Microbial and Geochemical Characterization of Wellington Oil Field, Southcentral Kansas, and Potential Applications to Microbial Enhanced Oil Recovery

By

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# Abstract

The aqueous geochemistry and microbiology of subsurface environments are intimately linked and in oil reservoir fluids. This interdependence may result in a number of processes including biodegradation of oil, corrosion of pipes, bioclogging of porous media, and biosurfactant production. During production of oil and reinjection of production water, surface exposed fluids are introduced to oxygen and exogenous microbes, both of which may alter reservoir biogeochemistry. In this study, production waters from six wells within the Wellington Field in SE Kansas, which has been water flooded continuously for 60 years, were sampled and analyzed for geochemistry, microbial ecology, microbial biomass, and biosurfactant production to better understand the relationship between the microbiology and oil production in the field.

Minor differences in aqueous geochemistry were detected among the five production wells and single injection well, and data analysis and modeling indicate that depth-specific water-rock reactions play a major role in controlling the major ion geochemistry in the field. Microbial diversity in fluids produced from the wells indicated a system that is in steady state, with microbial community composition linked to the stratigraphic location of pumping rather than injection of recycled fluids. Further, analyses of surface tension, a proxy for biosurfactant production, and its relationship to microbial biomass and oil production, suggests that while biosurfactants may be produced, biomass is likely clogging porosity and inhibiting oil recovery. Biociding practices, the injection of chemicals toxic to microorganisms, are implemented in the Wellington field to keep microbial biomass low. This study suggests their effectiveness

may need to be addressed further. Known biosurfactant-producing microbes isolated in this study may be targeted for *in-situ* stimulation to increase biosurfactant production through the introduction of nutrient and energy sources into the reservoir that can increase oil production.

## Acknowledgements

I would like to thank my advisors Jennifer Roberts and Dave Fowle for their direction, helpful conversations, and their excitement for this project. I would also like to acknowledge Lynn Watney for supporting my research and always being prompt to answer any question I had. I need to express gratitude to Belinda Sturm for her tremendous input on the DNA side of this project and her willingness to be available to train and put up with me as I learned completely new methods. I need to thank Christa Jackson, whom when I was away, struggled with the trials and tribulations of DNA work in all its frustration. Christa dedicated an entire summer to advancing my project and I am forever in her debt. I would like to acknowledge Masato Ueshima for being my field partner and analyzing samples I collected over two years. I want to show my gratitude to Karla Leslie for analyzing my samples, helping me with lab procedures and instruments and simply being always excited and available to talk science when I felt I had gone of course. I would like to thank Karen Peltier and Stephen Johnson from the Tertiary Oil Recovery Program. I want to acknowledge funding from the Kansas Geological Survey, the University of Kansas Department of Geology, the Geological Society of America, the Association of Women Geoscientists and the American Association of Petroleum Geology. Lastly, I need to thank Jessica Clatterbuck for all her love and unyielding support over the years and all of my friends and family who have always stood by me.

# Table of Contents

# List of Tables

# List of Figures

# Chapter 1: Introduction

The understanding of microbial diversity, metabolic processes and impacts on aqueous geochemistry in petroleum reservoirs is still incomplete. Analysis of microbiology in these subsurface environments may be useful as their metabolic activities can lead to detrimental impacts on oil quality and mobility, such as souring, biodegradation, and bioclogging, as well as contribute to the corrosion of pipelines. This same knowledge of the microbiology can in turn be used to enhance productivity and recovery efficiency (Youssef et al., 2009) through targeted bioclogging or by stimulation or bioaugmentation of biosurfactant-producing organisms. Additionally, studies on the impact of reservoir water flooding on the spatial distribution of aqueous geochemistry and microbiology are limited. Therefore, additional microbial investigations need to be conducted in oil reservoirs to contribute to the growing understanding of their impact in these systems.

## Microbial Ecology of Oil Field Brines

The scientific investigations of microorganisms can be traced to approximately 1677 and the studies of Anton van Leeuwenhoek (Chapelle, 2001). Microscopes that he contrived with his lenses could magnify objects up to 300 times. This gave him the ability to directly observe microorganisms. His interest in examining water samples from various sources provided the first example in human history, of microorganisms associated with ground water (Chapelle, 2001). In 1926, Edson S. Bastin and colleagues reported observations that can be considered the first study of the microbial ecology of oil

field production waters (Bastin, 1926). Their microbiological investigation of 67 wellhead samples from California and Illinois oil fields was conducted and sulfate-reducing bacteria were demonstrated to be widespread inhabitants of these systems (Bastin, 1926). These findings were the first to suggest that bacteria could thrive in deep subsurface oil reservoirs under highly saline conditions.

Oil reservoirs constitute uniquely extreme subsurface geological environments with diverse physicochemical *in situ* conditions (Ollivier and Magot, 2005). These environments were previously thought to be too severe to support life (due to high temperatures (35°C - >150°C) and high salinity ( > 300,000 ppm). Based on culture dependent and culture independent methods, a wide variety of microorganisms with a variety of metabolic requirements and terminal electron acceptors have been detected in a variety of oil reservoirs since the 1920's (Bastin, 1926; Li et al., 2007) including fermentative bacteria, sulfate-reducing bacteria (Rees et al., 1995; Leu et al., 1999; Magot et al., 2004), nitrate-reducing bacteria (Gieg et al., 2010), manganese-reducing bacteria (Gieg et al., 2010), iron-reducing bacteria (Semple and Westlake, 1987; Nazina et al., 1995), acetogens and methanogens (Li et al., 2007). Within the last two decades, culture-independent methods have revealed novel bacterial and archaeal species in these environments (Grabowski et al., 2005).

Although a considerable amount of work has been done in the past two decades, our present understanding of microbial diversity in petroleum reservoirs continues to have significant knowledge gaps. The application of molecular techniques, in particular the analysis of the 16S rRNA gene, has been significantly valuable in increasing knowledge of subsurface petroleum reservoir microbiology.

## Redox Conditions and Nutrient Availability of Oil Field Brines

The ability of microorganisms to alter the geochemistry of groundwater has long been known. As early as 1900 it was observed that groundwater associated with petroleum deposits omen lacked dissolved sulfate but contained abundant sulfide, whereas groundwater not associated with petroleum contained high levels of sulfate and little sulfide (Ollivier and Magot, 2005). The concept that the sulfide present in water produced from oil fields was microbial in origin was suggested in 1926, based on the detection of sulfate-reducing bacteria in water sampled from a number of oil fields (Bastin et al., 1926). Rosnes et al. (1991) demonstrated that sulfate-reducing bacteria were able to produce sulfide under simulated in situ reservoir temperatures and pressures, therefore showing that sulfide formation can be attributed to the activity of microorganisms. Subsequent work has demonstrated definitive isotopic signatures of sulfide attributed to biogenic sulfate reduction (Peters et al., 2010).

Direct microscopic counts in oil field brines most commonly indicate the presence of a moderate population range of microorganisms, from a few cells up to $10^6$ cells $mL^{-1}$ (Head et al, 2003). Although microbial biomass is not as abundant as in deep-sea hydrothermal vents where cell densities up to $10^9$ cells $mL^{-1}$ have been reported (Ollivier and Magot, 2005), these population densities suggest that most reservoir waters are a nutrient limited environment and/or that bacterial development is limited by other physiochemical parameters such as temperature, salinity, and the availability of electron acceptors and donors (Ollivier and Magot, 2005; Li et al., 2007). In most subsurface environments, nitrogen and phosphorous are the primary limiting nutrients (Ollivier and Magot, 2005). Electron donors in oil reservoirs include hydrogen, volatile fatty acids

such as acetate, propionate, and benzoate, petroleum hydrocarbons, and inorganic electron donors such as sulfide (Youssef et al., 2009). Some oil field microbial isolates use sulfate and iron (III) as electron acceptors (Greene et al., 1997; Nazina et al., 1995). As yet it is unclear how prevalent iron (III) is in oil reservoirs (Youssef et al., 2009) the concentration of which should be limited by the reservoir mineralogy (Machel and Foght, 2000). Deep subsurface petroleum reservoirs generally lack dissolved oxygen and therefore are anoxic habitats. Reinjection of produced brine as a secondary oil recovery technique, however, besides introducing exogenous microorganisms, could alter the geochemistry of the formation spatially or permanently, due to the introduction of sulfate or oxygen. This, in turn, may result in changes to the indigenous-microbial community structure (Youssef et al., 2009).

## Influence of Injection Waters on the Microbiology of Production Wells

Investigations on the spatial impact of the injection waters on the geochemistry and microbiology of production wells has not been extensively studied. Bacterial and archaeal community structure from injection and production waters was investigated by Tang et al. (2012). Clone library and terminal restriction fragment length polymorphism (T-RFLP) analysis were applied to samples from two oilfields (the Menggulin and Huabei Oilfields) in China. The sampling locations were 50 km apart and had different in-situ temperatures (37°C and 58.4°C respectively). Their findings from the Menggulin oilfield indicated that the microbiology of six production wells and one injection well were dissimilar. The bacterial community of the injection water was more diverse and had a higher species richness than the production waters. Additionally, the authors found that the variation in the microbial communities among the six production waters were

also significant. Archaeal communities isolated from the injection well and production waters were largely dominated by methanogens from the same taxa. Results from the Huabei oilfield indicate that again the injection water harbored a more diverse community than the production water although the bacterial and archaeal communities were fairly simple and the diversity was low in comparison to the Menggulin oilfield. The dominant bacteria and archaea in different production wells were very similar and *Methanomethylovorans* relatives were detected in high abundance in the injection water but detected in only one production well.

A study conducted by Ren et al. (2011) investigated the microbiology of the Gudao petroleum reservoir in the Yellow River Delta. The microbiology of one injection water and two production waters was examined using denaturing gradient gel electrophoresis (DGGE) and 16S rRNA gene clone library analysis. Results showed that the similarities of the bacterial communities between the injection water and production waters were lower than between the two production waters. The archaeal composition among all samples showed no significant difference. Only two out of 54 bacterial operational taxonomic units (OTUs) and five out of 17 archaeal OTUs in the injection water were detected in the production waters, indicating that most of the microorganisms introduced by the injection water may not have survived. Additionally, there were 55.6% and 82.6% unique OTUs in the two production waters suggesting that each production well has a specific microbial community structure.

These two studies suggest that petroleum reservoirs are environments that harbor diverse assemblages of microorganisms. While it seems probable that injection water will influence the microbiology of production waters, these studies show that injection water

microbes may not survive transport into the reservoir or are outcompeted by native microorganisms.

## Bioclogging and Biosurfactant Production by Microorganisms

There are a variety of mechanisms by which microorganisms can affect oil production from reservoirs. Laboratory experiments and field trials have demonstrated that microbial biomass can impact the hydraulic properties of porous media, an effect termed bioclogging. Microbial biomass can clog up pore spaces in reservoir rock and impact the mobility of the hydrocarbons in several ways, two of which will be discussed here. Controlled bioclogging of petroleum reservoirs may be applied to improve volumetric sweep efficiency (a measure of the effectiveness of an enhanced oil recovery technique that depends on the volume of the reservoir contacted by the injected fluid) by stimulating the growth of microbes in the reservoir. This method requires the injection of nutrients into the reservoir, which will enter the high permeability regions of the formation to encourage the growth of microbes. The increased biomass in the high permeability zones will reduce the movement of water in these areas and divert the water into zones of the reservoir with higher oil saturations (Youseff et al., 2009; Zhang et al., 2010) for the recovery of additional oil not previously targeted by the water flood.

The reduction in hydraulic conductivity due to bioclogging has been examined in column and core experiments. Cunningham et al. (1991) used a column inoculated with *Pseudomonas aeruginosa* that they cultured in their lab and applied a constant head difference between inflow and outflow. The authors reported a decrease of 90% in hydraulic conductivity and 50-90% in porosity. Studies by Raiders et al. (1989) and Bae et al. (1994) demonstrate that the growth of microorganism in sandstone cores results in

substantial decreases in permeability under complex reservoir flow conditions and the injection of various nutrient mixtures and injection schemes.

Brown et al. (2002) investigated the effect of bioclogging in the North Blowhorn Creek Oil Unit in Alabama. Production from this field was progressively declining and an estimated ten million barrels of oil would be lem unrecovered if enhanced recovery were not successful. The injection water was amended with phosphorus and nitrogen-containing nutrients to increase microbial growth in the reservoir. Bioclogging of the more porous zones in the reservoir resulted in the recovery of an additional 69,000 barrels of oil during the first three years with an estimated 400,000-600,000 barrels recovered over the next ten years (Brown et al., 2002). Microbial involvement was invoked by increased numbers of microbes amer treatment.

Additionally, microorganisms known to produce biopolymers or large amounts of microbial biomass such as *Enterobacter cloacae* and *Bacillus licheniformis*, can be injected into the reservoir to enhance oil recovery through bioclogging (Ohno et al., 1999; Maezumi et al., 1998).

In addition to the advantages of bioclogging highlighted above, biomass plugging of pore space can also lower the efficiency of oil production (Gittel et al., 2009; Aslam, 2009). Jack et al. (1989) identified two mechanisms of plugging near the bore hole in water flooded oil reservoirs that could hinder oil production (1) particulate plugging by the microbial cells themselves and (2) viable bacterial plugging through biofilm formation.

Secondary oil recovery techniques such as water flooding are employed to displace oil toward the wellbore. However, due to high capillary pressures, oil can

eventually become trapped within pore spaces in the rock matrix. The second way that hydrocarbon mobility is impacted by microbial biomass is through the production of biosurfactants. Biosurfactants are surface-active compounds produced by a wide variety of microorganisms that have been shown to decrease surface and interfacial tension between liquids and form microemulsions between two different phases (Banat et al., 2010). These molecules have both hydrophilic and hydrophobic domains that allow them to partition at the oil-water interface and at the oil-rock interface to promote the mobilization of oil (Youssef et al., 2009). Generally, biosurfactants are considered to be effective if they lower the surface tension between air and water from 72 to 35 dynes $cm^{-1}$ (Soberón-Chávez, 2010). Energy sources and nutrients and such as molasses, nitrogen, and phosphorous may be injected into the reservoir to enhance microbial metabolisms or exogenous organisms may be injected along with nutrients to increase cell biomass (Wang et al., 2012).

Gudiña et al. (2012) isolated biosurfactant-producing microorganism from crude oil samples from four wells in a Brazilian oil field. 16S rRNA gene analysis identified *Bacillus* strains capable of reducing the surface tension of water to 30 dynes $cm^{-1}$. An investigation into biosurfactant production and its influence on oil recovery was conducted by Xia et al. (2011) on three microbial strains isolated from the Xinjiang oil field in China, that belong to the families of *Pseudomonas*, *Bacillus* and *Rhodococcus*. Sand packed columns were used to conduct core flooding experiments. The cores were initially flooded with oil field brine to calculate pore volume. The cores were then flooded with crude oil from the reservoir to determine initial oil saturation. The sand pack was again flooded with brine until no oil flowed from the effluent. Biosurfactant was then

introduced into the column and allowed to rest for one day. Lastly, a second waterflood was applied and the discharge was collected to measure the amount of oil recovered. While all strains were successful in reducing the surface tension of their respective supernatants, *Pseudomonas* reduced the surface tension from 71.2 to 22.56 dynes $cm^{-1}$ and demonstrated a 14.3% additional oil recovery amer inoculating biosurfactant into the sandstone core.

## Overview of Thesis Chapters

The current study presents aqueous geochemical and microbiological data collected from the Wellington Oil Field, a mature field located in southcentral Kansas. The aqueous geochemistry and microbiology of an injection well, five producing wells, and a drill stem test are compared. Additionally, the spatial impact on the geochemistry and microbiology of the injection well on all other samples was assessed. Culture dependent and independent methods were used to characterize the microbial community structure and diversity. Correlations between microbial biomass concentrations and well performance were analyzed and the causation for presence and variations in the population of microorganisms was evaluated. Biosurfactant measurements, which correlate higher biomass concentrations with lower surface tension measurements, are explored.

The purpose of this study is to add to the growing but still limited knowledge of oil field microbiology. Understanding microbial activity in petroleum reservoirs is important when considering well performance. Additionally, studies investigating the impact of water flooding on the microbiology and aqueous geochemistry of production wells are sparse. Taken together this knowledge base will be of utility to develop

possible methods to increase well performance such as improving biocide treatments and targeting biosurfactant-producing microbes.

# Chapter 2: Field Site Description

## Site Description: Wellington Oil Field

The Wellington Oil Field occupies an area approximately two miles in width and three miles in length and is located in the central part of Sumner County in south-central Kansas (Fig. 2.1).

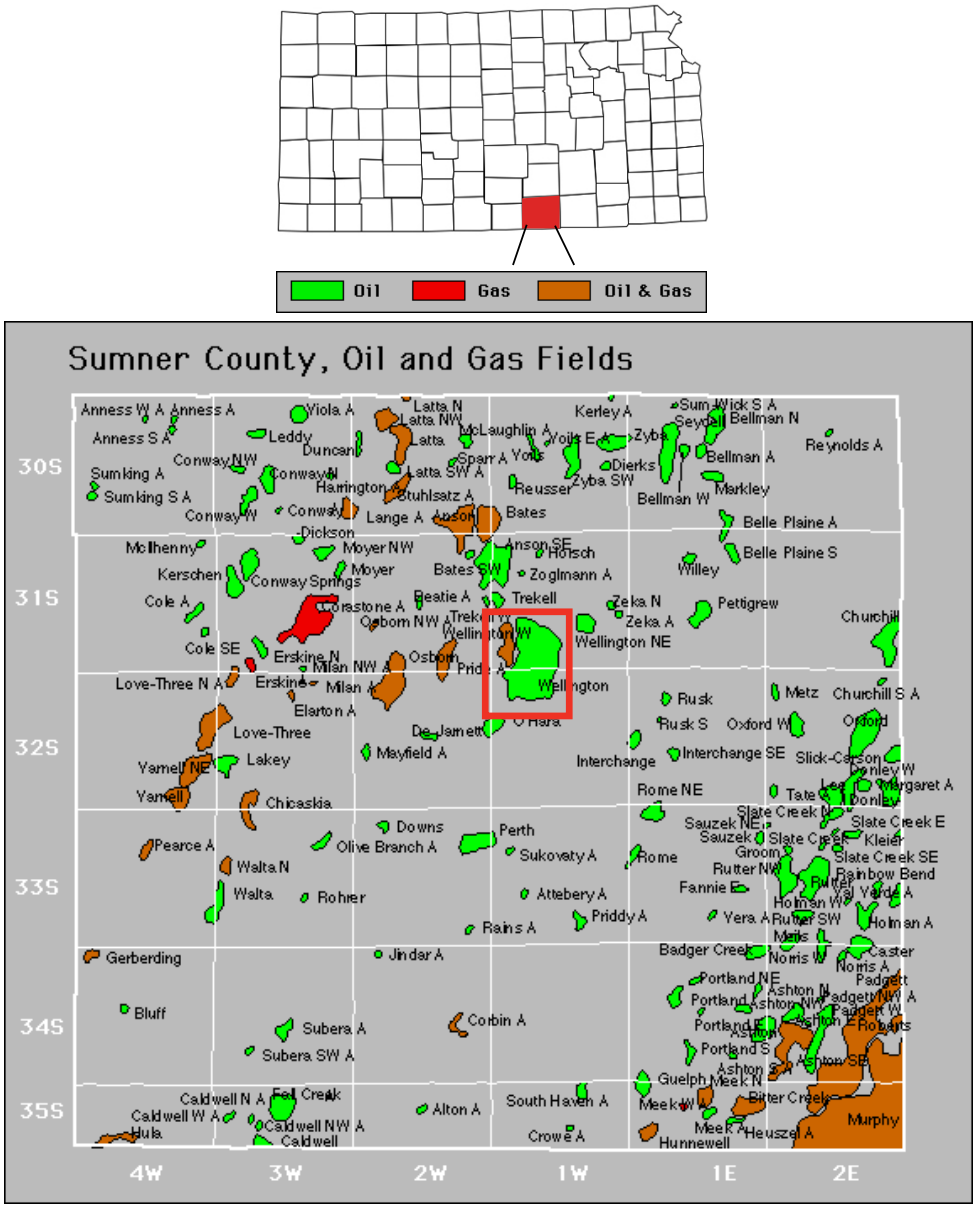



Figure 2.1. Oil and gas fields in Sumner County Kansas. Wellington Oil Field is highlighted in the red box (from the Kansas Geological Survey website, 2012).

From 1945 to 1950, 45 wells were drilled into the oil and gas-bearing zone of the Mississippian reservoir. This producing zone is approximately 9 meters thick and between the depths of 1119-1128 meters in the sampled location. Initial production of early wells ranged from 800 to 2,000 barrels of oil and 300,000 to 500,000 cubic meters of gas daily (Cooperative Refinery Association, 1949). The majority of wells, however, initially produced from 200 to 400 barrels of oil daily with undetermined quantities of gas. Production was short-lived and declined steadily with the depletion of the reservoir. As of 1949, many of the wells only yielded three to four barrels of oil daily with better wells producing 10 to 15 barrels (Cooperative Refinery Association, 1949). This decrease in productivity led to the undertaking of secondary methods to repressure the reservoir to enhance oil recovery. Water flooding of the Wellington field was initiated in February 1953. From February 1953 to December 1955 the field produced a total of 614,200 barrels of oil of which 262,250 barrels may be directly attributed to water flooding (Cooperative Refinery Association, 1955). To date, there are roughly 50 producing wells, three waterflood plants, and fourteen injection wells. Oil production from wells ranges from one to six percent (Berexco barrel test, Appendix E).

## Chemical Treatment of Wellington Oil Field

Injecting produced water amer it has been exposed to surface conditions can result in the inoculation of the reservoir with surface microorganisms (Youseff et al., 2009). To discourage microbial contamination and corrosion of the oil producing system, physical and chemical treatments are used to control bacterial growth and to keep pumping systems free from solid deposition. Producing wells in the Wellington Field are treated with two to five gallons of CRO195 approximately every three weeks (pers. comm. with

Jason Rush). CRO195 is an organic, film forming corrosion inhibitor in the form of an oil-soluble liquid with water dispersing properties (Baker Petrolite). Additionally, recovered water is treated with 3 gallons of WCW5827 daily before it is injected back into the subsurface. WCW5827 water treatment additive is a water soluble, liquid, quaternary ammonium compound that possesses surface active and corrosion inhibitive properties (Baker Petrolite). Composition and information on the ingredients of both chemical treatments can be found in Appendix A.

## Description of Sampled Wells

Production wells in the Wellington Oil Field target the upper 9 meters of an approximately 122 meters thick unit of Mississippian aged rocks. The production wells sampled in this study were open hole drilled to depths (below land surface) from approximately 1119 to 1129 meters. Oil production from the sampled wells varies from one to six percent (Table 2.1).

| Sample Name | Total Depth (m) | Oil Production (%) |
|---|---|---|
| Well 73 | 1119 | 1 |
| DST | 1121 | NA |
| Injection Well | 1122 | NA |
| Well 53 | 1126 | 1 |
| Well 69 | 1127 | 5.5 |
| Well 60 | 1128 | 1.5 |
| Well 11 | 1129 | 2 |

**Table 2.1. Depths and oil production percentages of sampled wells in this study. NA-Not applicable. DST-drill stem test from KGS well 1-32, sampled January 2011. (Berexco barrel test, Appendix E).**

Percent oil is determined by the ratio of the volume of oil and water produced during a ten-minute barrel test. An enlarged area of Wellington oil field with sampled wells highlighted is shown in figure 2.2. The boxes on figure 2.2 signify water injection plants (only the green box was sampled) and the circles represent the sampled producing wells with the exception of the non-producing well from which the drill stem sample was collected (black circle). The field is water flooded by three different water plants. To designate from which waterflood plant the injection water originates from, the sampled production wells are highlighted with the same corresponding colors as the waterflood plants.

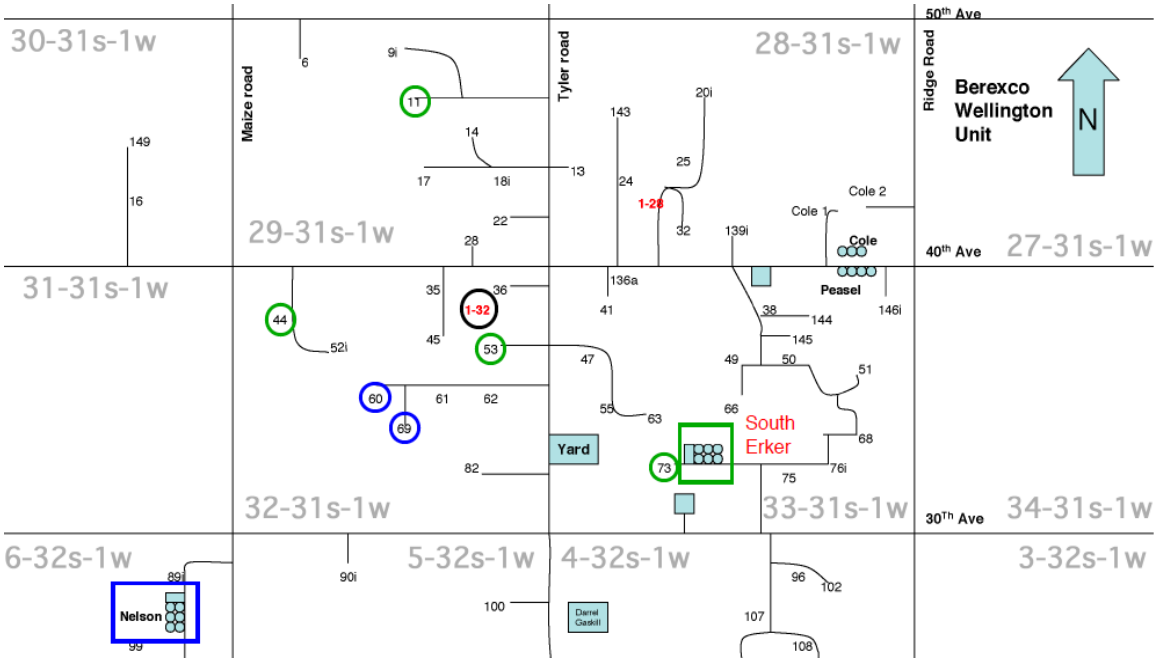



**Figure 2.2. Enlarged are of the Wellington oil field showing locations of sampled wells (circles), drill stem test, and waterflood plants. Boxes represent waterflood plants and circles represent sampled wells. The black circle illustrates where the drill stem test was collected. Green circles receive the injected water from the green squares and blue circles from the blue square. Well 11 is approximately 1.5 miles from the South Erker waterflood plant (data provided by Berexco).**

Structural Background of Kansas; Pre to Post-Mississippian

A broad northwest-southeast-trending structural high, termed the Central Kansas Arch, controlled the geology of Kansas in Ordovician and Devonian time (Fig. 2.3). The northwest and southeast extent of the Central Kansas Arch are known as the ancestral Central Kansas uplim and the Chautauqua arch, respectively (Fig. 2.4) (Merriam, 1963).

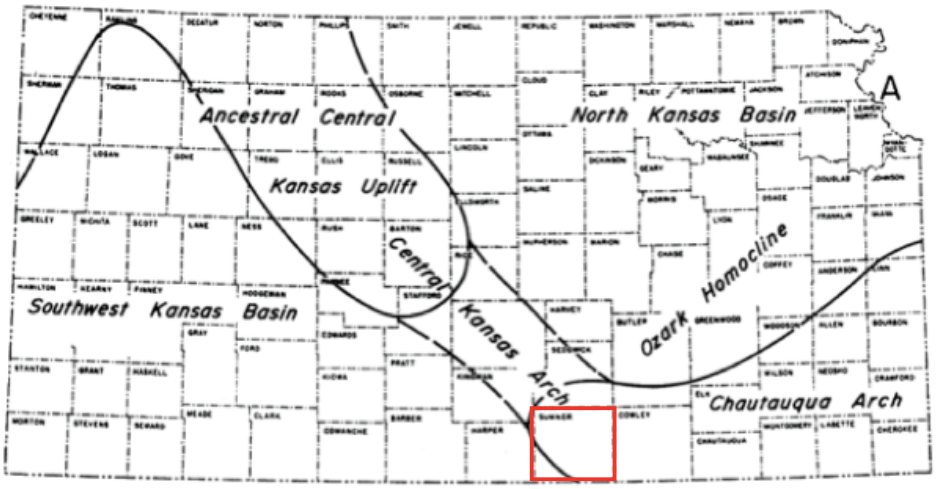



**Figure 2.3. Structural framework of Kansas during the Ordovician and Devonian time. Sumner County is highlighted in the red box (Merriam, 1963).**

A large change in structural development of the Kansas region occurred near the end of Mississippian time. Structures that began to develop near the end of the Mississippian and beginning of the Pennsylvanian would define the structural framework seen in Paleozoic beds (Merriam, 1963). The two primary post-Mississippian structural highs commonly observed in the subsurface geology in Kansas are the central Kansas and Nemaha uplims (Fig. 2.4). Sumner County is located in the region of the Sedgwick Basin, a shelf-like southerly plunging area in south-central Kansas (Fig. 2.4). The Sedgwick Basin is a pre-Desmoinesian post-Mississippian structural feature. The basin is bounded

by the Nemaha Anticline to the east and the Pratt Anticline to the west (Merriam, 1963). Strata in the basin have been described by facies change and increased thickness southward from the shelf area into the Anadarko Basin in Oklahoma (Merriam, 1963).

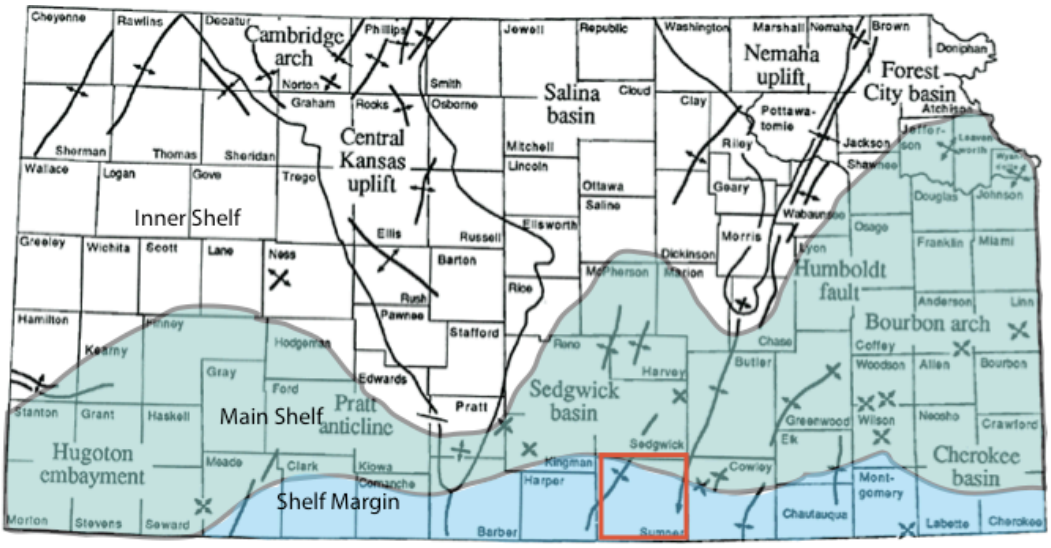



**Figure 2.4. Post-Mississippian structural highs and interpreted locations of the inner-shelf, main shelf, and shelf-margin environments. Sumner County is highlighted in the red box (Modified from Merriam, 1963 and Franseen, 2006).**

## Depositional Setting of Mississippian-Aged Rocks in Kansas

During the Carboniferous a carbonate shelf covered broad areas of the central U.S., including Kansas, with the outer shelf and shelf margin extended through southern Kansas (Fig. 2.4) (Witzke and Bunker, 1996). This regional setting resulted in deposition of extensive, laterally continuous carbonate and siliciclastic facies belts deposited in stacked stratal packages (Fig 2.5) (Watney et al., 2007). The Mississippi "Chat" (Osagean stage) is stratigraphically located as the top member at the unconformity between the Pennsylvanian and Mississippian aged rocks. (Fig. 2.6). The initial mudstones to sponge-spicule wacke-packstones were deposited during transgressive and regressive cycles on a shelf-to-shelf margin setting (Watney et al., 2001). Post Mississippian subaerial exposure,

amer early silicification, led to sponge spicule dissolution, vuggy porosity development and autobrecciation (Watney et al., 2001). Porosity varies from approximately four to 26 percent throughout the Mississippian reservoir.

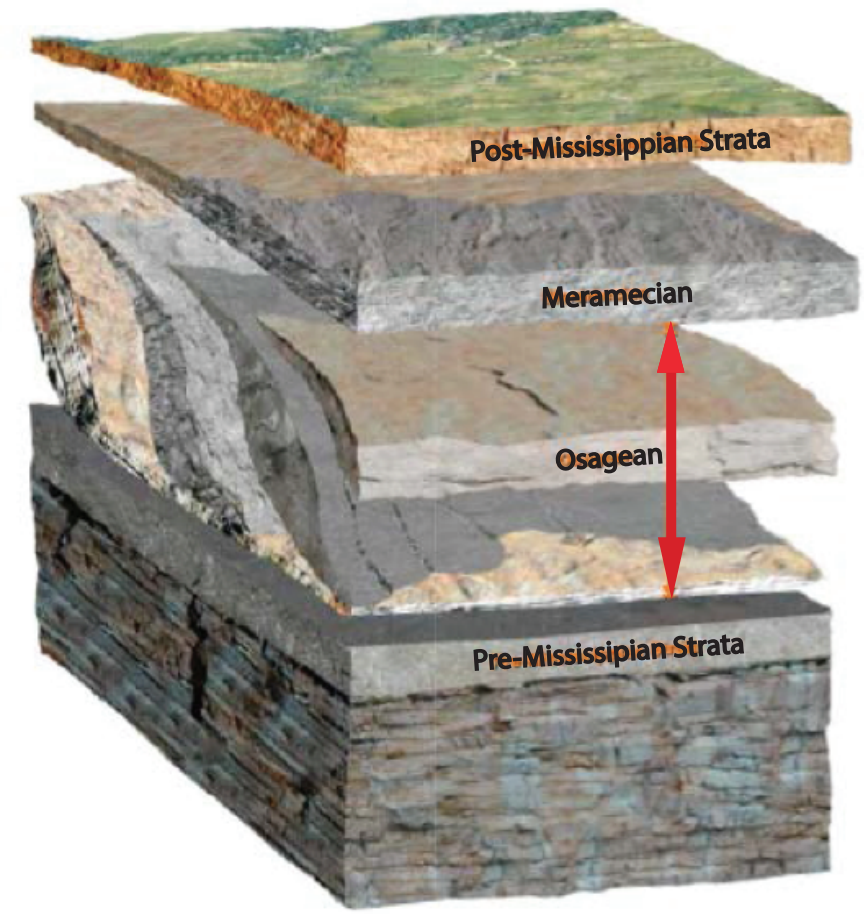



**Figure 2.5. Mississippian oil play-stacked and shingled Mississippian strata developed along southern Kansas and northern Oklahoma. The red arrow illustrates the Osagean interval sampled in this study (Watney et al, 2012) http://www.kgs.ku.edu/PRS/Ozark/pubs.html).**

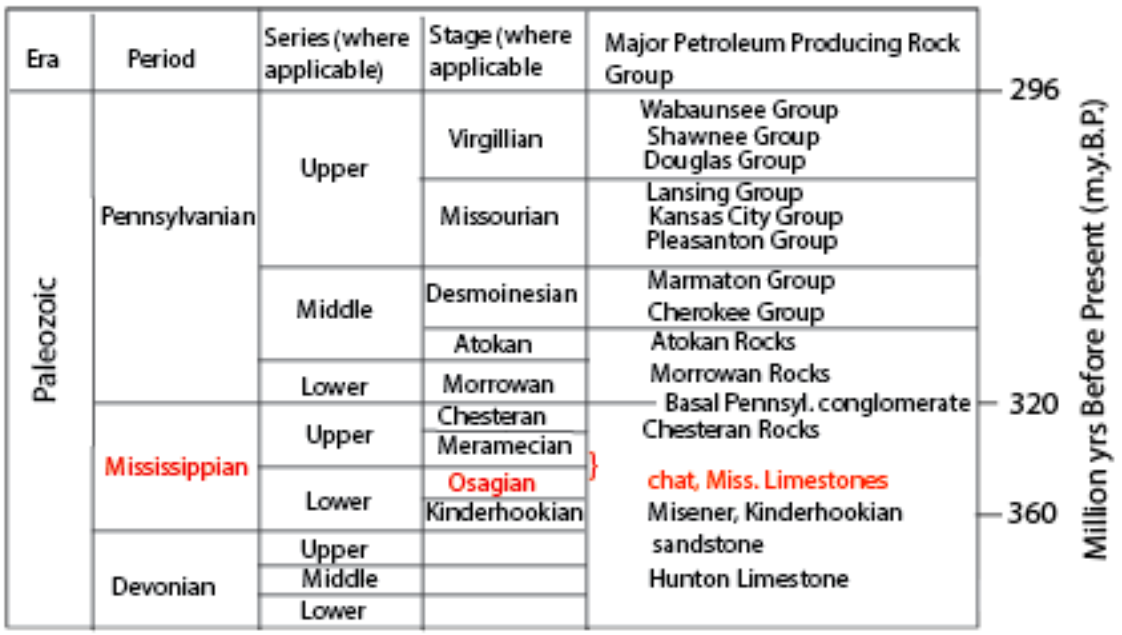
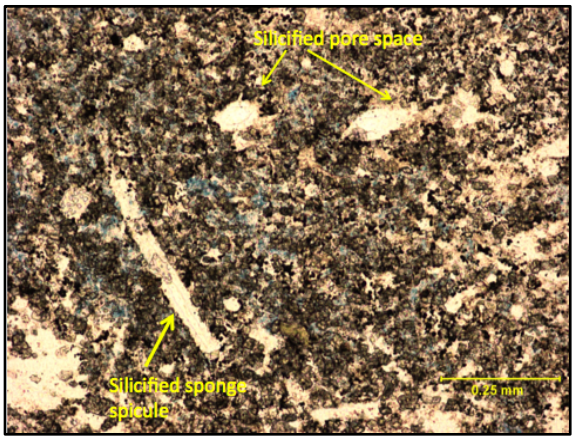



**Figure 2.6. Major petroleum producing rock units in Kansas. The Mississippian unit is highlighted in red (Modified from Stratigraphic and Spatial Distribution of Oil and Gas Production in Kansas by K. David Newell, W. Lynn Watney, Stephen W.L. Cheng, and Richard L. Brownrigg, 1987).**

## Mineralogy of the Mississippian Pay Zone

The lithology of the Mississippian pay zone includes chert, limestone, dolomite, and anhydrite with varying amounts of siliceous cement. Descriptions of core throughout the sampled interval were provided by Lynn Watney (pers. corresp.) and can be found in Appendix B. Thin sections from three depth intervals through the Mississippian pay zone were described by Barker (2012; Fig 2.7). Barker (2012) described the thin sections as exhibiting a fining upward trend with increasing silicification with depth. Mineralogy is dominated by fine-grained dolomite but included chalcedony, possible anhydrite, and silicified sponge spicules

A.

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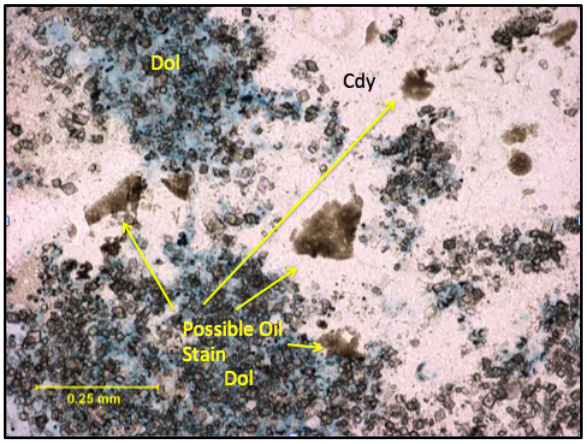


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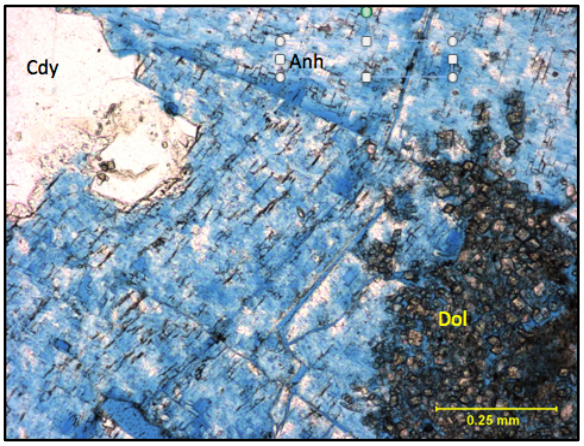



**Figure 2.7. Thin sections of three depth intervals through the Mississippian pay zone. A. 3670'. Plain light (10X zoom). Fine grained dolomite with silica cement. B. 1122'. Plain light (10X zoom). Dol-dolomite. Cdy-Chalcedony. C. 3687'. Plain light (10X zoom). Chalcedony and fine grained dolomite with anhydrite infilling large pore spaces (taken from Barker, 2012).**

# Chapter 3: Materials and Methods

## Research Approach

Wells were initially selected for sampling so that they included a representative array of daily oil production values to investigate microbially enhanced oil recovery. The reduction of surface tension in reservoirs has been correlated to the re-mobilization of trapped oil. Surface tension measurements on samples from the well heads were executed to examine the potential influence of biosurfactants in the reduction of surface tension. Additionally, one of the water injection wells was sampled to compare aqueous geochemistry and microbiology of the waterflood fluids to producing wells. Dates of sampling occurred just before wells were treated with biocide (~three weeks since treatment; pers corresp. Jason Bruns). Geochemical analyses were conducted on each sample and culture-based and non-culture based methods were used to investigate microbial community structure. Non-culture based DNA methods tend to be more robust due to the removal of microbial growth bias that comes with culture-based techniques. The DNA analysis approach for this project was to apply denaturing gradient gel electrophoresis (DGGE) and 16S rRNA gene methods. DGGE analysis would fingerprint similarities, differences, and diversity in the microbial communities among wells. 16S rRNA gene analysis would generate clone libraries and phylogenetic trees to infer evolutionary relationships among the samples. The samples for sequencing were selected to compare the injection well microbiology to producing wells and water phase microbiology to oil phase microbiology.

## Sample Collection and Geochemical Analysis

Brine samples for chemical analyses were collected from six producing wells, one drill stem test, and one waterflood plant during May and November 2011 (see figure 2.2). Production fluids were purged approximately three well volumes to remove stagnant fluid. Raw samples were collected in 1 L amber bottles. Temperature and pH were measured in the field, uphole with an infrared thermometer and an Accumet® portable pH meter. Production fluid rested in 5 gallon buckets for approximately ten minutes to separate the oil and aqueous phase. A Geosquirt purge pump was used to filter the production fluid through a 144 mm Polycarbonate In-Line Filter Holder holding a 1.2 um glass fiber prefilter and 0.2 um membrane filter. All samples were collected in triplicate and stored at 4°C until analyzed. Alkalinity was determined within 2 days by manual titration with 0.01 N sulfuric acid or automatically using a Fisher Titrimeter®II Automatic Titration System with 0.02 N sulfuric acid. Headspace gas samples were collected directly from the wellhead in 60 ml glass serum vials spiked with mercuric chloride and analyzed for methane and $CO_2$ on a gas chromatograph using a thermal conductivity detector using the following specifications: inlet screen front detector heater set at 120°C with a total flow of 32 ml/min, oven set at 90°C and the TCD front detector heater set at 160°C with a reference flow of 30 ml/min and a make up flow of 7 ml/min. Total carbon, dissolved organic carbon, and dissolved inorganic carbon concentrations were measured with a Torch Carbon Analyzer by Teledyne Tekmar. Cation concentrations were measured with a Perkin Elmer Optima 5300DV ICP-EOS on filtered and acidified samples (2% $HNO_3$). Anion concentrations were determined on a Dionex ICS-3000 Ion Chromatography System on filtered samples. Ferrous and ferric iron was

analyzed using the colorometric Ferrozine standard method on a spectrophotometer at 562 nm (American Public Health Association, 1989). Sulfide was measured on a spectrophotometer at 665 nm according to methylene blue 4500-$S^{2-}$ D standard method (American Public Health Association, 1989). Phosphate concentration was analyzed on a spectrophotometer at 880 nm following the USEPA PhosVer 3 (Ascorbic Acid) method 8048 (American Public Health Association, 1989).

## PHREEQC: Aqueous Geochemical Modeling

PHRQPITZ is a modification of the geochemical modeling code, PHREEQE, to permit equilibrium calculations of geochemical reactions in brines and other highly concentrated electrolyte solutions using the Pitzer approach for activity-coefficient corrections. (Langmuir, 1997). Reaction-modeling capabilities include calculation of (l) aqueous speciation and mineral-saturation index, (2) mineral solubility, (3) mixing and titration of aqueous solutions, (4) irreversible reactions and mineral-water mass transfer, and (5) reaction path (Parkhurst et al., 1988). In this study, measured geochemical data was entered into PHREEQC version 3.0, provided by the United States Geological Survey and includes the Pitzer approach, to calculate saturation indices of oilfield minerals in aqueous solution and to run mixing models between the production wells and the injection well.

## Most Probable Number Analysis (MPN)

MPN is a cultivation method used to estimate the number of viable microorganisms living in a test sample. It is based upon the application of probability to the number of observed positive growth responses to a series of serial dilutions in media formulated to target different physiologic types. In this study, production fluids were inoculated on-site

into sterile 10 ml serum vials at each well containing a known volume of 4 different media (Appendix C), specifically formulated to encourage the growth of fermentative bacteria, iron and sulfate reducing bacteria, and methanogens. The dilution factors used ranged from non-dilute to 1:100,000. Inoculated media was stored in an incubator set at 35°C for approximately two weeks and only removed for determination of positive growth. Sterile, non-inoculated media and filtered production fluid inoculated media were used as growth controls. Growth of fermenters was identified by visually inspecting the turbidity of the serum vials. Cloudy vials indicate positive results. Growth of sulfate reducing bacteria was determined by placing a drop of inoculated media onto lead acetate paper. The lead acetate paper turns black when sulfate reducers are present due to the production of sulfide. Iron reducing bacteria were positively identified via the reaction of inoculated media with a ferrozine reagent specific for $Fe^{2+}$. The reagent and sample were added to a cuvette and allowed to rest for 5 minutes. Positive growth was indicated by a color change from clear to purple. Methanogen growth was determined by injecting a subsample of headspace gas from the inoculated vials into a gas chromatograph that measured the $CH_4$ concentration. Results were positive when the concentration of $CH_4(g)$ was higher than the filtered inoculated control. The results were entered into an MPN calculator (Hurley and Roscoe, 1983) to estimate the original concentration of microorganisms in an undiluted sample.

## Lipid Phosphate Biomass Analysis

Methods for determining microbial biomass concentration in production wells and the injection well were carried out in duplicate according to Findlay et al., (1989). Approximately 11 liters of production fluid was filtered through an in-line filter holder at

each well. The filters were initially preserved on ice in the field, then transferred to a -80 °C freezer in the lab to prevent phospholipid degradation. Orthophosphate standards made from glycerol phosphate were processed in duplicate. In preparation for analysis, samples and standards were combined with ammonium molybdate and malachite green solutions and decanted into a cuvette where absorbance was recorded at 610 nm with a Spectronic GENESYS 20 spectrophotometer. The phosphate content of each sample was related to cell mass using a conversion factor from Findlay et al. (1989) of 4 x $10^9$ cells per 100 nmol lipid bound phosphate.

## Microbial Biosurfactant Experiments-Surface Tension Measurements

Raw samples were collected from the well heads in May of 2010 and stored in 1L glass amber bottles at 4°C until analysis approximately three days amer collection. Approximately 50 ml of each sample was placed in a glass beaker. Surface tension measurements were taken in triplicate with a Fisher Surface Tensiot Model 21 tensiometer from Fisher Scientific. The surface tension of samples was compared to the surface tension of distilled water which is 72 dynes $cm^{-1}$ at 25°C. Surface tension values lower than 72 dynes $cm^{-1}$ indicated the presence of biosurfactant.

## DNA Extraction

Production fluids from all sampled wells were collected from the well head into 5 gallon buckets and allowed to rest for 20 minutes with a lid on to separate the oil and water phases. The aqueous phase DNA was then collected by placing a Geosquirt purge pump into the base of the bucket and filtering approximately 11 liters of sample through a 0.2 μm sterilized membrane filter. The membrane filter was then removed using ethanol rinsed tweezers, placed into a sterile sample baggie and stored on dry ice for

transportation to the lab. The DNA filters were placed in an -80°C freezer until the extraction process. The filter apparatus was washed with ethanol between well sampling.

Samples for oil phase DNA were collected at the production well by skimming the oil phase off of the top of the production fluids into 50 ml sterile, polypropylene, ethanol rinsed Falcon® tubes. These samples were stored on dry ice for transportation to the lab and then placed into an -80°C freezer. Before the extraction process, oil phase samples were thawed and 25 ml of oil was removed and transferred into a new, ethanol washed 50 ml Falcon® tube. The presence of oil in a sample has been proven to inhibit the downstream polymerase chain reaction process, a technique used to amplify DNA. To encourage the removal of cells from the oil phase, 25 ml of Tween 80, a nonionic surfactant, was added to each Falcon® tube. These tubes were then vigorously vortexed for 30 minutes and centrifuged for 20 minutes. The Tween 80 phase with the oil DNA was transferred to new Falcon® tubes, and the oil phase was discarded. The samples were filtered using a glass funnel, vacuum pump, and 0.2 μm MediaKap hollow fiber membrane filters. These filters were stored in an -80°C freezer until the extraction process.

PowerMax® Soil DNA kits from MO BIO Laboratories were used to extract DNA from both the aqueous and oil phases of the production fluids. Using this kit, DNA filters from both aqueous and oil phases are added to bead beating tubes with a kit supplied proprietary buffer for rapid and thorough homogenization. Cell lysis and DNA exposure occurs by mechanical and chemical methods. Extracted DNA is captured on a silica membrane in a spin column format. DNA is washed and eluted from the membrane and is ready for other downstream applications.

## Polymerase Chain Reaction (PCR) and Gel Electrophoresis

Oil field bacteria and archaea were characterized by cloning of PCR amplified 16S rRNA genes. 5 ml of DNA from the extraction step was transferred into 5 separate 2 ml autoclaved microcentrifuge tubes for concentration (1 ml extracted DNA per 2 ml microcentrifuge tube). All well samples were placed into a Vacufuge Concentrator 5301 to evaporate the liquid and concentrate the DNA. The samples were then resuspended in 500 µl of autoclaved MilliQ water and placed into an -80°C freezer until the PCR process was initiated.

Extracted DNA gene fragments were amplified using primer pairs BAC8F (5'-AGAGTTTGATCMTGGCTCAG) and BAC519R (5'-GTATTACCGCGGCTGCTGG) and ARC 344F (5'-ACGGGGNGCAGCAGGCGCGA ) and 919R (5'-GTGCTCCCCCGCCAATTCCT) from Sigma-Aldrich®. Approximately 500 base pairs were targeted on the 16S rRNA gene. Reaction mixtures of 50 µl consisted of 31.8 µl distilled DNA grade water, 5 µl of template DNA, 5 µl of 5x Go Taq buffer, 5 µl $MgCl_2$ (25 mM), 0.2 µl of Hotstar Taq DNA Polymerase, 1 µl of dNTP mix (10mM), and 1 µl each of forward and reverse primers (10uM). The thermocycler program used to denature and synthesize the DNA is as follows: 95 °C for 2 minutes, 15 cycles of 95 °C for 30 seconds, 65 °C for 30 seconds, and 72 °C for 30 seconds, followed by 15 cycles of 95 °C for 30 seconds, 50 °C for 30 seconds, and 72 °C for 30 seconds, with a final extension at 72 °C for 5 minutes. Positive and negative controls were used to determine successful binding of primers and contamination, respectively. 1 ul of each PCR product was combined with 6X MassRuler Loading Dye and pipetted into successive lanes of a 1.7% (wt./vol.) gel of agarose. A MassRuler™ Low Range DNA Ladder, 80-1031 kb, was

loaded to flank the samples for comparison of DNA fragments. 100 Volts was applied through the gel for 45 minutes. The gel was placed in a tray with 1x TAE buffer and SYBR® Gold nucleic acid stain on a darkened rotator table for 20 minutes. The gel was then visualized and imaged under UV light with an AlphaImager® HP imager from Alpha Innotech for DNA band visualization.

## Denaturing Gradient Gel Electrophoresis (DGGE) Analysis

Genetic fingerprinting techniques provide a pattern or profile of the genetic diversity in a microbial community (Muyzer and Smalla, 1998). DGGE analysis of the PCR products was performed to separate DNA fragments of different sequences and determine bacterial diversity. Gels were made with 8% acrylamide solutions containing a gradient of 40 (40% Acrylamide/Bis, 50X TAE Buffer, formamide, and Urea) to 60% DNA denaturant agent. Gels were poured between two plates using a Bio-Rad Model 475 Gradient Delivery System. Gradients were formed from 20 ml of each acrylamide solution which contained 100 ul, 10 ul N,N,N',N'-Tetramethylethylenediamine (TEMED), and 10% ammonium persulfate (APS). 20 µl of PCR product were then mixed with 20 µl of 2x loading dye and pipetted into the wells of the gradient gel. 1 ul GeneRuler™ 100 bp Plus DNA Ladder (Fermentas) was combined with 4 ul deionized water and 5 ul 6X DNA loading dye and loaded into the first and last lanes to serve as molecular weight markers. Electrophoresis was ran at 100V and 60°C for 16 hours. 1x TAE buffer and SYBR® Gold nucleic acid stain were mixed in a tray, the gel was added and placed on a darkened rotator table for 20 minutes. The gel was rinsed with deionized water and was visualized and imaged under UV light with an AlphaImager® HP imager.

## DGGE Quantitative and Qualitative Analysis

Digital images were analyzed in GelCompar II somware (Applied Maths, Belgium). Each image was inverted and enhanced by adjusting the contrast. Individual genomic sequences were signified by their varying positions and intensities in the lanes. Different bands at varying positions were taken to a separate species. Aligning reference ladders on the outside lanes of each gel normalized band positions in consecutive lanes. Visual and statistical methods were used to identify bands present in the gels. A profile of the number and intensity of the bands was produced for each lane in the form of densitometric curves. Jaccard similarity coefficients were calculated and used for comparing the similarity and diversity of the samples. Peak intensities were assessed statistically by taking the ten values proceeding and following it and calculating the average intensity ± 1 times the standard deviation. It was identified as a peak if the resulting value exceeded that number. The Shannon-Weaver diversity index and the Species Richness values were calculated for all gels (Washington, 1984).

## DNA Cloning Methods

Five samples were selected for cloning reactions. Cloning of the PCR products was accomplished using a TOPO® TA Cloning® Kit from Invitrogen. The recombinant vector was transformed into One Shot® Top 10 Chemically Competent *E. Coli* cells in agar plates containing LB Media and ampicillin. Positive and negative controls were incorporated throughout the cloning process. Plates were incubated for 4 days at 37 °C. Autoclaved toothpicks were used to transfer single colonies into 96-well plates containing LB media and ampicillin. The 96-well plates containing the transformants were incubated until cloudy and then stored in the refrigerator. Plasmids were extracted

from each well using Invitrogen Purelink™ Quick Plasmid Mini Prep Kits. An Eon™ Microplate Spectrophotometer from BioTek® was used to quantify the DNA concentration before samples were sent off for sequencing.

## DNA Sequencing and Phylogenetic Tree Construction

Oil field microbiology was characterized by sequencing of 16S rRNA genes. Five samples using 96 clones were sent to MacrogenUSA in Maryland for sequencing using M13 universal sequencing primers for the bacterial samples and ARC314F for the archaeal samples. The resulting sequences for each sample were trimmed to approximately 500 base pairs in 4Peaks (*nucleobytes.com/**4peaks***). The trimmed sequences for each sample were then checked for duplicates and aligned in Geneious (http://www.geneious.com/). The aligned sequences were exported to Grenegenes (DeSantis et al, 2006) and aligned for a second time and chimera checked. Aligned sequences were then classified in Greengenes to determine their closest phylogenetic relatives. Sequences were aligned to their nearest neighbor in Geneious. Phylogenetic trees were constructed using the Tamura-Nei genetic distance model and the neighbor-joining tree building method. Bootstrap analysis with 100 replicates was applied to assign confidence levels to the nodes in the trees.

# Chapter 4: Results

## Aqueous Geochemistry of Production Fluids, Injection Well and Drill Stem Test

### *Major element geochemistry*

Variations in selected aqueous geochemical and physical parameters in producing wells, the injection well, and the drill stem test are reported in Table 4.1. Complete reports of aqueous geochemistry results can be found in Appendix D. Sampling of the injection well and production wells occurred in August and October 2010 and May and November 2011. The drill stem sample was collected in January 2010. Anion analysis was not run in August and October 2010, therefore data from those trips are not included. Differences in major element values between May and November 2011 trips range up to 12 percent with the highest occurring in magnesium concentration in well 11 (68 percent). Fluoride, nitrate, phosphate, and sulfide were not measured in May 2011. Data presented in this section is from November 2011 when a more complete chemical

| Sample I.D. | Depth (m) | pH | Temp (°C) | Salinity (ppm) | Ionic Strength | Total Carbon (mmol $L^{-1}$) | DOC (mmol $L^{-1}$) | DIC (mmol $L^{-1}$) | Alkalinity (mmol $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| 73 | 1119 | 5.64 | 32.3 | $2.07x10^5$ | 4.19 | 0.42 | 0.31 | 0.11 | 0.95 |
| DST | 1121 | 5.88 | 42.1 | $1.86x10^5$ | 3.65 | 2.66 | 1.70 | 0.96 | 1.34 |
| IW | 1122 | 5.84 | 28.2 | $1.98x10^5$ | 3.83 | 1.04 | 0.81 | 0.23 | 1.12 |
| 53 | 1126 | 5.45 | 31.1 | $2.04x10^5$ | 4.02 | 0.78 | 0.29 | 0.48 | 1.03 |
| 69 | 1127 | 5.98 | 21.5 | $1.88x10^5$ | 3.76 | 1.96 | 1.54 | 0.42 | 1.56 |
| 60 | 1128 | 5.48 | 23.3 | $1.94x10^5$ | 3.77 | 0.90 | 0.52 | 0.38 | 1.05 |
| 11 | 1129 | 6.35 | 25.8 | $1.97x10^5$ | 3.79 | 1.02 | 0.43 | 0.59 | 0.99 |

**Table 4.1. Aqueous geochemical parameters of production wells, injection well (IW), and drill stem test (DST). DOC-dissolved organic carbon, DIC-dissolved inorganic carbon.**

analysis was conducted. Field pH ranges from 5.45 and 6.35 and field temperature varies from 21.5°C to 42.1°C between wells. Salinity is fairly consistent ranging from 186,000 and 200,700 ppm. Ionic strength, total carbon, dissolved organic carbon, dissolved inorganic carbon, and alkalinity results indicate small to moderate variations among the samples. Charge balance ranges from two to 8 percent and was calculated by dividing the difference between cations and anions by the sum of the cations and anions. The dissolved major cation aqueous geochemistry is shown plotted as a function of depth in figure 4.1. When the standard deviation is taken into account significant variations in concentration among samples are generally not present. Ca to Mg molar ratios are generally consistent among sample wells (2.25-3.45 mmol $L^{-1}$) with the exception of well 11 (5.31 mmol $L^{-1}$). The injection well and DST concentrations are within the ranges of the producing well concentrations. Silica concentrations tend to increase with increasing depth. Silica was not measured for the drill stem test. Geochemical data points from wells 73 and 11 tend to have values outside of the cluster of data for Ca/Mg and Si in figure 4.1. Magnesium concentrations are highest in well 73 and lowest in well 11. Additionally, silica concentrations are lowest in well 73 and nearly highest in well 11. Well 73 potassium concentrations are the lowest out of all samples. Complete reports of aqueous geochemistry results can be found in Appendix D.

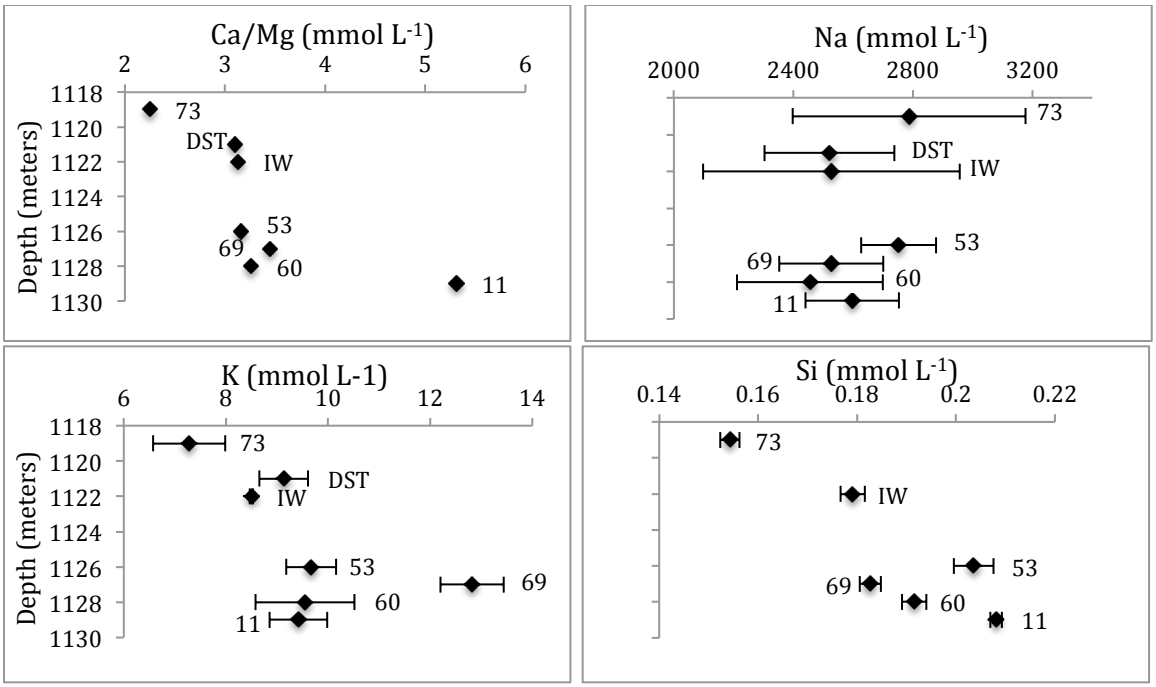



**Figure 4.1. Results of major cation concentrations in mmol $L^{-1}$ plotted against depth. Significant variations in concentrations are generally not present. Silica increases with depth and was not recorded for the drill stem test. DST-drill stem test and IW-injection well.**

## *Saturation indices of oilfield minerals in aqueous solution*

PHREEQC 3.0 using the Pitzer database, was used to calculate saturation indices of oilfield minerals (Parkhurst et al., 1988) (Table 4.2) The downhole temperature (42.1°C) collected during the drill stem test was used for all wells. Results indicate that the oilfield brine is supersaturated with respect to aragonite, calcite, and dolomite in the injection well, drill stem test, and wells 11 and 69. Well 73 is supersaturated with respect to calcite and dolomite and undersaturated with respect to aragonite. Well 53 is undersaturated with respect to aragonite and calcite. Well 60 is undersaturated with respect to aragonite, calcite, and dolomite. All sample wells are undersaturated with respect to anhydrite.

| Sample I.D. | Depth (m) | SI (aragonite) | SI (anhydrite) | SI (calcite) | SI (dolomite) |
|---|---|---|---|---|---|
| 73 | 1119 | -0.01 | -0.27 | 0.17 | 0.54 |
| DST | 1121 | 0.20 | -0.13 | 0.37 | 0.78 |
| Injection Well | 1122 | 0.21 | -0.10 | 0.38 | 0.82 |
| 53 | 1126 | -0.18 | -0.06 | -0.01 | 0.02 |
| 69 | 1127 | 0.46 | -0.13 | 0.63 | 1.26 |
| 60 | 1128 | -0.20 | -0.11 | -0.03 | -0.02 |
| 11 | 1129 | 0.62 | -0.08 | 0.80 | 1.61 |

**Table 4.2. Saturation indices reported from sampled wells. DST-drill stem test.**.

## *Biogeochemistry of production fluids*

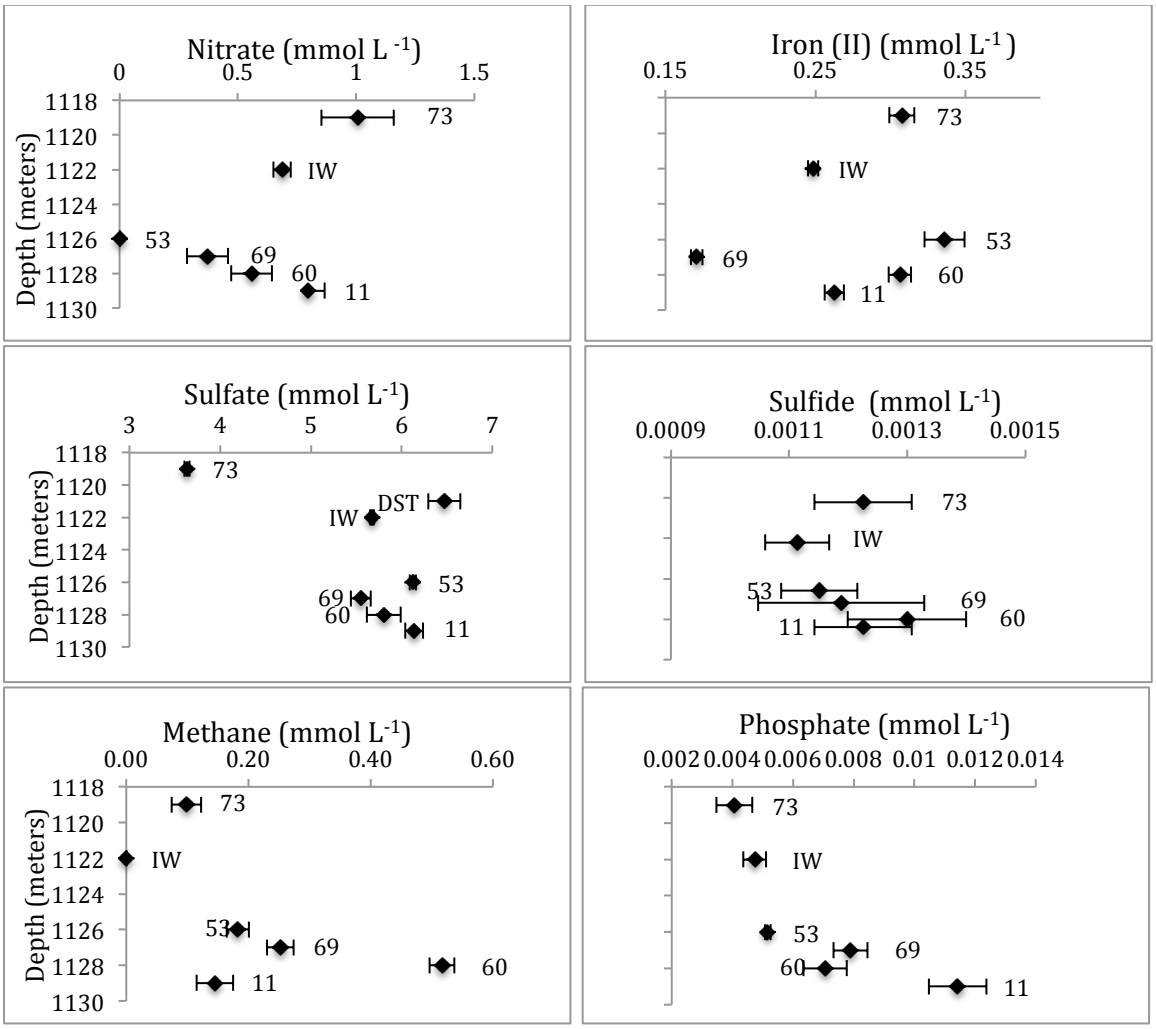



**Figure 4.2. Biogeochemistry plotted with depth from producing wells and injection well. These species were not measured in the drill stem test. Nitrate tends to decrease with depth while Fe(II), methane, and phosphate generally increase with depth. Concentrations in mmol $L^{-1}$. IW-injection well.**

Nutrients, electron donors and electron acceptor concentrations are plotted against depth in figure 4.2. Nitrate ranges from approximately 1 mmol $L^{-1}$ in well 73 and decreasing to below detection ($2x10^{-4}$ mmol $L^{-1}$) in well 53. Fe (II) concentrations range from approximately $1.7x10^{-1}$ to $3.4x10^{-1}$ mmol $L^{-1}$. Sulfate and sulfide show no real trend with depth and ranges from 3.64 to 6.47 mmol $L^{-1}$and $1.1x10^{-3}$ and $1.3x10^{-3}$ mmol $L^{-1}$ respectively. There is a general increase in methane with depth and concentrations range from below detection to $5.2x10^{-1}$. Phosphate ranges from $4.0x10^{-3}$ to $1.1x10^{-2}$ mmol $L^{-1}$ and generally increases with depth.

## Microbiology of Wellington Oil Reservoir

### *Most Probable Number Analysis-Cultured Reservoir Microbes*

Microorganisms that were successfully cultured in the lab from the Wellington oil field are represented by their relative percent abundance in figure 4.3. The only successfully cultured microorganisms from the drill stem sample were anaerobic fermentative bacteria. Iron reducing bacteria were detected in small abundances from the injection well and wells 69 and 60 and ranged from $1.2 \times 10^2$ to $4.6 \times 10^2$ cells $ml^{-1}$. Methanogens were successfully cultured from wells 73, 69, 60, and 11 and ranged from $1.2 \times 10^2$ to $4.6 \times 10^2$ cells $ml^{-1}$. Aerobic and anaerobic fermenting bacteria make up the largest percentages of the community structure in all samples and range from $2.4 \times 10^3$ to $4.6 \times 10^4$ cells $ml^{-1}$.

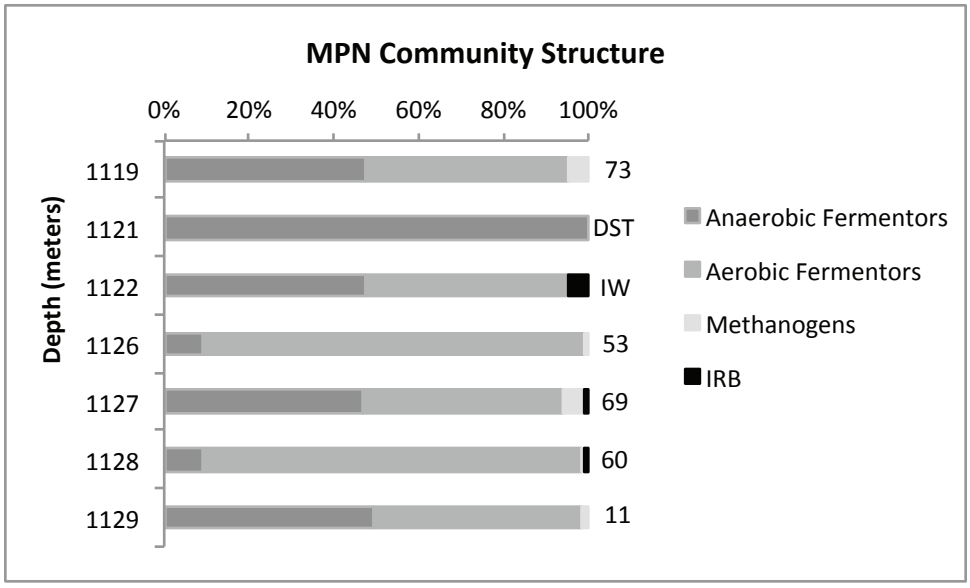



**Figure 4.3. Most probable number community structure results plotted versus depth. Relative percentages of lab cultured field microorganisms represented in shades of gray and black. DST-drill stem test and IW-injection well.**

## *Microbial Biomass and Oil Production*

Biomass concentrations and percent oil production measured from the sample wells are displayed in bar graph form in figure 4.4. Sampled production wells produce approximately one to six percent oil and tends to be fairly stable from month to month based on monthly barrel tests (Appendix E; pers. Comm. Berexco LLC). Biomass concentrations range from $1.10x10^5$ to $1.41x10^6$ cells $ml^{-1}$. Results indicate that wells that produce higher amounts of oil are associated with lower concentrations of biomass. Conversely, wells that produce lower amounts of oil are associated with higher concentrations of biomass. Figure 4.5 displays the same data set graphed using a

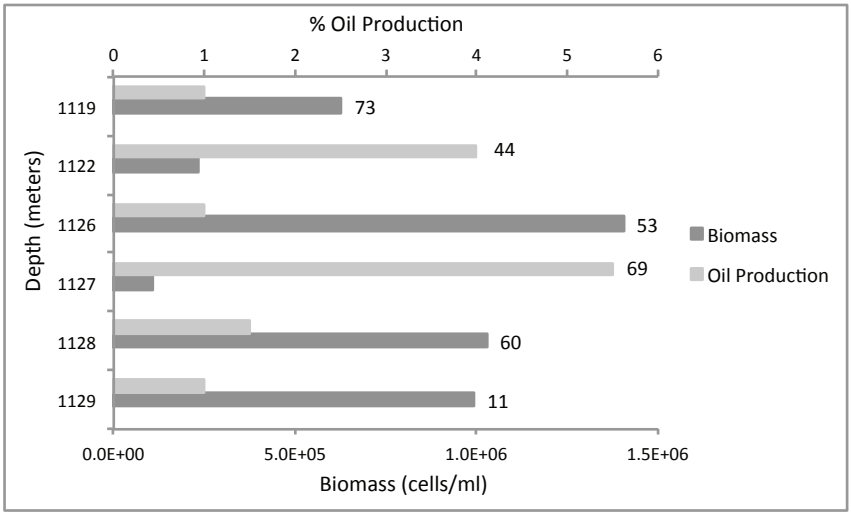



**Figure 4.4. Bar graph representing biomass concentrations and percent oil production from sampled wells. Higher concentrations of biomass are associated with lower producing wells and lower concentrations of biomass are found in higher producing wells.**

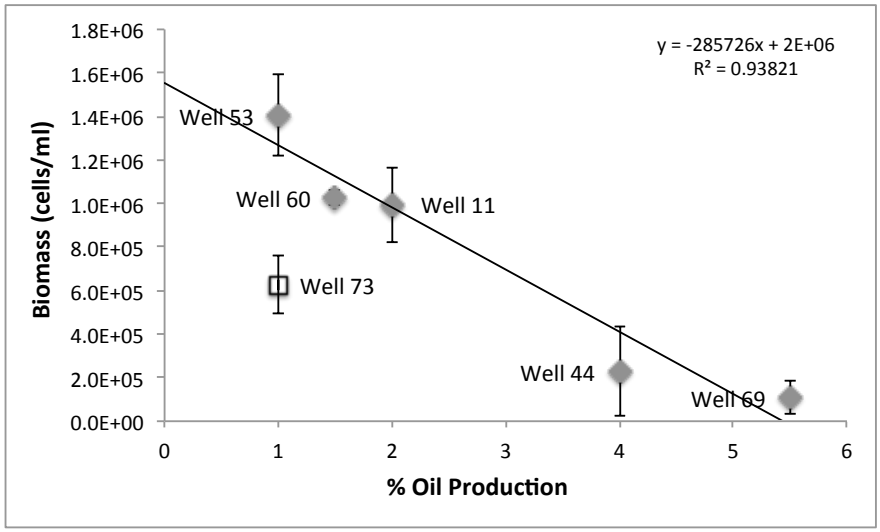



**Figure 4.5. Linear regression analysis of biomass concentration and percent oil production from sampled wells. Well 73 has not been included in the regression analysis. This plot suggests that a strong relationship exists between oil production and biomass.**

regression analysis to estimate the relationship between biomass concentration and percent oil production. Well 73 is not factored into the regression analysis and is plotted only as a solitary point. Well 73 appears to be an outlier in more than one measurement in this study and this issue will be addressed in the Discussion section.

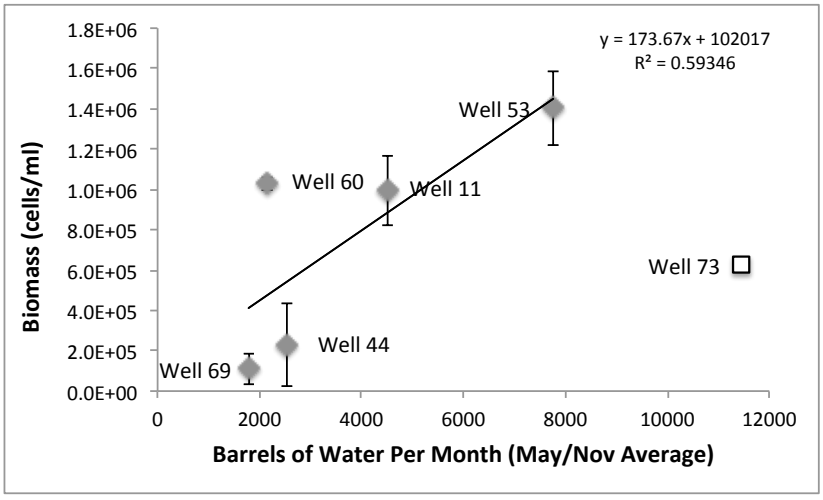



**Figure 4.6. Linear regression analysis of water production versus biomass concentration. This suggests that biomass concentration and water production increase together. Well 73 has not been included in the regression analysis.**

Figure 4.6 displays the relationship between biomass and barrels of water produced per month. If there is a relationship between biomass concentration and oil production as demonstrated in figure 4.5, then there should also exist a relationship between biomass concentration and water production from each well. Well 69 produces the lowest amount of water out of all samples and has the lowest biomass concentration. Alternatively, Well 53 produces the most water and has the highest biomass concentration. This trend is the opposite of the relationship of biomass concentration versus oil production.

## *Surface Tension Measurements on Raw Production Fluid*

Surface tension measurements are plotted versus biomass concentration in figure 4.7. Surface tensions of the samples range from approximately 64.6 dynes $cm^{-1}$ in well 60 to 53.9 dynes $cm^{-1}$ in well 53. The surface tension of pure water at room temperature (~20°C) is approximately 72 dynes $cm^{-1}$. The injection well and well 73 were not

included in the regression analysis.

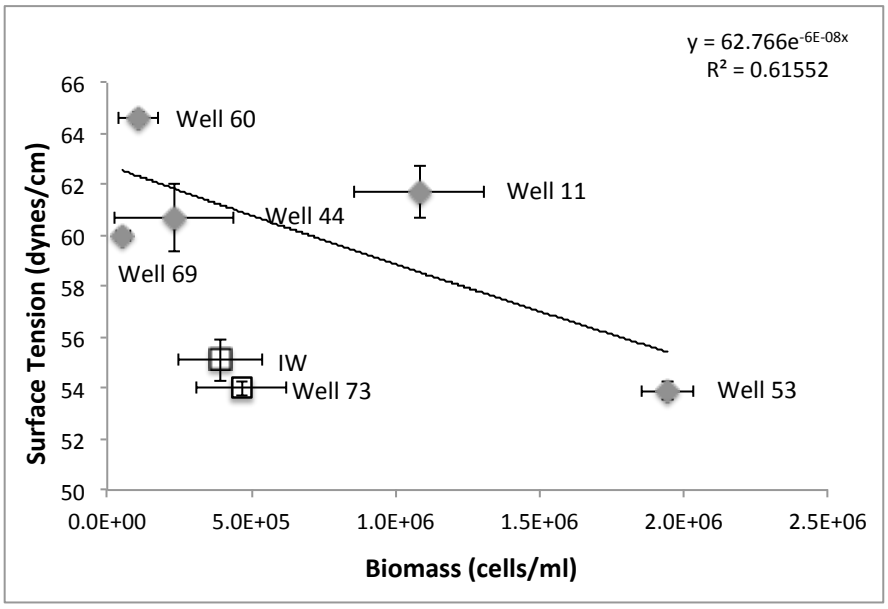



**Figure 4.7. Graph of surface tension versus biomass concentration. This trend may suggest that surface tension is lowest where biomass concentrations are highest. IW-injection well.**

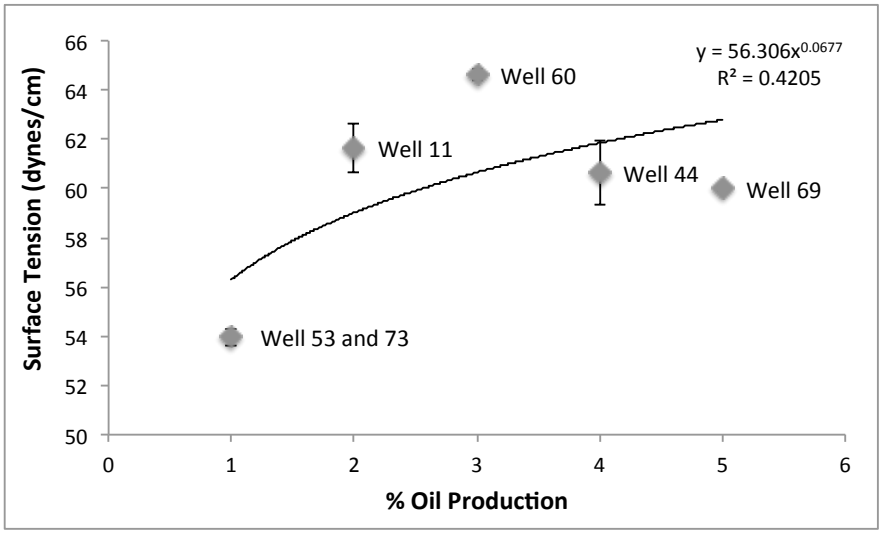



**Figure 4.8. Graph of surface tension versus oil production. Wells 53 and 73 have the lowest surface tensions and percent oil production. The highest surface tensions are found in the higher producing wells. Surface tension and oil production generally increase together and then level out.**

Surface tension versus percent oil production is plotted in Figure 4.8. Well 53 has the lowest surface tension and oil production. As oil production increases, surface tension

follows a similar trend before reaching a maximum with higher oil production rates.

## Community Diversity of Oilfield Microorganisms

Samples from producing wells, an injection well and a drill stem test were analyzed using DGGE and DNA sequencing. Table 4.3 lists all samples and the DNA analysis performed on them. DGGE analysis shows the presence of many distinguishable bands in the separation pattern, which represent the bacterial species that most likely comprise the microbial populations (Fig. 4.9). Each separate band in a single lane signifies a unique species while bands that are in the same position in consecutive lanes represents shared species amongst the samples. The relative intensity of each band signifies the relative abundance of that particular species. Figure 4.9A shows the DGGE gel comparing the

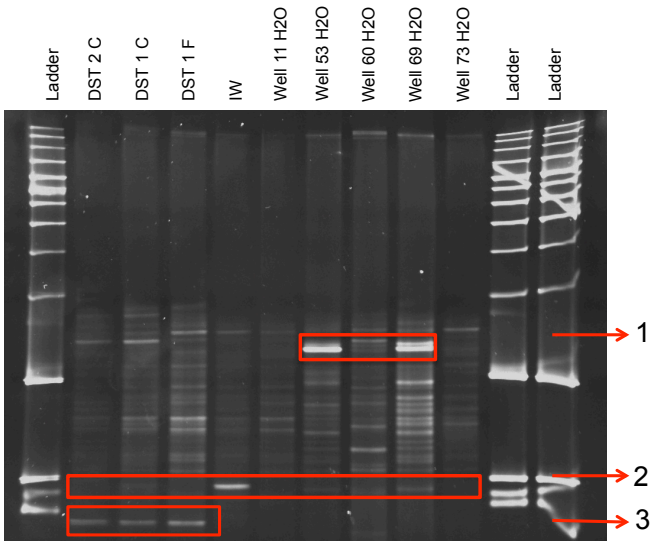
| | | Analysis | |
|---|---|---|---|
| **Sample** | **Depth (m)** | **DGGE** | **DNA** |
| IW | 1122 | X | X |
| DST | 1121 | X | X |
| Well 11 $H_2O$ | 1129 | X | |
| Well 11 Oil | 1129 | X | |
| Well 53 $H_2O$ | 1126 | X | |
| Well 53 Oil | 1126 | X | |
| Well 60 $H_2O$ | 1128 | X | X |
| Well 60 Oil | 1128 | X | X |
| Well 69 $H_2O$ | 1127 | X | |
| Well 69 Oil | 1127 | X | |
| Well 73 $H_2O$ | 1119 | X | |
| Well 73 Oil | 1119 | X | |

**Table 4.3. Sample wells and analytical procedure list. Not every sample was analyzed for DNA. Well 60 water phase was analyzed using both bacterial and archaeal primers. H2O-water phase, Oil-oil phase, IW-injection well, DST-drill stem test.**

microbial communities between the injection well and producing wells. The drill stem

test is also included to compare to all samples. The number of bands present in each sample suggests that the microbial communities are fairly diverse, however similarities in band positioning between samples signifies that these species are present in the majority of the samples. Higher intensity bands are highlighted and represent a higher abundance of species in a particular sample. The drill stem test shows a band near the bottom of the lane not common in the other samples (highlighted by red box). Microbial communities in the injection well are fairly similar to those present in the producing wells, yet there is a band present in figure 4.9A (highlighted by red box) that does not appear to be in the same position as any other band. Figure 4.9B compares the microbial communities of the oil and water phases collected from the producing wells. Again, there are several shared bands between the oil and water phases but higher microbial abundances are highlighted by the red box and are also represented by the higher intensity bands. Well 53 oil has the most intense bands and shares one of those with its water phase (lower red box) as does well 60 (upper red box).

A.




B.

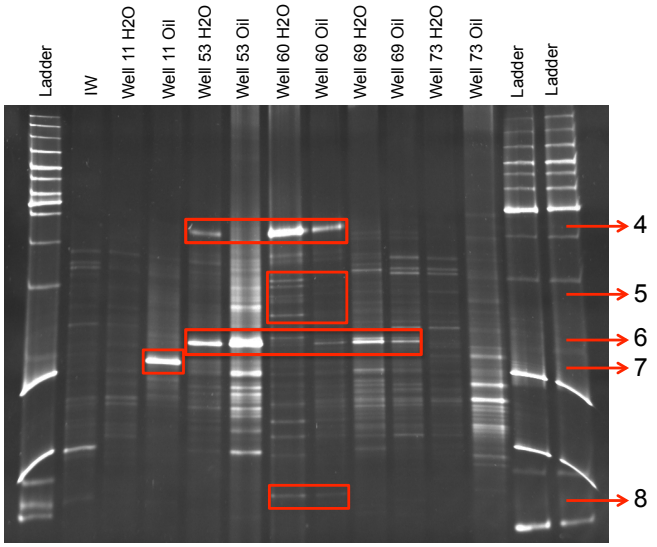



**Figure 4.9. Analysis of DGGE profile showing separation patterns of PCR products. A. Comparison of the injection well (IW) to water from producing wells and the drill stem test (DST 2C, 1C, 1F; DST C-drill stem test raw sample centrifuged in lab to collect pellet containing DNA, DST F-drill stem test raw sample filtered in lab to collect DNA). While all samples are fairly diverse, similarities in band positioning are evident between all wells, suggesting fairly similar microbial communities. Box 1 demonstrates higher intensity bands, 2 of which are shared by wells 53 and 69 indicating higher abundance of that species. Box 2 demonstrates that the injection well has a higher abundance of one species but it is shared by most samples. Box 3 shows a DST band not shared with other samples. B. Comparison of the water and oil phases. Wells 53 $H_2O$ and 60 oil and water phases share one fairly intense band (box 4), as does well 53 oil and water phases (box 6). Box 5 shows uncommon bands between well 60 water and oil phases. Well 11 oil (box 7) has a very intense band when compared to the other samples, suggesting a higher abundance of that species. Box 8 is an example of shared species between oil and water phases. Well 53 oil has a higher abundance of shared species than all other samples. $H^2O$-water phase.**

## Microbial Community Diversity as a Function of Distance from the Injection Well

DGGE gels were analyzed for similarity, diversity, and species richness among the microbial assemblages in each sample. One aspect of this study is to investigate the

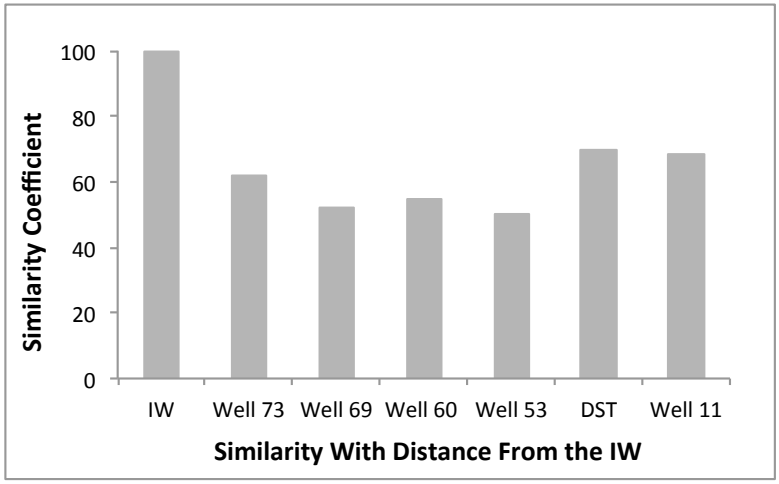



**Figure 4.10. Jaccard similarity coefficients plotted with distance away from the injection well. Results from samples indicate similarity to the injection well. IW-injection well, DST-drill stem test.**

spatial impact of the injection well on the microbiology of the producing wells and the drill stem test. To accomplish this, Jaccard similarity coefficients were generated to assess the similarities and difference amongst samples and in particular, for comparison of all samples to the injection well. Figure 4.10 demonstrates the similarity of the production wells and drill stem test when compared to the injection well. The similarity coefficient of the injection well is 100% because it is being compared to itself. Well 73 is approximately 61% similar to the injection well and well 11 and the drill stem test are approximately 70% similar to the injection well. Similarity amongst samples generally decreases with distance away from the injection well.

Similarity coefficients were plotted with depth in figure 4.11. Wells 73 (1119 meters) and 11 (1129 meters) and the drill stem test share more similarities with the injection well than do the other samples. Wells deeper than the injection well generally are less similar to the injection well, with the exception of well 11.

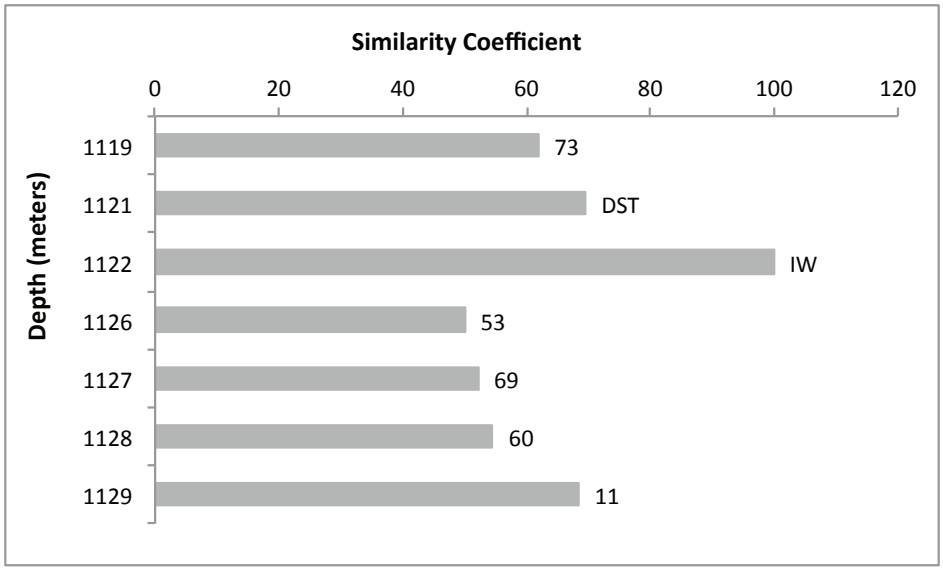



**Figure 4.11. Similarity coefficients plotted with depth. Similarity results are generated from comparing all samples to the injection well.**

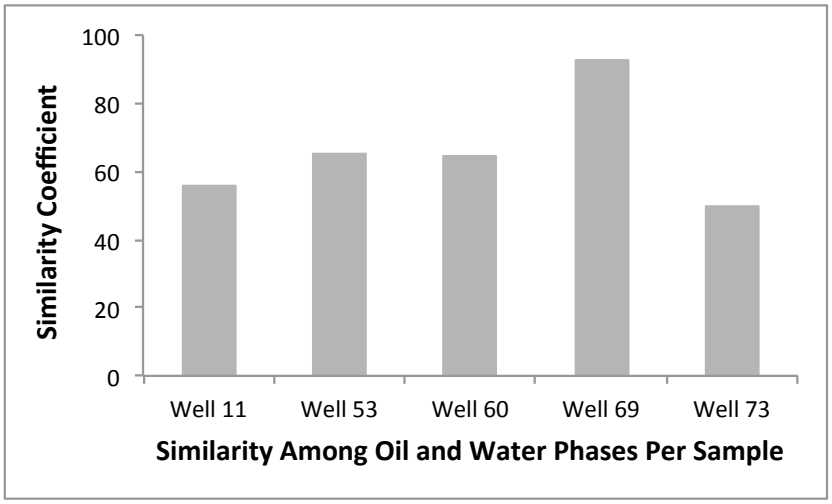



**Figure 4.12. Jaccard similarity coefficients between oil and water phases in each sample. The oil and water phases in well 60 share the most similarities in microbial communities while well 73 share the least.**

Another goal of the present study was to determine differences in the microbiology of water and oil phases. Figure 4.12 illustrates how similar the microbial communities are in the oil and water phases in each particular sample. Results indicate that the largest similarities exist between the two phases in well 69, while well 73 harbors the least similar microbial communities between phases.

## Microbial Community Diversity and Species Richness

The Shannon-Weaver index is widely used to determine species diversity and richness between samples (Shannon, 1948; Pratt et al., 2012). If only a few species account for most of the biomass than the index is low. Higher index values signify a more diverse microbial community (Peet, 1974). Typical values range from zero to ~ 4.6 (Cheyne, 2006). A result of zero indicates that every species in the sample is the same, whereby a value of 4.6 suggests that the number of individuals is distributed evenly between all species (Cheyne, 2006). Species richness values indicate the number of different species detected in a sample. Results from the water phase are illustrated in

| Sample | Shannon Index | Species Richness |
|---|---|---|
| IW | 1.16 | 15 |
| Well 53 $H_2O$ | 1.14 | 14 |
| Well 69 $H_2O$ | 1.14 | 14 |
| DST | 1.11 | 13 |
| Well 11 $H_2O$ | 1.08 | 12 |
| Well 73 $H_2O$ | 1.08 | 12 |
| Well 60 $H_2O$ | 1.04 | 11 |

**Table 4.4. Shannon Weaver diversity index and species richness values for water phases. Low values indicate low diversity amongst samples. The injection well waters have the highest species richness value but is followed closely by wells 53 and 69 and the drill stem test. DST-drill stem test, IW-injection well.**

table 4.4. The injection well harbors the most diverse and richest microbial communities. However, the Shannon index values for all samples are fairly similar suggesting each have a rather low diversity of species in terms of abundance. More species were detected in the injection well, followed by wells 53 and 69 and the drill stem test.

Shannon Weaver index and species richness values for oil and water phases are listed in table 4.5. Well 73 oil has the largest diversity and species richness when compared to all samples. Additionally, the difference between diversity and species richness from oil and water phases is largest in well 73.

| Sample | Depth (meters) | Shannon Index | Species Richness |
|---|---|---|---|
| Well 73 $H_2O$ | 1119 | 1.08 | 12 |
| Well 73 Oil | 1119 | 1.19 | 17 |
| Well 53 $H_2O$ | 1126 | 1.14 | 15 |
| Well 53 Oil | 1126 | 1.16 | 15 |
| Well 69 $H_2O$ | 1127 | 1.11 | 14 |
| Well 69 Oil | 1127 | 1.19 | 16 |
| Well 60 $H_2O$ | 1128 | 1.14 | 15 |
| Well 60 Oil | 1128 | 1.09 | 13 |
| Well 11 $H_2O$ | 1129 | 1.08 | 12 |
| Well 11 Oil | 1129 | 1.07 | 13 |

**Table 4.5. Shannon Weaver diversity index and species richness values for water and oil phases. Diversity indices indicate a similarly low diversity in all samples. Well 73 exhibits the largest species richness gap between oil and water phases.**

## Phylogeny of Oilfield Microbes

## Statistical Analysis of the 16S rRNA Gene Clone Libraries

Four bacterial and one archaeal clone library were generated from sequencing the 16S rRNA gene. For comparison of microbial ecology, the injection well, drill stem test, and well 60 water and oil phases were sequenced using bacterial primers. The injection

well, and well 60 water and oil phases produced a positive PCR product using archaeal primers, however only well 60 water phase was sequenced.

A total of 301 bacterial clones were sequenced (Table 4.6). 299 sequences were lem amer removing chimeras. Percent coverage of the four clone libraries was calculated from dividing 299 sequences into 51 operational taxonomic units (OTU's). The coverage ranged from 89.6% to 97.9%. These results indicate that the majority of the 16S rRNA gene sequences represent the majority of the bacterial community of the samples. A total of 89 archaeal clones were sequenced and five chimeras were removed. 84 archaeal sequences were divided into two OTU's and the resulting coverage indicates that 100%

| | **Bacterial library** | | | | **Archaeal library** |
|---|---|---|---|---|---|
| **Library Name** | **Well 60 $H_2O$** | **Well 60 Oil** | **DST** | **IW** | **Well 60 $H_2O$** |
| Number of raw sequences | 94 | 68 | 68 | 71 | 89 |
| Number of Chimeras | 0 | 1 | 0 | 1 | 5 |
| Number of analyzed sequences | 94 | 67 | 68 | 70 | 84 |
| Coverage (%) [a] | 97.9 | 89.6 | 89.7 | 85.7 | 100 |
| OTU number | 8 | 15 | 11 | 17 | 2 |

**Table 4.6. Statistical analysis of 16SrRNA gene clone libraries from oil field samples. Operational taxonomic units (OTU's) are listed for sample comparison. [a] $C_x=(1-(n_x/N)) \times 100$, where $n_x$ is the number of singletons that appear only once in a clone library and N is the total number of clones (Good, 1953). DST-drill stem test, IW-injection well.**

of the 16S rRNA gene sequence for the archaeal community in this sample is represented. This result suggests that archaeal diversity in this producing well is extremely low.

## Microbial Ecology of the Injection Well, Drill Stem Test and Production Wells-Phylogenetic Tree Analysis

Figures 4.13, 4.14, 4.15, 4.16 and 4.17 show the results of the phylogenetic tree

analysis generated from 16S rRNA gene sequencing. Four clone libraries were targeting the bacterial communities of the injection well, the drill stem test and well 60 oil and water phases. One clone library was targeting the archaeal community of well 60 water results illustrate the genetic relatedness of this sample to halophilic sulfate reducers. Well 60 water phase (Fig. 4.13) was dominated by two distinct phyla: Proteobacteria (82%) and Firmicutes (18%). At the genus level, 5% of the sequences could not be assigned to known genera. The compositions of the known genera in the sample are: Desulfonatronospira (44%), Desulfovermiculus (18%), Desulfosalina (12%) Halanaerobium (12%), Desulfovibrio (6%), and Desulfohalobium (3%).

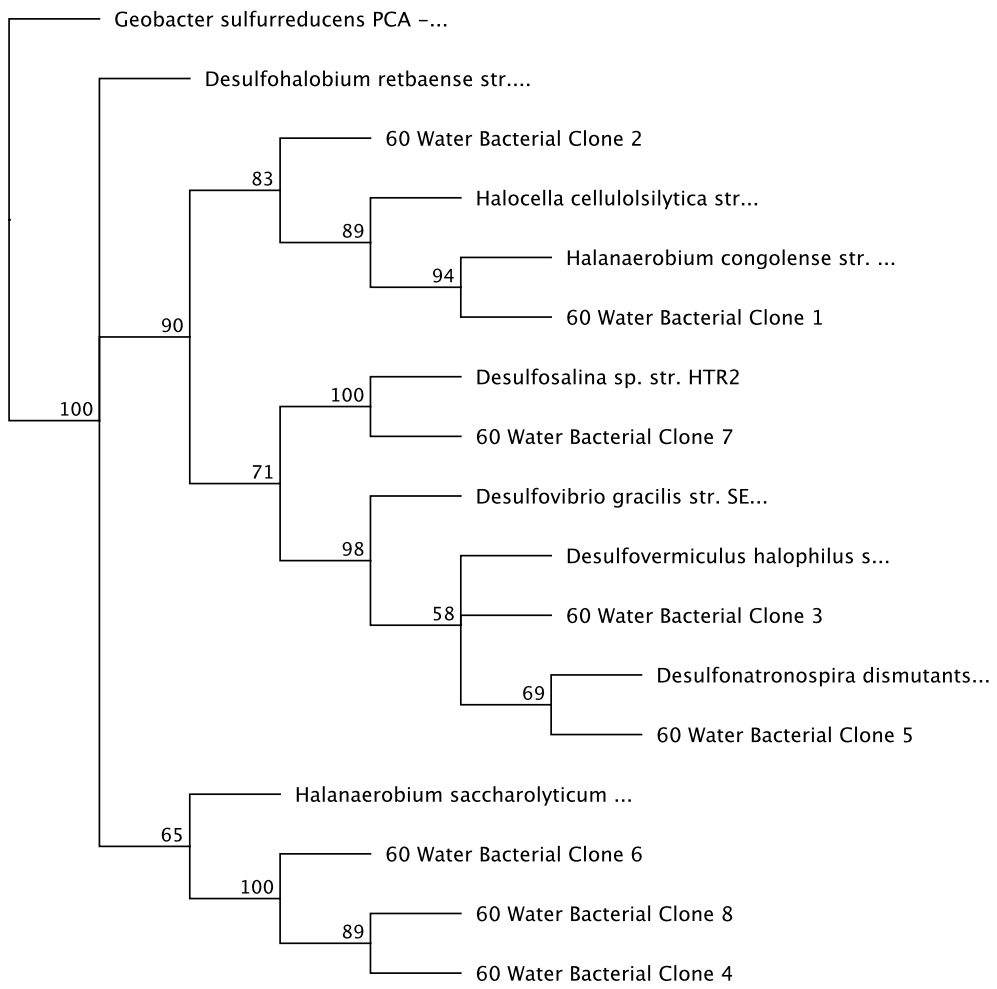



**Figure 4.13. Phylogenetic tree of the 16*S* rRNA gene-based library obtained from well 60 $H_2O$ phase using the neighbor-joining method. Numbers on nodes represent bootstrap analyses performed with 100 repetitions and only values higher than 50% are shown. The clones are mostly related to halophilic sulfate reducers. *Geobacter sulfurreducens* is the outgroup.**

Phylogenetic analysis of well 60 oil phase (Fig. 4.14) resulted in sequences of three phyla; Proteobacteria (86%), Firmicutes (10%) and Acidobacteria (4%). At the genus level 17% of the sequences could not be assigned to known genera. The dominant genera were Desulfonatronospira (36%), followed by Alkanindiges (13%), Halomonas (6%), Alicyclobacillus (6%), Psedoxanthomonas (6%), Sphingomonas (4%), Haloanaerobium (4%), Desulfovibrio (2%), Methylobacterium (2%), and Desulfosalina (2%). This phylogenetic tree shows the interrelatedness of a clade of clones that are the closest

related to *Alkanindiges illinoisensis*, which has been isolated from oil field soils and described as hydrocarbonoclastic (oil degrading; Bogan et al, 2003).

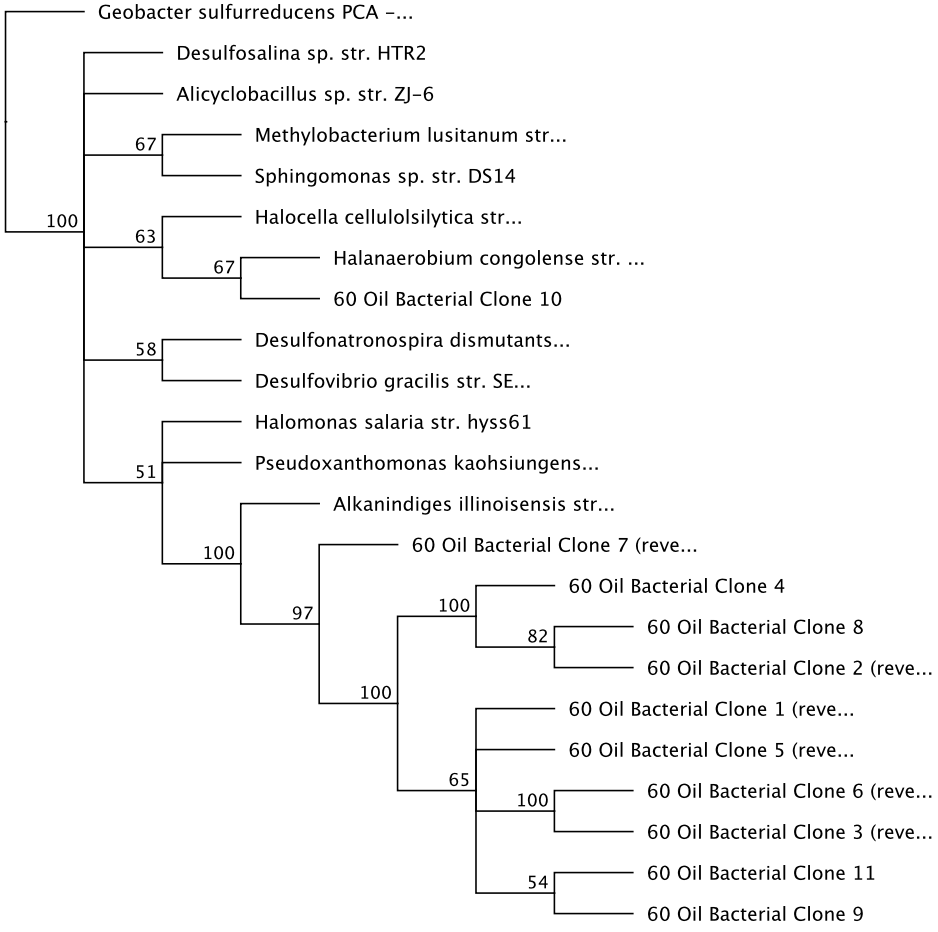



**Figure 4.14. Phylogenetic tree of the 16S rRNA gene-based library obtained from well 60 oil phase (96 clones) using the neighbor-joining method. The majority of these clones are interrelated and les evolutionarily related to the nearest neighbors in the tree. Geobacter *sulfurreducens* is the outgroup**

The phylogenetic tree constructed for well 60 oil phase, specifically targeting the archaeal community, is illustrated in figure 4.15. Only two OTUs (operational taxonomic units) were identified indicating that the archaeal community in this sample lacks diversity. The dominant genera in this sample are Methanohalophilus (72%) and Methanolobus (28%).

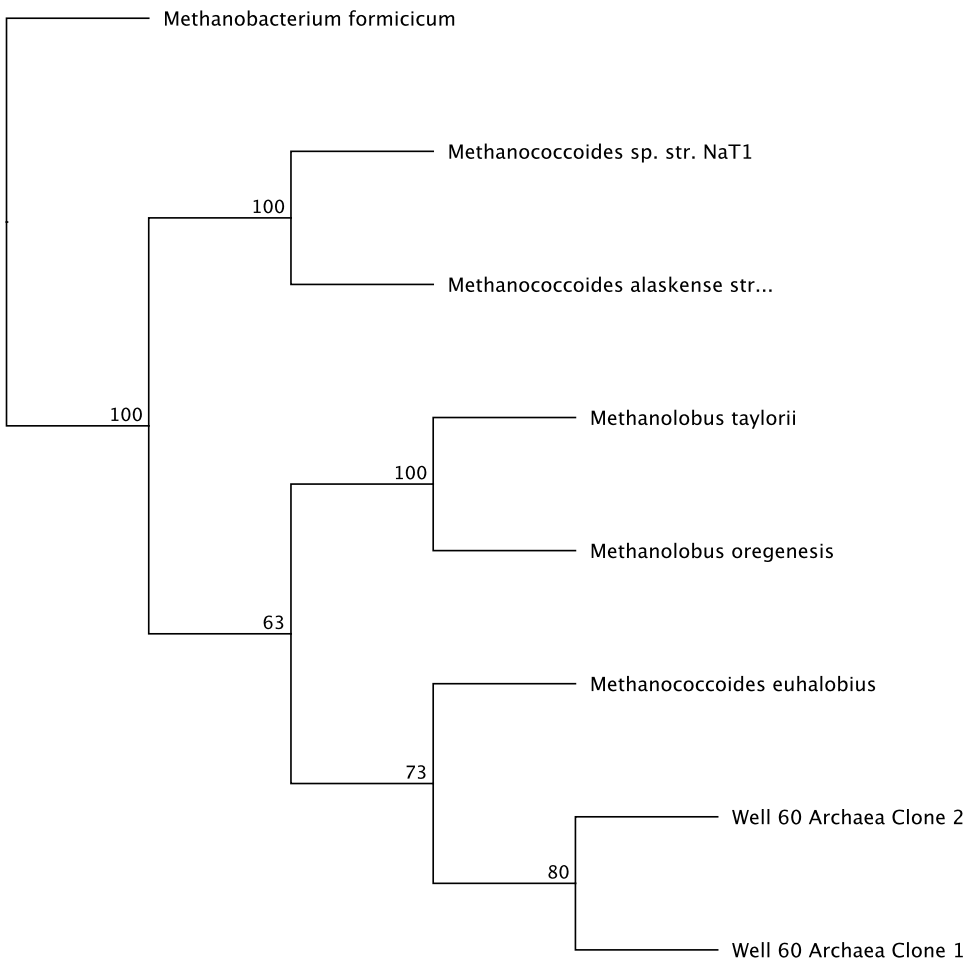



**Figure 4.15. Phylogenetic tree of the 16S rRNA gene-based library obtained from well 60 water phase archaea using the neighbor-joining method. These clones are related to methanogens. *Methanobacterium formicicum* is the outgroup.**

Phylogenetic analysis of the drill stem test (Fig. 4.16) identified sequences from five phyla: Actinobacteria (65%), Firmicutes (15%), Proteobacteria (12%), Bacteroidetes (4%) and Chloroflexi (4%). Only 6% of these sequences were not identified at the genus level and originate from the Chloroflexi and Bacteroidetes phyla. The genera in this sample include: Xylanimicrobium (62%), Anaerobacillus (15%), Acinetobacter (8%), Isoptericola (4%), Spirosoma (2%), Diaphorobacter (2%) and Aquabacterium (2%).

Figure 12 illustrates that the clade of clones two, three and six are more closely related to each other. Clones one, four and five are closely related to Diaphorobacter and Xylanibacterium, which have been isolated as a denitrifying, ammonia oxidizer and a bacterium recovered from an elm tree (Hiraichi et al, 2005; Kroppenstedt et al, 2004).

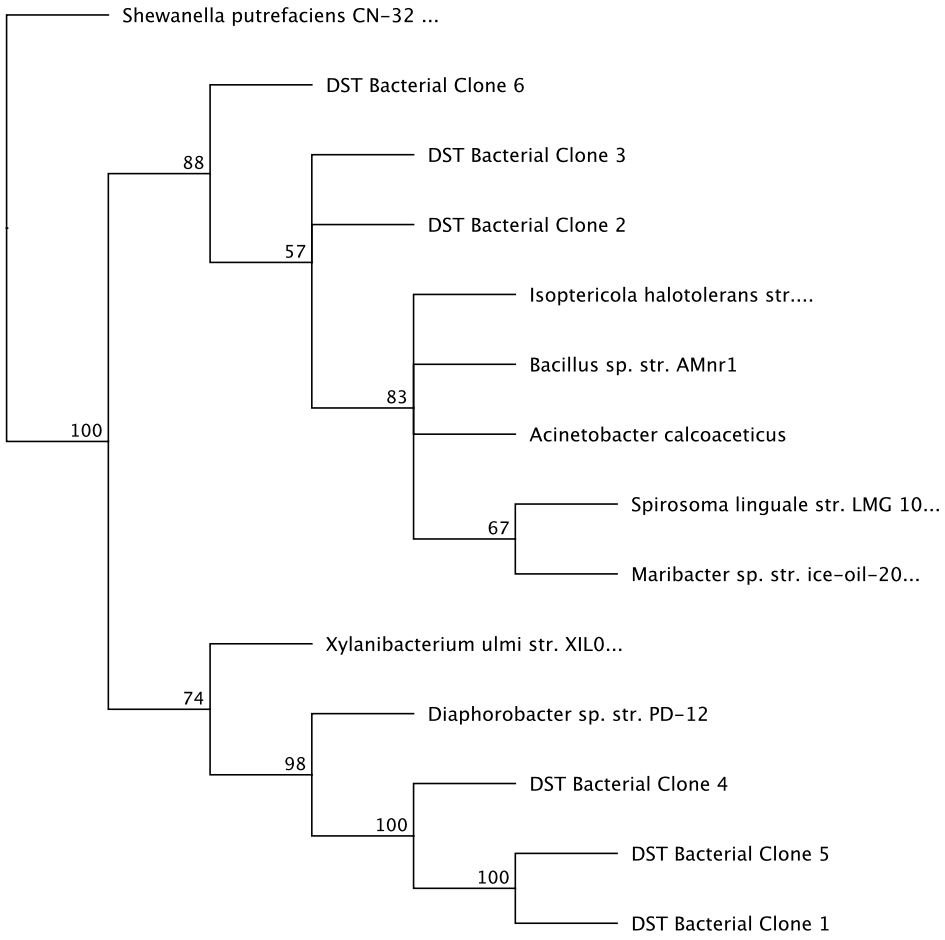



**Figure 4.16. Phylogenetic tree of the 16S rRNA gene-based library obtained from the drill stem test using the neighbor joining method. The clades of clones are generally less related to their nearest evolutionary neighbors.**

The phylogenetic tree constructed for the injection well (Fig. 4.17) demonstrates the diversity of the sample. The sequences are distributed among three phyla but are dominated by Proteobacteria (80%), followed by Firmicutes (16%) and Acidobacteria (4%). At the genus level, 10% of the sequences could not be assigned to known genera.

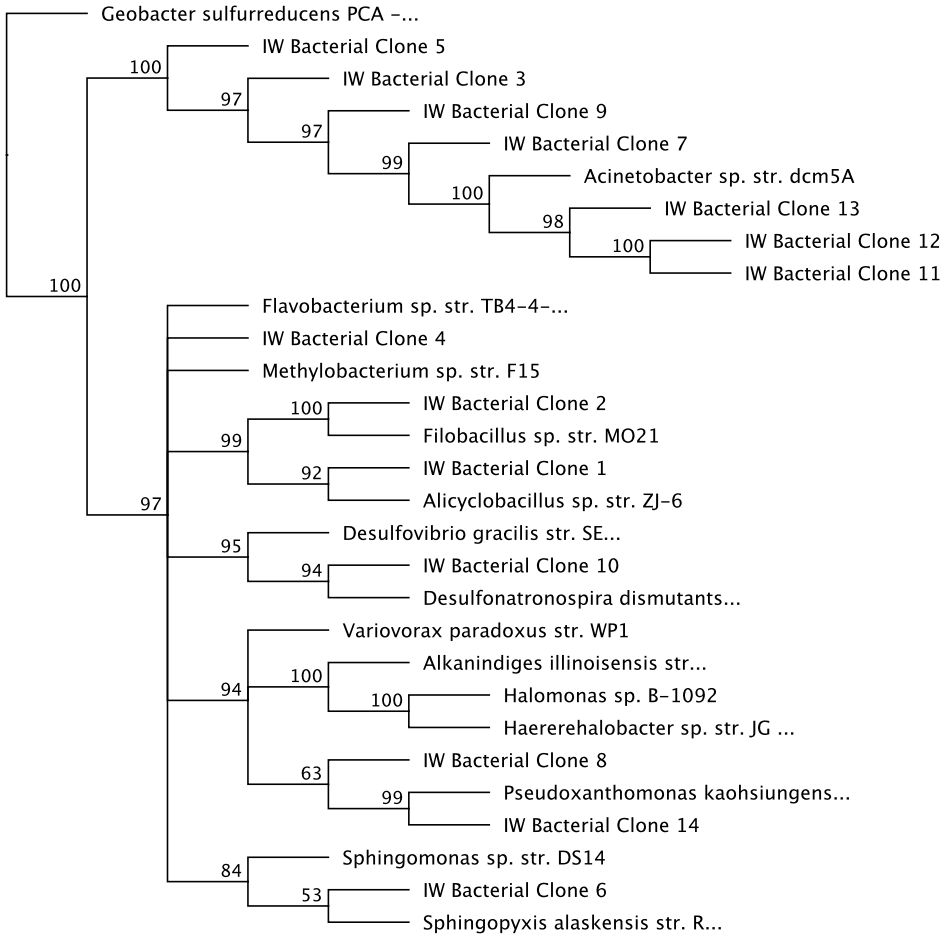



**Figure 4.17. Phylogenetic tree of the 16S rRNA gene-based library obtained from the injection well using the neighbor-joining method. The injection well clones are diverse and include at least four nearest neighbors identified from soils.**

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Sequences at this level were assigned to Desulfonatronospira (22%), Alkanindiges (18%), Desulfovibrio (16%), Alicyclobacillus (13%), Tepidomonas (4%), and 2% of Methylobacterium, Sphingomonas, Sphingopyxis, Delmia, Haererehalobacter, Acintobacter and Psuedoxanthomonas. Although the microbial diversity of the injection well is greater than all other samples based on the number of unique OTU's, the bulk of the community are known sulfate reducing bacteria. Additionally, a large amount of the

clones are related to Acinetobacter, a soil microbe. The other clones are related to halotolerant heterotrophs, soil clone isolates, isolates from oil polluted sites (*Pseudoxanthomonas kaohsiungensis* str. J36-produces extracellular surface activity), and a hydrocarbonoclastic bacterium from an oilfield soil (hydrocarbon degrading bacterium, Bogan et al. 2003).

# Chapter 5: Discussion

## Spatial influence of Injection Well on the Aqueous Geochemistry of Producing Wells

### Major Ion Geochemistry

The spatial influence of the injection well on the aqueous geochemistry of the drill stem test and producing wells was investigated in this study. Research exploring this possible impact is extremely limited in the literature. Our results indicate that major element chemistry does not differ significantly among samples (Appendix D) and all are dominantly enriched in NaCl, with salinities from 186,000 to 200,700 ppm. Due to the high ionic strength of the reservoir brine, dilutions of 1:1500 were made before analyzing the samples. This effectively diluted any minor elements, which could not be measured in this study.

Geochemical logs of total chemical concentrations on a continuous basis with depth were used on well 1-32 to estimate mineral abundances in the producing zone. Well 1-32, drilled in 2011, is the location of the drill stem test collected in this study. Solid phase calcium and magnesium abundance from well 1-32 were provided by researchers from the Kansas Geological Survey (Lynn Watney, pers. comm.) and are plotted as weight percent abundance in figure 5.1. Aqueous phase calcium and magnesium concentrations from wells sampled in the study are plotted as filled in symbols in figure 5.1. Solid phase calcium and magnesium trend opposite of their dissolved phases. The increase in solid phase magnesium abundance and depletion of dissolved phase magnesium (~1128 meters) may be explained by the increase in dolomitization of limestone with depth in the reservoir (figure 5.2). Saturation indices

determined in this study also support the precipitation of dolomite in the majority of wells sampled. Modeling results from wells 73, 69, 53, and 11 along with injection well fluid and the drill stem test were oversaturated with respect to dolomite (Table 4.2).

Stueber et al. (1993) analyzed formation water samples from Mississippian and Pennsylvanian carbonate and siliciclastic strata in the Illinois basin and they suggested that brine compositions were modified through water-rock interactions that included

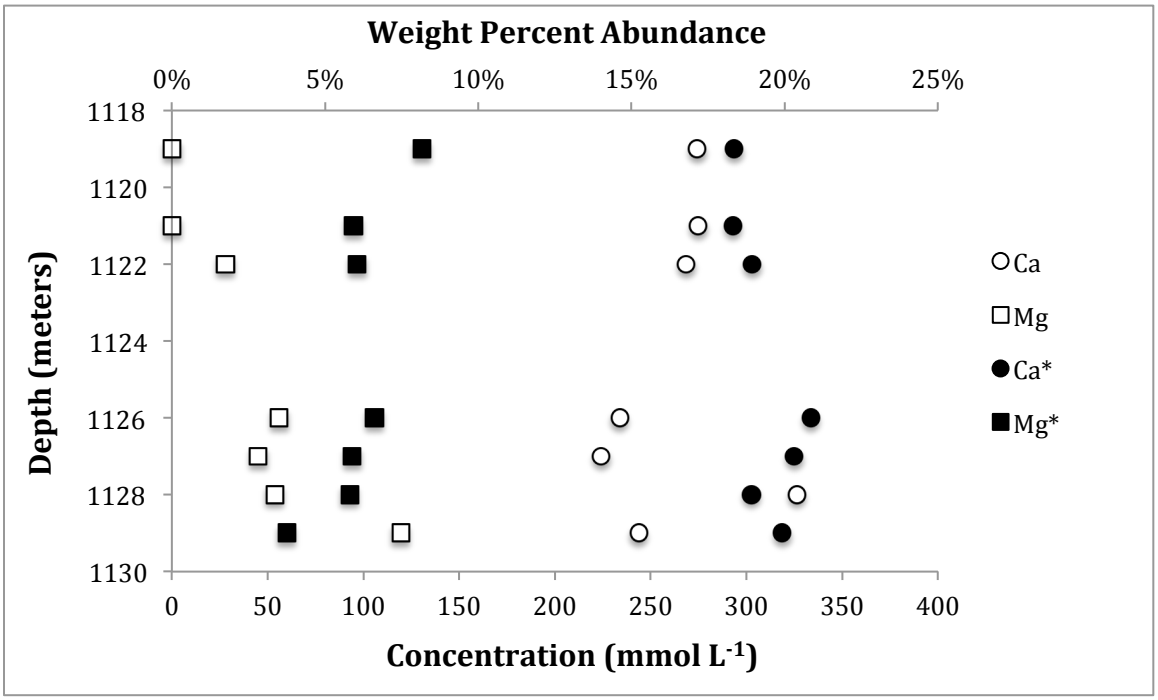


**Figure 5.1. Calcium and magnesium solid phase weight percent abundance (open, unfilled symbols) are plotted with depth from well 1-32 (DST) as determined by researchers at the Kansas Geological Survey. Dissolved phase concentrations (filled symbols) from wells sampled in this study are plotted with depth.**

potassium depletion through interaction with clay minerals and calcium enrichment and magnesium depletion as a result of dolomitization of limestones. Our results show potassium concentrations increasing to approximately 1127 meters then decreasing to 1129 meters (Fig. 4.1) where dolomite is interpreted to increase in the reservoir (Fig.

5.2). Dolomite [CaMg $(CO_3)_2$] forms through a dissolution-reprecipitation reaction in which a calcium carbonate precursor (i.e. limestone) is replaced by dolomite through interaction with magnesium-rich fluids (Kaczmarek and Sibley, 2011) (Eq. 1).

$$2CaCO_3 + Mg^{2+} \text{ è } CaMg(CO_3)_2 + 2Ca^{2+} \quad (1)$$

Our results indicate that dissolved calcium concentrations generally increase with depth while dissolved magnesium concentrations don't fluctuate much between the depths of 1121 and 1128 meters yet begin to decrease at 1128 meters (Fig. 5.1) with the appearance of increased dolomite in the reservoir.

Although major ion geochemistry is similar among wells and saturation indices have been presented in this paper, it is not clear whether this system is in steady state as the injected water flows through the reservoir and is modified by reservoir mineralogy and in turn, modifies the geochemistry of the production wells sampled in this study. In inverse modeling using PHREEQC, one aqueous solution is assumed to react with minerals to produce the observed composition of a second aqueous solution. It is possible to determine mixing fractions and the mass transfer of minerals necessary to produce the

| Solution 1 | Solution 2 | | | | |
|---|---|---|---|---|---|
| **Injection Well** | **Well 73** | **Well 53** | **Well 69** | **Well 60** | **Well 11** |
| Calcite | -9.18E-02 | -2.41E-02 | 9.17E-03 | 1.05E-02 | 1.53E-03 |
| Dolomite | 4.58E-02 | 1.19E-02 | -4.42E-03 | -5.47E-03 | -5.87E-04 |
| Anhydrite | -2.43E-03 | 6.11E-04 | -2.19E-04 | 1.28E-04 | 5.66E-04 |

**Table 5.1 Results of the inverse modeling mixing the injection well aqueous solution with wells 73, 53, 69, 60, and 11. Major mineral reacted in the modeling are calcite, dolomite, and anhydrite. Positive values indicate dissolution reactions and negative values indicate precipitation reactions. Phase mole transfers in millimoles.**

composition of another aqueous solution (Parkhurst et al., 1988). The injection well elemental concentrations were modeled against each of the producing well elemental concentrations using calcite, dolomite, and anhydrite as the major mineral assemblages to

react within the reservoir. Possible precipitation and dissolution reactions that would need to take place to result in the elemental concentration differences between the two solutions as the injected water flowed through the reservoir and mixed with production water were determined and summarized in table 4.7. Output results are phase mole-transfer values. Positive phase mole transfer values indicate dissolution and negative values indicate precipitation.

The injection well sampled in this study, located at the South Erker tank battery (fig 2.2) floods wells 73, 53, and 11. As the water flood travels approximately 61 meters to the location of well 73, the difference in chemical concentrations between the injection well and well 73 suggest that calcite and anhydrite should be precipitating along this flow path while dolomite is dissolving. Well 53 is approximately 873 meters away from the injection well. Inverse modeling results along this flow path indicate that calcite needs to precipitate and dolomite and anhydrite need to dissolve. These results suggest that as the injection water flows through the reservoir it is reacting with reservoir mineralogy which is responsible for the changes we see in chemical composition between the injection well and production well fluids. Lastly, well 11 is approximately 2,385 meters from the injection well. To account for the changes in chemical concentrations between these wells, calcite and anhydrite should dissolve and dolomite should precipitate. The precipitation of dolomite is unlike the two previous scenarios where dolomite was modeled to be dissolving. Decreases in magnesium concentrations as the injected water flowed to well 11 support dolomite precipitation which is also supported by an increase in solid weight percent abundance of magnesium (fig. 5.1) and increased dolomitization of the reservoir from mineralogy interpretations at that depth (fig. 5.2, 1129 meters).

Additionally, a separate water flood location, (fig. 2.2) the Nelson, floods wells 69 and 60. Although this injection water was not sampled in this study, mixing of the three water injection plants is expected to some degree. Wells 69 and 60 that are flooded by the Nelson plant show opposite trends in the dissolution and precipitation of calcite and dolomite when compared with wells 73 and 53 that are flooded by the South Erker (injection well sampled in this study). Flow paths to wells 69 and 60 model dolomite precipitation and calcite dissolution. Again, these results correspond with increased dolomitization of the reservoir with depth (wells 60 and

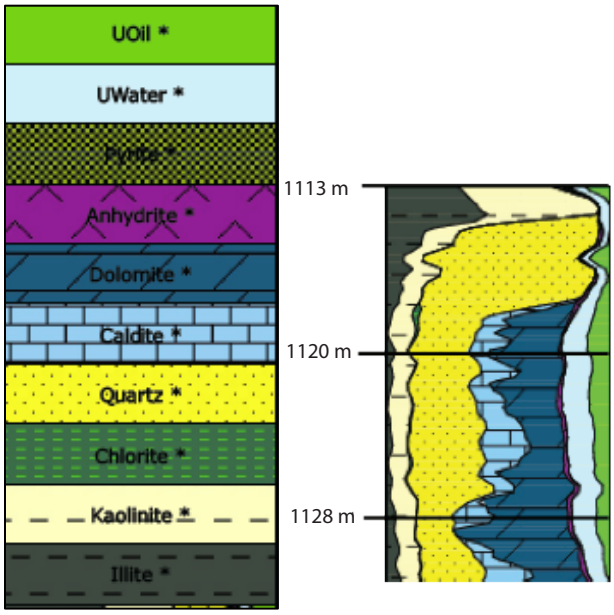


**Figure 5.2. Mineralogy interpretation from the combination of geochemical and conventional logs taken from well 1-32. A chlorite lens is present at a depth of approximately 3660-3674 meters. Provided by the Kansas Geological Survey. UOil-unflushed oil, UWater-unflushed water.**

meters, respectively) Additionally, anhydrite is modeled to precipitate in well 69 and dissolve in well 60. Our interpretation of these results is that the heterogeneity in reservoir mineralogy along the flow path from injected water to producing well plays a large role in the differences we see in major element concentration between samples at

different depths and spatial distances. These water and rock interactions combined with the probable mixing of injection fluids will never necessarily be at a steady state but constantly trying to reach equilibrium between solid and dissolved phases.

The abundance of solid phase silica from well 1-32 is shown in figure 5.3. Weight percent abundance of silica tends to generally trend the opposite of dissolved silica, decreasing with depth and correlates with the increase of dolomite in the reservoir. Unfortunately, dissolved silica concentrations were only measured on the first trip in this study when anions were not measured. Only complete data (anions, cations) collected in November 2011 was entered into PHREEQC for saturation determination. Therefore, the saturation index for silica is unknown, however, figure 5.2 shows a sharp decrease in quartz around 1125 meters which correlates well with the decrease in solid phase abundance and general increase in dissolved silica shown in figure 5.3.

The Mississippian reservoir has been described as heterogeneously complex in chert and dolomite content (Montgomery et al., 1998). The trends in concentrations with depth observed in the major aqueous phase elements collected in this study and compared to mineral abundance determined by researchers at the KGS supports the heterogeneity of the reservoir suggested by Montgomery et al., (1998). Unfortunately, there are no well logs available from the wells sampled in this study to be used for lithological comparison at varying depths. However, as discussed above, research conducted on oil field brines, along with the data presented above, supports our interpretation that these are systems where water-rock interactions play a large role in major element composition of the brine.

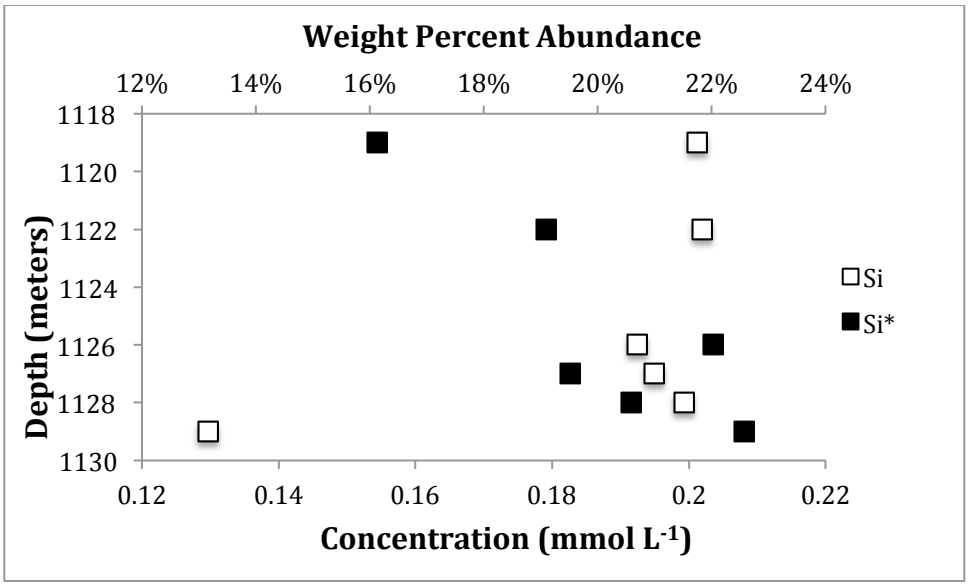


**Figure 5.3. Weight percent abundance silica determined from well 1-32 (unfilled squares) and dissolved silica concentrations determined from wells sampled in this study (filled squares) plotted with depth.**

Additionally, the similarity in major element chemistry between all wells sampled in this study suggests that lithological variations in the reservoir most likely control the vertical distribution of silica and magnesium concentrations and mixing of the Nelson and South Erker water floods combined with lithological heterogeneity of the reservoir controls the precipitation and dissolution reactions modeled in this study. Na and Cl in well 73, closest to the injection well, are approximately ten percent higher than Na and Cl in the injection well. It is hypothesized that the injection waters bypass the shallower well 73.

## Biogeochemistry

Microorganisms have the ability to transform and recycle both organic and inorganic substances for energy and growth of which two important sources are nitrogen, and phosphorous. Our results show that dissolved nitrate ranges from below detection to approximately 1 mmol $L^{-1}$ and generally decreases with depth. Phosphate generally increases with depth and ranges from $4.0x10^{-3}$ to $1.1x10^{-2}$ mmol $L^{-}1$. Zhao et al., (2012)

identified phosphate and nitrate concentrations ranging from below detection to $5.3x10^{-4}$ and $1.8x10^{-2}$ mmol $L^{-1}$ respectively, from four water flooded blocks of two oil fields in northern China. Li et al., (2010) described phosphate concentrations between 3.8 $x10^{-3}$ and 2.9 $x10^{-2}$ mmol $L^{-1}$ and nitrate concentrations ranging from 1.8 $x10^{-2}$ to 1.6 $x10^{-1}$ mmol $L^{-1}$, respectively. Nitrate concentrations from the present study are slightly higher when compared to Zhao et al. (2012) but within the range described by Li et al., (2010). Phosphate concentrations from this study are comparable to Li et al., (2010). In 1958, Alfred Redfield analyzed samples of oceanic marine biomass and proposed they were composed of carbon, nitrogen, and phosphorous in a consistent molar ratio of 106:16:1 (Cleveland and Liptzin, 2007). These so called Redfield ratios inform our understanding of nutrient limitation for microbial populations in terrestrial ecosystems. Our ratios of N:P range from 69.8 in well 11, 79 in well 60, 47.2 in well 69 to 145 in the injection well and 247 in well 73. Petroleum reservoirs are highly complex systems and the availability of phosphate has a large impact on microbial ecology. The apparent phosphate limitation in well 73 may play an important role in microbial diversification. For example, Souza et al. (2008) argued that high microbial endemism could occur as a result of geographical isolation and strong phosphorous limitation, which acts to reduce the intensity of horizontal gene transfer. Additionally, other analyses performed on the sample from well 73 suggest that this zone behaves differently than the others. Sulfate and phosphate concentrations are both lowest in this sample and nitrate concentrations are highest. Well 73 produces the most water and only produces about 1% oil. Figure 5.2 indicates that chlorite is present at the depth of well 73. It is possible that this represents a laterally extensive isolated zone that may have varying effects on the microbiology,

biogeochemistry and reservoir properties that we see. Specific physiologic microbial groups may be partitioning themselves at the oil water interface in this zone and effectively clogging pore space and inhibiting oil recovery.

Nitrate is considered both a nutrient for microorganisms and a terminal electron acceptor. When oxygen is not available, microorganisms can reduce nitrate to nitrite in a process known as denitrification. Nitrate concentrations decrease down to 1126 meters, followed by an increase in concentration to 1129 meters. Culture media was not made for the cultivation of nitrate-reducing microorganisms, however, three clone sequences isolated from the drill stem test show 98% similarity to a known denitrifying bacterium. Hubert and Voordouw (2007) isolated nitrate-reducing bacteria from the Coleville oil field in western Canada. Salinas et al., (2004) isolated a novel nitrate reducing bacterium from an Australian oil reservoir. These results combined with our DNA work suggest that this metabolic pathway may play a role in the reservoir.

Fe(II) concentrations show no general trend with depth in the reservoir. Microbes with the ability to reduce Fe(III) to Fe(II) were cultured in the lab from the injection well and wells 60 and 69. Additionally, sulfate-reducing microbes from the genera Desulfonatronospira have been documented to use Fe(III) as an electron acceptor with Fe(II) formation (Ryzhmanova et al., 2013). DNA analysis in this study (Fig. 5.5) isolated microbes from the genera Desulfonatronospira in the injection well and well 60. These results suggest that iron reduction by microbes may be active in the oil field.

Sulfate concentrations from this study generally do not trend with depth in the reservoir. Figure 4.2 shows that sulfate concentrations don't vary between sample wells much but it is lowest in well 73. Well 73 was drilled to a depth of 1119 meters and figure

5.2 suggests that anhydrite may not be present at this depth as much as the other sampled well depths. Sulfate-reducing microbes were isolated once in this study from well 60.

Results indicate that methane concentrations increase with depth in the reservoir. Methanogens were cultured in the lab for all samples except the drill stem test as identified by measurements of methane on a gas chromatographer. Additionally, DNA sequencing results from well 60 where methane concentrations are the highest suggest the presence of methanogenic archaea. The increase in methane concentrations with depth follows the redox zonation described in many field studies (Ho et al., 2004; Postma and Jakobsen, 1996) where sequential depletion of the major electron acceptors and the appearance of the reduced products generally occur in the order of decreasing free energy yield (Froelich et al., 1979). The typical zonation begins with aerobic respiration, followed by denitrification, manganese reduction, iron reduction, sulfate reduction, and methanogenesis. It has been well documented that methanogens are present in oil reservoirs. Methylotrophic, acetoclastic, and hydrogenotrophic methanoarchaea are thought to have a large role in these environments (Ollivier et al., 1997; Davidova et al., 1997; Obraztsova et al., 1987). Carbon isotopes would help confirm the biogenicity of the methane gas, however, this was beyond the scope of this project.

In sum, the geochemistry suggests that water-rock interaction such as dissolution and precipitation of minerals such as calcite, dolomite, and anhydrite controls the bulk composition of the brines. The biogeochemistry may indicate depth controlled microbial processes such as nitrate-reduction, iron-reduction, sulfate reduction, and methanogenesis rather than input from the injection waters. The major elemental chemistry may be similar between samples but the differences in the biogeochemistry, biomass, and

microbiology with depth suggest that differences in lithology and mineralogy control the distribution of these in the reservoir.

## Biosurfactant Production and Bioclogging

Many processes can lead to decreased permeability in oil reservoirs, including but not limited to, physical and chemical processes that can clog pore spaces within the rock matrix. If secondary recovery techniques such as waterflooding are employed for additional oil recovery, the waterflood can carry fine, suspended solids, that can be deposited in pore spaces that may become clogged over time (Baveye et al., 1998).

Chemical processes that can contribute to pore clogging include precipitation and dissolution reactions. Saturation indices calculated in this study indicate that calcite, anhydrite and dolomite are supersaturated in some of the samples. However, the lowest oil-producing wells, 53 and 60, are undersaturated with respect to these minerals suggesting that precipitation may not be actively contributing to pore clogging in the field.

It is important to identify possible mechanisms that can contribute to decreases in permeability that affect oil production within the Wellington field. Some but not all of these mechanisms have been discussed in the previous paragraphs, however, the present study investigated the relationships between microbial biomass, oil production and biosurfactant production from the Wellington oil field. Our results indicate that microbial biomass is anticorrelated to oil production (Fig. 4.5). Our data strongly supports that wells with lower volumes of oil production harbor higher concentrations of microorganisms and wells producing larger volumes of oil have lower concentrations of microorganisms associated with them. These results suggest that microbial biomass may

be clogging pore space near the well bore environment and impeding oil recovery at the well head (Fig. 5.4).

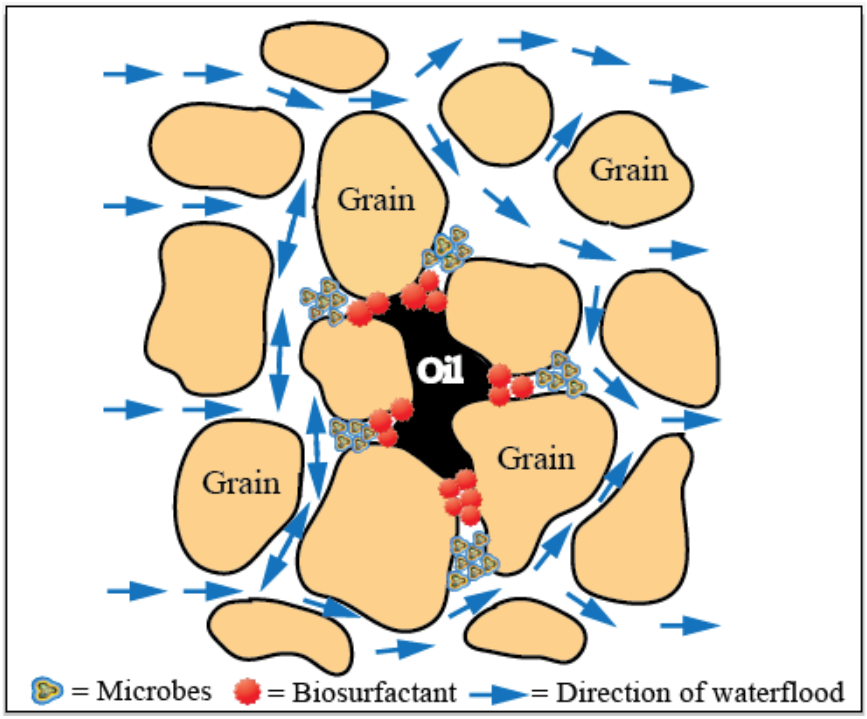


**Figure 5.4. Our interpretation of how microbial biomass may be clogging pore throats in the reservoir and impeding the movement of oil for recovery at the well head. Biosurfact production by microbe (red spheres) has been documented in this study but has no affect on oil production because the oil cannot move due to bioclogging of pore space. The waterflood may not be able to penetrate these zones in the reservoir and may just bypass them without effectively reaching the additional oil trapped in these bioclogged zones.**

Additionally, this study demonstrated the presence of microbial biosurfactants in the Wellington Oilfield, microbial metabolic byproducts that have been demonstrated to increase oil recovery. These surface-active compounds have an ability to lower surface and interfacial tension of liquids and form microemulsions between two different phases (Banat et al., 2010). Specifically, our results indicate that the greatest surface tension reduction (54 dynes $cm^{-1}$ compared to the surface tension of water, 72 dynes $cm^{-1}$) was observed in well 53, where microbial biomass is the highest and oil production is the lowest. This suggests that although biosurfactant is being produced, biomass is clogging

pore space and impeding oil flow to the well head (Fig. 5.4).

Alternatively, well 73 measurements indicate low surface tension but also low percent oil production and the highest water production. Well 73 produces from a zone that may be isolated by the presence of an extensive chlorite lens (Fig. 5.2). This lens may have a significant impact on oil and water production. Microorganisms in this zone may partition themselves at the oil water interface and although surface tension is low, the water flood may not be able to penetrate these zones due to microbial clogging of pore space (Fig. 5.4). There is also evidence for lowered permeability in this zone. Permeability results from core analysis by Weatherford® Laboratories on well 1-32 (Fig 2.2) indicates a reduction in permeability from 60.27 mD at 1118 meters to 8.11 at 1119 meters. Alternatively, the oil could just be located somewhere else where the water injection cannot reach. This hypothesis is supported by the difference in water chemistry between well 73 and the injection well that is so close to it.

Lab experiments have been carried out to identify microorganisms that produce biosurfactants efficiently with the aim of producing these compounds offsite and then delivering them into the subsurface to increase oil recovery. Suthar et al., (2008) screened a species of Bacillus for biosurfactant production and enhanced oil recovery in a lab setting. Results indicate a 43% additional oil recovery amer water extraction in a sand packed column. An alternate method would be to stimulate in-situ production of biosurfactant by either stimulating the indigenous microbial population or injecting known biosurfactant-producing microbes. Youssef et al., (2012) injected nutrients and two strains of Bacillus species into two wells within the Viola formation. Monitoring of production waters indicated in-situ growth of the injected strains, metabolism of the

nutrients, and biosurfactant production. An additional 52.5 $m^3$ of additional oil was recovered during the first 60 days of sampling.

Clogging of porous media by microbial biomass has been documented in the literature from a variety of environments (Brown et al., 2002; Cunningham et al., 1991; Bae et al., 1994; Schillig et al., 2011). Microbial clogging may be caused by the growth of microbial cells in the pore space, by the production of extracellular polymeric substances (EPS), through the microbial generation of gasses, and by the microbially mediated accumulation of insoluble precipitates (Baveye at al., 1998). Bioclogging can cause significant changes in the physical properties of porous media including porosity and permeability changes that influence fluid flow and transport properties (Baveye et al., 1998). Many studies have documented the importance of the EPS in microcolony formation, cohesion, and adhesion (Hand et al., 2008; Baveye et al., 1998). Vandivivere and Baveye (1992) used sand packed columns to demonstrate that biomass accumulation decreased the saturated hydraulic conductivity by four orders of magnitude. EPS production was not responsible for the resulting decrease and instead it was attributed to aggregates of cells that formed plugs within the pores.

Multiple investigations on microbial biodegradation of hydrocarbons have demonstrated that microorganisms position themselves at the oil-water transition zone and that this property is related to cell surface hydrophobicity (Röling et al., 2003; Oldenburg et al., 2009). In addition to strong adhesion to the oil-water interface, Dorobantu et al. (2004) discovered that hydrophobic bacteria possess an affinity for each other, leading to the self-assembly of bacteria at the oil-water interface, which resists coalescence and deformation and may be important for fluid flow through porous media,

such as in petroleum production.

As with many complex systems, pore plugging in the reservoir may be a combination of the biological/chemical/physical mechanisms. However, our results support a model of biologically mediated clogging in the Wellington field. Our data demonstrate a correlation between microbial biomass and oil production. If this correlation did not exist, then other mechanisms aside from microbial activity must be investigated. Baveye et al. (1998) suggests that another way to assess biological involvement is to apply treatments to the wells in question to suppress microbial biomass, which theoretically should result in increased hydraulic activity or, in our study, increased oil production. Production and injection wells in the Wellington oil field receive daily to tri-weekly applications of anti-corrosion compounds with a biocide component. A plausible scenario is that the treatments are not having a desired effect on the microbiology due to built up resistance to the treatment, subtle changes in the community composition or ineffective delivery or concentrations of the treatment. It may be beneficial to collect more well samples or perhaps intact cores and conduct experiments with varying concentrations of the biocide to deduce what is the most effective.

These data lend insight into new strategies to increase oil recovery from the Wellington oil field. As mentioned earlier, biocide treatments may need to be fine-tuned through laboratory investigations on types of treatment compounds and their effective concentrations using biomass concentrations determined in this study. If biomass numbers could be effectively reduced then other options such as injecting nutrients to stimulate biosurfactant production could be applied to enhance additional oil recovery.

Additionally, more experiments to identify reservoir microbes that produce biosurfactants must be carried out to aid in the stimulation process.

## Culture-Based vs. Non Culture-Based Microbiological Techniques

The majority of cultured microbes in this study were distributed between aerobic and anaerobic fermentative bacteria, followed by lower concentrations of methanogens and iron reducing bacteria. Most probable number and direct plate count techniques have been used to quantify active cells in environmental samples (Stevik et al., 1998; Olson et al., 1981). These culture-based approaches are useful for understanding the physiological potential of isolated organisms and can help clarify biogeochemical data. For example, the cultivation of methanogens in this study is supported by the presence of methane gas in the sampled wells. However, these methods can introduce bias through the selection of certain organisms that thrive in the applied cultivation conditions, which may not be suitable for other species (Orphan et al., 2000; Amann et al., 1995; Röling et al., 2003). Due to this disparity between cultivatable and *in situ* diversity, it can be difficult to evaluate the significance of cultured members in resident microbial communities (Orphan et al., 2000; Orphan et al., 2003; Amman et al., 1995). Additionally, sulfate-reducing bacteria (SRB) were only cultured from well 60 once out of two sampling trips, yet DNA work indicates that SRB make up a large portion of the microbial community structure in the reservoir. For this reason, culture independent methods such as DGGE and DNA sequencing were applied in this study to characterize microbial communities.

## DGGE and Phylogenetic Analysis of Microbial Communities

The application of water flooding to petroleum reservoirs helps maintain reservoir pressure and is widely used to enhance oil recovery. Through this process,

microorganism can be introduced into the reservoir with the injected water. Potential applications of microbial enhanced oil recovery have increased investigations into the microbial ecology of petroleum reservoirs and broadened our knowledge of microbial diversity in these environments. Yet, investigations into the microbiology of the injected water and its potential influence on the microbiology of the production fluids are inadequate to date. Zhang et al. (2012) reported that 7 co-existing operational taxonomic units (OTUs) in samples from an injection well and a production well were isolated from a low temperature oil reservoir, indicating related but not necessarily identical microbial community members. Similarities in the microbial ecology between two production wells and an injection well were also reported by She et al. (2005). They found that 30% and 20% of production well sequences were shared with the injection well at the family level. Alternatively, Yuan et al. (2007) used T-RFLP to demonstrate a higher microbial diversity in an injection well compared to production water. Results from field investigations concerning the influence of injected water on the microbiology of production fluids is still very limited, therefore one goal of the present study is to contribute to increasing understanding of this issue. Additionally, studies on the similarities and differences between oil and water phase microbial community structures are scarce and published investigations are typically concerned with biodegraded oil reservoirs. Maia de Oliveira et al. (2008) used DGGE to compare oil and water phase bacterial communities from a non-biodegraded and a biodegraded petroleum reservoir. DGGE results indicated very similar profiles in each phase however, 16S rRNA gene analysis revealed major differences between the phases, suggesting that specific species are responsible for oil biodegradation. It is apparent that the combination of DGGE and

16S rRNA gene analysis is important in characterizing microbial structure and ecology.

DGGE methods were applied in this study to compare similarities and differences in the microbial communities between the injection well and production wells and between the oil and water phases. Qualitative results of a DGGE gel comparing the injection well to producing wells and a drill stem test indicate a high similarity between the injection well and producing wells. Varying intensities of the bands suggest that while there are similar species in common between wells, their relative abundances differ. The drill stem test has a distinct band that is absent in the other samples suggesting it harbors a unique species. The drill stem test microbial community had not been impacted by oil production at the time of sampling. The differences in bands between the producing wells and the drill stem test may be an indication that production processes control the distribution of microbial ecology surrounding the borehole. One explanation for these differences is that production processes are introducing oxygen near the borehole of producing wells, which would influence the microbial ecology and biomass concentrations (Zhao et al., 2012). Unfortunately, microbial biomass numbers were not determined for the drill stem test for comparison.

Qualitative analysis on a gel comparing production wells oil and water phases indicates that there are several species in common to both phases however, again the relative abundances of species are variable. Bands in well 60 water phase are absent in well 60 oil phase suggesting that the microbial ecology is different between phases. Our results qualitatively indicate that both comparisons between the injection well and producing wells as well as the oil and water phases are very similar but also indicate unique differences in the microbial ecology. This initial data suggests that the injection

well does not necessarily influence the species distribution in the producing wells although there are similarities. Oil and water phase DGGE results show some common similarities between wells and phases, however, differences between phases have been demonstrated in this study. One way to understand these differences and identify microbial species more associated with the oil phase would be to excise specific DGGE bands and apply additional DNA sequencing methods. Results could be compared to examples in the literature for comparison to known metabolic pathways and possibilities as to why they are more associated to the oil phase.

Quantitative analysis using GelCompar II somware generated similarity and diversity indices and species richness values based on the detection, number, and intensity of bands. To understand the spatial influence of the injection well microbiology on producing well microbiology, Jaccard similarity coefficients were represented on bar graphs with increasing distance from the injection well and increasing depth. There is a trend of decreasing similarity with distance and depth from the injection well. This not only suggests that only a portion of the microbial community in the injection well is part of the microbial ecology in the producing wells but proposes that there may be a microbial community that has established itself over time and is not impacted by the microbial ecology of the injection water. It is probable that the microbial communities in the producing wells and drill stem test are depth specific and controlled by lithological interactions with reservoir rock. Additionally, surface microorganisms introduced into the physical and chemical conditions of the reservoir may not be able to survive transport (Ren et al., 2011).

Similarities and differences in microbial ecology between oil and water phases are

not readily apparent from gel visualization, however calculated Jaccard coefficients suggest that variances in microbial structure do exist. Shannon-Weaver indices of 1.04 to 1.19 and species richness values from 11 to 17 indicate that the diversity of these samples is fairly low. Although results indicate that there are 17 species present in well 73 oil phase, the low Shannon-Weaver index indicates that the microbial community is dominated by only a few species. There is evidence supporting a chlorite lens (Fig 5.2) at this depth and it is possible that well 73 is located in a zone that has remained undisturbed from microbial input from the injection well. Future work should include DNA work to support the possible endemic nature of the microbial community in well 73 as opposed to the other samples at depths not in the chlorite zone.

Low community diversity is not necessarily atypical in petroleum reservoirs. Ren et al., (2011) used 16S rRNA gene sequencing to demonstrate the relative abundance of sequences at the family level and found that Pseudomonadaceae, Rhodocyclaceae, and Alteromonadaceae represented 73% of the microbial community sampled from an oilfield injection well. Additionally, Pseudomonadaceae and Moraxellaceae represented 100% of the microbial ecology in a producing well. Conversely, Zhao et al. (2012) published Shannon-Weaver indices of 3.46-3.87 as determined from two waterflooded oil fields in China; however, these authors used nearly 200 more clones in their analysis.

It is apparent that significant differences in microbial structure can be difficult to measure using only statistical indices, thus this study applied 16S rRNA gene analysis to better understand compositional differences and similarities in microbial ecology. Oliveira et al., (2008) observed the same discrepancy in results when using DGGE and 16S rRNA methods on oil field samples. The authors suggested it was due to the non-

representativeness of low abundance DNA templates amer PCR amplification of the samples, which are below the detection level of the DGGE technique.

## Phylogeny of Oilfield Microbes

16S rRNA gene analysis was applied to five samples from the Wellington oil field. The resulting phylogenetic analysis provided a more detailed description of the microbial ecology when compared to the DGGE analysis. 17 bacterial OTUs were identified in the injection well, 11 in the drill stem test, eight in well 60 water phase, and 15 in well 60 oil phase. Additionally, two archaeal OTUs were identified in well 60 water. DGGE analysis was not performed on the archaeal communities in the reservoir although PCR revealed the presence of archaea in the injection well and well 60 oil phase.

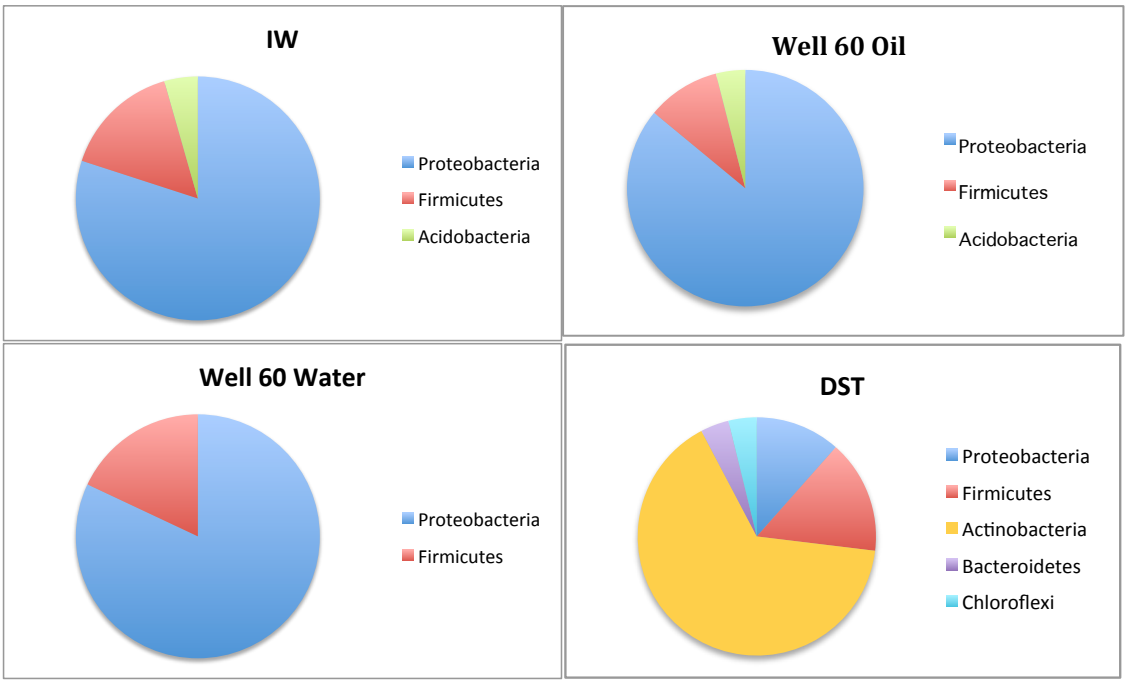


**Figure 5.5. Distribution of bacterial phyla from sampled wells and drill stem test. Proteobacteria dominates the injection well and well 60 oil and water phases. Actinobacteria are the majority in the drill stem test. Methanosarcinales is the only identified archaeal phylum from well 60 water phase (data not shown). IW-injection well, DST-drill stem test.**

Figure 5.5 illustrates the various bacterial phyla identified from the Wellington oil field. Other investigations were found to compare 16S rRNA results from similar oil reservoir environments. Species from phyla Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Chloroflexi, and Methanolobus were identified from two 27°C oil reservoirs (Pham et al., 2009; Wang et al., 2012). Acidobacteria, Firmicutes, Proteobacteria, and Methansarcinales were isolated from a 20°C petroleum reservoir in Alberta, Canada (Grabowski et al., 2005). These same phyla were also identified in the Wellington oil field fluids and will be discussed in more detail. Sequences from all samples were organized into phyla and genera in figures 5.5 and 5.6, respectively. On the genus level, results show that the injection well has greater species richness and is generally more diverse than the production wells and drill stem test. This is most likely due to the introduction of surface microbes during injection that do not survive transport throughout the reservoir. The drill stem test has more phyla represented, however, the diversity is dominated by the genus Xylanimicrobium (figure 5.5), known facultative anaerobes that are carbon degraders (Stackebrandt and Schumann, 2004).

The injection well sample showed relation to *Acinetobacter sp. str. Dcm5A* that was isolated from an estuarine environment and able to degrade dichloromethane (Gonzalez et al., 2001). *Alkanindiges illinoisensis* was identified from crude-contaminated soil and demonstrated an ability to degrade hydrocarbons (Bogan et al., 2003). Genera within the Acidobacteria phylum represent a metabolically and genetically

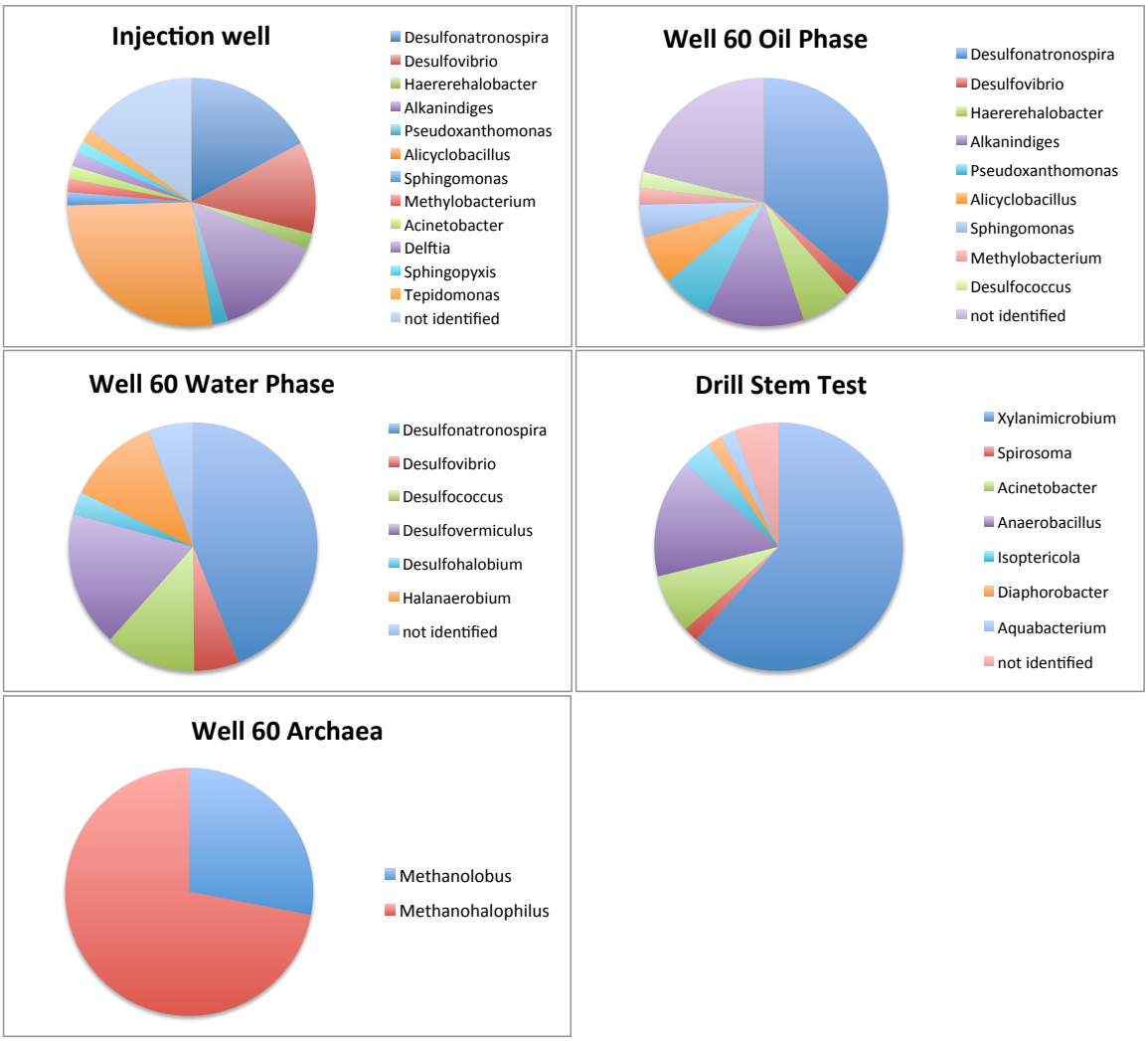


**Figure 5.6. Pie charts showing the distribution of genera in the Wellington oil field samples. Injection well is slightly more diverse than well 60 oil phase and clearly more diverse than well 60 water phase. The drill stem test does not share any genera with the other samples. The arcaeal diversity in well 60 is extremely low.**

diverse group and are found in soils, freshwater habitats, hot springs, wastewaters and others (Miqueletto et al., 2011). *Alicyclobacillus sp. str. ZJ-6* was isolated from a copper mine and is capable of ferrous and sulfur oxidation. (Guo et al., 2009). Members of the Bacillus genera have also demonstrated the ability to produce biosurfactants (Ruez-Garcia et al., 2005; Yakimov et al., 1995). The genus Tepidomonas is related to a thermophilic bacterial isolate from a bioreactor (Mohn and Yu, 1999). Lastly, a fairly

large percentage of genera distribution is known sulfate-reducers (Sorokin et al., 2008; Feio et al., 2004). The large variety of genera isolated from the injection well compared to production wells and their known association with surface environments suggests that these are most likely introduced during the process of oil/water separation in above ground tanks and re-entry of the water flood into the subsurface. The sulfate-reducing isolates, however, are most likely indigenous to the reservoir as they are common in all producing wells. Additionally, isolates from the Bacillus genera that are known biosurfactant producers may be stimulated in-situ to increase oil production.

The drill stem test does not share OTUs with any of the samples. This sample represents an environment not affected by the oil production process and provides an opportunity to compare microbiology between the production wells. One interesting observation is that there is a cluster of clones on the phylogenetic tree that is more closely related to each other and share less identity to reference sequences in the database. This suggests that these sequences may represent new phylotypes that have not been entered into the DNA databank. This is not uncommon in petroleum reservoirs where new species are sequenced frequently and may represent long-term isolation for this clade of species and potential microbial endemism (Souza et al., 2008; Ramette and Tiedje, 2007). For example, Li et al., (2007) used 16S rRNA gene analysis to describe bacterial and archaeal community structure from a high temperature oil field and found that 50% of archaeal sequences and 16.7% of bacterial sequences showed less than a 97% sequence similarity with known sequences. Li et al., (2010) identified six phylotypes from an oil field in China that showed less than 92% sequence similarity to their nearest database entries and suggested they may belong to an unknown phylotype. The majority of the

sequences belong to the phylum Actinobacter and more specifically to the genus Xylanimicrobium. Xylanimicrobium are known facultive anaerobes that are carbon degraders (Stackebrandt and Schumann, 2004). It is possible that this genus is utilizing the oil as a carbon source as it is readily bioavailable. The second largest distribution of genera is Anaerobacillus. Bacillus species strain AMnr1 is a known dissimilatory manganese reducing haloalkaliphile from Soda Lakes (Sorokin and Muyzer, 2010).

It has been proposed that drilling muds introduce exogenous organisms into the reservoir (Struchtemeyer et al., 2011) rendering drill stem tests an unlikely representation of native microbial community structure. Struchtenmeyer et al., (2010) identified many anaerobic sulfate-reducing lineages such as *Desulfovibrio* from drilling muds used in drilling processes in the Barnett Shale in northern central Texas. In their study, they used the presence of sulfate-reducing bacteria in the production water to have originated from the drilling mud. In this study, sulfate-reducing bacteria are present in all samples except for the drill stem test. Additionally, another study isolated *Xylanimonas* from a drill stem that was taken from the same well sampled in this study. Researchers ran batch studies at reservoir temperatures and pressures with the isolated organisms and *Xylanimonas* was able to survive throughout the study (Djuna Gulliver, pers. comm.). These results suggest that *Xylanimicrobium* is indigenous to the reservoir and not a contaminant of the mud or a surface microbe. Based on the uniqueness of the OTUs from the drill stem test when compared to the injection and production wells, it is likely that production processes in the field have an affect on the microbial communities within the reservoir.

Well 60 oil phase is the second most diverse sample aside from the injection well. Although this sample shares eight OTUs with the injection well, clones on the

phylogenetic tree near the bottom cluster together and are not closely related to databank sequences. This suggests that they represent a special, interrelated group that have adapted to the oil phase environment. They may represent new species or diverging strains as 21% of the sample could not be identified at the species level in Greengenes. *Alkanindiges illinoisesis*, the closest relative of this group of clones has been isolated from oil field soils and is a known degrader of hydrocarbons. Additionally, *Pseudoxanthomonas kaohsiungensis* was isolated from an oil-polluted site and has demonstrated the ability to produce biosurfactant and reduce surface tension from 68 to 32.6 dynes/cm (Chang, 2005). The relatedness of the clones to known biosurfactant producers and oil biodegraders may present an opportunity to exploit them *in-situ* for enhanced oil recovery techniques. The largest distribution (36%) of the microbial community is related to sulfate-reducing microorganisms (Sorokin et al., 2008). Well 60 water phase shares two OTUs with the injection well and three OTUs with its oil phase. All of the closest relatives identified are known halophilic sulfate-reducing bacteria or halophilic fermentative bacteria. Approximately 80% of the OTUs identified are known to be active sulfur cycling bacteria.

These data demonstrate that sulfate-reduction is a major metabolic pathway in production and injection wells. Our sulfate data, however, show no definitive trend with depth. Our sulfide measurements are may be unreliable due to interferences in the colorometric analysis caused by the high concentration of salt ions. Additionally, elemental sulfur was not measured due to the high dilution factor applied to the samples before analysis on the ICP-OES. Therefore, it is not possible to assess microbial S-cycling with depth. Researchers at the Kansas Geological Survey used geochemical and

conventional logs for mineralogical interpretation and both their data and ours show evidence for increasing $CaSO_4$ (anhydrite) with depth. Changes in anhydrite abundance most likely controls the concentration of sulfate in the reservoir. Nevertheless, it is apparent that sulfate-reducing microorganisms are present in the reservoir because they make up the bulk of the microbiology of the injection well and well 60 oil and water phases.

The presence of archaea in oil reservoirs has been documented in many microbiological investigations of oil field brines (Ren et al., 2011;Orphan et al., 2000; Li et al., 2007; She et al., 2005; Pham et al., 2009). PCR with archaeal primers was applied to the oil and water phases of the production wells, the drill stem test and the injection well. Positive PCR products were recovered from the injection well and the oil and water phases of well 60. Unfortunately, the PCR products of the injection well and well 60 oil phase were too weak and could not be used in cloning reactions. Therefore, well 60 water phase is the sole archaeal sample that was sequenced. Only two OTUs were identified and belong to the genera *Methanolobus* and *Methanohalophilus*. *Methanolobus* and *Methanohalophilus* have been identified as obligate methylotrophs that metabolize methanol and methylamines to methane and ammonium (Cornish-Shartau et al., 2010; Orphan et al., 2000). Methane concentrations generally increase with depth in the Wellington oil reservoir and specifically, methane concentrations in well 60 are the highest. It is evident that archaeal diversity is low in the reservoir, however methanogenesis is still an important microbial pathway.

Comparison of the injection well microbiology to a drill stem test and oil and water phases of a production well have demonstrated their similarities and differences in

microbial ecology. One explanation that the injection well is slightly more diverse in microbial structure than the producing wells and drill stem is the amount of dissolved oxygen, nutrient availability and temperature between the groundwater recirculation system and subsurface oil strata (She et al., 2005; Yuan et al., 2007). Oxygen can be introduced with injected water during water flooding, leading to the detection of abundant aerobic bacteria in oil reservoirs (Zhao et al., 2012). It is unknown how far oxygen penetrates into the reservoir due to scavenging by organic matter and redox sensitive dissolved metals, however, dissolved oxygen was not measured in this study because of the sampling protocol that led to up hole oxygenation. Down hole temperature was also not measured in this study, however, injected water measured at the surface before re-injection is likely to be cooler than production water. Additionally, when the injected water is in the oil-water separation tanks, microorganisms associated with the tanks and pipes are most likely re-introduced into the subsurface but are not detected in producing wells because they do not survive transport.

The injection well shares eight OTUs with well 60 oil phase and two OTUs with well 60 water phase. The common microbiology between the injection and production wells may represent a unique community that has been able to survive and evolve in the reservoir conditions over time. The three species identified to only be present in the injection well are most likely surface microorganisms that do not survive transport through the reservoir.

# Chapter 6: Conclusions

Samples were collected for microbial and geochemical analysis on five production wells, one injection well, and one drill stem test from the Wellington oil field. Geochemical analyses suggest that dissolution and precipitation of calcite, dolomite, and anhydrite controls the bulk composition of the brines and the biogeochemistry indicates depth controlled processes mediated by methanogenesis and sulfate reduction rather than input from the injection waters.

Microbial biomass concentrations and oil production values from each well suggest that bioclogging near the borehole may be a major process inhibiting oil recovery.

Surface tension measurements indicate the presence of microbial biosurfactants where microbial biomass concentrations are highest and oil production is lowest. These microbial byproducts are known to increase oil production, however, due to bioclogging, the biosurfactants have no effect on oil recovery.

DGGE and DNA results indicate that the microbial similarities between the production and injection wells may represent species that have been able to thrive and survive and evolve in this environment over time. Species isolated from the injection well but not from any of the production wells likely represent exogenous species introduced at the surface and that cannot survive transport into the reservoir. The drill stem test harbors a unique microbial community, only sharing two operational taxonomic units on the phylum level and none on the genus level. This result is likely due to the effect of oil production processes on the microbial ecology near the borehole and are consistent with geochemical and biogeochemical data.

Lastly, biosurfactant-producing microbes identified in the literature have been isolated in this study such as *Pseudoxanthomonas kaohsiunge0nsis* and species of Bacillus. It may be possible to exploit these organisms in-situ through the introduction of nutrients and energy sources into the reservoir that will increase biosurfactant production and consequently improve oil recovery. Prior to this the bioclogging issue needs to be addressed by trying to target the microbial communities responsible through the testing of various biocides. Additionally, more lab studies need to be applied to better understand biosurfactant-producing indigenous microbes to target those who are most efficient at doing so. Once this is better understood, steps can be taken to initiate field trials to stimulate those communities and to monitor whether this application could be beneficial to additional oil recovery in the Wellington oil field.

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# Appendix

## Appendix A: Chemical Treatment Compositions

CRO195 Corrosion Inhibitor (from Baker Petrolite)

| Composition and Information on Ingredients | | |
|---|---|---|
| **Name** | **CAS #** | **% by Weight** |
| Light aromatic naptha | 64742-95-6 | 30-60 |
| 1, 2, 4-Trimethylbenzene | 95-63-6 | 10-30 |
| Phosphates | Trade secret | 5-10 |
| Quaternary ammonium chloride | Trade secret | 5-10 |
| 1, 3, 5-Trimethylbenzene | 108-67-8 | 5-10 |
| Amine derivative | Trade secret | 1-5 |
| 1, 2, 3-Trimethylbenzene | 526-73-8 | 1-5 |
| Methanol | 67-56-1 | 1-5 |
| Xylene | 1330-20-7 | 1-5 |
| Fatty Acids | Trade secret | 1-5 |
| Fatty Acids | Trade secret | 1-5 |
| 1, 4-Dioxane | 123-91-1 | 0.1-1 |

WCW5827 Wetting Agent (from Baker Petrolite)

| Composition and Information on Ingredients | | |
|---|---|---|
| **Name** | **CAS #** | **% by Weight** |
| Quaternary ammonium compounds | Trade secret | 10-30 |
| Methanol | 67-56-1 | 10-30 |

Appendix B: Core Descriptions of the Mississippian Reservoir Provided by Lynn Watney, Kansas Geological Survey.

**3707; 3698.4**; 10YR 6/2; pale yellowish brown; silt sized dolomite; microcrystallized chert tripolite; oil stained; microcrystalline porosity increasing upward; massive; scattered vugs; pinpoint to sum-cm vugs; no body fossils or skeletal debris; no black chert or chert nodules; gradational contact

**3698.4; 3691**; 10YR 6/2; pale yellowish brown; matrix is fine microporous dolomite/chert (tripolite); alternating cm-size bedding with darker bands with increased gray banded chert; irregular complex bands; pore space/vugs in chert bands; fractures concentrated in the chert bands; lenticular dolomite has associated vuggy porosity; tripolite is uniformly porous and oil stained; discontinuous chert nodules; gradational contact

**3691; 3667**; 10YR 6/2; pale yellowish brown; chert breccia; matrix is microporous chert (tripolite); brecciated chert clasts; gray nonporous porcelain chert clasts angular infilled with tripolite; massive; fresh chert surrounded by interclast porosity; same chert seen in nodules below; interclast porosity; coarse breccia; pore space/vugs in chert bands; (cave fill?); very porous and permeable; fractures; tripolite looks dissolved; preserving more porcelain chert; **3668-3667.4** is infilled between chert with grayish yellow green 5GY 7/2 clay; **3667.4-3667** is breccia with cm-size chert clasts partially silicified less connected porosity a brickwork with grayish yellow green 5GY 7/2 shale fill; sharp contact

**3667; 3665**; 10YR 6/2; pale yellowish brown; massive chert; composed of cm-size clasts and fine breccia clasts all silicified together; scattered cavities subhorizontal lenses of grayish yellow green 5GY 7/2 shale; few fractures; clasts of oil stained tripolite giving a brown mottled appearance; shale laminae increase upwards; sharp contact
**3665; 3664.2**; 10YR 6/2; pale yellowish brown; massive; chert; multi-cm chert clasts infilled with grayish yellow green 5GY 7/2; chert clasts are corroded; autoclastic breccia; bed of grayish yellow green 5GY 7/2 shale

**3664.2-3662.2**; 10YR 6/2; pale yellowish brown; siliceous autoclastic breccia; tripolite; nodules and lenses of porcelain gray-green chert in a matrix of tripolitic chert; oil stained; incipient autoclastic breccia; subvertical fractures common; tripolitic chert is incipient autoclastic breccia; matrix is a more broadly distributed tripolite in which autoclasts have formed; oil staining in tripolites; sharp contact
**3662.2; 3662.1**; grayish yellow green; 5GY 7/2; shale; thinly bedded with chert; opaque very pale orange 10YR 8/2 chert; module of pelleted silicified wackestone to packstone; bioclasts within chert; sharp contact

**3662.1; 3658.3**; grayish yellow green; 5GY 7/2; massive; chert; several-cm- sized brick like rectangular chert with corroded edges in matrix; chert; peloidal crinoidal peloid packstone to grainstone; autoclastic breccia; chert is rimmed with 10YR 6/2; light

brownish gray and grades out to N3 dark gray; matrix is mortar-like silt sized quartz and chert grains; **3660-3658.6** grades up from incipient to autoclastic breccia; sharp contact

**3658.3; 3657.7**; 10YR 6/2; light brownish gray; conglomerate; isolated chert clasts; calcareous siltstone matrix; sharp contact

# Appendix C: Medium Formulations For Most Probable Number Analysis (Bekins et al., 1999)

Aerobic and Anaerobic Difco Marine Agar 2216 Composition
Peptone…….5.0 g
Yeast Extract……5.0 g
Ferric Citrate…….0.1 g
Sodium Chloride……..19.45 g
Magnesium Chloride……....8.8 g
Sodium Sulfate…….3.24 g
Calcium Chloride……1.8 g
Potassium Chloride……..0.55 g
Sodium Bicarbonate…….....0.16 g
Potassium Bromide……..0.08 g
Strontium Chloride……...0.034 g
Boric Acid……....0.022 g
Sodium Silicate…….0.004 g
Sodium Fluoride.……..0.0024 g
Ammonium Nitrate……...0.0016 g
Disodium Phosphate…….0.008 g
Sodium Chloride……...50 g
Distilled Water……..dilute to 1000 ml

Autoclave media at 121°C

Anaerobic media; followed formulation above and prepared in an anaerobic chamber.

For Cultivation of Iron-Reducing Bacteria
Sodium Acetate……1.5071 g
NaHCO3……...2.5 g
CaCl2*H2O……..0.1 g
KCl……...0.1 g
NH4Cl……..1.5 g
KH2PO4……...1.0 g
MgCl2*6H2O……...0.1 g
MgSO4…….0.0488 g
Sodium Chloride……..50 g
Trace Metal Solution……....9 ml (see formulation below)
Vitamin Solution.…….……5 ml (see formulation below, add amer autoclaved)
0.5 M Fe3+ ions……...200 ml
Distilled Water…….dilute to 1000 ml

Prepare 0.5 M Fe3+ ions by making a 0.5 M FeCl3 solution in distilled water, titrate to pH 7 using HCl and NaOH. Prepare media anaerobically in anaerobic chamber. Autoclave for 15 minutes at 121°C. Add the vitamin solution via filter syringe amer

media has cooled. Amer inoculation, aseptically pressurize the MPN bottles using a 70:30 mix of H2:CO2 for ~10 seconds.

Trace Metal Solution
ZnSO4*7H2O……...0.10 g
MnCl2*4H2O….....0.03 g
H3BO3……..0.3 g
CoCl2*6H2O…….....0.2 g
CuCl2*2H2O…..……..0.01 g
NiCl2*6H2O……0.02 g
Na2MoO4*2H2O……..0.03 g
Distilled Water……..dilute to 1000 ml

Adjust the final pH of Trace Metal Solution to 3.4 with HCl.

Wolfe's Vitamin Solution
Biotin……2.0 mg
Folic Acid…….2.0 mg
Pyridoxine Hydrochloride……10.0 mg
Thiamine*HCl……..5.0 mg
Riboflavin…….5.0 mg
Nicotinic Acid……..5.0 mg
Calcium D-(+)-pantothenate……5.0 mg
Vitamin B12…….0.1 mg
p-Aminobenzoic Acid……..5.0 mg
Thioctic Acid……5.0 mg
Distilled Water…….dilute to 1000 ml

Filter-sterilize into Iron-Reducing Bacteria Media amer it has been autoclaved.

For Cultivation of Sulfate Reducing Bacteria
Na2SO4…….3.0 g
KH2PO4……....0.2 g
NH4Cl……...0.3 g
KCl……0.5 g
CaCl2*2H2O…….0.15 g
NaCl……...5.0 g
MgCl2*6H2O……0.4 g
Sodium Acetate……1.688 g
Trace Metal Solution……9.0 ml
Vitamin Solution……...4.0 ml (add amer autoclaved)
Distilled Water……..dilute to 1000 ml

Prepare anaerobically with resazurin and cysteine. Autoclave for 15 minutes at 121°C and filter-syringe the vitamin solution in the anaerobic chamber. Amer inoculation, aseptically pressurize the MPN vials with a 70:30 mix of H2:CO2 for ~10 seconds.

For Cultivation of Methanogens
KH2PO4……..0.75 g
K2HPO4……..0.89 g
MgCl2*6H2O……..0.36 g
NH4Cl……..0.9 g
NaCl…….5.0 g
Trace Metal Solution……9.0 ml
Vitamin Solution……..3.0 ml
Sodium Acetate……1.5071 g
Sodium Formate……...2.5 g
Distilled Water…….dilute to 1000 ml

Prepare anaerobically with resazurin and cysteine. Autoclave for 15 minutes at 121°C. Add the vitamin solution by filter-syringe in the anaerobic chamber amer media has been autoclaved. Amer inoculation, aseptically pressurize MPN vials with a 70:30 mix of H2:CO2 for ~10 seconds.

# Appendix D: Aqueous Geochemistry Data

Data concentrations are in mmol $L^{-1}$, except as noted. Non-measurements denoted with an asterisk. Analyses from November 2011. Silica data from August 2010.

| Sample Well | Total Depth (m) | pH | Temperature °C | Salinity (ppm) | Ionic Strength | Total Carbon | DOC | DIC |
|---|---|---|---|---|---|---|---|---|
| 73 | 1119 | 5.64 | 32.3 | $2.07x10^5$ | 4.19 | 0.42 | 0.31 | 0.11 |
| DST | 1121 | 5.88 | 42.1 | $1.86x10^5$ | 3.65 | 2.66 | 1.70 | 0.96 |
| IW | 1122 | 5.84 | 28.2 | $1.98x10^5$ | 3.83 | 1.04 | 0.81 | 0.23 |
| 53 | 1126 | 5.45 | 31.1 | $2.04x10^5$ | 4.02 | 0.78 | 0.29 | 0.48 |
| 69 | 1127 | 5.98 | 21.5 | $1.88x10^5$ | 3.76 | 1.96 | 1.54 | 0.42 |
| 60 | 1128 | 5.48 | 23.3 | 1.94x105 | 3.77 | 0.90 | 0.52 | 0.38 |
| 11 | 1129 | 6.35 | 25.8 | $1.97x10^5$ | 3.79 | 1.02 | 0.43 | 0.59 |

| Sample Well | Total Depth (m) | Ca | K | Mg | Na | Si |
|---|---|---|---|---|---|---|
| 73 | 1119 | 293 | 7.3 | 130 | 2787 | 4.3 |
| DST | 1121 | 292 | 9.1 | 94 | 2520 | * |
| IW | 1122 | 303 | 8.5 | 96 | 2526 | 5.0 |
| 53 | 1126 | 333 | 9.7 | 105 | 2751 | 5.6 |
| 69 | 1127 | 325 | 12.8 | 94 | 2526 | 5.1 |
| 60 | 1128 | 302 | 9.5 | 92 | 2455 | 5.8 |
| 11 | 1129 | 318 | 9.4 | 60 | 2596 | 6.0 |

| Sample Well | Total Depth (m) | Cl | Br | Fl | Alkalinity (meq/L) |
|---|---|---|---|---|---|
| 73 | 1119 | 3868 | 6.10 | 0.11 | 0.95 |
| DST | 1121 | 3193 | 4.72 | * | 1.34 |
| IW | 1122 | 3504 | 4.73 | 0.11 | 1.12 |
| 53 | 1126 | 3479 | 5.56 | 0.11 | 1.03 |
| 69 | 1127 | 3274 | 3.84 | 0.21 | 1.56 |
| 60 | 1128 | 3453 | 5.27 | 0.40 | 1.05 |
| 11 | 1129 | 3421 | 5.22 | 0.12 | 0.99 |

| Sample Well | Total Depth (m) | $Fe^{2+}$ | $SO_4$ | $NO_3$ | $PO_4$ | $S^2$ | $CH_4$ | $CO_2$ |
|---|---|---|---|---|---|---|---|---|
| 73 | 1119 | 0.31 | 3.64 | 0.658 | 0.004 | 0.0012 | 0.10 | 11.23 |
| DST | 1121 | 0.34 | 6.47 | * | * | * | * | * |
| IW | 1122 | 0.25 | 5.68 | 0.534 | 0.005 | 0.0011 | 0.00 | 4.59 |
| 53 | 1126 | 0.34 | 6.12 | 0.000 | 0.005 | 0.0012 | 0.18 | 13.93 |
| 69 | 1127 | 0.17 | 5.55 | 0.286 | 0.008 | 0.0012 | 0.25 | 6.39 |
| 60 | 1128 | 0.31 | 5.80 | 0.393 | 0.007 | 0.0013 | 0.52 | 5.35 |
| 11 | 1129 | 0.26 | 6.14 | 1.055 | 0.011 | 0.0012 | 0.15 | 7.97 |

# Appendix E: Berexco Barrel Test Data

BEREN/BEREXCO - BARREL TESTS **2011/05/01** To **2011/11/31**

**TF**-Total Fluid

| Date | Well | Pump Size | TF | % Oil | Total Oil |
|---|---|---|---|---|---|
| 2011/05/05 | 11 | 2.00 | 172.00 | 2.00 | 3.44 |
| 2011/09/07 | 11 | 2.00 | 172.00 | 2.00 | 3.44 |
| 2011/11/15 | 11 | 2.00 | 129.00 | 2.00 | 2.58 |

| Date | Well | Pump Size | TF | % Oil | Total Oil |
|---|---|---|---|---|---|
| 2011/05/05 | 44 | 1.50 | 86.00 | 3.00 | 2.58 |
| 2011/09/07 | 44 | 1.50 | 86.00 | 3.00 | 2.58 |
| 2011/11/15 | 44 | 1.50 | 86.00 | 3.00 | 2.58 |

| Date | Well | Pump Size | TF | % Oil | Total Oil |
|---|---|---|---|---|---|
| 2011/05/05 | 53 | 1.75 | 257.00 | 1.00 | 2.57 |
| 2011/09/07 | 53 | 1.75 | 229.00 | 1.00 | 2.29 |
| 2011/11/15 | 53 | 1.75 | 257.00 | 1.00 | 2.57 |

| Date | Well | Pump Size | TF | % Oil | Total Oil |
|---|---|---|---|---|---|
| 2011/05/05 | 60 | 1.50 | 72.00 | 1.50 | 1.08 |
| 2011/09/07 | 60 | 1.50 | 114.00 | 2.00 | 2.28 |
| 2011/11/15 | 60 | 1.50 | 72.00 | 1.50 | 1.08 |

| Date | Well | Pump Size | TF | % Oil | Total Oil |
|---|---|---|---|---|---|
| 2011/05/05 | 69 | 1.50 | 58.00 | 5.00 | 2.90 |
| 2011/09/07 | 69 | 1.50 | 62.00 | 5.00 | 3.10 |
| 2011/11/15 | 69 | 1.50 | 66.00 | 6.00 | 3.96 |

| Date | Well | Pump Size | TF | % Oil | Total Oil |
|---|---|---|---|---|---|
| 2011/05/05 | 73 | 2.00 | 386.00 | 1.00 | 3.86 |
| 2011/09/07 | 73 | 2.00 | 229.00 | 1.00 | 2.29 |
| 2011/11/15 | 73 | 2.00 | 372.00 | 1.00 | 3.72 |