral media was made up of NH_4Cl, K_2HPO_4, KH_2PO_4, $MgCl_2*6H_2O$, $MgSO_4*7H_2O$, $CaCl_2*2H_2O$, NaCl, KCl, yeast extract, and a trace mineral elements solution (Gales, et. al., 2011). The media used by Kuppardt, et. al., was sulfate, sulfide, ammonium, phosphate, potassium, magnesium, sodium, and calcium (Vogt, et. al., 2006 and Kuppardt, et. al., 2014). The temperatures *Clostridia spp.* was grown at varied a bit, they were 43°C, 25°C, and 30°C for Gales, et. al., Kuppardt, et. al., and Lara-Martin, et. al., respectively (Gales, et. al., 2011, Kuppardt, et. al., 2014, and Lara-Martin, et. al., 2007).

2.4.15 *Desulforomonas spp.*

Lastly, *Desulfuromonas spp*. is another sulfur reducing bacteria group that is able to dechlorinate PCE and TCE (Loffler, et. al., 1999). The carbon source used by Loffler, et. al., and Sung, et. al. was acetate (Loffler, et. al., 2010 and Sung, et. al., 2003). The studies by Lee, et. al. and Dowideit, et. al., however used lactate and sodium pyruvate as

the carbon source for enriching *Desulfuromonas spp*. (Lee, et. al., 2010 and Dowideit, et. al., 2009). The mineral medium used by Loffler, et. al., is called a "basal salts medium" consisting of 1 g/L of NaCl, $MgCl_2$,*$6H_2O$, KH_2PO_4, NH_4Cl, KCl, $CaCl_2$*$2H_2O$, resazurin, and trace elements (Loffler, et. al., 1996 and Loffler, et. al., 1999). The study done by Lee, et. al., used a marine potassium phosphate buffer with 3% NaCl (Lee, et. al., 2010). The Sung, et. al., studies had a mineral medium consisting of $MgSO_4$*$6H_2O$, NH_4SO_4, K_2SO_4, $CaSO_4$*H_2O, resazurin, trace element solution, Na2S*9H2O, L-cysteine, $NaHCO_3$ (Sung, et. al., 2009). This medium is particularly interesting when looking at what is enriched in a salty environment. The medium used by Dowideit, et. al. is made up of KH_2PO_4, NH_4Cl, .5 g/L of NaCl, $MgCl_2$*$6H_2O$, KCl, $CaCl_2$*$2H_2O$, trace elements, and vitamins (Dowideit, et. al., 2009). The temperatures used to grow *Desulfuromonas* were 25C for Lee, et. al. and Sung et. al. and 20 for Dowideit, et. al. (Lee, et. al., Sung, et. al., and Dowideit, et. al.).

2.5 Summary

PCEs are chlorinated pollutants that can cause a variety of toxic effects to both humans and animals. It and its daughter products are toxic and potentially carcinogenic. They are mobile in water and can quickly contaminate the groundwater and sediments. Naturally occurring organochlorines are compounds that are abundant and naturally produced by many organisms by the enzyme chloroperoxidase (CPO). They could potentially be used to grow organochloride respiring microorganisms quickly for bioaugmenting contaminated sites. There are many types of organohalide respiring bacteria found in a few separate phyla: *Proteobacteria, Firmicutes*, and *Chloroflexi*. *Dehalococcoides* is the most studied organohalide respiring bacteria because of its ability

to completely degrade PCE to the non-toxic ethane. Therefore, most studies are conducted by trying to grow *Dehalococcoides* and other similar bacteria and only changing the electron acceptor, the pollutant or chlorinated compound. There has not been much research on testing the growth conditions such as temperature or using a different carbon source.

Growth conditions such as carbon source, salinity, and temperature that were used tended to be pretty much the same across many different studies with a few exceptions. When looking at carbon source, acetate was used the most often. However, pyruvate and lactate were also used commonly. Some particular differences were when looking at *Acinetobacter spp*., they used 4-CBA and guaiacol, and the growth of *Clostridia spp*. used glucose and toluene as carbon sources. The salinity was all pretty much based on the mineral media that was used for the particular experiment. They all basically had the same basic components of NH_4Cl, K_2HPO_4, KH_2PO_4, $MgCl_2*6H_2O$, $MgSO_4*7H_2O$, $CaCl_2*2H_2O$, NaCl, KCl, yeast extract, and a trace mineral elements solution. This solution varies of course, but not by much. There were some solutions however that were described as "marine" or "estuarine" that should be mentioned. They were for the growth of *Dehalobium chlorocoercia, Desulfomonile spp., Desulfovibrio spp., Clostridia spp*., and *Desulfuromonas spp.* The temperatures as well, were all mostly the same around room temperature. However, again, there were some that were grown at a slightly higher temperature. *Dehalococcoides mccartyi, Desulfomonile spp., Desulfovibrio spp., Gopher Group,* and *Clostridia spp*. all were grown at temperatures above 35°C. *Clostridia spp*. in particular was grown at 43°C, which was noticeably higher than the others.

CHAPTER III

MEDTHODOLOGY

3.1 Soil and Sediment Collection

The microbial seed material used in each microcosm was collected from a slow running stream at Ray Harrell Nature Park in Broken Arrow, Oklahoma in March 2014. The material was collected one foot from the stream bank in six inches of running water. The sediment was shoveled to about four inches of depth and funneled into a 500 mL bottle until it was filled completely. The park has no known history of contamination of chlorinated compounds. The soil used for organochlorine synthesis was collected from two separate areas. About two kg of surface soil was collected from two different forests one with oak tree cover and the other with pine tree cover in Payne County, Oklahoma. Both sites were rich with decaying organics and were collected only from the top one inch of the soil horizon.

3.2 Extraction of Organic Matter

To synthesize organochlorines, the organic matter must first be extracted from the soil samples, both the oak and pine, from Payne County, Oklahoma. Approximately 30 mL of soil was added to a 45 mL centrifuge tube then the rest of the tube is filled

with the solvent. The extraction of the organic material was done with sequential extractions with dichloromethane, hexane, and acetone. For each solvent, the tube is mixed by vortex for 5 minutes, incubated at 35°C overnight, and then mixed by a Cole-Parmer sonic oscillator for 15 minutes with heat. The tube was allowed to settle then the solvent was transferred to 250 mL Erlenmeyer flasks with a salinized glass pipette. The solvents from the oak soil were equally distributed in to four separate flasks and the solvents from the pine soils were distributed into two flasks. A total of 20 aliquots of 30 mL of soil were extracted from the oak soil and 10 aliquots of 30 mL of soil from the pine soil. Two of the oak flasks and one of the pine flasks were used for the synthesis of organochlorines and the other two oak flasks and pine flask as the control. The flasks were blown down to dryness using a stream of compressed air.

3.3 Synthesis of Organochlorines

To synthesize the orgnochlorines, 100 mL of a phosphate buffer is added to each flask with the dried organic extracts. The buffer has 0.1 M KH_2PO_4 and 20 mM KCl. After these were added the pH was adjusted for each beaker to 3.0 and was maintained from 2.8 to 3.2 throughout the chlorination reaction. For the reaction, 100 µL of a 0.1 M solution of H_2O_2 was added to all the reactors and 10 µL of CPO enzymes was added to the two flasks of oak organic extracts and the single flask of pine organic extracts for chlorination. Another 100 µL of the H_2O_2 solution was added after 30 minutes and then again after another 30 minutes. After each addition, the flasks were swirled by hand gently to mix. The flasks were left overnight and the CPO and H_2O_2 addition was repeated for four total days. After the reaction, the chlorinated compounds were extracted with dichloromethane by adding 100 mL to each of the flasks, mixed by hand, and settled

into two phases. The bottom phase, the dichloromethane and organochlorines, was transferred to a large beaker and pooled together keeping the CPO oak extracts, the CPO pine extracts, the control oak extracts, and the control pine extracts separate in their own beakers. This extraction was repeated 3 times. Once pooled into their respective beakers, the organics were separated evenly into 160 mL microcosm bottles and then dried under a stream of compressed air.

3.4 Microcosms

The microcosms were constructed in salinized 160 mL serum bottles capped with Teflon stoppers and aluminum crimps. Each microcosm contained either the organochlorine or control extract, five grams of soil from the Ray Harrell Nature Park sediment, 100 mL of anaerobic mineral media, and 50 mg of potassium acetate. The microcosms were constructed in an anaerobic glovebag with a 3% H_2/97% N_2 headspace.

For this particular experiment, three different variables were changed: salinity, carbon source, and temperature. Each microcosm was operated in duplicate and each variable has a non-chlorinated (no CPO-produced organochlorines) control, also operated in duplicate. A summary of each set of microcosms can be seen below in **Tables 1**, **2**, and **3**. Unless otherwise stated, the control parameters are: no extra salt added after the mineral media, a carbon source of acetate, and a temperature of 25°C (room temperature).

Table 1. Summary of Salinity Microcosms

Salinity		
Name	**Organics**	**NaCl added**
Control	Oak	No NaCl added
Pine Control	Pine	No NaCl added
Medium Salinity	Oak	500 mg
High Salinity	Oak	1000 mg
Pine High Salinity	Pine	1000 mg

Table 2. Summary of Carbon Source Microcosms

Carbon Source		
Name	**Organics**	**Carbon Source added**
Control	Oak	50 mg Potassium Acetate
Methanol	Oak	10 µL Methane
Butyrate	Oak	10 µL Butyrate
Bicarbonate	Oak	Nothing (Bicarbonate is in mineral media)

Table 3. Summary of Temperature Microcosms

Temperature		
Name	**Organics**	**Temperature**
Control	Oak	25°C (Room Temperature)
Pine Control	Pine	25°C (Room Temperature)
30°C	Oak	30°C by incubator
37°C	Oak	37°C by water bath
45°C	Oak	45°C by water bath
45°C Pine	Pine	45°C by water bath

3.5 Sample Collection

Samples were collected at Days 0, 14, 27, 42, 56, 70, and 92 for DNA analysis. Bottles were vigorously hand shaken for thirty seconds before they were opened in anaerobic chamber and 500 µL of sediment slurry was transferred to microcentrifuge tubes. The samples were then centrifuged at 10,000 g for three minutes and the

supernatant removed to a separate microcentrifuge tube. The pellet was transferred to bead-beating tubes for DNA extraction with the PowerSoil DNA isolation kit from MoBio Laboratories and the DNA extracted according to manufacturer's recommendations for the kit. These and the supernatant were then stored at -20°C until further analysis.

3.6 Polymerase Chain Reaction (PCR)

For the polymerase chain reaction, or PCR, analysis 1 µL of extracted DNA is injected into the middle of 24 µL of a master mix solution in a PCR tube for each DNA sample. The master mix solution consists of 52 µL of $MgCl_2$, 260 µL of 5X buffer, 65 µL of BSA, 20.8 µL of a dNTP mixture, 16.25 µL of both the forward and reverse primers, 6.5 µL of the Taq enzyme, and 811.2 µL of PCR water. The forward and reverse primers used for the PCR reaction are ITSF and ITSF Reub – HEX, respectively. This mixture of the DNA and master mix was constructed in duplicate and then transferred to a thermocycler where it underwent a series of temperatures to replicate the DNA added. The cycle used was 45 seconds at 94°C, 60 seconds at 55°C, and then 2 minutes at 72°C. This cycle was repeated 32 times for DNA replication. The PCR product was stored at -20°C until further analysis.

Electrophoresis was then performed to test the success of the PCR. A gel was prepared by combining 50 mL of 1X TAE buffer and 0.5 g of agarose. This was then heated and stirred until boiling then removed and allowed to cool slightly. Once the solution was cool to the touch, but not yet a gel, 15 µL of DNA stain from EnviroSafe was added. Then it was poured into a gel mold and allowed to cool and solidify

completely in the dark. The gel was then loaded with a well-mixed combination of 7 µL of PCR product and 3 µL of loading buffer. The gel was run at 100V for 15 minutes and then read by a gel imager from Bio-Rad. If there were bands, the PCR was considered a success and stored at -20°C for further analysis. If there were no visible bands, the PCR was re-done and tested again until bands could be seen.

3.7 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

After the PCR product was confirmed using electrophoresis, the product was prepared for automated ribosomal intergenic spacer analysis, or ARISA. The duplicates were combined into a single PCR tube. The samples were prepared by injecting 1 µL of combined PCR product into the center of 24 µL of a master mix solution composed of 0.5 µL of MapMarker 1000 dye and 8.5 µL HiDi Formamide in a 96-well plate. The plate is then covered with a 96-well plate seal and sent to the DNA/Protein Core Facility at Oklahoma State University for fragment size analysis on an ABI Model 3730 DNA Alalyzer.

The data obtained from the DNA/Protein Core Facility was analyzed with a software called Peak Scanner available online from Life Technologies. This data was then copied to Excel where it was binned together. The binning process starts with eliminating all peak areas larger than 1,000 and less than 50 and then normalizing as a percentage of total the remaining data. Then all data that's normalized value is less than 0.5% is deleted. The remaining normalized peak areas can then be binned together according to size; they were binned together within about 0.4 bp of each other.

3.8 Quantitative Real-Time PCR (qPCR)

Quantitative polymerase chain reaction (qPCR) was done to quantify several 16S rRNA genes from known organohalide respiring bacteria. The phylogenetic targets for each primer, the primers, and the references for each qPCR primer are listed in **Table 4**.

Table 4. Summary of phylogenetic targets, primer pairs, and references

Pylogenetic Target	Primer Pair	Reference
Dehalococcoides mccartyi	Dhc582F//Dhc728R	Duhamel, et. al., 2004
Dehalobium chlorocoercia DF1	Dhbm866F//Dhbm1265R	Fagervold, et. al. 2005 and Watts, et. al., 2005
Dehalogenimonas spp.	Dhg634F//Dhg799R	Yan, et. al., 2009
Dehalobacter spp.	Dhb447F//Dhb647R	
Desulfitobacterium spp.	Dsb406F//Dsb619R	Smits, et. al., 2004
Geobacter lovleyi	Geo564F//Geo840R	Cummings, et. al., 2003 and Sanford, et. al., 2007
Desulfomonile spp.	Dsm205F//Dsm628R	El Fantroussi, et. al., 1997
Desulfovibrio spp.	Dsv691F//Dsv826R	Fite, et. al., 2004
Sulfurospirillum spp.	Sulfuro114F//Sulfuro421R	Loffler, et. al., 2005 and Duamel and Edwards, 2006
Gopher Group	Gfr163F//Gfr441R	Krzmarzick, et. al., 2014
Bacteria	Eub341F//Eub534R	
Acinetobacter	UC6-244F//UC-480R	Lim, et. al., 2015
Sedimentibacter	Sdm787F//Sdm992R	Lim, et. al., 2015
Acetobacteria	Ace572F//Ace784R	Duhamel and Edwards, 2006
Clostridia	Cls115F//Cls202R	
Desulforomonas	Dsf205F//Dsf1020R	Loffler, et. al., 2000

The qPCR was done on a CFX Connect Real Time System from Bio-Laboratories and the analysis software was Bio-Rad CFX Manager. Each well in the 96-well plate contained 9.5 µL of a master mix solution and 0.5 µL of DNA extract. The master mix solution was made up of 28.125 µL of 2 X iTaq SyberGreen Supermix with Rox, 1.25 µL of BSA, .444 µL of each the forward and reverse primers, and 9.26 µL of water. Each plate was put through a thermocycling protocol that was 95 ºC for 3 min followed by 40 cycles of 95 ºC for 15 s and 60 ºC for 30 s. Standards for each qPCR were prepared from known concentrations of plasmid extracts containing the 16S rRNA gene of interest.

CHAPTER IV

FINDINGS

4.1 Growth of Organohalide Respirers using qPCR

The amount of particular organohalide respiring bacteria were measured using qPCR to determine the concentration of that bacteria group or species over time. The 16S rRNA gene was quantified of different bacteria or bacteria groups. The measurement of the more common dechlorinating species, or species most commonly found in a dechlorinating community, were measured first: this includes *Dehalococcoides mccartyi, Dehalobacter spp., Desulfovibrio spp*., and *Sulfuropirillum*. The differences between the effects of the different salinity concentrations, the different carbon source, and the different temperatures were compared to each other. Also, the same bacteria are compared when looking at the effects of growth on the organochlorines produced with the organic matter extracted from under an oak tree versus the effects of growth on the organochlorines produces with the organic matter extracted from under a pine tree. In each of these conditions, the differences in growth between the reactors with the CPO produced organochlorines and those with just organic matter in the same conditions were also compared. The quantity for the 16S rRNA shown has been normalized with the amount of DNA quantified in the extract sample.

4.1.1 Growth of Organohalide Respirers in Different Salt Concentrations

The first comparisons for each bacteria or bacteria group, are the differences in growth in different salinity concentrations. There is "Low," "Medium," and "High" salinity which corresponds to just the salt added by the mineral medium, an additional 500 mg of NaCl added per reactor, and an additional 1000 mg added per reactor, respectively. When looking at *Dehalococcoides mccartyi* in particular for differences in growth because of salinity concentrations, the amount of bacteria grew in each microcosm for each condition, then at Day 56 the microcosm with a medium amount of salinity seemed to be higher than that of the other two levels, but not significantly higher, however the error bars are very large here as can be seen in **Figure 1**. *Dehalobacter spp*. on the other hand, was enriched and grew in concentration after Day 0 and stayed around Log 8.5 copies of 16S rRNA genes per ng of DNA until Day 56. The concentrations in each reactor then fell until Day 70 as can be seen in **Figure 2**. *Sulfurospirillum spp*. grew relatively quickly, as can be seen in **Figure 3**, up to Day 14, but then declined in concentration to about the same amount, around Log 5 copies of 16S rRNA genes per ng of DNA, on Day 42. Then had another spike of growth on Day 56 before dropping again by Day 70. *Desulfovibrio spp*.was enriched as well and only the microcosms with high salinity fell in concentration after Day 56 as can be seen in **Figure 4**.

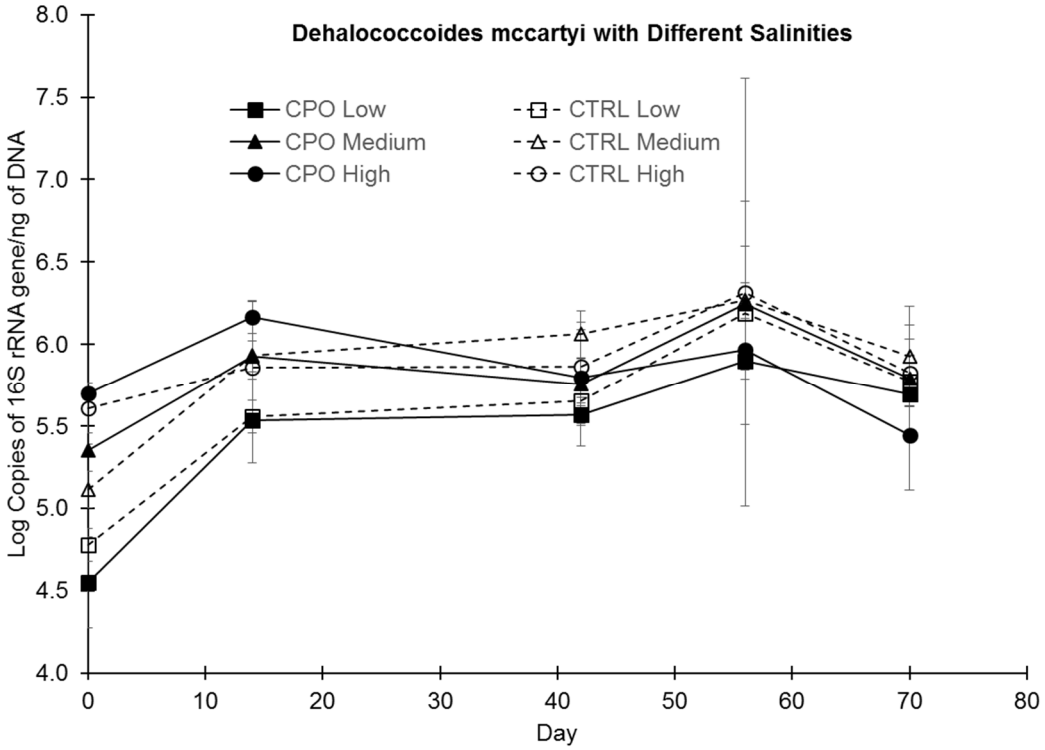

Figure 1. The concentration of *Dehalococcoides mccartyi* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines at different salinities: CPO Low (■), CPO Medium (▲), and CPO High (●) with their respective non-chlorinated controls: CTRL Low (□), CTRL Medium (Δ), and CTRL High (○).

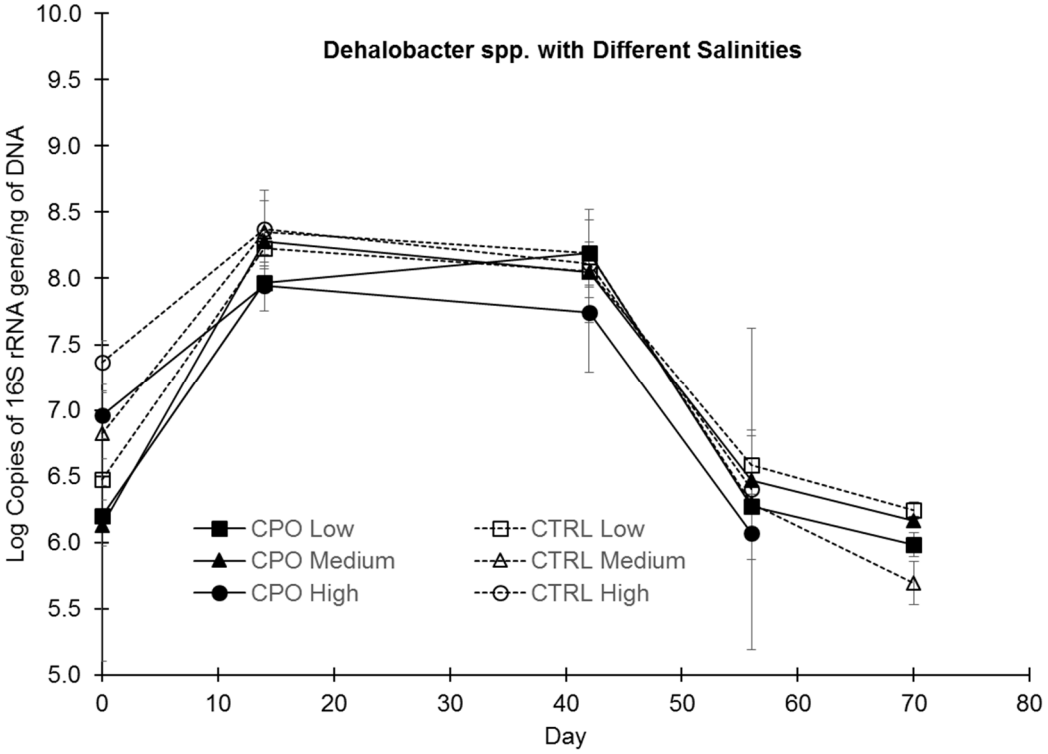

Figure 2. The concentration of *Dehalobacter spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines at different salinities: CPO Low (■), CPO Medium (▲), and CPO High (●) with their respective non-chlorinated controls: CTRL Low (□), CTRL Medium (Δ), and CTRL High (○).

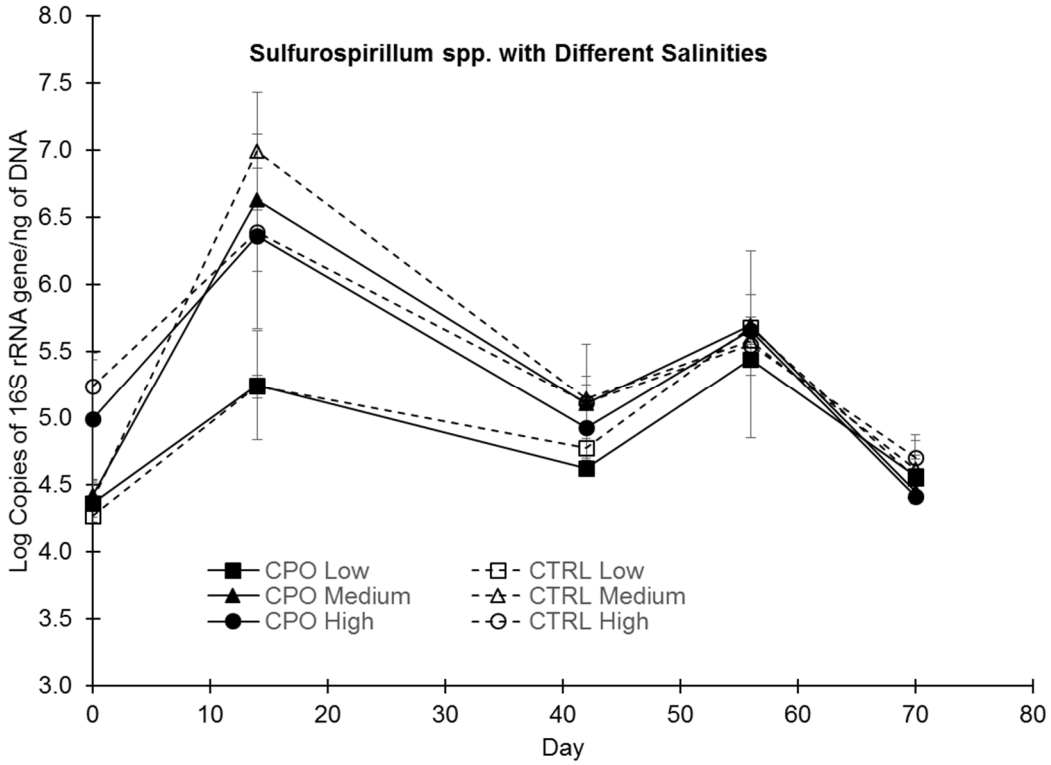

Figure 3. The concentration of *Sulfurospirillum spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines at different salinities: CPO Low (■), CPO Medium (▲), and CPO High (●) with their respective non-chlorinated controls: CTRL Low (□), CTRL Medium (Δ), and CTRL High (○).

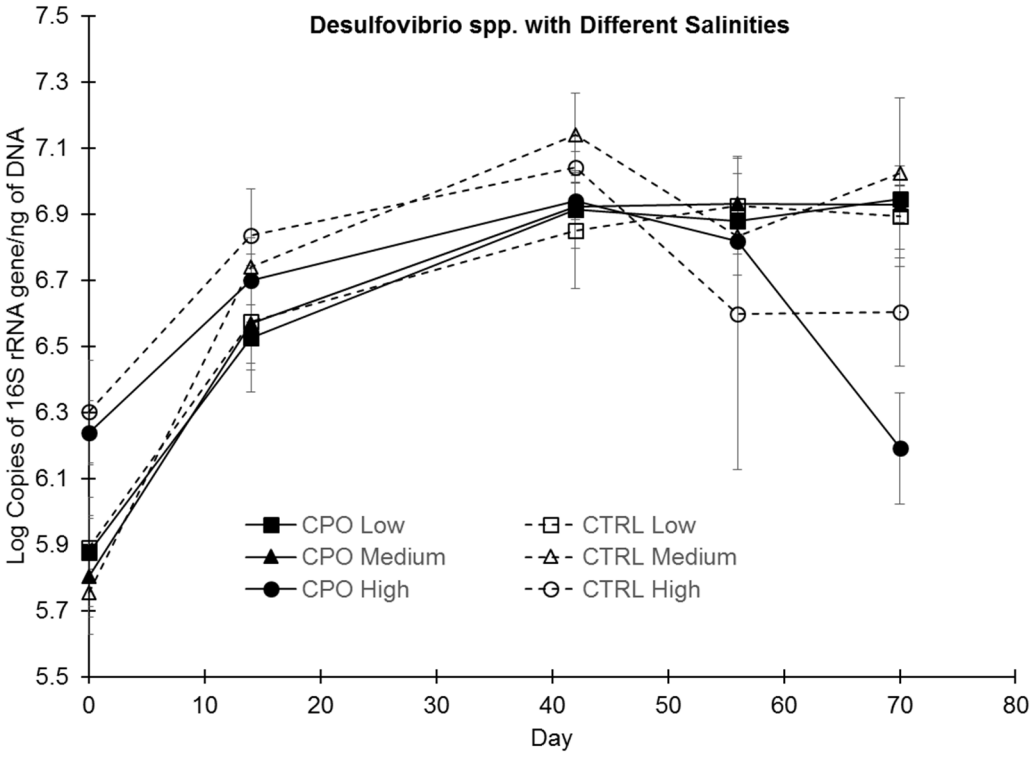

Figure 4. The concentration of *Desulfovibrio spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines at different salinities: CPO Low (■), CPO Medium (▲), and CPO High (●) with their respective non-chlorinated controls: CTRL Low (□), CTRL Medium (Δ), and CTRL High (○).

4.1.2 Growth of Organihalide Respirers using Different Carbon Sources

The differences in growth among those grown by using different carbon sources are shown below in **Figures 5-8**. The different carbon sources used were acetate as the control, methanol, butyrate, and bicarbonate. *Dehalococcoides mccartyi* seemed to start to have some growth from Day 0 to Day 14 as can be see in **Figure 5**, but by Day 28 each reactor dropped to roughly Log 6.0 copies of 16S rRNA gene per ng of DNA and stayed around that concentration until Day 70. *Dehalobacter spp*. seemed to increase in concentration quickly by Day 14, particularly the reactor with CPO produced enzymes grown on methanol and bicarbonate, but then dropped below those grown without the addition of CPO produced organochlorines, **Figure 6**. *Sulfurospirillum spp*., also grew relatively quickly from Day 0 to Day 14, then decreased until Day 70. It seemed to increase in concentration faster when grown on methanol, as can be seen in **Figure 7**. Lastly, *Desulfovibrio spp*. grew steadily until Day 70. However, again when looking at the microcosms grown with methanol, from Day 0 to Day 14 it increased in concentration rapidly, **Figure 8**.

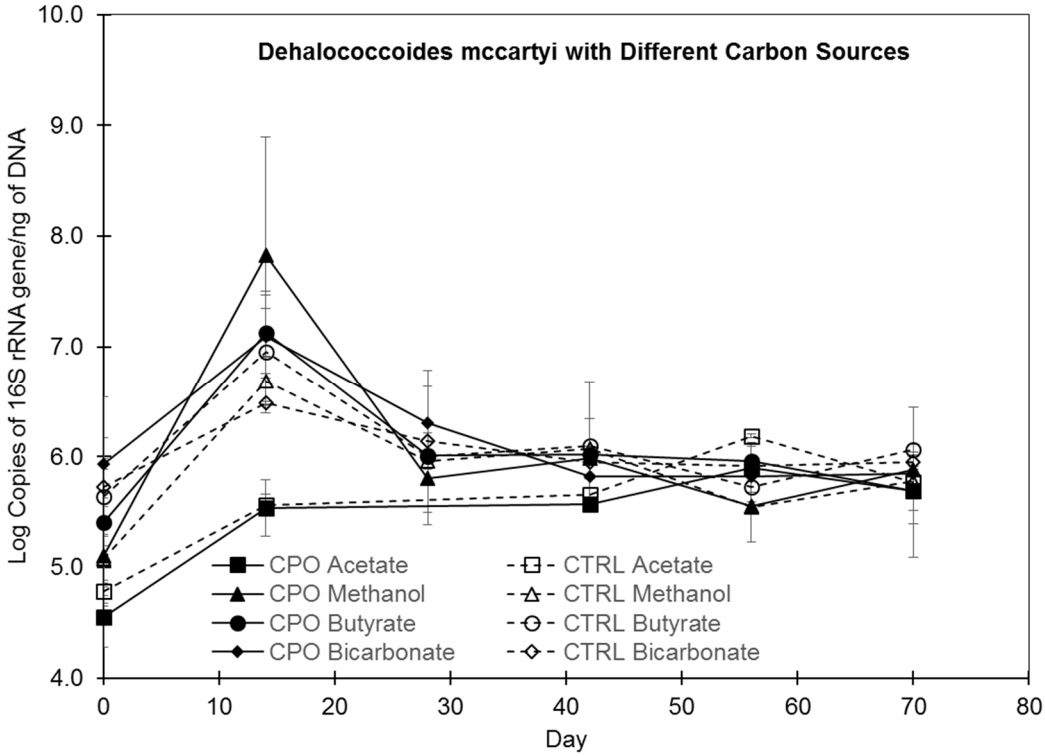

Figure 5. The concentration of *Dehalococcoides mccartyi* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines with different carbon sources: CPO Acetate (■), CPO Methanol (▲), CPO Butyrate (●), and CPO Bicarbonate (♦) with their respective non-chlorinated controls: CTRL Acetate (□), CTRL Methanol (Δ), CTRL Butyrate (○), and CTRL Bicarbonate (◊).

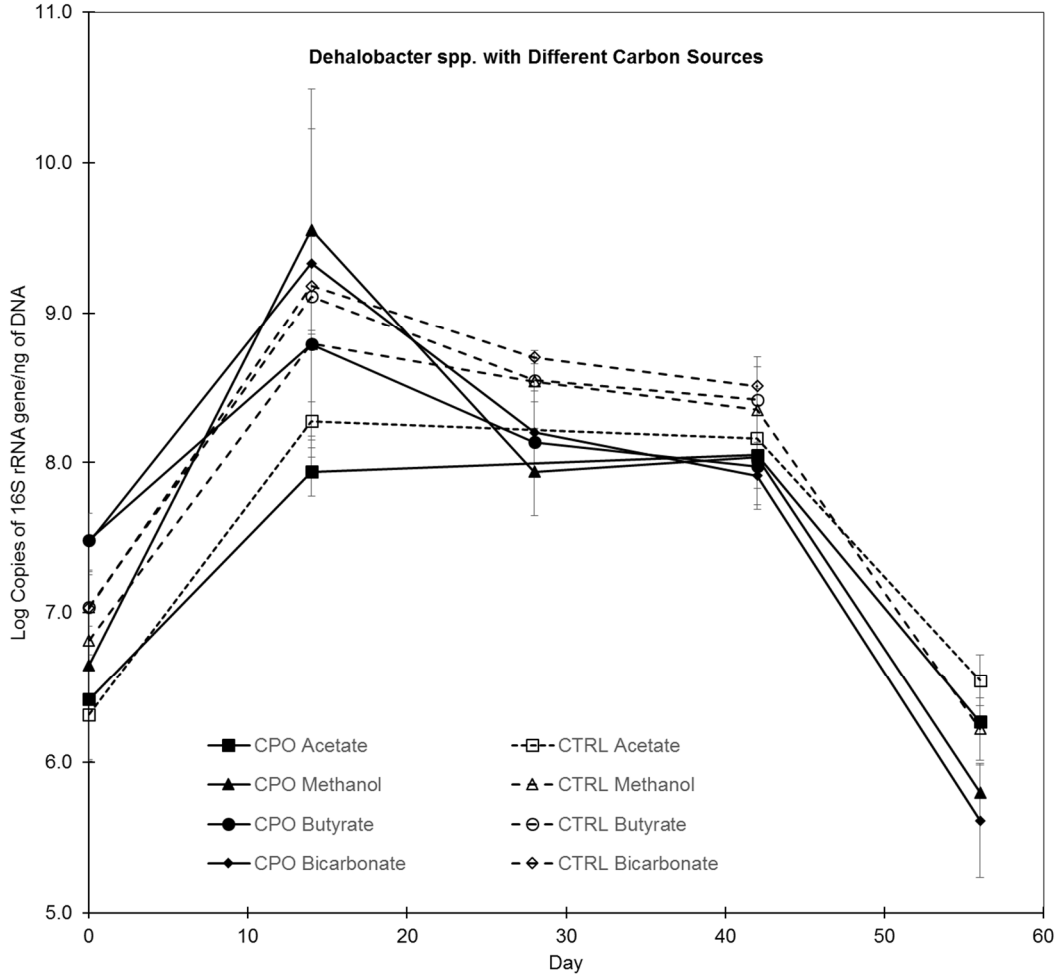

Figure 6. The concentration of *Dehalobacter spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines with different carbon sources: CPO Acetate (■), CPO Methanol (▲), CPO Butyrate (●), and CPO Bicarbonate (♦) with their respective non-chlorinated controls: CTRL Acetate (□), CTRL Methanol (Δ), CTRL Butyrate (○), and CTRL Bicarbonate (◊).

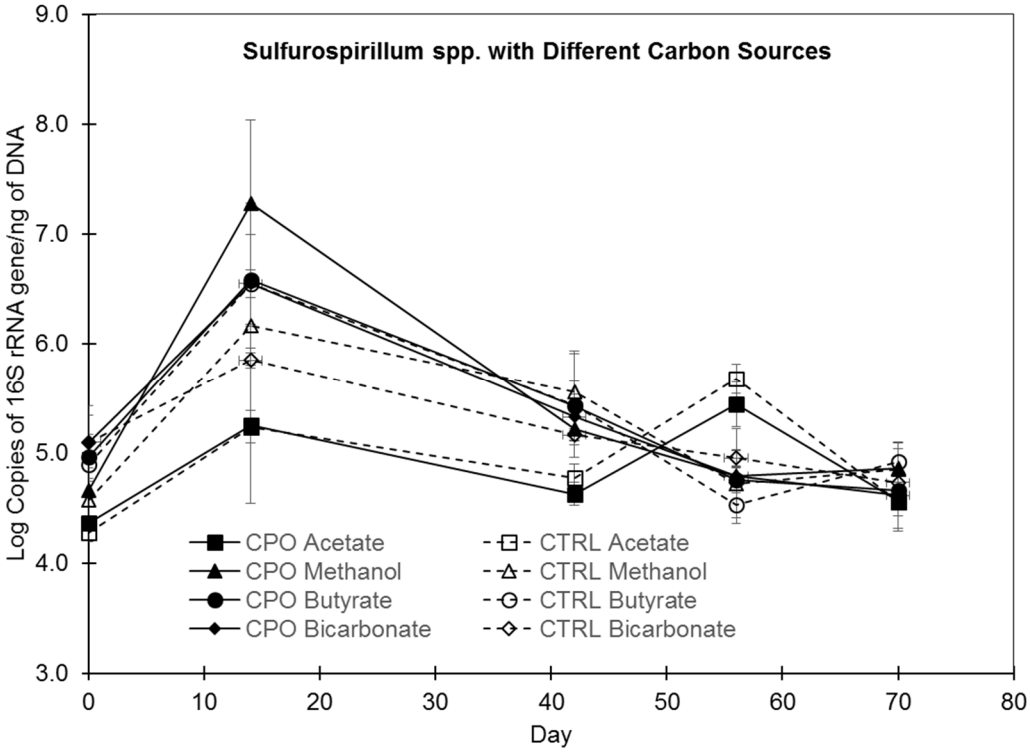

Figure 7. The concentration of *Sulfurospirillum spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines with different carbon sources: CPO Acetate (■), CPO Methanol (▲), CPO Butyrate (●), and CPO Bicarbonate (♦) with their respective non-chlorinated controls: CTRL Acetate (□), CTRL Methanol (Δ), CTRL Butyrate (○), and CTRL Bicarbonate (◊).

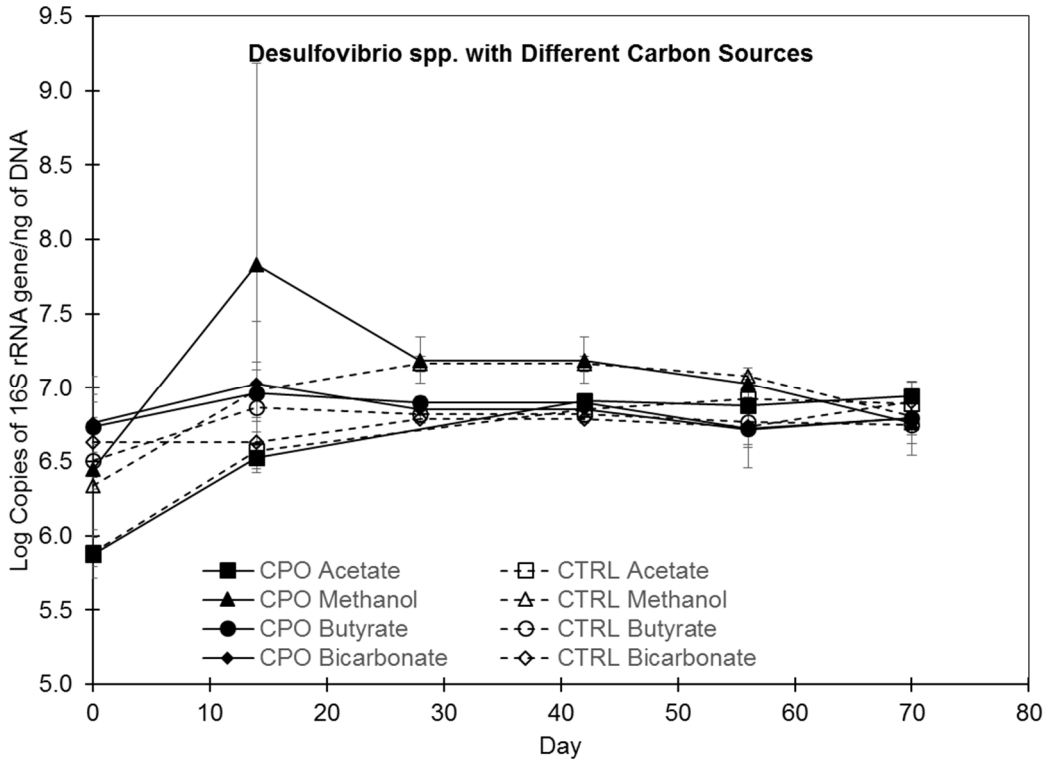

Figure 8. The concentration of *Desulfovibrio spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines with different carbon sources: CPO Acetate (■), CPO Methanol (▲), CPO Butyrate (●), and CPO Bicarbonate (♦) with their respective non-chlorinated controls: CTRL Acetate (□), CTRL Methanol (Δ), CTRL Butyrate (○), and CTRL Bicarbonate (◊).

4.1.3 Growth of Organohalide Respirers at Different Temperatures

The temperature at which the reactors were kept seems to have the largest effect on the growth of different species. The temperatures at which they were kept was 25°C (room temperature) for the control, 30°C, 37°C, and 45°C. *Dehalococcoides mccartyi* had a largely different growth at the highest temperature, 45°C, as well as at 37°C. However, what was grown differently was in the microcosm without the CPO produced organochlorines, as can be seen in **Figure 9**. *Dehalobacter spp*. that was grown all basically had the same trend in growth where they grew from Day 0 to Day 28, then remained around Log 10.5. copies of 16S rRNA until Day 42 where the concentrations dropped and remained until Day 70. Again, the bacteria grown at 45°C and 37°C were the most abundant, but the most enriched *Dehalobacter spp*. was found in the microcosm that contained no CPO produced organochlorines, **Figure 10**. *Sulfurospirillum spp*. has a similar trend, with the most enriched microcosm was at 45°C, but at Day 14, the concentration of 16S rRNA genes in the microcosm with the CPO produced organochlorines was found to be slightly higher, than that of the microcosm without it, **Figure 11**. Something else that should be mentioned about this result in particular is that the organisms grew in concentration more and more rapidly at progressively higher temperatures, which in itself makes logical sense as microorganisms grow more rapidly at higher temperatures because their metabolisms work faster at higher temperatures. *Desulfovibrio spp*. too has a similar trend where the most enriched microcosm was the one without the CPO produced organochlorines at 45°C, **Figure 12**.

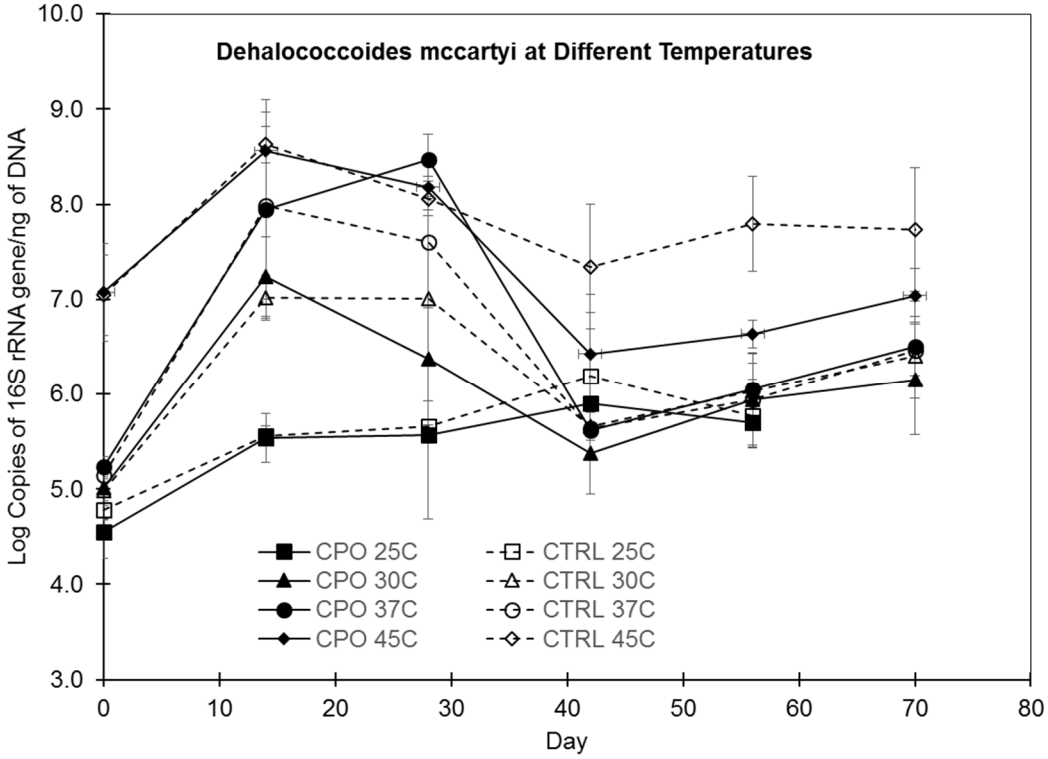

Figure 9. The concentration of *Dehalococcoides mccartyi* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines at different temperatures: CPO 25°C (■), CPO 30°C (▲), CPO 37°C (●), and CPO 45°C (♦) with their respective non-chlorinated controls: CTRL 25°C (□), CTRL 30°C (Δ), CTRL 37°C (○), and CTRL 45°C (◊).

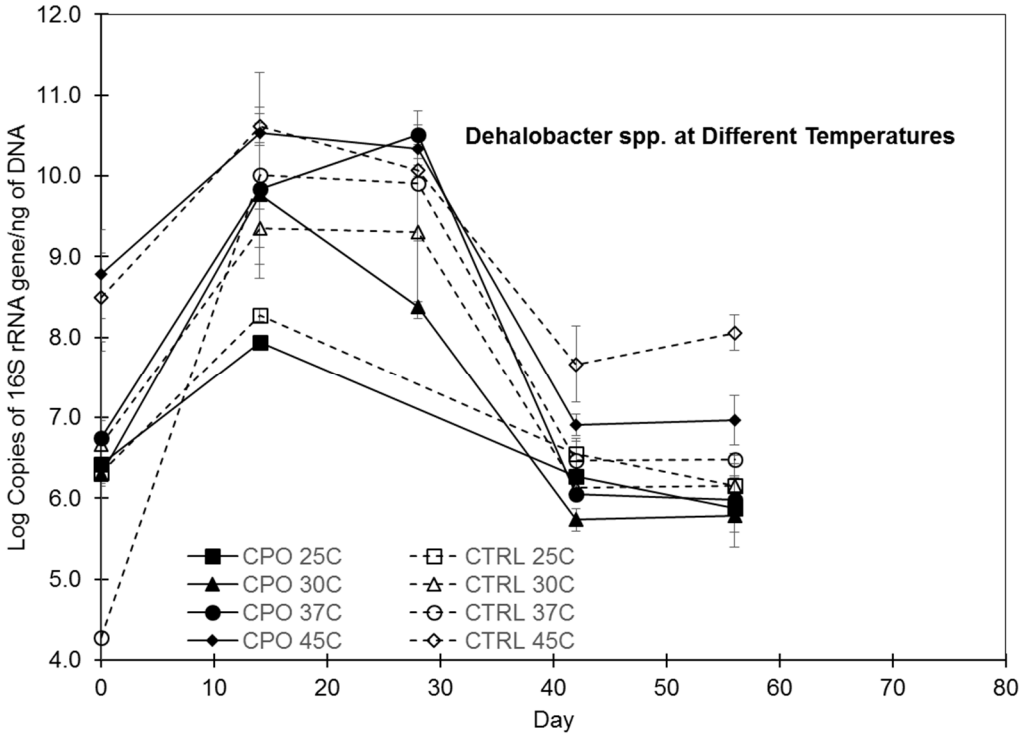

Figure 10. The concentration of *Dehalobacter spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines at different temperatures: CPO 25°C (■), CPO 30°C (▲), CPO 37°C (●), and CPO 45°C (♦) with their respective non-chlorinated controls: CTRL 25°C (□), CTRL 30°C (Δ), CTRL 37°C (○), and CTRL 45°C (◊).

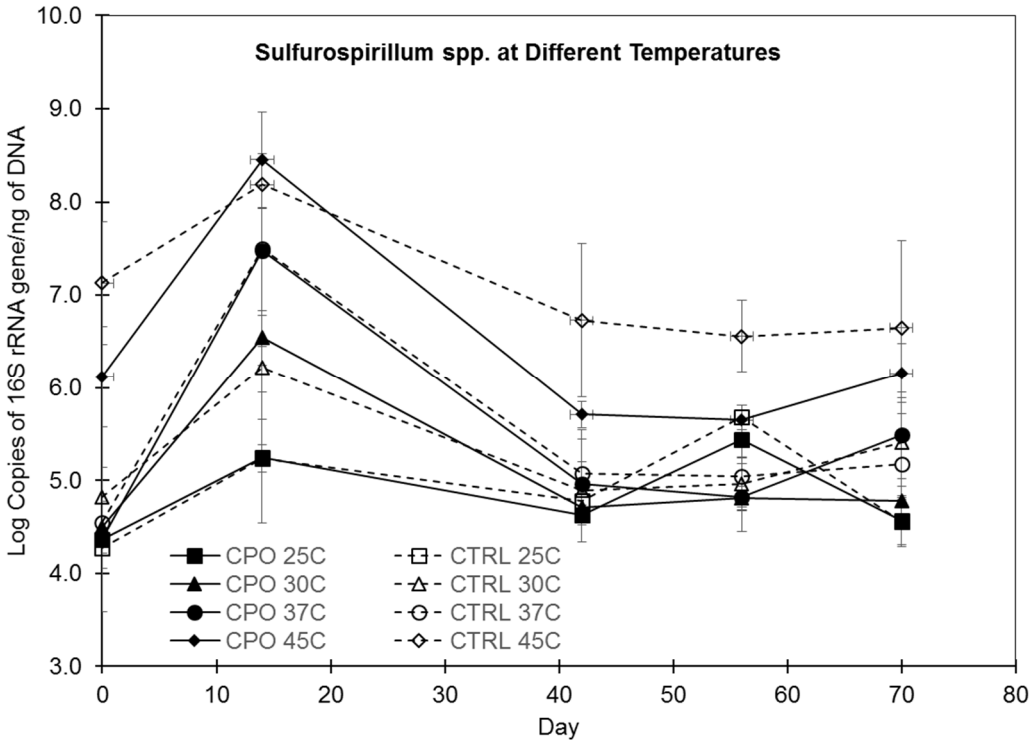

Figure 11. The concentration of *Sulfurospirillum spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines at different temperatures: CPO 25°C (■), CPO 30°C (▲), CPO 37°C (●), and CPO 45°C (♦) with their respective non-chlorinated controls: CTRL 25°C (□), CTRL 30°C (Δ), CTRL 37°C (○), and CTRL 45°C (◊).

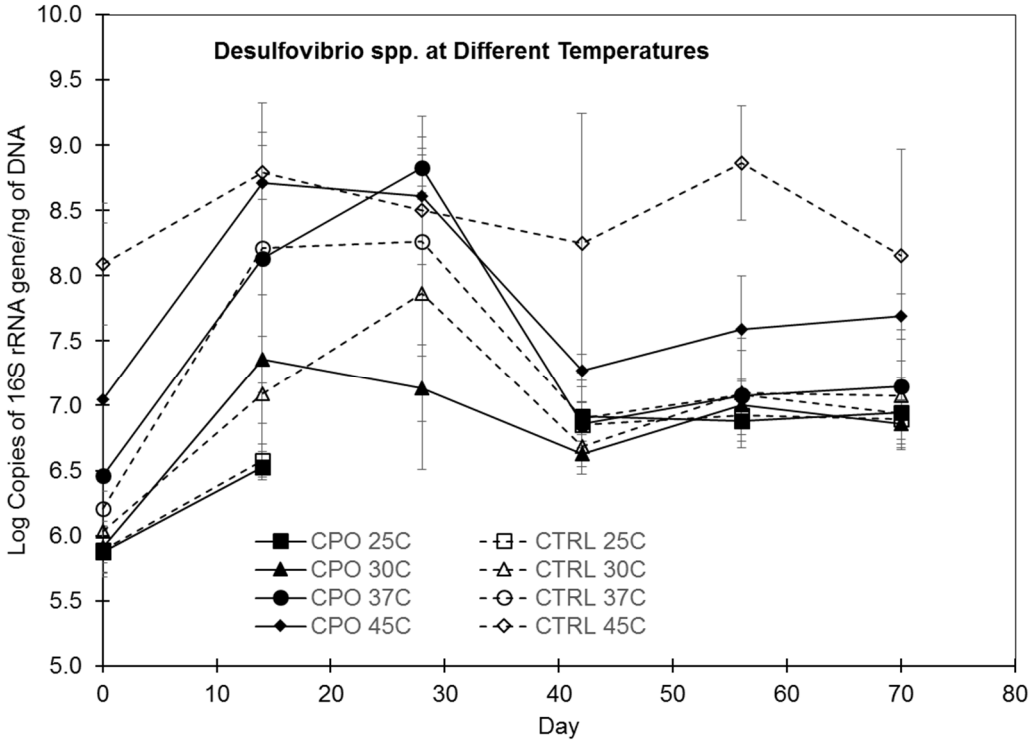

Figure 12. The concentration of *Desulfovibrio spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines at different temperatures: CPO 25°C (■), CPO 30°C (▲), CPO 37°C (●), and CPO 45°C (♦) with their respective non-chlorinated controls: CTRL 25°C (□), CTRL 30°C (Δ), CTRL 37°C (○), and CTRL 45°C (◊).

4.1.4 Growth of Organohalide Respirers using Oak and Pine Based Organochlorines

When the organochlorines were enzymatically produced, both organic matter from under oak trees and from under pine trees was used. This difference in organic matter used for organochlorines was then tested in the controls (low salinity, acetate, and 25°C), the highest salinity parameter, and the highest temperature parameter. The growth of *Dehalococcoides mccartyi* at standard conditions was observed and shown below in **Figure 13**. It grew slowly over the 56 days, but by Day 70, it had declined slightly. The growth of *Dehalobacter spp*. at normal conditions can be seen in **Figure 14**. The concentration of the *Dehalobacter spp*. increased after Day 0 and basically stayed around Log 8.0 of 16S rRNA genes per ng of DNA until Day 42 where it decreased until Day 70. *Sulfurospirillum spp*. had a more erratic growth pattern than that of *Dehalococcoides mccartyi* or *Dehalobacter spp*. when grown at control conditions, **Figure 15**. However, for both oak and pine based organochlorines, the reactors without the enzymatically produced organochlorines had a slightly higher, growth. Lastly, *Desulfovibrio spp*. steadily increased in concentration until Day 56 then decreased, **Figure 16**.

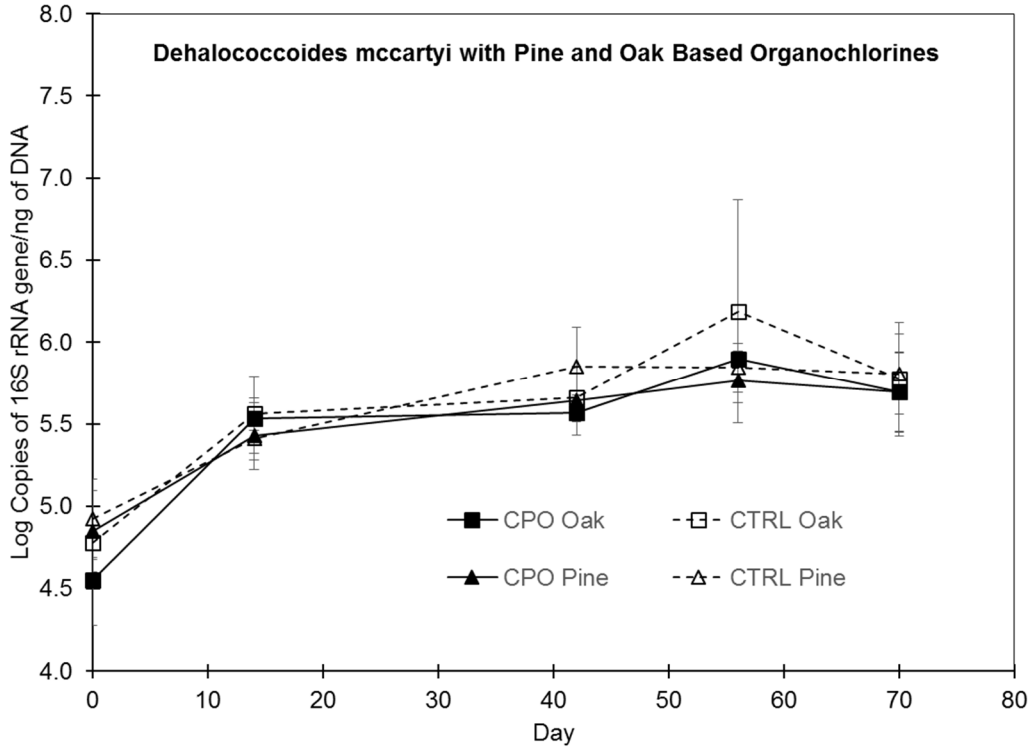

Figure 13. The concentration of *Dehalococcoides mccartyi* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines produced from different organic matter: CPO Oak (■) and CPO Pine (▲) with their respective non-chlorinated controls: CTRL Oak (□) and CTRL Pine (Δ).

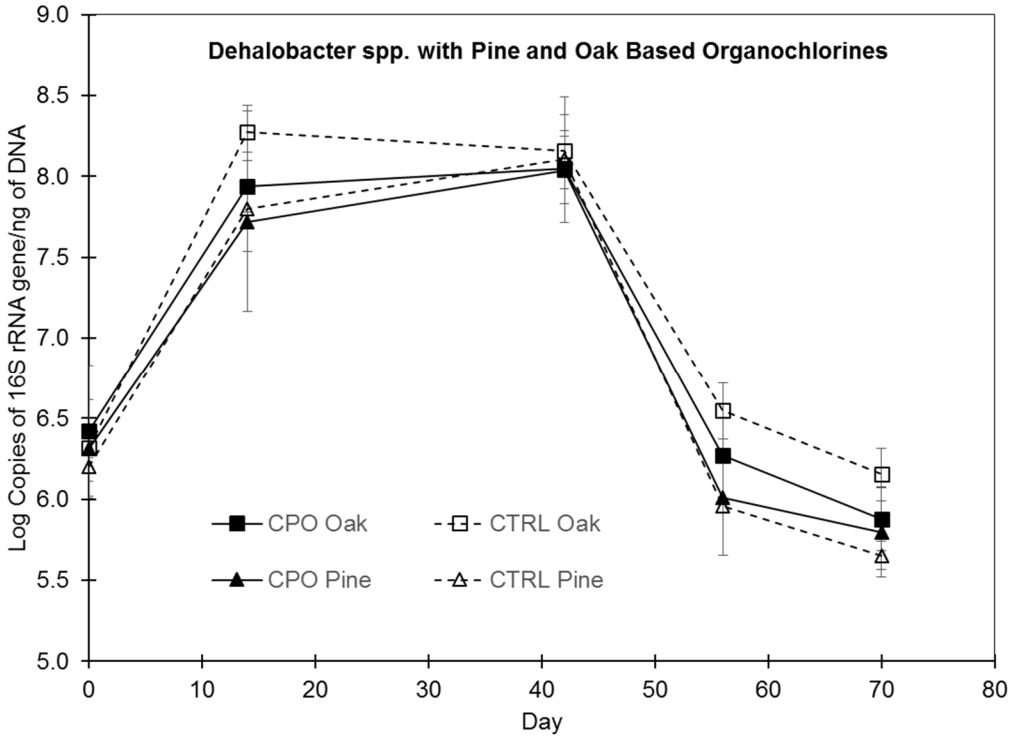

Figure 14. The concentration of *Dehalobacter spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines produced from different organic matter: CPO Oak (■) and CPO Pine (▲) with their respective non-chlorinated controls: CTRL Oak (□) and CTRL Pine (Δ).

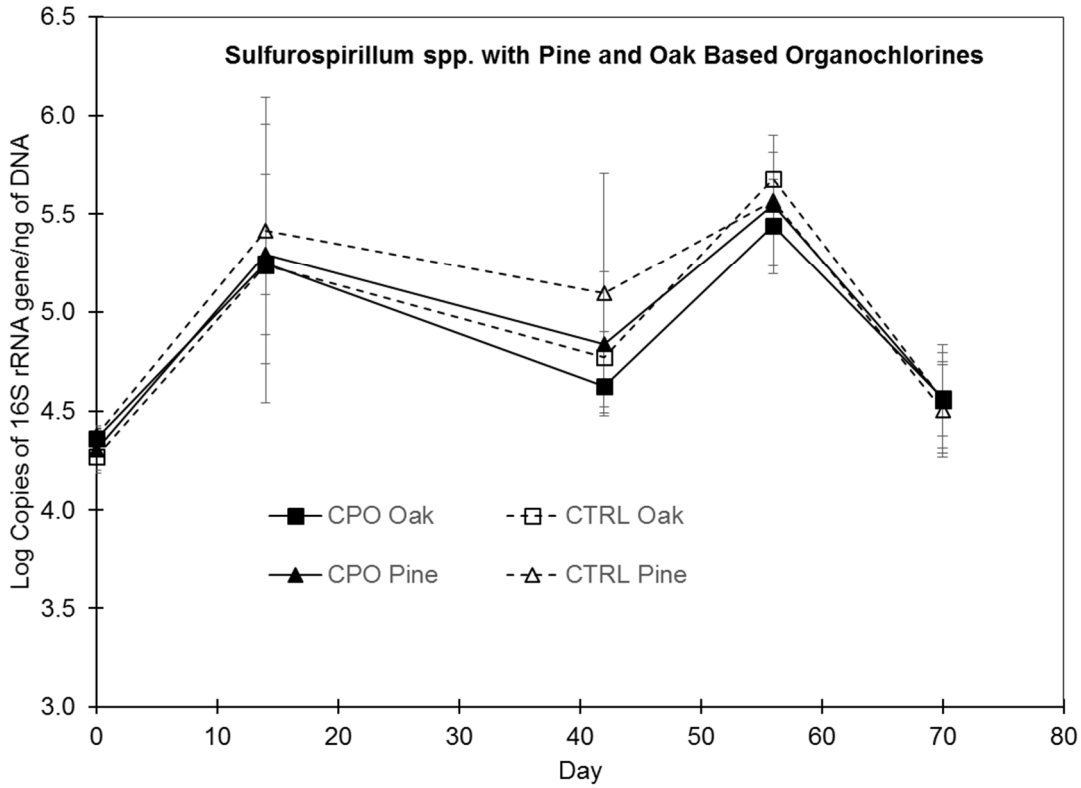

Figure 15. The concentration of *Sulfurospirillum spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines produced from different organic matter: CPO Oak (■) and CPO Pine (▲) with their respective non-chlorinated controls: CTRL Oak (□) and CTRL Pine (Δ).

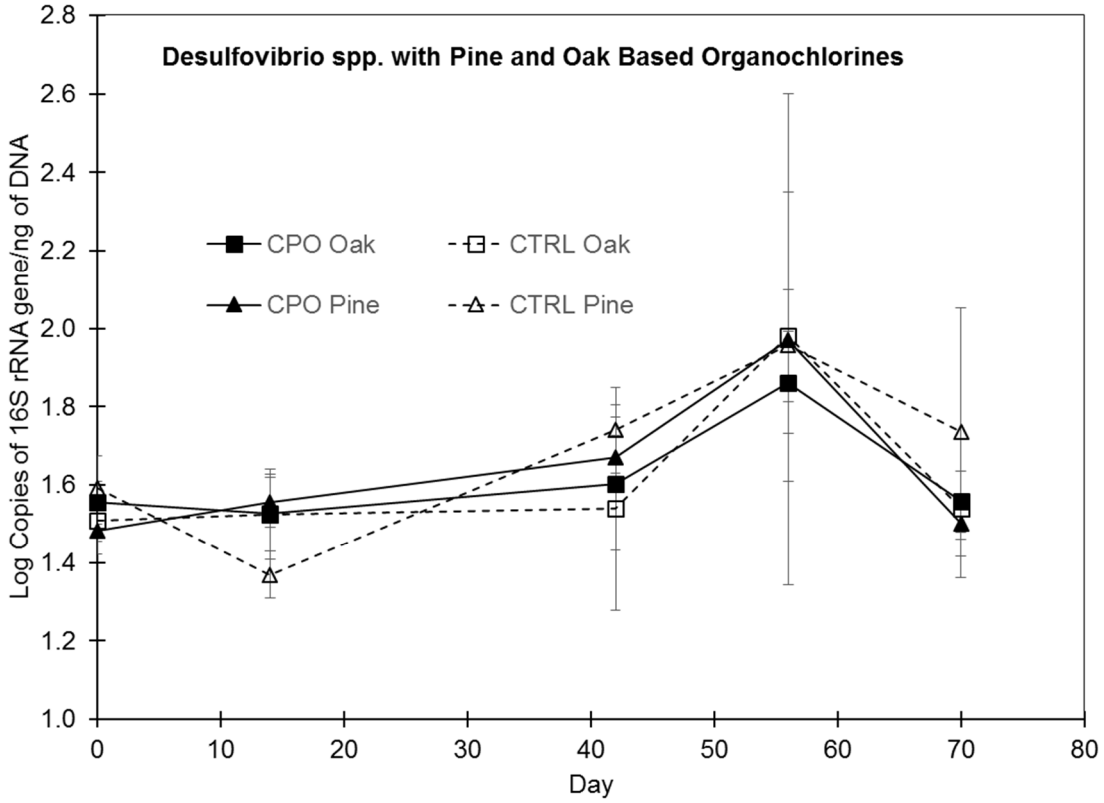

Figure 16. The concentration of *Desulfovibrio spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines produced from different organic matter: CPO Oak (■) and CPO Pine (▲) with their respective non-chlorinated controls: CTRL Oak (□) and CTRL Pine (Δ).

4.1.5 Growth of Organohalide Respirers using Oak and Pine Based Organochlorines at High Salinity

Microcosms were made with a "High" salinity content, an additional 1000 mg of NaCl, with organochlorines produced from organic matter from under oak trees and from under pine trees. The concentration of *Dehalococcoides mccartyi* 16S rRNA genes in these microcosms basically stay around Log 6.0 copies of 16S rRNA genes per ng of DNA until Day 56 where the pine based organochlorines increase and the oak based organochlorines decrease, **Figure 17**. *Dehalobacter spp*. rises until Day 14 then stays basically the same concentration until Day 42 where it drops to around Log 6 copies of 16S rRNA per ng of DNA, **Figure 18**. *Sulfuropirillum spp*. has a similar trend to when it was grown at low salinity, but with a higher overall concentration at the beginning of the experiment, than at the end, though it is still slightly irratic, **Figure 19**. *Desulfovibrio spp*. decreases in concentration dramatically until Day 14 where it then slowly rises until Day 56, **Figure 20**.

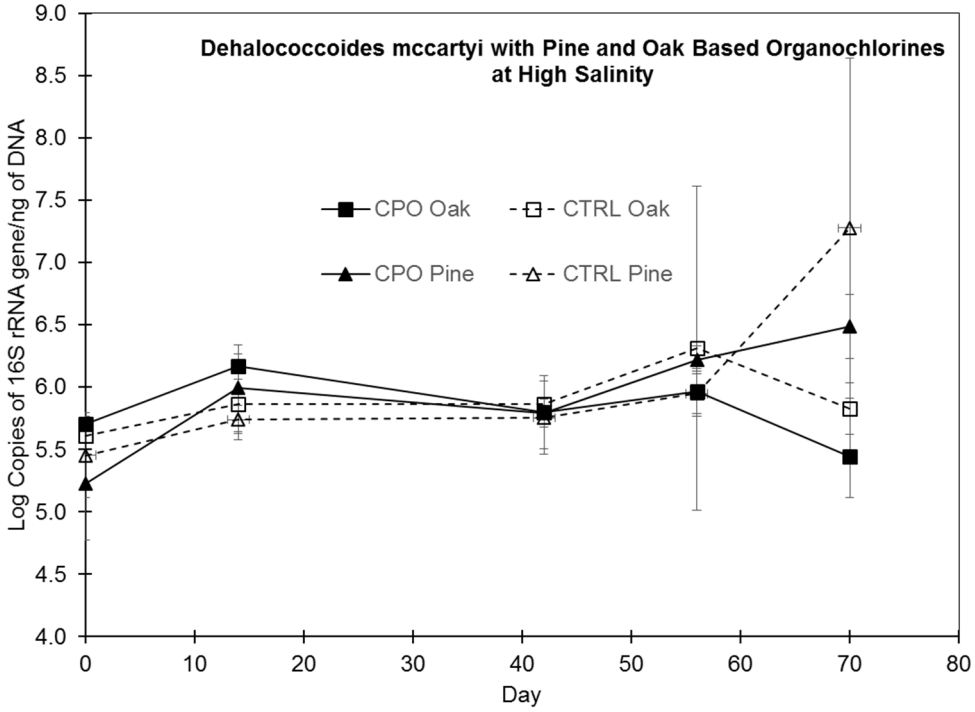

Figure 17. The concentration of *Dehalococcoides mccartyi* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines produced from different organic matter at high salinity: CPO Oak (■) and CPO Pine (▲) with their respective non-chlorinated controls: CTRL Oak (□) and CTRL Pine (Δ).

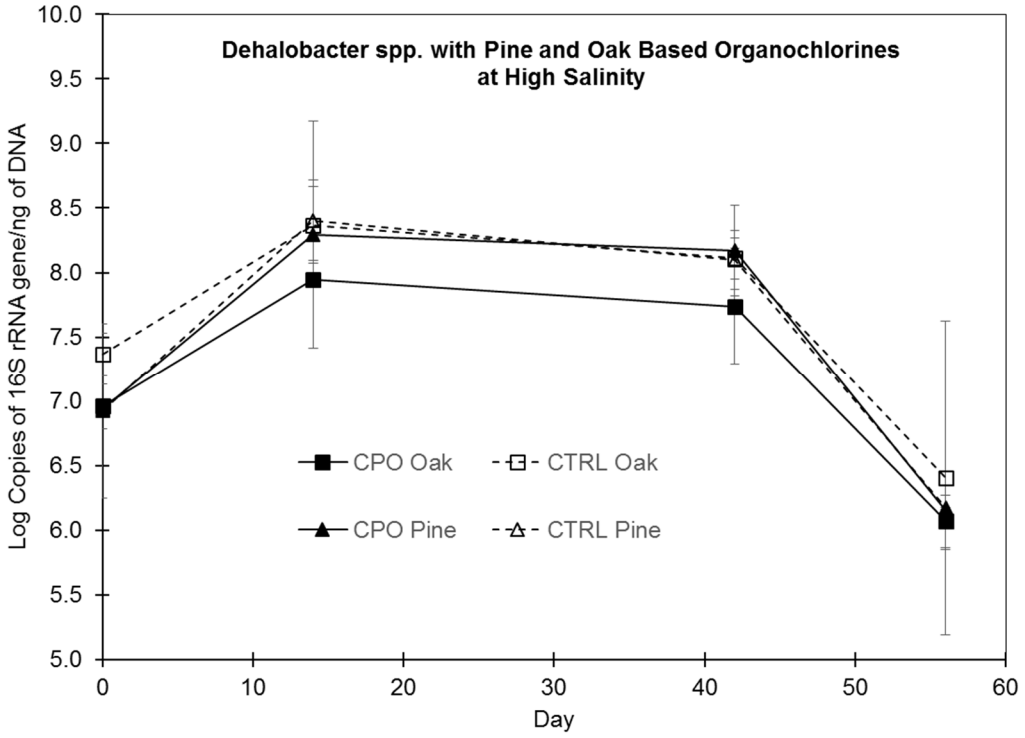

Figure 18. The concentration of *Dehalobater spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines produced from different organic matter at high salinity: CPO Oak (■) and CPO Pine (▲) with their respective non-chlorinated controls: CTRL Oak (□) and CTRL Pine (Δ).

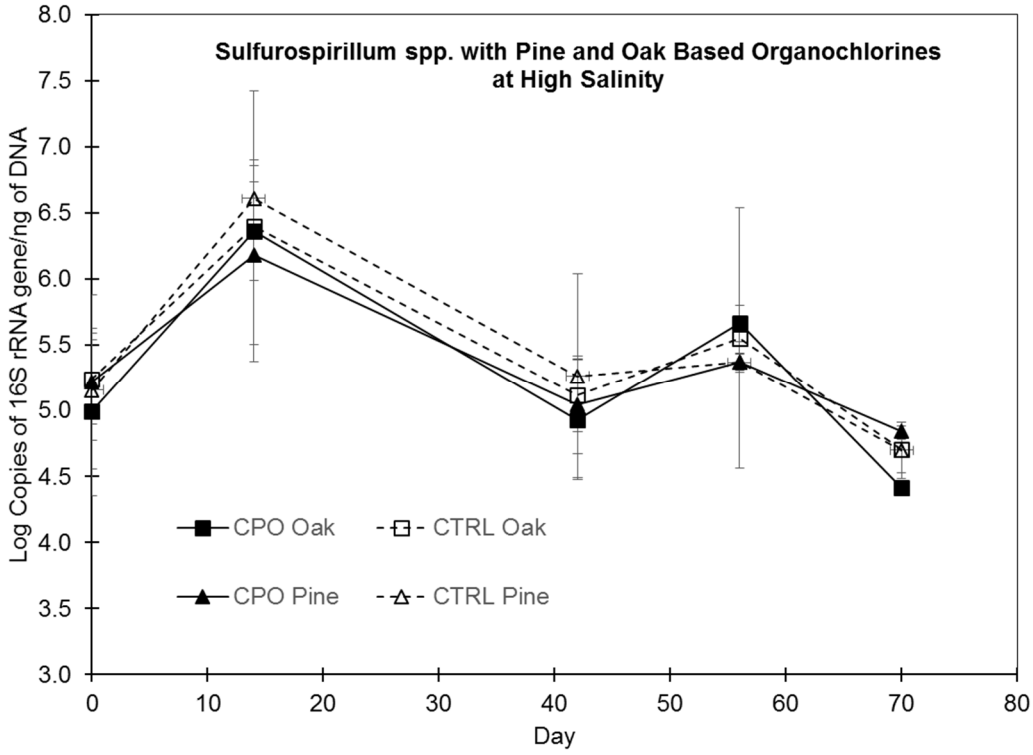

Figure 19. The concentration of *Sulfurospirillum spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines produced from different organic matter at high salinity: CPO Oak (■) and CPO Pine (▲) with their respective non-chlorinated controls: CTRL Oak (□) and CTRL Pine (Δ).

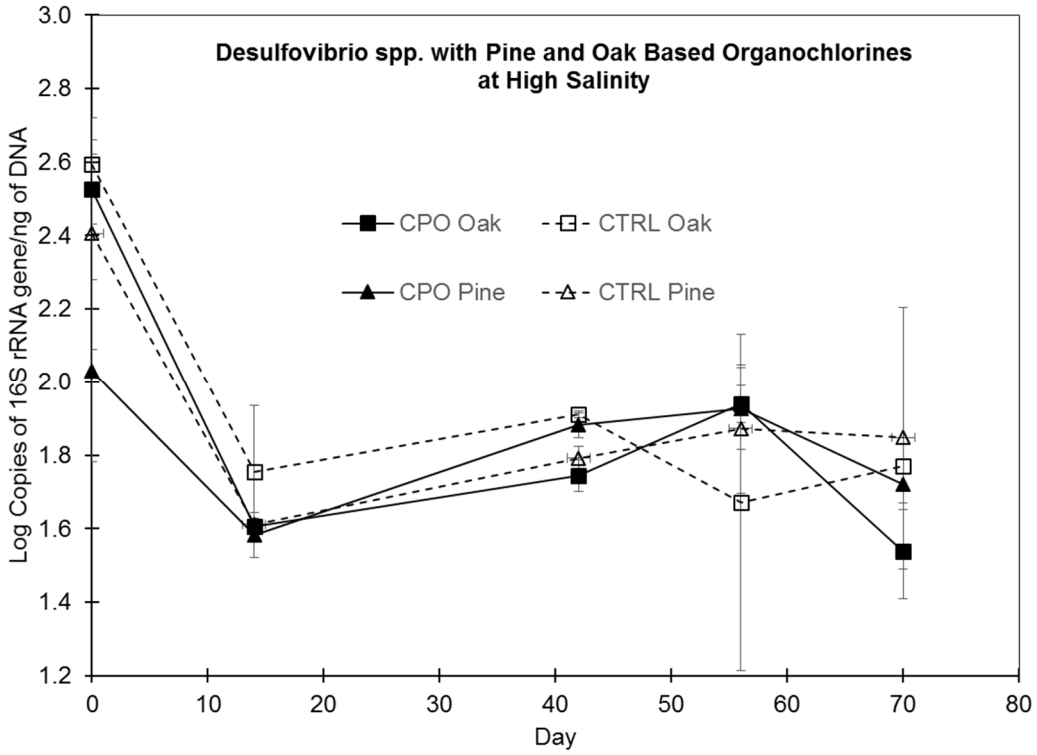

Figure 20. The concentration of *Desulfovibrio spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines produced from different organic matter at high salinity: CPO Oak (■) and CPO Pine (▲) with their respective non-chlorinated controls: CTRL Oak (□) and CTRL Pine (Δ).

4.1.6 Growth of Organohalide Respirers using Oak and Pine Based Organochlorines at High Temperature, 45°C

The last set of comparisons were among microcosms that were kept at 45°C and had either CPO produced organochlorines made from organic matter obtained from soil under oak trees or made from organic matter obtained from soil under pine trees. The *Dehalococcoides mccartyi*, as seen in **Figure 21**, grew in concentration until Day 14, then dropped until Day 42 where it slowly rose again. The concentration of 16S rRNA genes in microcosms without the CPO produced organochlorines fell more rapidly than those with them. *Dehalobacter spp*. grew until Day 14 then stayed at that concentration of Log 11 copies of 16S rRNA genes per ng of DNA until Day 28 where it fell, the bacteria concentration in the CPO produced microcosms more quickly again, **Figure 22**. *Sulfurospirillum spp*. also grew quickly in concentration from Day 0 to Day 14, then decreased until Day 56 where it grew again until Day 70. The concentrations in the CPO produced organochlorine microcosms fell more quickly again, **Figure 23**. Lastly, *Desulfovibrio spp*. had the greatest difference between the microcosms with CPO produced organochlorines added and the microcosms without. They all grew until Day 14, then decreased steadily, the organochlorine amended microcosms more quickly, however on Day 42, the organochlorine amended microcosms started to increase in concentration again, whereas the ones without did not, **Figure 24**.

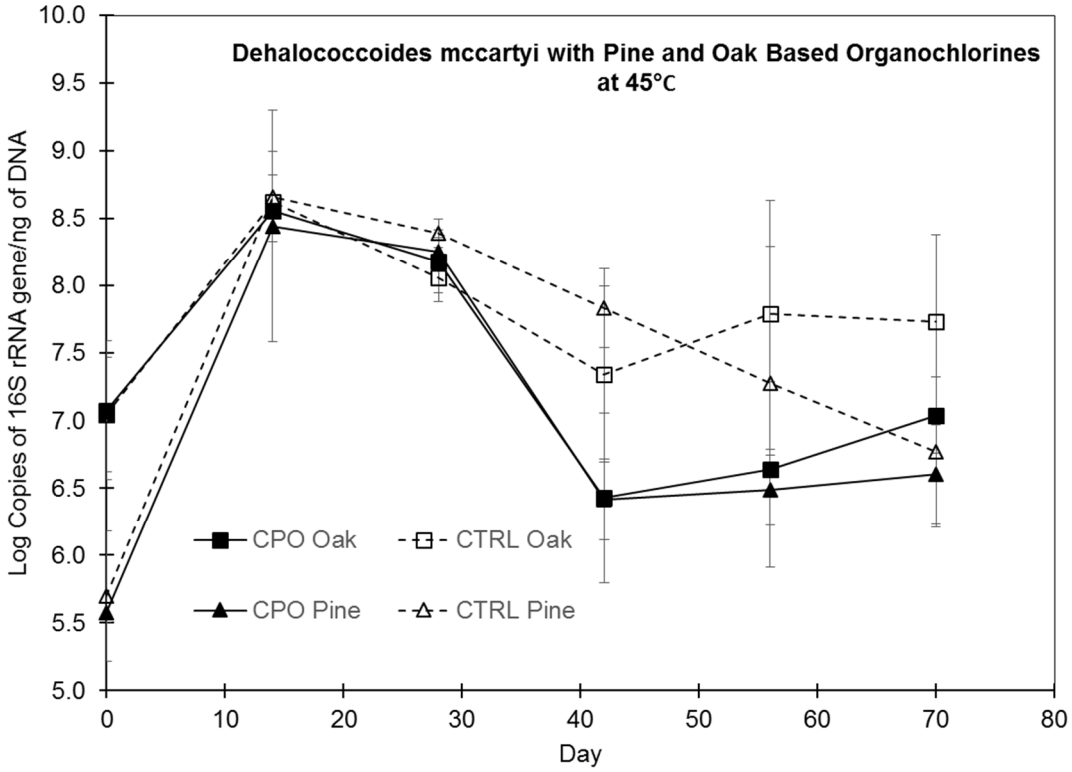

Figure 21. The concentration of *Dehalococcoides mccartyi* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines produced from different organic matter at 45°C: CPO Oak (■) and CPO Pine (▲) with their respective non-chlorinated controls: CTRL Oak (□) and CTRL Pine (Δ).

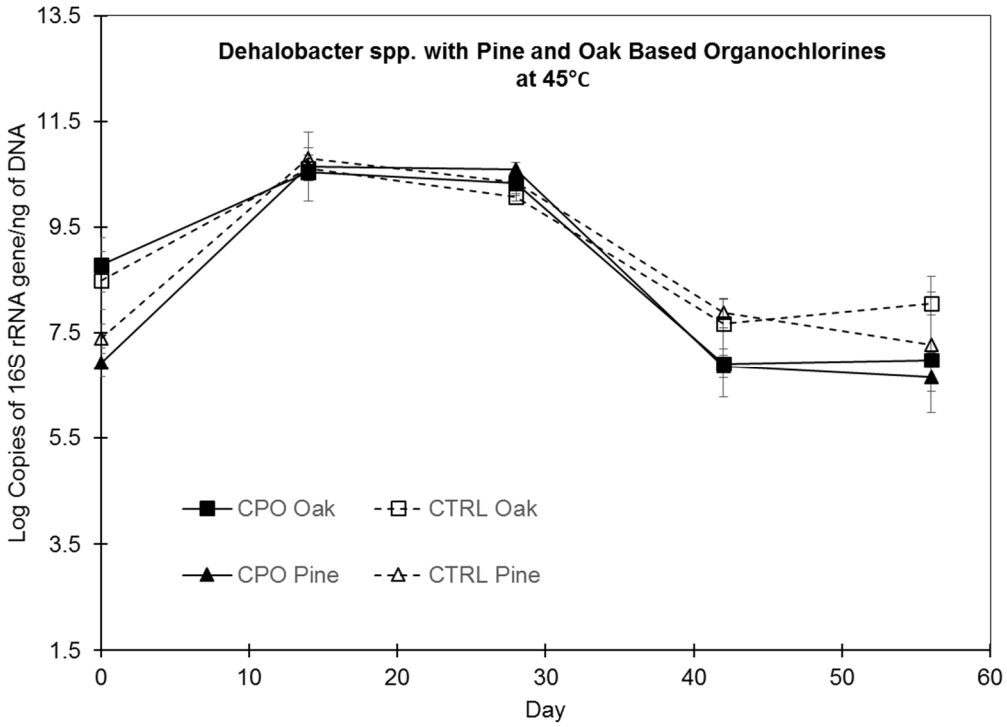

Figure 22. The concentration of *Dehalobacter spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines produced from different organic matter at 45°C: CPO Oak (■) and CPO Pine (▲) with their respective non-chlorinated controls: CTRL Oak (□) and CTRL Pine (Δ).

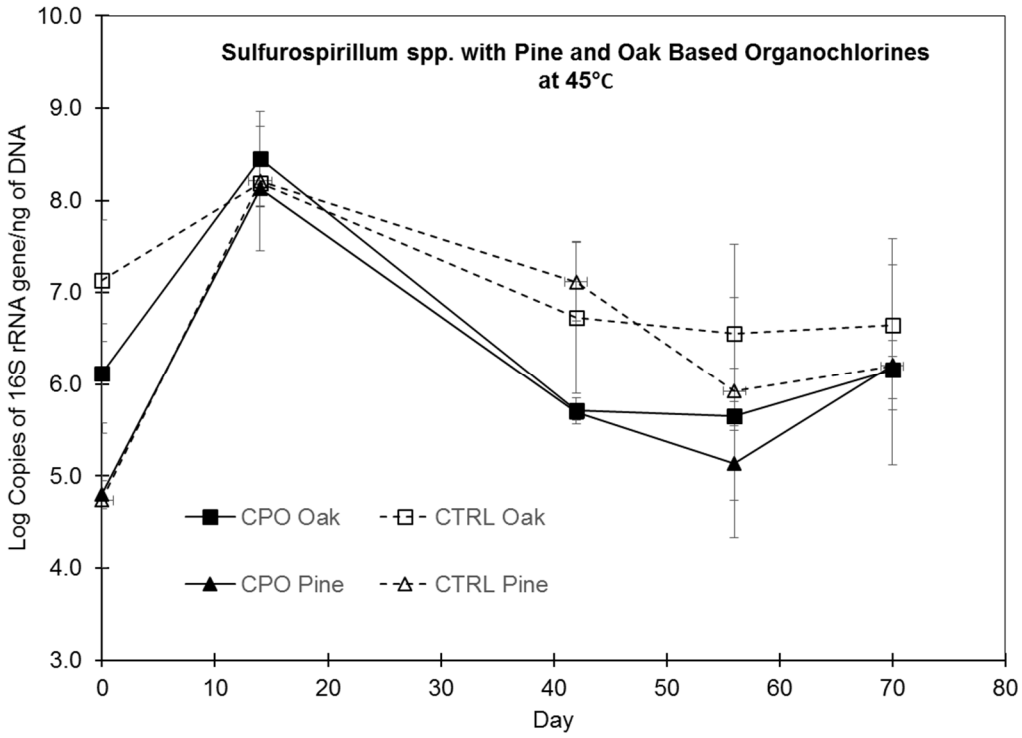

Figure 23. The concentration of *Sulfurospirillum spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines produced from different organic matter at 45°C: CPO Oak (■) and CPO Pine (▲) with their respective non-chlorinated controls: CTRL Oak (□) and CTRL Pine (Δ).

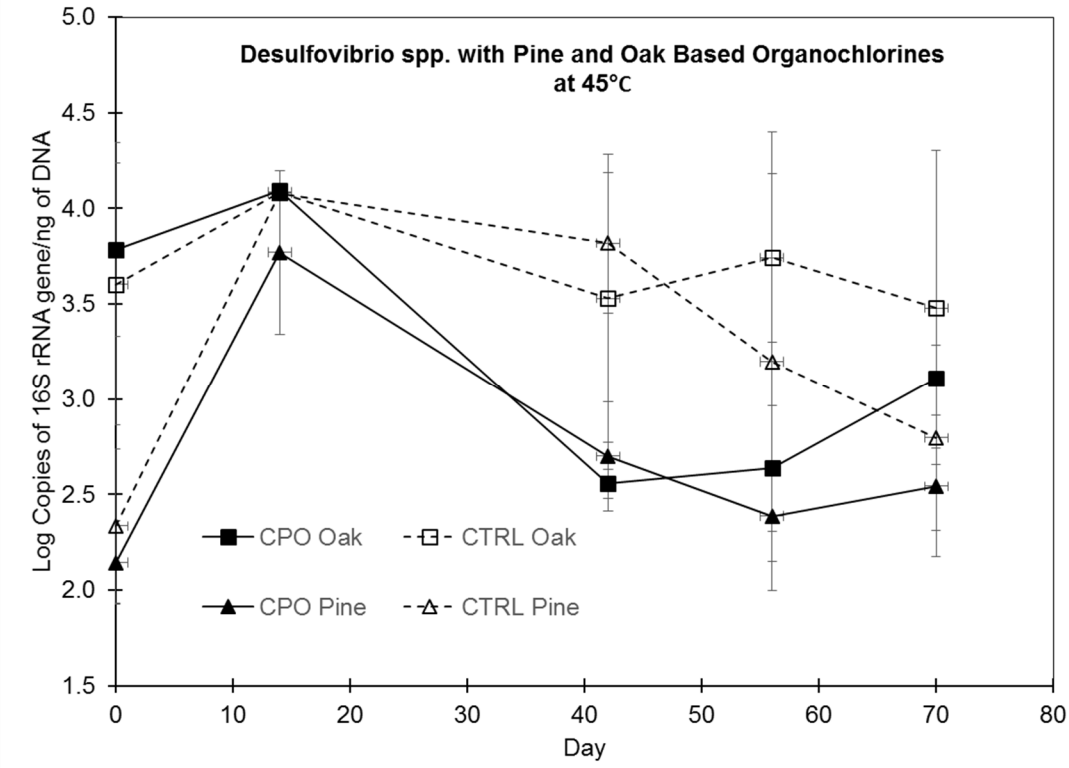

Figure 24. The concentration of *Desulfovibrio spp.* in Log Copies of 16S rRNA genes/ng of DNA in microcosms amended with CPO produced organochlorines produced from different organic matter at 45°C: CPO Oak (■) and CPO Pine (▲) with their respective non-chlorinated controls: CTRL Oak (□) and CTRL Pine (Δ).

4.2 ARISA Analysis

The results from the ARISA analysis were able to show the presence of unique OTUs at different conditions. This will help make clear what differences there are to the communities on a broad scale instead of looking at specific organisms. When looking at this data, the results that show something unique to a reactor that contained CPO produced organochlorines can be speculated to be because something was enriched in these reactors due to the addition of the CPO produced organochlorines. Once this idea is observed, the data in **Table 5** below becomes relevant. It shows a summary of what OTUs were present and at what conditions for OTUs found uniquely in the reactors containing CPO produced organochlorines. There is a large set at an OTU of 301.5 where the carbon source given or temperature it was kept at doesn't seem to have any effect on its growth, but salinity does. There is another large set at an OTU of 573.5 where the salinity and carbon source given has no effect on its growth, but temperature does. Excluding the OTUs mentioned before, the saline reactors had some unique OTUs at 227, 437.5, 468.5, 563, and 577. The OTUs that were found to only grow on a specific carbon source are 350.5, 307, 468.5, 522, 578, and 595.5. The OTUs that grew at 45°C seemed to be the most unique of all the temperatures. There were a fair amount that were present at 45°C and not at other temperatures, such as: 220, 222, 287, 440, 470, 485, and 498. This could potentially mean that there is a whole group of bacteria that is enriched from CPO produced organochlorides at higher temperatures. Lastly, the organochlorines produced from oak and pine organic matter showed some differences too. At the control conditions, the OTUs grown on organochlorines based on oak organic matter and not on

the organochlorines based on pine organic matter were 202.5 and 540. However, at 45°C, the reactors with pine based organochlorines had OTUs at 222, 287, 470, and 498. And the oak based organochlorines had OTUs at 220 and 485.

Table 5: Presence of OTUs unique to reactors with CPO produced organochlorines

OTU	202.5	220	222	227	287	301.5	307	350.5	399	437.5	440	468.5	470	485	498	522	540	563	573.5	577	578	595.5
Oak 'Normal'	×	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	×	-	×	-	-	~
Pine 'Normal'	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	×	-	-	×
Oak Medium Salt	-	-	-	-	-	-	-	-	-	×	-	×	-	-	-	-	-	-	×	-	-	-
Oak High Salt	-	-	-	×	-	-	-	-	-	-	-	×	-	-	-	-	-	+	×	+	-	-
Pine High Salt	-	-	-	×	-	-	-	-	-	-	-	~	-	-	-	-	-	+	×	+	-	-
Oak Methanol	-	-	-	-	-	×	-	×	-	-	-	~	-	-	-	-	-	-	+	-	-	×
Oak Butyrate	-	-	-	-	-	+	×	-	-	-	-	-	-	-	-	~	-	-	×	-	×	×
Oak Bicarbonate	-	-	-	-	-	+	×	-	-	-	-	-	-	-	-	+	-	-	+	-	~	-
Oak 30	×	-	-	-	-	-	-	-	-	-	-	×	-	-	-	-	-	-	-	-	-	-
Oak 37	-	-	-	-	-	+	×	-	×	-	-	×	-	-	-	-	-	-	-	-	-	-
Oak 45	-	×	~	-	-	+	-	-	-	-	×	~	~	×	+	-	-	-	-	-	-	-
Pine 45	-	-	+	-	×	+	-	-	-	-	×	~	×	-	×	-	-	-	-	-	-	-

× Present only in CPO

+ Present in both CPO and CTRL, but abundance in CPO greater than CTRL ($P<0.05$)

- Not present in either

~ Present in both CPO and Control at similar levels

Bold symbols signifies OTU reaches over 3% of area in duplicate samples

CHAPTER V

CONCLUSION

Chlorinated compounds such as PCE are present in many waterways and are toxic to both organisms in the environment and to humans. Remediation of such contaminated sites is performed mostly by microorganisms. Naturally occurring organochlorines are found everywhere and can be dechlorinated by a group of microorganisms called organohalide respiring bacteria. These bacteria have been studied in the past, but most research is conducted by changing the electron donor, the chlorinated compound, of the bacteria and growing it in anaerobic conditions promoting the dechlorination of the pollutant. This study was to observe what could possibly grow in different conditions that would dechlorinate. This is important because these techniques could be used to grow a quickly grown and highly dense amount of dechlorinating bacteria efficiently that can then be bioaugmented to contaminated sites to remove the toxic compounds.

The qPCR results showed trends for specific organisms. For example, when looking at the different carbon sources, the organisms tended to like methanol over the other carbon sources particularly around Day 14, near the beginning of the experiment. Organisms tended to like the higher temperatures as well, specifically at 45°C. The temperatures seemed to effect the organism's growth more than the other parameters.

The ARISA data shows a much broader sense of what is happening in the microcosms. There are unique OTUs that grew only or mostly in the microcosms with the CPO produced

organochlorines. This can be speculated to mean that they were enriched by the addition of the CPO produced organochlorines. In this particular group that was enriched with the organochlorines, there were unique OTUs found where the carbon source or salinity had no effect on their growth, but any temperature above room temperature they didn't grow. There were also OTUs where the temperature and carbon source didn't have any effect, but if there was a higher salinity than was provided by the mineral media solution, they didn't grow. Some OTUs were only present for microcosms grown with a specific carbon source. There is a fair amount of OTUs present that are unique to the microcosms that were grown at 45°C. This could potentially mean that there's a group of bacteria that could potentially dechlorinate only at higher temperatures. There is also some differences between the CPO produced organochlorines that were made using either organic matter from under oak trees or pine trees. This shows that this difference in the organochlorines has an effect on what can be enriched off of it.

Looking at overall conclusions however, methanol seems to be a better carbon source to use when trying to enrich organohalide respiring bacteria quickly and the carbon source that seemed to enrich the bacteria the least was acetate. Also, the temperature of 30°C seems to be the best temperature in which to enrich the bacteria as well, shown both by the results here and by the studies observed. While the qPCR doesn't show much difference between the CPO produced organochlorine amended microcosms and the non-chlorinated controls, **Table 5** from the ARISA results were made strictly from microcosms that had an enrichment in the CPO produced organochlorine amended microcosms. This shows that there was, in fact, an effect from the amendment of organochlorines compared to the non-chlorinated controls.

Additional research for this project would include additional qPCR runs for the remaining bacteria to see if other types of organohalide respiring bacteria had specific effects from the different physiological changes. Also, additional research for this experiment could also include sending the samples that produced unique OTUs for Illumina sequencing for identification of what these particular OTUs are.

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APPENDICES

Appendix A: ARISA Sample Data

Size	Oak CPO Day 0
195	0.789
225	0.735
273	0.850
274	1.163
335	0.802
343	1.103
402	0.544
420	0.994
432	2.546
433	0.518
438	0.679
531	2.660
565	0.511
571	0.556
594	0.707
595	1.422
597	2.319
599	33.369
600	39.939
603	1.731
617	2.284
623	0.726
629	0.634
637	1.130
749	1.289

Size	Oak CPO B Day 0
195	0.919
204	0.558
225	1.142
273	1.138
274	1.856
335	1.036
343	1.554
389	0.520
402	0.712
420	0.817
432	1.923
438	0.933
521	0.542
564	0.602
565	0.755
571	1.464
589	0.920
594	2.966
597	1.565
599	33.587
600	41.251
603	0.518
617	0.772
637	0.655
749	1.297

Size	Oak CPO C Day 0
81	0.788
82	0.889
87	1.107
100	0.563
108	1.405
110	1.123
121	0.773
273	0.611
274	1.971
335	1.034
343	1.737
389	0.562
402	0.807
417	0.721
420	1.173
425	0.706
429	0.803
432	3.692
433	0.927
438	0.772
565	0.634
571	0.751
597	3.311
599	29.563
600	37.415
608	0.665
610	1.178
617	1.480
623	1.303
637	0.804
749	0.731

Size	Oak CTRL A Day 0
195	0.870
204	0.533
225	1.001
273	1.150
274	1.914
327	0.510
335	1.104
343	1.551
389	0.539
402	0.697
420	1.069
432	2.790
433	0.585
438	0.821
500	0.504
564	0.512
565	0.676
571	1.404
594	0.665
597	1.833
599	34.323
600	40.924
603	0.511
610	0.563
623	0.852
637	0.939
749	1.160

Size	Oak CTRL B Day 0
82	0.596
86	0.513
87	0.874
108	1.067
110	0.961
115	0.558
121	0.965
274	1.000
343	0.843
402	0.506
432	1.457
438	0.541
562	20.194
564	2.083
567	1.632
571	0.844
595	1.721
597	0.785
599	29.384
600	33.478

Size	Oak CTRL C Day 0
81	0.818
82	0.984
87	1.248
100	0.523
108	1.674
110	1.373
121	0.983
195	0.547
225	0.542
273	0.713
274	2.245
335	1.096
343	1.947
389	0.670
402	0.980
420	1.392
429	0.502
432	3.367
438	0.801
565	0.714
571	1.073
594	2.015
595	0.644
597	1.643
599	28.768
600	35.806
603	0.599
607	1.044
610	0.718
617	1.034
622	0.662
623	0.851
637	1.244
749	0.777

Size	Oak CPO Day 14
385	0.725
420	1.764
425	0.560
429	0.649
432	4.271
433	0.989
531	3.666
586	0.682
594	1.956
595	3.149
597	3.540
599	27.141
600	35.577
603	3.113
610	0.777
617	0.968
620	0.652
622	1.065
623	1.361
637	2.365
665	0.666
749	2.011
773	0.599
819	0.549

Size	Oak CPO B Day 14
121	0.707
195	0.526
225	0.771
274	0.955
355	1.039
414	0.544
420	2.151
423	1.017
429	0.772
432	4.802
433	1.089
437	0.632
529	0.533
531	0.663
571	0.538
586	0.958
594	1.389
595	1.306
597	4.210
599	27.882
600	37.831
603	0.705
610	1.074
622	1.115
623	1.525
637	2.126
661	0.504
665	0.709
749	1.929

Size	Oak CPO C Day 14
121	0.646
225	0.669
274	0.830
286	0.830
417	1.179
419	0.726
420	1.850
425	1.217
429	1.524
430	0.883
432	7.104
433	2.057
595	0.881
597	8.449
599	23.827
600	31.357
603	0.884
607	0.641
610	2.105
617	1.516
620	1.330
622	0.673
623	3.054
637	1.798
665	0.996
749	2.050
773	0.924

Size	Oak CTRL Day 14
121	0.731
195	0.573
225	0.711
274	0.907
320	2.762
343	0.605
420	1.594
432	3.203
433	0.633
469	6.359
571	0.585
586	0.874
594	7.610
597	2.430
599	25.051
600	36.918
603	0.608
610	0.508
622	0.792
623	0.825
627	2.389
637	1.630
749	1.702

Size	Oak CTRL B Day 14
121	0.651
225	0.600
274	0.760
296	1.168
420	1.130
429	0.537
432	2.878
562	29.482
564	1.999
567	1.659
586	0.949
594	4.173
597	1.820
599	19.402
600	27.532
603	0.844
610	0.540
622	0.556
623	0.829
637	0.970
665	0.723
749	0.798

Size	Oak CTRL C Day 14
274	0.622
290	0.598
296	1.617
320	4.107
385	0.649
410	0.549
420	2.627
425	0.513
429	0.823
432	6.785
469	9.704
562	0.595
586	0.648
594	8.796
595	0.979
597	3.693
599	18.953
600	24.697
603	0.810
610	1.009
617	0.663
622	1.515
623	1.217
627	3.616
637	2.828
749	1.386

Size	Oak CPO Day 42
51	0.552
56	0.712
61	0.684
65	0.633
87	0.600
93	0.814
189	10.523
195	1.004
203	0.504
204	0.908
225	0.718
265	0.602
273	0.622
274	1.714
335	0.858
343	1.016
402	0.598
419	0.524
432	0.830
471	0.815
565	0.664
599	34.979
600	36.716

Size	Oak CPO B Day 42
51	0.645
61	0.557
195	0.812
204	0.874
225	0.891
256	0.747
273	0.721
274	2.850
335	1.093
343	1.434
364	0.527
402	0.765
432	0.632
565	0.887
571	1.091
595	0.607
599	39.544
600	45.321

Size	Oak CPO C Day 42
51	0.577
61	0.591
87	0.584
195	0.609
204	0.754
225	0.731
226	0.632
273	0.605
274	2.411
335	1.005
343	1.187
385	0.604
402	0.634
420	0.506
432	1.339
565	0.751
571	0.872
597	0.623
599	41.250
600	43.735

Size	Oak CTRL Day 42
51	0.523
61	0.577
87	0.538
189	0.969
195	0.694
204	0.841
225	0.798
265	0.709
273	0.591
274	2.530
335	1.111
343	1.295
385	0.621
402	0.727
420	0.714
432	1.176
565	0.789
571	1.073
591	0.575
594	0.549
599	40.143
600	42.456

Size	Oak CTRL B Day 42
51	0.569
189	0.524
204	0.593
225	0.604
226	0.636
274	2.330
335	1.040
343	1.211
364	0.501
385	0.671
402	0.598
420	0.544
432	1.170
562	2.755
564	0.790
565	0.676
567	0.530
571	0.839
591	1.116
594	1.266
599	37.932
600	43.107

Size	Oak CTRL C Day 42
51	0.558
56	0.609
61	0.588
87	0.633
110	0.571
195	0.582
204	0.693
225	0.572
226	0.506
273	0.538
274	1.777
335	0.807
343	0.955
385	0.710
402	0.502
420	1.650
432	3.152
565	0.637
571	0.719
594	1.734
595	0.537
597	1.133
599	39.531
600	38.756
622	0.583
637	0.965

Size	Oak CPO Day 56
51	0.848
61	0.795
108	0.629
110	0.622
195	2.379
203	0.813
204	0.898
225	1.994
245	0.651
259	1.098
273	1.854
274	1.582
327	0.716
335	1.392
339	0.527
343	2.289
364	0.686
367	0.951
385	1.482
389	0.725
395	0.650
402	1.254
410	0.512
414	0.590
417	0.603
420	2.620
432	4.396
438	1.492
500	0.847
521	0.979
531	1.070
540	0.805
571	1.005
574	5.915
592	2.859
596	1.622
599	22.960
600	23.266
611	0.624
623	0.709
637	1.539
749	0.751

Size	Oak CPO B Day 56
51	0.698
58	0.568
61	0.543
92	0.539
195	1.724
203	0.596
204	0.559
225	1.338
245	0.603
259	0.977
273	1.435
274	1.294
296	1.662
302	0.591
326	0.604
327	0.712
335	1.274
343	1.891
364	0.872
367	0.642
385	1.312
389	0.813
390	1.419
395	1.161
402	0.965
420	1.056
432	2.096
438	1.267
500	0.741
521	0.951
540	0.732
564	0.673
565	0.543
571	1.528
583	1.479
591	1.250
592	11.196
597	0.571
599	18.125
600	30.002
637	0.745
749	1.505

Size	Oak CPO C Day 56
51	0.632
61	0.597
108	0.530
195	1.725
203	0.538
204	0.524
225	1.302
245	0.508
259	0.873
273	1.461
274	1.205
296	1.470
327	0.697
335	1.243
343	1.897
364	0.577
385	2.151
389	0.670
395	0.725
402	0.942
414	0.682
417	1.541
419	0.737
420	2.004
425	0.684
429	1.786
432	5.569
433	1.162
438	1.258
500	0.759
521	1.109
540	0.738
564	0.628
567	0.524
571	1.697
586	0.616
589	1.712
596	0.763
597	3.459
599	16.453
600	26.746
611	1.860
617	0.855
620	1.363
623	0.764
624	1.641
637	1.389
749	1.234

Size	Oak CTRL Day 56
58	0.533
195	1.111
225	0.909
259	0.735
273	0.960
274	0.865
296	1.292
320	1.415
327	0.512
335	0.909
343	1.402
385	1.283
389	0.577
395	0.882
402	0.755
420	1.941
432	4.122
438	0.896
469	1.733
500	0.612
521	0.721
564	0.502
571	1.238
586	0.944
591	1.418
592	19.283
595	2.149
596	0.598
597	1.577
599	16.666
600	25.335
611	0.536
617	0.537
623	0.846
624	0.591
627	0.565
637	1.645
749	1.402

Size	Oak CTRL B Day 56
51	0.502
195	0.896
225	0.714
259	0.635
273	0.771
274	0.778
296	0.663
335	0.935
343	1.438
364	0.518
385	1.484
386	0.640
389	0.578
390	1.066
395	1.257
402	0.629
414	0.511
417	0.744
420	1.684
432	3.610
438	0.824
521	0.617
562	1.431
564	0.606
571	1.013
586	0.581
592	24.794
595	0.670
597	2.176
599	14.839
600	27.300
611	0.560
617	0.932
620	0.544
623	0.617
624	0.522
637	1.017
650	0.905

Size	Oak CTRL C Day 56
195	1.415
225	1.073
259	0.764
273	1.225
274	0.846
276	0.509
296	0.509
320	2.061
335	0.909
343	1.381
385	2.201
389	0.634
390	0.517
395	1.084
402	0.816
410	0.545
414	0.650
417	0.948
420	4.399
429	0.762
432	8.874
433	0.690
438	0.952
469	2.996
500	0.748
521	1.029
540	0.590
564	0.614
571	1.227
591	0.557
592	1.075
595	7.836
596	1.534
597	4.117
599	13.166
600	19.105
611	0.896
617	0.532
620	0.886
623	2.135
624	1.235
627	0.883
637	3.272
749	1.272

Appendix B: qPCR Results Example Process

$$\frac{\text{Log (qPCR concentration for first duplicate)} + \text{Log (qPCR concentration for second duplicate)}}{2}$$

Then take this number and divide it by the DNA concentration to get the normalized numbers below. The final steps are shown below.

Dehalococcoides mccartyi	0	14	42	56	70
Oak CPO	4.333307	5.827624	5.628063	5.906133	5.941701
Oak CPO B	4.457686	5.355991	5.554867	5.842107	5.462876
Oak CPO C	4.85959	5.420341	5.522163	5.941727	5.682563
Oak CTRL	4.685208	5.462186	5.658722	6.65433	5.514431
Oak CTRL B	4.767272	5.557425	5.672713	6.49922	5.636869
Oak CTRL C	4.885986	5.659981	5.639457	5.408344	6.167677
M Oak CPO A	5.378462	5.862953	5.489458	6.494703	5.891288
M Oak CPO B	5.3246	5.995353	6.023462	5.993196	5.695505
M Oak CTRL A	5.195226	6.164101	6.162698	6.342886	6.145335
M Oak CTRL B	5.036382	5.699476	5.961482	6.190124	5.715134
H Oak CPO A	5.719921	6.094687	6.00269	6.093985	5.678577
H Oak CPO B	5.683094	6.237998	5.587882	5.839493	5.208338
H Oak CTRL A	5.501642	6.058477	5.899621	7.231663	5.970631
H Oak CTRL B	5.719136	5.66101	5.831386	5.395592	5.680264
CPO Average	4.550194	5.534652	5.568364	5.896656	5.695713
Stdev.	0.275067	0.255753	0.054225	0.050482	0.239684
CTRL AV	4.779489	5.559864	5.656964	6.187298	5.772992
Stdev.	0.100945	0.09892	0.016697	0.679037	0.347246
CPO Med. Avg.	5.351531	5.929153	5.75646	6.24395	5.793397
Stdev.	0.038086	0.093621	0.377598	0.354619	0.13844
CTRL Med. Avg.	5.115804	5.931789	6.06209	6.266505	5.930234
Stdev.	0.11232	0.328539	0.142281	0.108019	0.304198
CPO High Avg.	5.701507	6.166342	5.795286	5.966739	5.443457
Stdev.	0.026041	0.101336	0.293313	0.179953	0.332509
CTRL High Avg.	5.610389	5.859744	5.865504	6.313628	5.825448
Stdev.	0.153792	0.281052	0.048249	1.298299	0.20532

VITA

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Master of Science

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